COST Action CM1201

Biomimetic Radical Chemistry

Scientific Meeting

WG3 Membrane Stress, Signalling and Defences

Date:	September 11th - 12th, 2013
Venue:	Hotel Kolovare
	Bože Peričića 14
	23 000 ZADAR, CROATIA
Local Organizer:	Branka Mihaljević
	Ivana Tartaro Bujak
	Iva Džeba
	Radiation Chemistry and Dosimetry Laboratory
	Division of Materials Chemistry
	Ruđer Bošković Institute

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1st WG3 meeting

September 11th-12th, 2013

Zadar, CROATIA

Main Programme

Tuesday, September 10th

17:00 – 19:00	Arrival and check-in
19:00 – 20:30	*Welcome cocktail*

Wednesday, September 11th

08:30 – 09:30	Arrival and check-in
09:30 – 09:40	Welcome **Branka Mihaljević** *Ruđer Bošković Institute, Zagreb, Croatia* Introductory words **Chryssostomos Chatgilialoglu** *ISOF, Consiglio Nazionale delle Ricerche, Bologna, Italy*

Morning Session Chairperson: **Carla Ferreri**

09:40 – 10:20	Protein Disulfide Isomerase modification, ER stress and apoptosis in atherosclerosis. **Anne Negre-Salvayre** *Université Paul Sabatier UMR 1048, Institut des Maladies Métaboliques et Cardiovasculaires, Toulouse Cedex 4, France*
10:20 – 11:00	Coffee break

11:00 – 11:40	The origin of TRANS fatty acids: exogenous versus endogenous contribution. **Chryssostomos Chatgilialoglu** *ISOF, Consiglio Nazionale delle Ricerche, Bologna, Italy*
11:40 – 12:20	Cell membrane phospholipid adaptation to nutrient overload. **Shlomo Sasson** *Institute for Drug Research, Department of Pharmacology, Faculty of Medicine, The Hebrew University, Jerusalem, Israel*
12:20 – 12:45	On the control of NADPH oxidase by the lipid content **Chantal Houée-Lévin** *Université Paris Sud/CNRS, Centre Universitaire, 91405 Orsay, France*
12:45 – 14:30	*Lunch break*

Afternoon Session Chairperson: **Shlomo Sasson**

14:30 – 14:55	Structural and functional characterization of HDL particles isolated from morbidly obese patients before and after biliopancreatic diversion by Roux en Y **Kyriakos E. Kypreos** *Pharmacology laboratory, Department of Medicine, University of Patras Medical School, Greece*
14:55 – 15:20	Effects of Bleomycin and Antioxidants on FattyAcid Profile in Human Testicular Cancer Cell Membranes. **Tomris Őzben Tomasi** *Akdeniz University Medical Faculty, Department of Biochemistry, Antalya, Turkey*
16:30 – 20:00	*Guided tour to the city center*
20:00 –	*Festive Dinner*

Thursday, September 12th

Morning Session Chairperson: **Branka Mihaljević**

9:00 – 09:40	Lipidomic profiles and nutrilipidomics: representative steps from bench to bedside. **Carla Ferreri** *ISOF, Consiglio Nazionale delle Ricerche, Bologna, Italy*
09:40 – 10:05	Noise induced oxidation impairs outer hair cell's function through membrane fluidity loss. **Giuseppe Maulucci** *Istituto di Fisica, Università Cattolica del Sacro Cuore, Roma, Italy*
10:05 – 10:30	Lipidomic studies on lipoprotein: insights on the cis/trans C16 monounsaturated fatty acid content. **Anna Sansone** *ISOF, Consiglio Nazionale delle Ricerche, Bologna, Italy*
10:30 – 11:00	*Coffee break*
11:00 – 11:25	4-Hydroxynonenal-histidine adducts: Major bioactive marker of oxidative stress, lipid peroxidation and oxidative homeostasis **Neven Žarković** *Ruđer Bošković Institute, Zagreb*
11:25 – 12:05	Protein and nucleotide advanced glycation endproducts as biomarkers for diagnosis, disease progression and therapeutic intervention. **Naila Rabbani** *Warwick Medical School, Clinical Sciences Research Institute, University of Warwick, University Hospital, Coventry CV2 2DX, UK*
12:05 – 12:45	Electrochemistry – Mass Spectrometry: A Tool to Study Oxidative Modifications in Drugs and Proteins. **Rainer Bischoff** *University of Groningen, Department of Pharmacy, Groningen, Netherlands*
12:45 – 14:00	*Lunch break*

