EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

COST Action CM1201

Biomimetic Radical Chemistry

WG1 & WG3 Meeting

WG1: Radical enzymes

WG3: Membrane stress, signalling and defences

Programme & Abstract book

Lodz (Poland), 9 - 11 September 2015

GENERAL INFORMATION

REGISTRATION DESK / VENUE

Focus Hotel Lodz
Lakowa str. 23/25
90-554 Lodz, Poland
phone: +48 42 637 12 00, e-mail: lodz@focushotels.pl

Registration desk opening since 08:00 on 09.09.2015

ORGANIZING COMMITTEE

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TIMETABLE

PLEASE NOTE: THE FORMAT OF LECTURES IS EITHER 45 + 15 MIN OR 20 + 10 MIN

Timing	*Wednesday 09.09*	*Thursday 10.09*	*Friday 11.09*
8:00 – 8:45	Registration	-	-
8:45 – 9:00	Opening remarks	-	-
9:00 – 10:00	**C. Ferreri**	**B. Golding**	**D.M. Smith**
10:00 – 10:30	**G.R. Mendeluk**	**F.J. Dekker**	**P. Paneth**
10:30 – 11:00	**E.E. Pohl**	**A. Sikora**	**G. Ionita**
11:00 – 11:30	*Coffee break*	*Coffee break*	*Coffee break*
11:30 – 12:00	**S. Sasson**	**A. Cort**	**C.M. Jäger**
12:00 – 12:30	**G. Giacometti**	**J. Krych-Madej**	**N. Rabbani**
12:30 – 13:00	**B. Mihaljević**	**A. Dudzik**	**I. Smonou**
13:00 – 14:30	*Lunch*	*Lunch*	*Lunch*
14:30 – 15:00	**L. Valgimigli**	**M. Seemann**	Discussions
15:00 – 15:30	**B. Gemici**	**M. Číž**	
15:30 – 16:00	**N. Rohr-Udilova**	**R. Silaghi-Dumitrescu**	
16:00 – 16:30	*Coffee break*	*Coffee break*	*Coffee break*
16:30 – 17:30	**M. Orfanopoulos**	*Guided tour and dinner*	*Free time*
17:30 – 18:00	**G. Gescheidt**		
18:00 – 18:30	**C. Houée-Levin**		

CONFERENCE PROGRAMME

Wednesday, September 9th

8:00 – 8:45	Registration
8:45 – 9:00	Opening remarks
	Chair: L.WOZNIAK
9:00 – 10:00	**Carla Ferreri** Membrane lipidomics of cancer cells: dietary-induced remodeling and cell fate
10:00 – 10:30	**Gabriela Ruth Mendeluk** Sperm lipidomic in view of recent transcriptomic profiling studies
10:30 – 11:00	**Elena E. Pohl** Regulation of membrane transporters by reactive aldehydes
11:00 – 11:30	*Coffee break*
	Chair: C. HOUÉE-LEVIN
11:30 – 12:00	**Shlomo Sasson** Altered metabolism of polyunsaturated fatty acids in diabetes
12:00 – 12:30	**Giorgia Giacometti** Application of biophotonics to an integrated approach with membrane lipidomics and clinical evaluation for early diagnosis of autism
12:30 – 13:00	**Branka Mihaljević** Resveratrol and its reactions with biologically relevant free radicals
13:00 – 14:30	*Lunch*
	Chair: F. DEKKER
14:30 – 15:00	**Luca Valgimigli** The catalytic antioxidant behaviour of nitroxides under mild acidic conditions: surprising role of the oxoammonium ion
15:00 – 15:30	**Burcu Gemici** Effects of hydrogen sulfide on gastric acid secretion and gastric emptying in rats
15:30 – 16:00	**Nataliya Rohr-Udilova** Impact of trans fat on liver disease: a project concept
16:00 – 16:30	*Coffee break*
	Chair:C. CHATGILIALOGLU
16:30 – 17:30	**Michael Orfanopoulos** Singlet oxygen: Discovery, reaction mechanisms and applications in organic synthesis

17:30 – 18:00	**Georg Gescheidt** Radicals and radical ions in the action of anitoxidants
18:00 – 18:30	**Chantal Houée-Levin** Induction of oxidative stress through interaction of TiO_2 nanoparticles with NADPH oxidase

Thursday, September 10th

	Chair: C. FERRERI
9:00 – 10:00	**Bernard T. Golding** How do enzymes tame radicals?
10:00 – 10:30	**Frank J. Dekker** Novel chemical biological methods for detection of enzyme activity in inflammation
10:30 – 11:00	**Adam Sikora** Detection of reactive oxygen and nitrogen species with the use of boronate probes – the mechanistic aspects
11:00 – 11:30	*Coffee break*
	Chair: B.GOLDING
11:30 – 12:00	**Aysegul Cort** Effects of bleomycin and antioxidants on the fatty acid profile of testicular cancer cell membranes
12:00 – 12:30	**Justyna Krych-Madej** Reactive oxygen species-mediated catalase inhibition and its biological consequences
12:30 – 13:00	**Agnieszka Dudzik** Stickland-reaction enzymes from *Clostridium sporogenes*
13:00 – 14:30	*Lunch*
	Chair: S. SASSON
14:30 – 15:00	**Myriam Seemann** Mechanistic investigations of a $[4Fe\text{-}4S]^{2+}$ enzyme involved in the MEP pathway, a target for the development of new antimicrobials
15:00 – 15:30	**Milan Číž** Interactions of serotonin with myeloperoxidase
15:30 – 16:00	**Radu Silaghi-Dumitrescu** (Per)oxidation cascades induced by metallo proteins: analytical uses, mechanistic insights
16:00 – 16:30	*Coffee break*
16:30 –	*Guided tour and dinner*

Friday, September 11th

Chair: R. SILAGHI-DUMITRESCU

9:00 – 10:00	**David M. Smith** Non-enzymatic ribonucleotide reduction in the prebiotic context
10:00 – 10:30	**Piotr Paneth** Theoretical study of the *cis*-dihydroxylation of nitrobenzene catalyzed by nitrobenzene dioxygenase
10:30 – 11:00	**Gabriela Ionita** Spin labelled polysaccharides – suitable probes for investigating supramolecular assemblies
11:00 – 11:30	*Coffee break*

Chair: G. MENDELUK

11:30 – 12:00	**Christof M. Jäger** The radical-mediated deazapurine ring contraction in QueE – the enzyme's and the metal's role
12:00 – 12:30	**Naila Rabbani** Application of glycation markers for clinical diagnostics
12:30 – 13:00	**Ioulia Smonou** Enzyme-catalyzed reductions for the synthesis of valuable chiral intermediates
13:00 – 14:30	*Lunch*
14:30 – 16:00	Discussion
16:00 – 16:30	*Coffee break*

Abstract book

Texts of abstracts have not undergone any linguistic correction.

Talks are presented by time order.

Membrane lipidomics of cancer cells: dietary-induced remodeling and cell fate

Carla Ferreri,[a] Anna Sansone,[a] Roberta Scanferlato,[a] Letizia Polito,[b] Andrea Bolognesi[b]

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It is known that nutritional status influences the cell membrane composition, inducing the remodeling of the fatty acid components and consequently the vitality and metabolic fate of the cells. We reported the neuroblastoma cell line behavior treated with a saturated fat diet (palmitic acid) which suggested the efficacy of a nutritional strategy to assist with the antitumoral effects.[1] On this basis, the cell line of colon cancer cell (Caco-2) was used to study in more detail the lipid remodeling, especially the saturated fatty acid pathway occurring under normal and various dietary conditions, with a specific attention to the saturated (SFA) vs. monounsaturated fatty acid (MUFA) pathways monitoring the two main biomarkers, palmitoleic (9*cis*-16:1) and sapienic (6*cis*-16:1) fatty acids. New insights on the metabolism of cancer cells will be provided, with the role of metabolic transformations involving the effect of sapienic acid as a newly discovered biomarker of MUFA biosynthesis. Also, the role of phospholipase A2 enzyme will be highlighted as immediate cell response to the nutritional stimuli, which is activated and causes a profound membrane lipid remodeling, having different course under the various experimental conditions.
These results are expected to have an impact on the research of vesicle interaction with natural membranes and metabolic effects of the anticancer drugs, taking into account the participation of cell membrane organization and signaling in the whole cell reactivity.

References:
[1] Bolognesi, A. et al. *PLoSOne* **2013**, *8(2)*: e55537; doi: 10.1371/journal.pone.0055537.
[2] Sansone, A., et al. *Chem. Res. Toxicol*. **2013**, *26*, 1703-1709

Notes

Sperm lipidomic in view of recent transcriptomic profiling studies

Gabriela Ruth Mendeluk[a], Julia Ariagno[a], Mariano Isaac Cohen[b], František Liška[c], Carla Ferreri[d], Chryssostomos Chatgilialoglu[e]

a) Laboratory of Male Fertility, Hospital de Clínicas "José de San Martín", INFIBIOC, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina; b)Urology Division, Hospital de Clínicas "José de San Martín", University of Buenos Aires, Buenos Aires, Argentina; c) Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Czech Republic; d) ConsiglioNazionaledelleRicerche (CNR), ISOF Bio Free Radicals, Bologna, Italy; e)Institute of Nanoscience and Nanotechnology, National Center of Scientific Research, Athens, Greece.

Decreasing the number of men affected by infertility has become a top priority for many health organizations, including Healthy People 2020. The aim of this presentation is to approach the pathogenesis of male infertility from a lipidomic point of view either environmental, namely nutritional, and genetical, employing a transcriptomic design.

To evaluate the effect of nutrition we studied a group of ten "idiopthatic infertile" patients having a typical "Western pattern diet", who did not consume fish. They were examined by the andrologyst and nutritionist staff, and in parallel by lipidomics, evaluating erythrocyte and sperm cell membranes fatty acids before and after 3 months supplementation with omega-3.Conventional sperm assay was performed according to WHO criteria- 2010, a CASA system was employed to assess kinetic parameters. Low levels of EPA and DHA and increased values of the saturated fatty acid/monounsaturated fatty acid and ω6/ω3 ratios in red blood cells membrane phospholipids were revealed. EPA, DHA, the ω6/ω3 ratio and arachidonic acid in erythrocytes were good predictors of EPA in sperm (R: 0, 9882) (β: -0,422; 2,307; 1,498; 0,571 respectively). Omega-3 supplementation for three months was then indicated. After treatment an increase in EPA ($p<0$, 000002) and DHA ($p<0$, 02) was clearly observed in erythrocytes while no changes in sperm parameters nor in sperm membrane lipidomic profile were observed. Only two out of the studied patients were asthenospermic, the rest were severely comprised being oligoasthenoteratospermic

For our genetic study,108 semen samples were considered for molecular biological studies. We analyzed the transcriptome of 12 sperm samples using Affymetrix HuGene2.1ST microarrays. 4 participants were fertile donors, 4 samples were from infertile patients that presented low sperm motility (asthenospermia), and 4 samples with low motility as well as abnormal morphology (asthenoteratospermia).When comparing the asthenoteratospermic to controls samples, most of the 11 included meaningful differentially controlled genetic networks involved in regulation of sperm development and morphology. Comparison of asthenospermic to controls samples revealed partial overlap with the former. However, there were more genes associated with intermediate metabolism that includes some pathways associated to lipids, mainly cholesterol.

To our knowledge this is the first report on a mathematic equation that could predict EPA in sperm by EPA, DHA, the ratio ω-6/ω-3, as compared with optimal values found in literature andarachidonic acid in erythrocyte membranes, thus reflecting the real impact of nutrition on individual male reproductive health in regard to fatty acid intake. Transcriptomic profiling is a promising tool that may allow to identify common deregulated genetic pathways implied in sperm membrane lipidomics.

Notes

Regulation of membrane transporters by reactive aldehydes

Elena E. Pohl

Institute of Physiology, Pathophysiology and Biophysics, University of Veterinary Medicine, Vienna, Austria

Reactive oxygen species and its derivatives are involved in a variety of physiological and pathophysiological cellular processes. The molecular mechanisms of this universality are elusive. We discuss a novel mechanism by which reactive aldehydes (RA) influence a transport function of membrane proteins. We employ electrophysiological methods, mass spectrometry, EPR, molecular dynamic simulations, etc. to show that the activity of uncoupling protein 1, valinomycin and CCCP depends on the formation of phosphatidylethanolamine – RA-adducts, their position in the lipid bilayer and biophysical properties of the membrane affected by RA. Taken together, our results explain the diversity of aldehyde action on cytosolic and membrane cell proteins.

References:

1. Malingriaux EA, Rupprecht A, Gille L, Jovanovic O, Jezek P, Jaburek M, Pohl EE (2013) Fatty Acids are Key in 4-Hydroxy-2-Nonenal-Mediated Activation of Uncoupling Proteins 1 and 2. PLoS ONE 8:e77786

Notes

Altered metabolism of polyunsaturated fatty acids in diabetes

Shlomo Sasson

Institute got Drug Research, Dept. of Pharmacology
Faculty of Medicine, The Hebrew University of Jerusalem, Israel

The roles polyunsaturated fatty acids (PUFA) in cells is dual: they are incorporated in membrane bi-layers in cells when acylated to the *sn-2* position in phospholipids. PUFA are also the source of myriad metabolites that have numerous signaling functions via interaction with specific and selective receptors. These metabolic pathways include cyclooxygenase enzymes, various lipoxygenase isoforms and cytochrome P450 epoxidases and hydrolases. Their action gives rise to many metabolites that further undergo metabolic transformation by other enzymes to generate over 100 different bioactive lipids in cells. PUFA are also subjected to non-enzymatic transformation, mainly hydroxyl radical-driven peroxidation that results in the generation of 4-hydroxyalkenals. When present at normal levels the latter exhibit signaling properties by modulating signaling pathways and by activating peroxisome proliferator-activated receptors and their transcriptional regulatory functions. However, severe oxidative environment augments the peroxidation flux of PUFA towards the generation of very high levels of 4-hydroxyalkenal that can damage cells by forming covalent adducts with macromolecules. The ability of cells to protect their intracellular environment against these deleterious effects determines their fate under extreme conditions like nutrient overload, oxidative stress and similar stressful stimuli. These pathways and protective mechanisms in insulin secreting beta cells under normal and diabetic conditions will be discussed.

Notes

Application of biophotonics to an integrated approach with membrane lipidomics and clinical evaluation for early diagnosis of autism

Giorgia Giacometti[a,b] and Carla Ferreri[a]

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Biophotonic methodologies have been applied to the study of morphological and cytoskeletal abnormalities of red blood cells (RBC) in spherocytosis[1], systemic lupus erythematosus[2], as well as in Rett Syndrome[3]. In this work, hyperspectral dark-field microscope (HDFM), currently used in biomedical and food analyses[4-6], was for the first time used to characterize the differences between RBCs of healthy and ASD (autism spectrum disorders) children aged 6 to 12 years. HDFM spectral signatures, characterized by a library of eight endmembers, were determined from RBCs of healthy children (n = 12) (**Fig 1)** using whole blood. The comparison with RBC spectral signatures of autistic children (n = 12) showed significant differences in one of the eight spectra. A significant correlation was also seen between the spectral signatures of healthy children and the membrane lipid asset, as expressed by the comprehensive indicator named unsaturation index (double bond contribution of fatty acids composing membrane phospholipids). Interestingly, also clinical variables (estimated by the collaboration with the Maggiore Hospital, Dr. Paola Visconti) such as CARS, ADOS, hyperactivity, stereotypies, correlated with some spectral signatures in ASD children. Altogether, these results suggest the possibility of a novel integrated diagnostic signature of the disease.

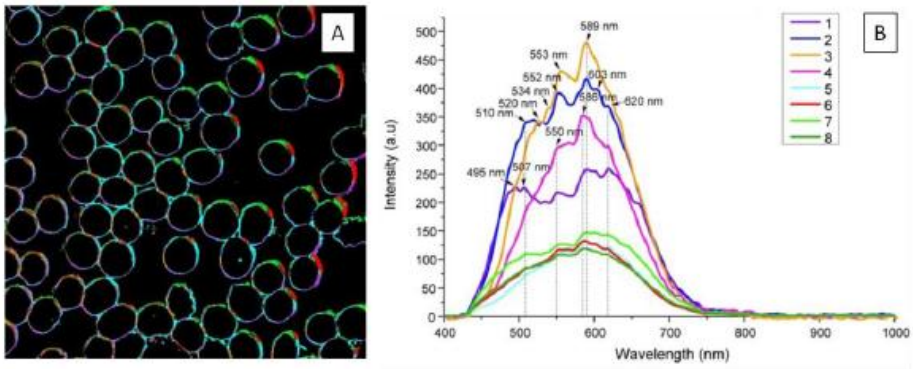

Fig 1. (A) A representative hyperspectral image of RBCs from healthy children; (B) Map of the spectral endmembers in the hyperspectral image of the sample obtained by SAM analysis.