Afternoon Session Chairperson: **Tomris Őzben Tomasi**

14:00 – 14:25	A biomimetic model for the study of the free radical-induced lipid modification processes. **Branka Mihaljević** *Ruđer Bošković Institute, Radiation Chemistry and Dosimetry Laboratory, Zagreb, Croatia*
14:25 – 14:50	Uncoupling proteins: the mystery about their function(s). **Elena Pohl** *Institute of Physiology, Pathophysiology and Biophysics, University of Veterinary Medicine, Vienna, Austria*
14:50 – 15:15	Uncoupling proteins and reactive aldehydes. **Olga Jovanović** *University of Veterinary Medicine, Department of Molecular Physiology and Biophysics, Vienna, Austria*
15:15 – 15:40	A quantitative study on the pro-oxidant behavior of metal nanoparticles. **Riccardo Amorati** *University of Bologna, Dept of Chemistry "G. Ciamician", Bologna, Italy*
15:40 – 16:15	*Coffee break*
16:15 – 16:40	Selenenic Acids as chain-breaking antioxidants. Kinetics, Thermodynamics and Mechanisms. **Luca Valgimigli** University of Bologna, Dept of Chemistry "G. Ciamician", Bologna, Italy
16:40 – 17:05	Preventive effects of red wine polyphenols on oxidative stress and metabolic syndrom. **Manar Aoun** *INRA UMR 866, Dynamique Musculaire et Métabolisme, INRA, Montpellier, France*
17:05 – 19:00	Discussions and planning of collaborations Closing of the Meeting

List of participants

COST Action CM1201 / 1st WG3 meeting in Zadar, CROATIA, 11-12 September 2013

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Lecture Abstracts

Protein disulfide isomerase modification, ER stress and apoptosis in atherosclerosis

Anne Negre-Salvayre

INSERM UMR1048 – I2MC, Toulouse, France

Oxidized low-density lipoproteins (oxLDLs) and oxidized lipids are involved in atherogenesis. In atherosclerotic areas, the modification of cellular proteins by reactive carbonyl compounds, such as aldehydes derived from lipid peroxidation, suggests that the 'carbonyl stress' may contribute to the conformational change (misfolding) and impaired function of modified proteins. We recently reported that apoptosis of vascular cells induced by oxLDLs is associated with a sustained endoplasmic reticulum stress (ER stress) and unfolded protein response (UPR).

Protein disulfide isomerase (PDI) is an abundant endoplasmic reticulum (ER)-resident chaperone and oxidoreductase that catalyzes the formation and rearrangement (isomerisation) of disulfide bonds on newly formed proteins, thereby participating in protein folding. PDI is a target of S-nitrosylation and the resulting structural modifications are known to increase protein misfolding, ER stress, a loss of its protective properties, and neuronal apoptosis. The role of PDI in atherosclerosis and cardiovascular diseases is not known.

Since PDI modification and carbonyl stress are strong inducers of prolonged ER stress and apoptosis, we hypothesized that PDI could be targeted and inhibited by carbonyl compounds present in oxLDLs and in atherosclerotic lesions, and that PDI inhibition may result in increased protein misfolding and sustained ER stress, thereby promoting apoptosis and the progression of atherosclerotic lesions.

The aim of this study was to investigate whether PDI is a target of oxLDLs and of carbonyl stress, and whether PDI modification plays a role in oxLDL-induced ER stress and apoptosis in vascular cells.