References:

[1] Barcellini W. et al., *Blood Transfus.*, 9, 274-277 (2011)
[2] Ghiran I.C. et al., *Arthritis Rheum*., 63, 503-512 (2011)
[3] Cortelazzo A. et al., *PLoS ONE*, 9(3): e93181 (2014)
[4] Lu G. et al., *J. Biomed. Opt.*, 19, 010901 (2014)
[5] Siddiqi A.M. et al**.,** *Cancer Cytopathol.*, 114, 13-21 (2008)
[6] Sacco Verebes G. et al., *J. Biophotonics*, 11-12, 960-967 (2013)

Notes

Resveratrol and its reactions with biologically relevant free radicals

Branka Mihaljević,[a] Iva Džeba and Ivana Tartaro Bujak

Ruđer Bošković Institute, Radiation Chemistry and Dosimetry Laboratory, Bijenička 54., 10000 Zagreb, Croatia;

It is well known that natural compound *trans*-resveratrol (*trans*-3,5,4'-trihydroxystilbene) possesses significant biological activity. The paucity of studies of antioxidant mechanism of resveratrol suggests that further work is required to fully understand its protective role in living organisms. The main goal of our research is to investigate systematically reaction kinetics and mechanisms of *trans*-resveratrol (*trans*-3,5,4'-trihydroxystilbene) with rarely researched biologically relevant free radicals which form under conditions of oxidative stress and consequent induce damages of all macromolecules in cells. All radicals and excited states of resveratrol were generated with ruby or Nd:YAG laser flash or electron accelerator pulse. Short-lived intermediate species of resveratrol and its reaction kinetics with oxygen, sulphur and carbon-centered radicals and aromatic carbonyl triplets were determined with transient absorption spectroscopy.[1] Measurements were conducted at time scales from 10^{-6} s to 10^{-12} s in the organic solvents or in a solvent mixture water/alcohol. Results of this research could contribute to the understanding of the mechanism and reaction kinetics of resveratrol antioxidant activity with free bioradicals which are known to be responsible for the malfunction of the normal processes in living cells.

In order to expand this research towards reaction kinetics we have researched the influence of *trans*-resveratrol on the reactions induced by free radicals in lipid model systems, in which evaluation of the competition between radiation induced lipid peroxidation and *trans*-isomerisation process in the presence of thiol can be studied.[2] A model micellar system was developed in which radical processes of linoleic acid were selectively determined under conditions that, in contrast to the physiological, could be adapted to the experiments requirements.[3]

References:

[1] Džeba, Iva; Pedzinski, Tomasz; Mihaljević, Branka. Photophysical and photochemical properties of resveratrol. *Journal of Photochemistry and Photobiology A: Chemistry.* 299 (2015) 118-124.

[2] Mihaljević, Branka; Tartaro, Ivana; Ferreri, Carla; Chatgilialoglu, Chryssostomos. Linoleic acid peroxidation vs. isomerization : a biomimetic model of free radical reactivity in the presence of thiols. *Organic & biomolecular chemistry.* 9 (2011) 3541-3548.

[3] Mihaljević, Branka; Tartaro, Ivana; Filipović-Vinceković, Nada. Evaluation of a model system for the selective study of the lipid peroxidation process. *European journal of lipid science and technology*. 114 (2012) 1292-1303.

Notes

The catalytic antioxidant behaviour of nitroxides under mild acidic conditions: surprising role of the oxoammonium ion

Luca Valgimigli

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2,2,6,6-Tetramethylpiperidine-N-oxyl (TEMPO) is the best know nitroxyl radical. Being one electron away from hydroxylamines and oxoammonium ions, nitroxides have a privileged redox position that is responsible for their role as oxidation catalysts. The same chemistry is also the basis for the superoxide dismutase mimetic activity. Considerable interest has emerged in the biological activities of nitroxides, which have been ascribed to their antioxidant activity, but the mechanistic rationale behind it could be elucidated only recently.[1] It is due to formal hydrogen-atom transfer from the protonated nitroxyl radical to oxidative chain-carrying peroxyl radicals, hence it requires acidic conditions. In the presence of strong pTSA (10 mM) k_{inh} as high as 1.8×10^{8} $M^{-1}s^{-1}$ was recorded at 30°C in acetonitrile, while with weak carboxylic acids k_{inh} was up to 1.5×10^{6} $M^{-1}s^{-1}$ outperforming α-tocopherol.[1] An important point remained to be elucidated: when weak acids are used as the proton source, the TEMPO-inhibited autoxidations have apparently infinite inhibited period and the concentration of TEMPO shows nearly no decay during the reaction. This catalytic behavior implies that the oxidized TEMPOnium ion is recycled during the autoxidation, i.e. it is reduced back to the nitroxyl radical under oxidative conditions! We will provide evidence that this reduction is performed at diffusion controlled rate ($k \sim 10^{10}M^{-1}s^{-1}$) by alkyl radicals, which get oxidized to the corresponding carbocations. The reaction is sufficiently fast to outcompete the reaction of alkyl radicals with molecular oxygen to form the chain-carrying peroxyl radicals.

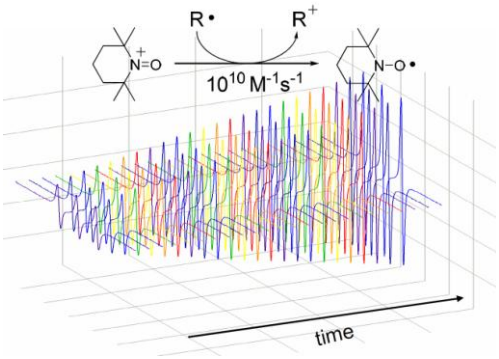

Figure. Growth of the EPR signal of TEMPO during the autoxidation of cumene at 30°C in the presence of TEMPOnium ion.

References:

1. Amorati, R.; Pedulli, G. F.; Pratt, D.A.; Valgimigli, L. *Chem. Commun.*, **2010**, *46*, 5139–5141

Notes

Effect of hydrogen sulfide on gastric acid secretion and gastric emptying in rats

Burcu Gemici & John L. Wallace

Near East University, Nicosia, Turkish Republic of Nothern Cyprus, McMaster University, Hamilton, Canada

Background: Hydrogen sulfide (H_2S) is a gaseous mediator with physiological and patho-physiological roles in many organs. In the gastrointestinal tract, H_2S exerts cytoprotective and anti-inflammatory effects, as well as modulating smooth muscle contraction and blood flow. The mechanisms for the cytoprotective actions of H_2S in the stomach are not clear, but it is well documented that H_2S-releasing nonsteroidal anti-inflammatory drugs (NSAIDs) do not produce damage in the stomach. The aim of the present study was to investigate the effects of an H_2S releasing derivative of naproxen, ATB-346 on gastric acid secretion and gastric emptying in rats.

Methods: Male, Wistar rats (n=5-6/group) were fasted overnight, then treated orally with vehicle, naproxen (20 mg/kg) or an equimolar dose of ATB-346 or of the H_2S-releaing moiety of the latter drug. Thirty min later, under anesthetic, the abdomen of each rat was opened and the pyloric sphincter was ligated. The rats were allowed to recover from the anesthetic and were left for 3 hours, after which they were euthanized and the contents of the stomach were collected for measurement of gastric acid secretion. In other experiments, fasted rats were allowed access to 1.6 g of food for 10 min, after which the food was removed and weighed. 90 minutes later, the rats were anesthetized and the stomach was carefully excised so that the amount of food remaining in the stomach could be determined. The amount of gastric emptying was the difference between the amount ingested and the amount recovered from the stomach.

Results: Treatment with naproxen did not significantly affect the volume or pH of gastric secretion. In contrast, both the volume and pH of gastric juice were reduced following treatment with ATB-346, such that titratable acidity was 87% lower in this group than in the naproxen-treated group ($p<0.05$). Treatment with the H_2S-releasing moiety of ATB-346 did not significantly reduce gastric titratable acidity. None of the treatments had a significant effect on gastric emptying. ATB-346 suppressed gastric prostaglandin synthesis to the same extent as naproxen (>90%; $p<0.01$).

Conclusions: The ability of ATB-346 to reduce the acidity of gastric juice may contribute to the lack of gastric damage in rats treated with this drug, despite its ability to markedly suppress prostaglandin synthesis.

Notes

Impact of trans fat on liver disease: a project concept

Nataliya Rohr-Udilova

Medical University of Vienna, Austria

Background / hypothesis: The prevalence of non-alcoholic fatty liver disease (NAFLD) in Western countries reaches 30%, with increasing tendency. The dramatic increase is facing limited therapeutic options. NAFLD evolves to non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma (HCC), liver transplantation being often the only cure. Overnutrition is a known risk factor. However, poor patient compliance in sustained lifestyle modifications reveals a great need for efficient drugs, which could not be developed from previously identified mechanisms.

NAFLD and the successive liver diseases represent common comorbidities of cardiovascular disorders, which are clearly linked to dietary trans fat. Besides dietary sources, trans fat was recently discovered to be formed endogenously by radical-triggered cis-trans isomerisation of fatty acids. Both radicals and cis fatty acids are elevated in NAFLD and NASH thus creating conditions favourable for endogenous hepatic trans fat formation.
We aim to investigate a causative role of trans fat in liver diseases. We hypothesize that hepatic trans fats - of both dietary and endogenous origin – change membrane structure and cell metabolism favoring cholesterol accumulation in microcrystals which induce sterile inflammation and radical generation. Radicals induce TFA by cis-trans fatty acid isomerization in a vicious cycle resulting in NAFLD, NASH and HCC.

Methods: Trans fatty acids (TFA) will be profiled in human NASH and HCC liver samples, then screened in cultured human and mouse liver cells. The most abundant and most active compounds will be further investigated in mouse NASH and HCC models. Cholesterol and microcrystals will be analysed using GC, fluorescent probes, and confocal laser-scanning microscopy. Mitochondrial respiration, cell proliferation, apoptosis, lipid peroxidation, migration, and cell cycle will also be studied. Activated pathways will be identified by microarray analysis.