The preincubation of human endothelial HMEC-1 with toxic proapoptotic concentration of oxLDLs induced an inhibition of the enzymatic activity of PDI inhibition, resulting from its modification by 4-hydroxynonenal (4-HNE, an aldehyde abundantly present in oxLDLs), as assessed by 4-HNE-PDI adducts formation. PDI inhibition may contribute to oxLDL-induced apoptosis, as supported by the effect of bacitracin (a PDI inhibitor) which potentiated ER stress (increased mRNA expression of CHOP and sXBP1) and apoptosis induced by oxLDLs. In

contrast, increased PDI activity by overexpression of an active wild-type PDI was associated with reduced oxLDL-induced ER stress and toxicity, whereas the overexpression of a mutant inactive form was not protective. PDI inhibition by oxLDLs was prevented by the carbonyl-scavengers N-acetylcysteine and pyridoxamine, which reduced ER stress (CHOP expression) and apoptosis. Interestingly, 4-HNE-modified PDI was detected in the lipid-rich areas of human advanced atherosclerotic lesions.

In conclusion, PDI modification by oxLDLs and carbonyl stress inhibits its enzymatic activity and potentiates both ER stress and apoptosis by oxLDLs. PDI modification by lipid peroxidation products in atherosclerotic lesions suggests that a loss of function of PDI may occur in vivo, and may contribute to local ER stress, apoptosis and plaque progression.

The origin of trans fatty acids: exogenous versus endogenous contribution

Chryssostomos Chatgilialoglu

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The configuration of isolated double bonds in naturally occurring lipids of eukaryotes is cis. An increasing number of studies have explored the presence of trans fatty acid residues in living systems. This is a very lively field of interdisciplinary research spanning from chemistry to microbiology, pharmacology, biology, and medicine.

Trans fatty acids in mammalian cells are well known to have an exogenous origin, after dietary supplementation of chemically-modified fats. The latter is important nutritionally, since cis/trans isomeric mixtures of fats result from vegetable and fish oils manipulated by partial hydrogenation or deodorization processes employed in the food-processing industry. In modified fats, the structures of trans fatty acid residues consist of geometrical and positional isomers having un-shifted and shifted double bonds, respectively, compared to natural cis compounds. Their biological role and adverse health effects have been evidenced, raising attention for the dietary intake.

On the other hand, some trans fatty acid residues found in living organisms can only be formed through an endogenous transformation of the naturally occurring cis structures. Cis-trans isomerization of lipid double bonds occurs in some bacteria enzymatically or by radical stress produced during physiological and pathological processes.

The biological consequences of the free radical production are the central subject of a very important scientific debate, focusing on the estimation of the type and extent of damage, as well as the efficiency of the protective and repair systems. Research is ongoing to establish the biological effects of free radical-catalyzed transformation of lipids from cis to trans configuration, together with the overall effect of radical stress within the body, focusing on the consequences for membrane structures, lipid metabolism and enzymatic processes.

Cell membrane phospholipid adaptation to nutrient overload

Shlomo Sasson

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Hyperglycemia and hyperlipidemia, which are characteristic of type 2 diabetes mellitus (T2DM), worsen through the progression of the disease. Adaptive responses in the early stages of T2DM compensate this nutrient overload to maintain glucose homeostasis below the diabetic threshold while protecting vulnerable tissues and organs against the development of peripheral complications of diabetes. We studied these adaptive responses in insulin secreting β-cells and vascular endothelial cells (VEC). Our findings indicate a prominent role for lipid peroxidation by-products in the adaptive and protective processes. These factors arise from enzymatic and non-enzymatic metabolism of polyunsaturated fatty acids (PUFA), which are liberated by phospholipase A2-mediated hydrolysis of *sn-2* bonds in phospholipids. We have also observed other prominent changes in the abundance of PUFA, monounsaturated and saturated fatty acids that affect β-cell and VEC functions. The impact of the nutrient overload induced-remodeling of membrane phospholipids in the early adaptive phase and the advanced detrimental stages of T2DM will be discussed and recent data on the cellular mechanisms we discovered in β-cells and VEC will be presented.

On the control of NADPH oxidase by the lipid content

R. Masud, T. Bizouarn, L. Baciou, C. Houée-Levin

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NADPH oxidase is a multi protein complex that delivers superoxide anions upon activation. It comprises two membrane proteins and several cytosolic ones that assemble upon activation. It has been shown that the superoxide ions produced by NADPH oxidase are at the origin of cellular communications induced by all events linked to low dose irradiation such as the bystander effect.

We have constructed a cell-free system, which allows us to study in detail its functioning and the activation process. We show that the lipid composition of the membrane modulates the superoxide production. For instance it is known that cis arachidonic acid activates it but we have shown that the trans isomer inactivates it. Conversely, Cholesterol modulates its activity. These data may provide a basis to conceive activators or inactivators of NADPH oxidase that might modulate the response to ionizing radiations.

Structural and functional characterization of HDL particles isolated from morbidly obese patients before and after biliopancreatic diversion by Roux en Y

Evangelia Zvintzou[1], Georgios Skroubis[2,] Angelika Chroni[3], Donald Gantz[4], Ioanna Mihou[1], Fotis Kalfarentzos[2], and Kyriakos E. Kypreos[1]

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Recently it became apparent that in addition to plasma HDL cholesterol levels, HDL particle functionality is also an important parameter in HDL physiology and pharmacology. Despite an established inverse correlation between obesity and plasma HDL cholesterol levels the effects that weight-loss exerts on HDL structure and functionality remain vague. Here we performed structural and functional characterization of HDL from morbidly obese patients undergoing biliopancreatic diversion by *Roux en Y* before and six months after the operation, and compared it to lean control subjects. Plasma was fractionated by KBr density gradient ultracentrifugation and lipoprotein fractions were isolated and analyzed. Reduction in body weight and body mass index (BMI) correlated with an increase in HDL cholesterol levels that was accompanied by a homogeneous decrease in apoA-I, apoE, and apoCIII of HDL which was accompanied by a concomitant increased in HDL apoA-I/apoCIII or apoA-I/apoE ratios. Despite a decrease in plasma LCAT activity, results from nondenaturing two-dimensional electrophoresis analysis indicated that surgery led to a reduction in pre-β particles and an increase in mature HDL forms that had a profoundly higher antioxidant potential. We are currently in the process of analyzing the lipid content of the HDL in these patients. Overall, our data indicate that malabsorptive *Roux an Y* procedure may trigger significant alterations in the levels, structure and functions of HDL which correlate with significant changes in the protein component of the particle.

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

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Bleomycin is used in chemotherapy regimens in the treatment of patients having testicular germ-cell tumor (TGCT). There is no study in the literature investigating effects of bleomycin and N-Acetyl-L-Cysteine (NAC), and curcumin (Cur) on membrane lipid profile in testicular cancer cells. For this reason, membrane fatty acids were isolated, derivatized and analysed by gas chromatography in NTera-2 cells incubated for 24h with bleomycin (Bleo), N-Acetyl-L-Cysteine (NAC), curcumin (Cur), Bleo+NAC or Bleo+Cur. We studied the MAPK pathway and measured EGFR levels. Bleomycin increased saturated fatty acid (SFA) percentage of membrane lipids, whereas decreased the percentage of monounsaturated (MUFA) and polyunsaturated fatty acid (PUFA). Bleomycin and curcumin combination led to a significant increase in trans lipid isomers of oleic and arachidonic acids. Bleomycin increased p38 MAPK and JNK levels, but NAC attenuated these effects of bleomycin. NAC upregulated bleomycin induced EGFR down regulation. These results highlight the role of the membrane asset for fatty acid remodeling and suggest the potential of lipid-based strategies for influencing cell response and fate in human diseases, such as testicular germ cell tumors.

Keywords: Bleomycin; curcumin; membrane lipid profile, N-Acetyl-L-Cysteine; testicular cancer cells.

Lipidomic profiles and nutrilipidomics: representative steps from bench to bedside