Innovation: Our proposal combines novel ideas with innovative methods of investigation. Endogenous formation of trans fats and their suspected causal effects on NAFLD, NASH and HCC represent a completely new concept for disease understanding and prevention. We dispose of a complete, worldwide unique library of trans fatty acids. This library, in combination with newly developed resolution techniques, allows to profile hepatic TFAs in previously unmatched multiplicity and accuracy. The assumed key role of radicals, if proven, opens the possibility to prevent and treat disease by specific radical scavenging drugs. Overall, this project constitutes an innovative synergism between advanced chemical analytics and scrutiny of new concepts of liver disease.

Notes

Singlet oxygen: discovery, reaction mechanisms and applications in organic synthesis

Michael Orfanopoulos and Mariza N. Alberti

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Singlet molecular oxygen ($^1O_2$, $^1\Delta_g$)[1] plays a crucial role in the environment,[2] degradation of materials,[3] as well as in biological[4] and therapeutic processes.[5] Over the last several years, the use of $^1O_2$ as a reagent in organic synthesis has been receiving continuous and remarkable attention.[6]

The more classical $^1O_2$ reactions are: (1) the [4+2] cycloaddition with conjugated dienes or anthracenes to yield endoperoxides, (2) the [2+2] cycloaddition with enol ethers, enamines or electron-rich alkenes to yield 1,2-dioxetanes, and (3) the so-called ene or Schenck reaction with alkenes to form allylic hydroperoxides.

The mechanism of the singlet oxygen allylic oxygenation of alkenes[7] (the singlet oxygen *ene* reaction) was the subject of earlier[8] and more recent controversy.[9,10] The main question is whether this reaction is concerted, involves intermediates such as dipolar, biradical, perepoxide, or is a two- step no-intermediate mechanism.[9]

We present in-here the discovery and applications of singlet oxygen in organic synthesis and focus on mechanistic possibilities under the light of a) stereoisotopic studies of the singlet oxygen *ene* reaction with simple alkenes[11] and b) singlet oxygen photooxidations involving "free-radical clock" substrates. These and previous results show that this reaction is a highly stereospecific suprafacial process.[12]

References:

[1] Schweitzer, C.; Schmidt, R. *Chem. Rev.* **2003**, *103*, 1685.
[2] Scurlock, R. D., Wang, B., Ogilby, P. R., Sheats, J. R., and Clough, R. L. *J. Am. Chem. Soc.* **1995**, 117, 10194
[3] Ranby, B.; Rabek, J. F. In *Photodegradation, Photooxidation and Photostabilization of Polymers*; Ed.; Wiley: London, **1975**.
[4] Foote, C. S.; Clennan, E. L. In *Active Oxygen in Chemistry*; Foote, C. S., Valentine, J. S., Greenberg, A., Liebman, J. F., Ed.; Chapman and Hall: London, **1995**.
[5] (a) Kuimova, M. K.; Botchway, S. W.; Parker, A. W.; Balaz, M.; Collins, H. A.; Anderson, H. L.; Suhling, K.; Ogilby, P. R. *Nature Chem.* **2009**, *1*, 69. For selected reviews of photodynamic therapy, see: (b) Dougherty, T. J.; Gomer, C. J.; Henderson, B. W.; Jori, G.; Kessel, D.; Korbelik, M.; Moan, J.; Peng, Q. *J. Natl Cancer Inst.* **1998**, *90*, 889.
[6] (a) Hoffmann, N. *Chem. Rev.* **2008**, *108*, 1052. (b) Montagnon, T.; Tofi, M.; Vassilikogiannakis, G. *Acc. Chem. Res.* **2008**, *41*, 1001.
[7] Alberti, M. N.; M. Orfanopoulos, M. *Chem. Eur. J.* **2010,** *16*, 9414
[8] Orfanopoulos, M.; Smonou, I.; Foote, C. S. *J. Am. Chem. Soc.* **1990**, *112*, 3607
[9] Singleton, D. A.; Hang, C.; Szymanski, M. J.; Meyer, M. P.; Leach, A. G., Kuwata, A.T.; Chen, J. S.; Greer, A.; Foote, C. S.; Houk, K. N. *J. Am. Chem. Soc.* **2003**, *125*, 1319
[10] Sheppard, A. N.; Acevedo, O. *J. Am. Chem. Soc.* **2009**, *131*, 2530
[11] Alberti, M. N.; Orfanopoulos M. *Org. Lett.*, **2008,** *10*, 2465
[12] Alberti, M. N.; Vassilikogiannakis, G.; Orfanopoulos, M. *Org. Lett.*, **2008**, *10*, 3997

Notes

Radicals and radical ions in the action of anitoxidants

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The reactivity of various polyphenols in terms of their antioxidative potential has been investigated. Products formed upon deprotonation/odidation, established by assays and by time-resolved techniques (EPR, CIDNP) are compared and discussed.[1,2]

The regioselectivity of H-abstraction reactions will be addressed (Figure).

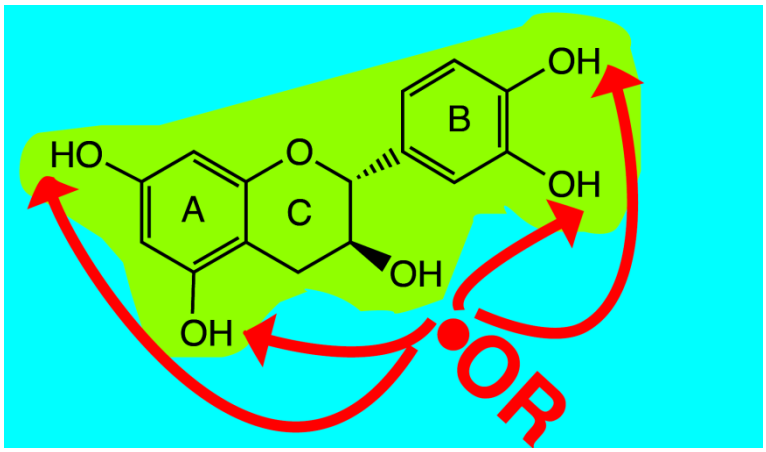

Figure. Regioselectivity in H-abstraction reactions from non-conjugated polyphenols.

References:

[1] Neshchadin, D.; Batchelor, S. N.; Bilkis, I.; Gescheidt, G., Short-lived Phenoxyl Radicals Formed from Green-Tea Polyphenols and Highly Reactive Oxygen Species: An Investigation by Time-Resolved EPR Spectroscopy. *Angew. Chem. Int. Ed.* **2014,** *53*, 13288-13292.

[2] Neshchadin, D.; Levinn, R.; Gescheidt, G.; Batchelor, S. N., Probing the antioxidant activity of polyphenols by CIDNP: From model compounds to green tea and red wine. *Chem.--Eur. J.* **2010,** *16* (23), 7008-7016.

Notes

Induction of oxidative stress through interaction of TiO_2 nanoparticles with NADPH oxidase

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Titanium dioxide (TiO_2) anatase nanoparticles (NPs) are metal oxide NPs commercialized for several uses of everyday life. However their toxicity has been poorly investigated. Cellular internalization of NPs has been shown to activate macrophages and neutrophils that contribute to superoxide anion production by the NADPH oxidase complex.

Using a cell-free system, we have investigated the influence of TiO_2 NPs on the behavior of the NADPH oxidase. In the absence of the classical activator molecules of the enzyme (arachidonic acid) but in the presence of TiO_2 NPs, no production of superoxide ions could be detected indicating that TiO_2 NPs were unable to activate by themselves the complex. However once the NADPH oxidase was activated (i.e., by arachidonic acid), the rate of superoxide anion production went up to 140% of its value without NPs, this effect being dependent on their concentration. In the presence of TiO2 nanoparticles, the NADPH oxidase produces more superoxide ions, hence induces higher oxidative stress.

This hyper-activation and the subsequent increase in ROS production by TiO_2 NPs could participate to the cell toxicity, which is strongly related to oxidative stress development.

Notes

How do enzymes tame radicals?

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Since the discovery of the trityl radical ($Ph_3C^{\bullet}$) by Moses Gomberg in 1900, an enormous array of species ('free radicals') with a single electron located on a carbon, nitrogen, oxygen, sulfur or other atom have been identified.[1] In the past two decades, the study of enzymes that catalyse reactions in which radicals participate as intermediates has blossomed.[2] Free radicals are usually short-lived, highly reactive species that may dimerise, abstract hydrogen atoms (e.g. from C-H or S-H groups), or add to π-systems or dioxygen. These properties make it remarkable that these species can be handled by some enzymes without severe damage to protein functional groups. And there seems to be no end to reports of new intriguing reactions of the radical enzymes class.

Structural biology has revealed a variety of modes of non-covalent binding of substrate molecules by enzymes with hydrogen, hydrophobic and ionic bonding being dominant motifs. Clearly, 'the taming of the radical' requires that these binding modes provide sufficient anchoring points to constrain the radical within an enzyme's catalytic site such that only the required chemistry occurs. Crystal structures of coenzyme B_{12}-dependent radical enzymes show typical binding modes for radicals: e.g. glutamate mutase exhibits three points of attachment between substrate radical and enzyme that involves both carboxylates and the amino group. The need for multiple effective anchoring points indicates why enzymes do not use the simplest radicals such as methyl or even hydroxyl, with the latter being generally associated with damage to biomolecules. The figure below summarises the criteria that a methylene carbon radical should fulfil for compatibility with a protein.

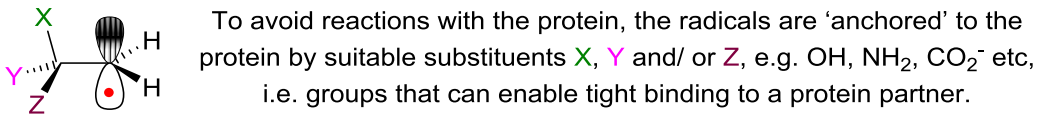

Figure: Desirable characteristics for a methylene radical as putative intermediate for a radical enzyme.

In a current research project at Newcastle we are surveying and quantifying the contribution of 'dual hydrogen bonding' in the binding of substrates, inhibitors and cofactors to enzymes. In the context of carbonyl groups this means determining the relative contributions of the two oxygen lone pairs as hydrogen bond acceptors. The possible role of dual hydrogen bonding in the binding of radical intermediates to enzymes will be discussed with reference to coenzyme B_{12}-dependent methylmalonyl-CoA mutase.

References:

[1] Bauer G, Chatgilialoglu C, Gebicki JL, Gebicka L, Gescheidt G, Golding BT, Goldstein S, Kaizer J, Merenyi G, Speier G, Wardman P, Biologically Relevant Small Radicals, *Chimia*, 2008, **62**, 704-712.

[2] Buckel W, Golding BT, Radical Enzymes in Chatgilialoglu C, Studer A, Eds, *Handbook of Radicals in Chemistry, Biology & Materials*, Wiley: New York, 2012.

Notes

Novel chemical biological methods for detection of enzyme activity in inflammation

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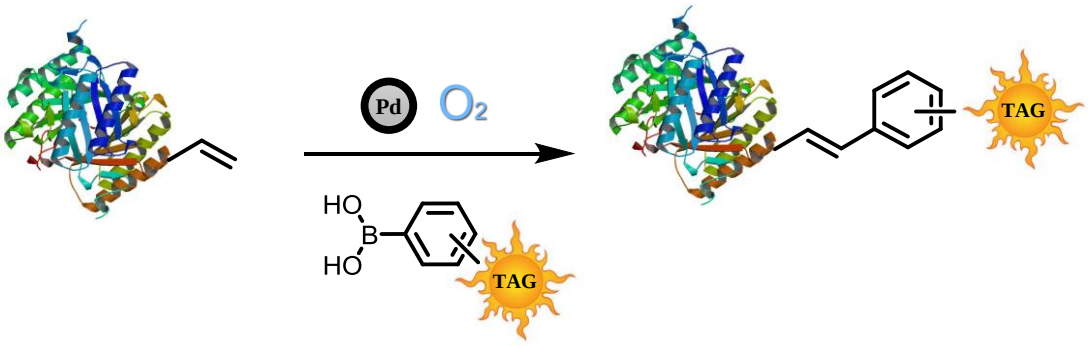
Bioorthogonal coupling of small organic molecules to protein-bound functional groups is a long-standing challenge with many implications for cell biology. Regretfully, the wealth of organic chemistry reactions is poorly translated into novel methods that are applicable in cell biology. Recently we developed the oxidative Heck reaction as a novel strategy for bioorthogonal coupling of arylboronic acids to protein-bound alkenes[1]. Further development of this reaction led to the identification of EDTA-Pd(II) as a novel catalyst for the oxidative Heck reaction on protein-bound alkenes, which can be employed in fully aqueous reaction conditions[2]. This reaction was employed to monitor histone lysine acylation *in vitro* after metabolic incorporation of olefinic carboxylates as chemical reporters[2]. Our current work is focussed on application of the oxidative Heck reaction in a novel class of activity-based probes for lipoxygenase enzymes. These probes will enable the identification and analysis of lipoxygenase activity in complex biological samples. Altogether these novel chemistry-based methods will be employed to study inhibition of enzyme activity in the context of inflammation.



References:

[1] Ourailidou ME, Dockerty P, Witte M, Poelarends GJ, Dekker FJ. Metabolic alkene labeling and in vitro detection of histone acylation via the aqueous oxidative Heck reaction. Org. Biomol. Chem. 2015, 13(12), 3648-3653.

[2] Ourailidou ME, van der Meer JY, Baas BJ, Jeronimus-Stratingh M, Gottumukkala AL, Poelarends GJ, Minnaard AJ, Dekker FJ. Aqueous oxidative Heck reaction as a protein-labeling strategy. Chembiochem. 2014, 15(2), 209-212.

Notes

Detection of reactive oxygen and nitrogen species with the use of boronate probes – the mechanistic aspects

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Boronates are boron-containing organic compounds with boron atom substituted with one alkyl or aryl group and two hydroxyl or ester groups. Recently boronate-based fluorogenic probes has emerged as a versatile tool for detection of reactive oxygen and nitrogen species (ROS/RNS). In the middle of past decade it has been proposed,[1] that boronate-derivatives of fluorescent dyes can be used as a convenient fluorogenic probes for the detection of hydrogen peroxide. Generally, other oxidants can also oxidize boronate compounds to corresponding phenols and that reaction is typical for hypohalous ions and nucleophilic peroxy oxidants like peroxynitrite or hydroperoxides.[2] The mechanism of oxidant detection is based on the oxidative transformation of nonfluorescent boronate probe into strongly fluorescent products.

Peroxynitrite reacts directly and stoichometrically with aromatic boronates to form the corresponding phenols (80−85% yield).[2] We showed that other minor products (yield ~ 10−15%) can be derived from radical intermediates - phenyl and phenoxyl radicals.[3,4] The first step of the peroxynitrite-derived oxidation of borates is the formation of anionic adduct of peroxynitrite to the boronate moiety. There are two pathways of the further transformation of that adduct. The first pathway (80-90%) leads via the heterolytic cleavage of the peroxide bond in the anionic adduct and results in the formation of the major, phenolic product. The second pathway leads via the homolytic cleavage (~10-20%) of the O-O bond. The homolytic cleavage results in the formation of transient radical anion $RB(OH)_2O^{\bullet -}$, that undergoes further spontaneous fragmentation with the formation of phenyl radicals.[3,4] The formation of phenyl radical-derived minor products is specific for the reaction of boronates with peroxynitrite. During the last few years formation of such peroxynitrite specific products was shown for simple arylboronate compounds,[2,3] mitochondria targeted isomeric arylboronates[4] as well as for fluorogenic boronate probe coumarin 7-boronic acid (CBA).[5]

References:

[1] Lo L.C., Chu C. Y. *Development of highly selective and sensitive probes for hydrogen peroxide.* Chem. Commun. (2003), 2728-9.

[2] Sikora A., Zielonka J. et al. *Direct oxidation of boronates by peroxynitrite: mechanism and implications in fluorescence imaging of peroxynitrite.* Free Radic. Biol. Med. (2009), **47**, 1401.

[3] Sikora A., Zielonka J. et al. *Reaction between peroxynitrite and boronates: EPR spin-trapping, HPLC Analyses, and quantum mechanical study of the free radical pathway.* Chem. Res. Toxicol. (2011), **24**, 687-97.

[4] Sikora A., Zielonka J. et al. *Reaction between peroxynitrite and triphenylphosphonium-substituted arylboronic acid isomers: identification of diagnostic marker products and biological implications.* Chem. Res. Toxicol. (2013), **26**, 856-67.

[5] Smulik R., Dębski D. et al. *Nitroxyl (HNO) reacts with molecular oxygen and forms peroxynitrite at physiological pH. Biological Implications.* J. Biol. Chem. (2014), **289**, 35570-81.

Notes

Effects of bleomycin and antioxidants on the fatty acid profile of testicular cancer cell membranes

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Bleomycin is used in chemotherapy regimens for the treatment of patients having testicular germ-cell tumor (TGCT). There is no study in the literature investigating the effects of bleomycin on membrane lipid profile in testicular cancer cells. We investigated membrane fatty acid (FA) profiles isolated, derivatised and analysed by gas chromatography of NTera-2 testicular cancer cells incubated with bleomycin (Bleo) for 24h in the absence and presence of N-Acetyl-L-Cysteine (NAC) and curcumin (Cur) as commonly used antioxidant adjuvants. At the same time the MAPK pathway and EGFR levels were followed up. Bleomycin treatment increased significantly saturated fatty acids (SFA) of phospholipids at the expense of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). Bleomycin also led to a significant increase in the trans lipid isomers of oleic and arachidonic acids due to its free radical producing effect. Incubation with bleomycin increased the p38 MAPK and JNK levels and downregulated EGFR pathway. Coincubation of bleomycin with NAC reversed effects caused by bleomycin. Our results highlight the important role of membrane fatty acid remodeling occurring during the use of bleomycin and its concurrent use with antioxidants which can adjuvate the cytotoxic effects of the chemotherapeutic agents.

Keywords: bleomycin; curcumin; membrane lipid profile; N-acetyl-L-cysteine; testicular cancer cell.

Notes

Reactive oxygen species-mediated catalase inhibition and its biological consequences

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Catalase is one of the most important antioxidant enzymes present in almost all aerobically respiring organisms. This enzyme not only decomposes hydrogen peroxide to water and molecular oxygen, but also catalytically scavenges peroxynitrite ($ONOOH/ONOO^-$) – a strong oxidizing and nitrating species.[1] Additionally, catalase can decrease peroxynitrite formation by scavenging nitric oxide ($^{\bullet}NO$), the precursor of peroxynitrite.[2]

Hypochlorous acid (HOCl), highly reactive oxidizing and chlorinating species, is formed in mammals' bodies in the immune response to invading pathogens. Although HOCl is one of the most important antibacterial agents it is also dangerous for host molecules at sites of inflammation. Since proteins are very abundant *in vivo* they are important targets for HOCl action.

In catalase-rich cells, hepatocytes, catalase is one of the main internal targets for HOCl.[3] Catalase inhibition in the host cells leads to the weakness of antioxidant defense system of the cell and may be detrimental to neighboring tissues. However, many tumor cells possess membrane-associate catalase that protects these cells against intracellular reactive oxygen species (ROS) signaling and in a consequence against apoptosis.[4] Additionally, some pathogenic bacteria, which are rich in catalase, are relatively resistant against the attack of neutrophils and other cells from the defense system. Hence, in these two cases catalase inhibition may be beneficial.

Presentation will be focused on the influence of selected ROS on catalase activity. Analysis of two major intracellular signaling pathways (the HOCl and the $^{\bullet}NO$/peroxynitrite pathway), which lead to the induction of tumor cells apoptosis, will be presented. Possible mechanisms of the reactions of HOCl with catalase will also be discussed.[5]

References:

[1] L.Gebicka, J.Didik, *Catalytic scavenging of peroxynitrite by catalase*, J. Inorg. Biochem. 103 (2009) 1375-79
[2] G.Brown, *Reversible binding and inhibition of catalase by nitric oxide*, Eur. J. Biochem. 232 (1995) 188-91
[3] M.G.Bonini, A.G.Siraki, B.S.Atanassov, R.P.Mason, *Immunolocalization of hypochlorite-induced, catalase-bound free radical formation in mouse hepatocytes*, Free Radic. Biol. Med. 42 (2007) 530-40
[4] G.Bauer, Targeting extracellular ROS signaling of tumor cells, Anticancer Res. 34 (2014) 1467-82
[5] J.Krych-Madej, L.Gebicka, *Do pH and flavonoids influence hypochlorous acid-induced catalase inhibition and heme modification?* Int. J. Biol. Macromol. 80 (2015) 162-69

Notes

Stickland-reaction enzymes from *Clostridium sporogenes*

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Aerobic and anaerobic bacteria usually oxidize amino acids to the corresponding α-oxo acids and further via the citric acid cycle to CO_2. In the absence of electron acceptors like oxygen, nitrate and sulfate, a few anaerobes such as *Clostridium sporogenes* can use amino acids for this purpose, a process called Stickland-reaction[1]. In this reaction one amino acid acts as electron donor and is oxidized to CO_2, ammonium and a carboxylic acid, which is one carbon atom shorter than the original. Examples of products formed are acetate from alanine, isobutyrate from valine, and 3-methylbutyrate from leucine, indoleacetate from tryptophan and phenylacetate from phenylalanine. Another amino acid serves as electron acceptor and is reduced to a carboxylic acid with same length as the original amino acid. Hence, glycine is reduced to acetate, tryptophan to indolepropionate, leucine to isocaproate, and proline to 5-aminovalerate. Later H. A. Barker discovered that most single amino acids can also serve for both, donor and acceptor[2].

The pathway of the fermentation of tryptophan to indoleacetate and indolepropionate in *Clostridium sporogenes* and the key enzyme indolelactyl-CoA dehydratase were investigated. Tryptophan disproportionates oxidatively via 3-indolepyruvate to 3-indolelacetate and reductively via (*R*)-3-indolelactate and 3-indoleacrylate to 3-indolepropionate (IPA). IPA, which is formed in the human intestine, is transported in the blood to the brain. In the brain IPA scavenges reactive oxygen species (ROS) and thus protects from Alzheimer's disease. Similarly, phenylalanine is reduced by *C. sporogenes* via (*R*)-phenyllactate and (*E*)-cinnamate to 3-phenylpropionate[3]. Studies were focused on the key enzyme indolelactyl-CoA dehydratase, which catalyzes a very unusual *syn*-elimination of a non-activated proton at the β-position and an OH-group at the α-position of the thioester carbonyl by employing radical intermediates.

C. sporogenes could be useful in a novel Nylon 6 biosynthesis pathway starting from bio renewable feed stocks. The crucial step in this pathway, reductive cleavage of pipecolic acid to 6-aminocaproic acid was proposed to be catalyzed by the selenium-containing D-proline reductase. Thus, the activity of this enzyme was tested towards D- and D,L-pipecolic acid. However, pipecolic acid was not accepted as substrate. An alternative way to 6-aminocaproic acid could be a reduction of lysine, analogous to the reduction of tryptophan to IPA.

References:

[1] Stickland, L. H. (1934) Studies in the metabolism of the strict anaerobes (genus *Clostridium*), Biochem J. 28, 1746–1759.

[2] Barker, H. A. (1961) Fermentations of nitrogenous organic compounds, p. 151-207. In Gunsalus I.C., Stanier R. Y. (ed.), The Bacteria, vol. 2. Academic Press, New York.

[3] Dickert, S., Pierik, A. J., Linder, D., Buckel, W. (2000) The involvement of coenzyme A esters in the dehydration of (*R*)-phenyllactate to (*E*)-cinnamate by *Clostridium sporogenes*, Eur. J. Biochem. 267, 3874-3884.

Notes

Mechanistic investigations of a [4Fe-4S]$^{2+}$ enzyme involved in the MEP pathway, a target for the development of new antimicrobials

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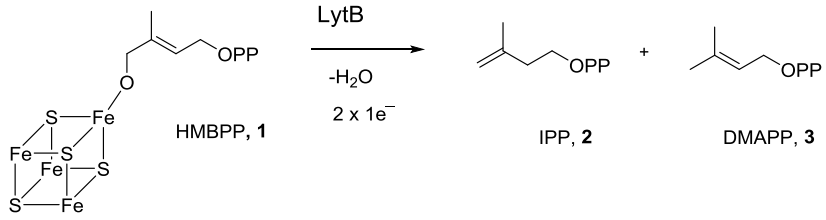
In many bacteria, including *Mycobacterium tuberculosis* responsible for tuberculosis, in the plant chloroplasts and in the malaria parasite *Plasmodium falciparum*, the biosynthesis of isoprenoids occurs according to the methylerythritol phosphate (MEP) pathway, an alternative to the well-known mevalonate pathway. The MEP pathway does not exist in humans and is therefore a valuable target for the development of new specific antibacterial and antiparasitic drugs.

IspH/LytB, the last enzyme of the methylerythritol phosphate pathway, converts (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate (HMBPP, **1**) into a mixture of isopentenyl diphosphate (IPP, **2**) and dimethylallyl diphosphate (DMAPP, **3**, Scheme 1). This reaction is catalyzed by a peculiar oxygen sensitive [4Fe-4S] cluster[1,2] and requires formally, removal of a hydroxyl group of the substrate, transfer of two electrons from the [4Fe-4S] cluster, and protonation of an anionic allylic intermediate.



Scheme 1: The reaction catalyzed by LytB

We will present our last results concerning the investigation of the IspH/LytB mechanism obtained from an approach combining crystallography, site-directed mutagenesis, enzymology, chemistry and Mössbauer spectroscopy.

References:

[1] A. Ahrens-Botzong, K. Janthawornpong, J. A. Wolny, E. Ngouamegne Tambou, M. Rohmer, S. Krasutsky, C.D. Poulter, V. Schünemann, M. Seemann (2011). Angew. Chem. Int. Ed. *50*, 11976.

[2] I. Faus, A. Reinhard, S. Rackwitz, J. A. Wolny, K. Schlage, H.-C. Wille, A. Chumakov, S. Krasutsky, P. Chaignon, C. D. Poulter, M. Seemann, V. Schünemann (2015). Angew. Chem. Int. Ed. (in press, DOI: 10.1002/anie.201502494)

Notes

Interactions of serotonin with myeloperoxidase

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We have already shown in our previous studies that serotonin inhibited the chemiluminescence response of neutrophils in human whole blood. The inhibition was partially due to the direct scavenging activity of serotonin towards individual reactive oxygen species and partially due to the inhibition of myeloperoxidase activity.

Serotonin acts as an alternative substrate for myeloperoxidase and therefore can undergo oxidation via peroxidase cycle of myeloperoxidase. Our recent data suggest that myeloperoxidase activity differs according to the pH value. Two different pH values were tested – pH = 5 which represents an environment in a neutrophil phagosome and physiological pH = 7.4. In case of pH = 5 serotonin decreased chlorinating activity of myeloperoxidase in a dose-dependent manner. This suggests that higher concentration of serotonin (10^{-5} M) compete sufficiently with halogenide and peroxidase cycle predominates. On the other hand dose-dependent potentiation of halogenating activity of myeloperoxidase was observed at pH 7.4. This finding could have substantial consequences in NET formation when neutrophil granular proteins including myeloperoxidase are attached.

Notes

(Per)oxidation cascades induced by metallo proteins: analytical uses, mechanistic insights

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Beyond the known purposefully-built enzymes that oxidatively process other organic molecules in our body (prostaglandin synthases, cytochromes P450, etc.), a few heme proteins are abundant enough for their oxidative *side*-reactions to become important. Such is the case of cytochrome *c*, whose pseudoperoxidase reactivity is linked to apoptosis. Similarly, several globins can engage in peroxidase reactivity in vivo, using hydrogen peroxide or lipid peroxide as oxidants towards small-molecule substrates such as ascorbate, urate, phenolics, lipids, thiols – but also peptides and even proteins.

On the analytical side, these processes can be turned into useful tools for assaying antioxidant and prooxidant activities. Lipid peroxidation assays with cytochrome *c* or hemoglobin, as well as hemoglobin ascorbate peroxidase assays, afford insight into antioxidant activity. A hemoglobin/copper-oxidase couple can be employed for assaying prooxidant reactivity. Recent applications on analyses of edible oils and of natural extracts or drug candidates will be illustrated. A variation of the liposome assay is also useful for testing blood substitute candidates – especially hemoglobin-based ones.

On the mechanistic side, peroxidase and pseudoperoxidase experiments with globins and laccases have allowed detection of previously unreported aromatic free radicals (mainly in the polyphenolics class), as well as of interaction modes between proteins and their small-molecule redox partners – including cases of covalent modification at the protein active site.

Notes

Non-enzymatic Ribonucleotide Reduction in the Prebiotic Context

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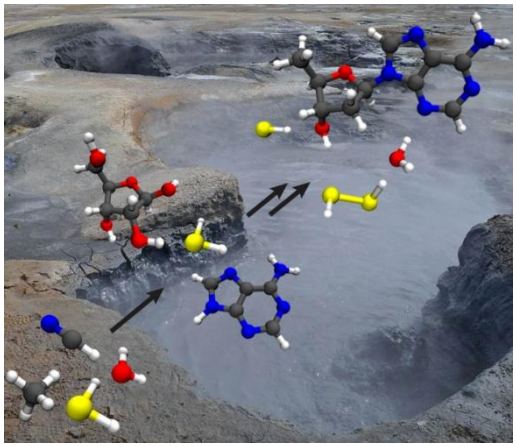
Model studies of prebiotic chemistry have revealed compelling routes for the formation of the building blocks of proteins and RNA, but not DNA.[1] Today, deoxynucleotides required for the construction of DNA are produced by reduction of nucleotides catalysed by ribonucleotide reductases,[2] which are radical enzymes.[3] This contributions considers potential non-enzymatic routes via intermediate radicals for the ancient formation of deoxynucleotides. In this context, several mechanisms for ribonucleotide reduction, in a putative $H_2S/HS^{\bullet}$ environment, are characterized using computational chemistry.[4] A bio-inspired mechanistic cycle involving a keto intermediate and HSSH production is found to be potentially viable. An alternative pathway, proceeding through an enol intermediate is found to exhibit similar energetic requirements. Non-cyclical pathways, in which HSS• is generated in the final step instead of HS•, show a markedly increased thermodynamic driving force (ca. 70 kJ mol^{-1}) and thus warrant serious consideration in the context of the prebiotic ribonucleotide reduction.



References:

[1] H. D. Bean, Y. Sheng, J. P. Collins, F. A. L. Anet, J. Leszczynski, N. V. Hud, *J. Am. Chem. Soc.* **2007**, *129*, 9556-9557.

[2] J. Stubbe, W. A. van der Donk, *Chem. Rev.* **1998**, *98*, 705-762.

[3] W. Buckel and B. T. Golding in *Encyclopedia of Radicals in Chemistry, Biology and Materials* (Eds.: C. Chatgilialoglu, A. Studer), John Wiley & Sons, Ltd., New York, 2012; Chapter 50.

[4] G. M. Sandala, D. M. Smith and L. Radom in *Encyclopedia of Radicals in Chemistry, Biology and Materials* (Eds.: C. Chatgilialoglu, A. Studer), John Wiley & Sons, Ltd., New York, 2012; Chapter 51.

Notes

Theoretical study of the *cis*-dihydroxylation of nitrobenzene catalyzed by nitrobenzene dioxygenase

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Nitroarene dioxygenases belong to the family of Rieskenonheme iron dioxygenases, which are able to oxidize a variety of aromatic compounds through direct incorporation of atmospheric oxygen into the substrate, leading to the formation of a cis-dihydrodiol. Nitroarene dioxygenases are uniquely able to oxidize the aromatic ring of nitroarene compounds, resulting in the elimination of the nitro group, which facilitates further metabolization. Nitrobenzene dioxygenase (NBDO), in particular, is the only nitroarene dioxygenase that can catalyze the denitration of all mono- and dinitrotoluenes.
NBDO, like other Rieske dioxygenases, is a three-component system that consists of an NADH-dependent flavoprotein reductase, a Rieske [2Fe-2S] ferredoxin, and terminal oxygenase. During the catalytic cycle two electrons are transferred one at a time from the reductase to the [2Fe-2S] cluster in ferredoxin and subsequently to the mononuclear iron(II) center located in the terminal oxygenase component, where the reaction takes place. The oxygenase component is an α3β3 heterohexamer with a mushroom-shaped quaternary structure, in which α subunits containing the active site and Rieske domains bear catalytic function, while β subunits, which are located more than 10 Å from the active sites, are believed to have a purely structural function. The active site domain in each α subunit hosts a high-spin mononuclear FeII bound to two histidines and a bidentate aspartate residue, forming the 2-his-1-carboxylate facial triad motif found in many mononuclear nonheme iron(II) oxygenases. Rieske domain contains [2Fe-2S] cluster coordinated by two cysteine and two histidine residues.

Being involved in bacterial metabolism, Rieske enzymes initiate degradation of many environmental contaminants, and thus remain viable targets for bioremediation platforms. Due to this potential use of these enzyme we are studying[1] the mechanism of their action both experimentally and theoretically using isotopic fractionation approach. In this communication, we present results of computational studies that include classical molecular dynamics simulation of the oxygenase component of the NBDO system in explicit water, DFT calculations of the alternative mechanistic scenarios performed at the cluster and QM/MM levels, as well as calculations of the resulting position-specific kinetic isotope effects that are used in elucidation of the actual reaction pathway.

[1]this work is supported by the grants PSRP-025/2010 from the Polish-Swiss Research Program

Notes

Spin labelled polysaccharides – suitable probes for investigating supramolecular assemblies

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The spin labelling EPR method provides structural and dynamic information on various systems based on theories describing the EPR line shapes of paramagnetic moieties covalently attached to diamagnetic structure [1-3]. In many systems, self-organisation processes occur depending on the balance between several supramolecular interactions whose energies are close to thermal energy (hydrogen bonds, electrostatic interactions π-stacking of aromatic systems. The most widely used spin probes or labels are nitroxides, which are stable paramagnetic molecules with a paramagnetic N-O· fragment bearing an unpaired electron. This fragment is surrounded by shielding substituents (usually methyl groups) [1].

This method has been applied to study the behaviour of the spin-labelled cyclic or linear polysacharides. Spin-labelled β-cyclodextrins [4, 5] were used to investigate various host-guest systems. The results suggest that by attaching the paramagnetic moiety to the cyclodextrin cavity, the binding ability decreases compared to the parent cyclodextrin. As a linear polysacharide, alginic acid has been selected, as this contain in the structure carboxy groups which can be easily linked to a paramagnetic moiety. The spin-labelled alginate has been used to investigate sol-to-gel phase transformation in pluronic/alginate systems and in solutions of alginate in the presence of divalent cations.

References:

[1] Advanced ESR Methods in polymer research, Ed. Shulamith Schlick, Wiley Interscience, 2006
[2] Spin Labeling. Theory and Application (Ed. L. J. Berliner) New York: Academic Press, 1976
[3] M. Lucarini, E. Mezzina, *Electron Paramag. Reson.* **2011**, *22*, 41-70
[4] G. Ionita, V. Chechik, *Org. Biol. Chem.* **2005**, ***3***, 3096–3098
[5] V. Chechik, G. Ionita, *Org. Biomol. Chem*. **2006**, *4*, 3505-3510

Notes

The radical-mediated deazapurine ring contraction in QueE - the enzyme's and the metal's role

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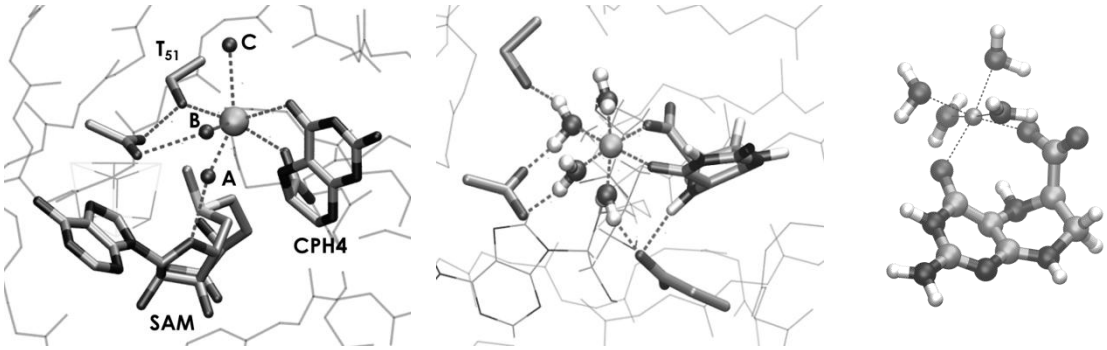
Radical reactions catalysed by enzymes using S-adenosyl methionine (SAM) as co-substrate or cofacor, have attracted recent interest because of their involvement in chemical processes leading to products of potential use for anti-viral, anti-cancer and antibiotic treatments. While a general framework for the initial catalytic mechanism has been established over the past years[1] much less is known about the subsequent chemical rearrangements in most cases. A thorough knowledge of these mechanisms can lay the foundation for rational protein engineering, with the potential for opening up a wealth of novel biologically-accessible chemistry.

The bacterial 7-carboxy-7-deazaguanine (CDG) synthase (QueE) catalyses the rearrangement of 6-carboxy-5,6,7,8-tetrahydropterin (CPH4) into CDG as a key step in queosine biosynthesis. This intermediate is a precursor to a number of interesting streptomycin antibiotics, and molecules with anti-viral and anti-cancer properties.[2] The radical rearrangement involves a very interesting ring-contraction step and additional metal cation binding that influences the catalysis.[3] Very recently, initial QM/MM calculations[4] have given further insights into the reaction mechanism, yet they still leave many open questions.

In particular the crucial role of the interplay of dynamic features of the enzyme - influencing reactivity and mechanism – and the outstanding role of metal coordination of the substrate with and without influences by the protein environment reveal fascinating details. We took a close look at the dynamic substrate-protein interactions, with specific consideration of the details of Mg^{2+} binding and coordination. Results will be presented in the context of DFT calculations and molecular dynamics simulations, with an outlook to the future and scope for engineering the QueE reaction.



Mg^{2+} and substrate binding in QueE in the crystal structure (left) and MD simulation (middle) and metal coordination in model system (right).

References:

[1] Broderick, J. B.; Duffus, B. R.; Duschene, K. S.; Shepard, E. M. *Chem. Rev*. **2014**, *114*, 4229.

[2] McCarty, R. M.; Bandarian, V. *Bioorg. Chem.* **2012**, *43*, 15.

[3] Dowling, D. P.; Bruender, N. A.; Young, A. P.; McCarty, R. M.; Bandarian, V.; Drennan, C. L. *Nat. Chem. Biol*. **2014**, *10*, 106

[4] Zhu, W., Liu, Y., *ACS Catal.*, **2015**, *5*, 3953.

Notes

Application of glycation markers for clinical diagnostics

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Glycation adducts of proteins have found diagnostic application in monitoring of blood glucose control in diabetes – particularly use of glycated haemoglobin A1C (measurement of early stage glycation adducts, N_{ε}-fructosyl-lysine and N_{α}-fructosyl-valine, of haemoglobin). Recently A1C has also been recommended for screening of people at increased risk of diabetes or pre-diabetes, impaired glucose tolerance and impaired fasting glucose, through a recommended range of A1C (5.7 – 6.4%). Measurement of advanced glycation endproducts (AGEs) has not yet found clinical translation. Other techniques that, in part, measure some AGEs – skin autofluorescence readers – have also been proposed.

Since glycation is implicated as a causative mechanism in ageing and disease, it is expected that measurements of glycation adducts may contribute mechanism-based biomarkers for improved health screening and clinical diagnosis. Minimal and non-invasive sampling for direct biochemical measurement has been considered: blood, urine and saliva. Glycation adducts, free or residues of proteins, may be quantified. Diagnostic information thereby obtained depends on the analyte measured, its half-life, quantitative change and factors producing it. Particular glycated proteins may be detected and identified by high resolution Orbitrap mass spectrometry proteomics. For clinical translation techniques with moderate/high throughput and available to the non-specialist are likely to be most valuable.

Immunoassays have been development for AGEs. They require careful validation and corroboration with stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry (LC-MS/MS). Immunoassays find favour generally in clinical diagnostic measurements for their high throughput and established high level automation in the clinical setting. Where multiple glycation adduct measurements are required, immunoassays may be multiplexed. Concerns remain, however, on cross-reactivity to antigens and markedly increased cost of immunoassay multiplexing. To date, few multiplex immunoassays for any analyte have been validated for clinical application.

Stable isotopic dilution analysis LC-MS/MS is the analytical method of choice for detection and quantitation of glycation adducts. It has robust, cross-reaction-free multiplexing for combinations of analytes which can be done at minimal additional cost. It operates at moderate sample throughputs – similar to methods for A1C. It is also being developed for multiplexed protein analysis by quantitation of tryptic peptides. There are few LC-MS/MS-based clinically-regulated and approved diagnostic procedures. A major barrier to clinical use is lack of high level automation to for use by the non-specialist.

Combination of biomarkers and clinical features in a diagnostic algorithm is often the most powerful approach to clinical diagnostics. Algorithms are developed by the process of machine learning where the weighting of different features is optimized or trained on experimental clinical data and then validated on independent subject/patient datasets.

We recently developed diagnostic algorithms for screening, detection and typing of early-stage arthritis. Similar techniques are in development for other health screening and disease diagnostics. We combine protein glycation, oxidation, nitration and other markers in a data-dependent process. Such approaches will likely produce valuable clinical diagnostic applications of carbonyl stress.

Reference: Ahmed *et al*., (2015) Nature Scientific Reports 5: 9259.

Notes

Enzyme-catalyzed reductions for the synthesis of valuable chiral intermediates

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The role of Biocatalysis in the development of simple and straightforward methodology for the stereoselective synthesis of pharmaceutically interesting compounds will be presented. More specifically, ketoreductase-catalyzed reductions of various carbonyl compounds have resulted in the synthesis of chiral synthons in high optical purities (>99% de, >99% ee) and chemical yields. These molecules are key intermediates for the synthesis of many natural products,[1] pharmaceuticals [2] and other valuable compounds.[3]

Ketoreductases are chemo-, stereo- and regioselective biocatalysts that often reduce a wide range of ketones and have been proved very efficient catalysts for the preparation of optically active keto alcohols, diols and hydroxy esters.[4]

More importantly, biocatalytic cascade processes consisting of two consecutive steps, which have been designed for the synthesis of chiral diols and β,δ-dihydroxy esters will be presented. [5]

Acknowledgement

Part of this work was supported by the project "IRAKLITOS II - University of Crete" of the Operational Programme for Education and Lifelong Learning 2007 - 2013 (E.P.E.D.V.M.) of the NSRF (2007 - 2013), which is co-funded by the European Union (European Social Fund) and National Resources. The Research Secretariat of the University of Crete (ELKE) is also acknowledged for financial support (KA 4039, KA 4065) and the COST Action CM1201.

References:

[1] (a) K. Tatsuta, Hosokawa, Seijiro. *Chem. Rev.* **2005**, *105*, 4707 (b) R. N. Patel, *Adv. Synth. Catal*. **2001**, *343*, 527

[2] R. N. Patel, *ACS Catalysis* **2011**, *1* (9), 1056-1074

[3] M. M. A. Mondol, J. H. Kim, M. Lee, F. T. Shahidullah, H-S. Lee, Y-J. Lee, H. J. Shin, *J. Nat. Prod*. **2011**, *74*, 1606

[4] (a) D. Kalaitzakis, J. D. Rozzell, S. Kambourakis, I. Smonou, *Org. Lett* **2005**, 7, 4799 (b) D. Kalaitzakis, J. D. Rozzell, I. Smonou, S. Kambourakis, *Adv. Synth. Catal.* **2006,** 348, 1958 (c) D. Kalaitzakis, I. Smonou, *Tetrahedron* **2010**, *66*, 9431-9439

[5] (a) D. Kalaitzakis and I. Smonou, *J. Org. Chem*., **2010**, *75* (24), 8658–8661 (b) A. Bariotaki, D. Kalaitzakis, I. Smonou, *Org. Lett.* **2012**, *14*(7), 1792–1795

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