Carla Ferreri and Chryssostomos Chatgilialoglu

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Fatty acid-based lipidomics, in particular focusing on the cell membrane compartment, can contribute to research in pharmacology, biology and medicine giving information on the important role of membranes in the metabolic response.[1] Membrane fatty acids, acting not only as structural components but also as precursors of signalling and mediators, have well known protective and regulatory effects. Using cell, animal and human models our group contributed to investigations on membrane fatty acid composition in health and disease conditions.[2] Membrane fatty acid lipid remodelling is deeply involved in cell adaptation processes, and this knowledge can be either used at the "bench side" for molecular biology and pharmacological studies, and applied at the "bed side" using nutrition and nutraceutical supplementation for the optimal balance of membrane composition in the subjects. This approach called nutrilipidomics combines the detailed erythrocyte membrane fatty acid analysis, carried out with an original high-throughput device for cell selection and work-up, with a personalized strategy in order to restore the membrane fatty acid profile found in the subject to the optimal values, thus recovering an important compartment for the homeostatic and metabolic control. Nutrilipidomics as science innovation born from the CNR research was developed for market innovation in the areas of molecular diagnostics for personalized health care and nutraceuticals/functional food industry, experiencing an innovative productive chain directed toward consumers' needs.

References:

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Noise induced oxidation impairs outer hair cell's function through membrane fluidity loss

Giuseppe Maulucci

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Noise-induced hearing loss is due to damage of outer hair cells (OHCs) which are the source of active amplification in the cochlea and the most vulnerable cells to sound exposure. The common basis for OHC loss by acoustic over-stimulation is triggered by the unbalance of cellular redox status due to reactive oxygen species (ROS) overload. As membrane structural organization is strongly affected by ROS, OHC electromotility may be altered by increased metabolic activity. To address this issue, we investigated in OHC different functional zones the mechanisms linking metabolic functional state (NAD(P)H intracellular distribution) to the generation of lipid peroxides and to the physical state of membranes. In the OHCs of control animals, a more oxidized NAD(P)H redox state is correlated with a less fluid membrane structure. In noise exposed animals, excessive noise induces a topologically differentiated NAD(P)H oxidation in OHC rows, which is damped between 1 and 6 h. Peroxidation occurs after ~ 4h from the noise insult, while ROS are produced in the first 0.2h and damages cells for a period of time after the noise exposure has ended (~ 7.5h) when a decrease of fluidity of OHC plasma membrane occurs. In control animals a functional relation is established between membrane fluidity and NAD(P)H distribution. In noise exposed animals, the time course of NAD(P)H oxidation, lipid peroxidation and membrane fluidity indicates that a perturbation of OHC metabolism triggers lipid peroxidation, which impairs OHC cell function through membrane fluidity loss. OHCs belonging to inner rows, characterized by a lower metabolic activity with respect to other rows, show a less severe metabolic impairment before the onset of membrane destructuration. On the whole, membrane fluidity is regulated by NAD(P)H redox state and lipid peroxidation which represent therefore key targets for a therapeutic rescuing plan from noise insults.

Lipidiomic studies on lipoprotein: insights on the cis/trans C16:1 monounsaturated fatty acid content

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Lipidomic analytical methodologies enable detailed characterizations of different lipid classes identifying their role and recognizing potential lipid biomarkers to establish preventive, therapeutic or nutritional approaches for human health. In our study we have applied a protocol to analyze the lipid fractions of commercial human LDL (Low Density Lipoprotein) and used the gas chromatography (GC) and GC/mass spectrometry (GC-MS) methods to examine the full fatty acid profile. We focused our attention on the hexadecenoic fatty acids which are emerging as interesting products of lipid biosynthesis and metabolomics signalling pathways. The application of combined chemical synthesis and GC-MS analytical protocol produced a satisfactory resolution of positional and geometrical isomers of this monounsaturated fatty acid family, allowing for their recognition in the lipids of human LDL for the first time.

Protein and nucleotide advanced glycation end products as biomarkers for diagnosis, disease progression and therapeutic intervention

Naila Rabbani and Paul J Thornalley

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Cellular and extracellular proteins suffer significant damage *in vivo* by glycation, oxidation and nitration. These processes form glycation, oxidation and nitration adduct residues in proteins. Glycated, oxidized and nitrated proteins undergo cellular proteolysis and release glycation, oxidation and nitration free adducts. Oxidation and glycation of cellular DNA forms oxidised and glycated nucleotides. Nucleotide excision repair releases corresponding oxidised and glycated nucleosides. These adducts are quantified using liquid chromatography with tandem mass spectrometric detection by stable isotopic dilution analysis. Free adducts are determined by analysis of ultrafiltrates of plasma, urine and other physiological fluids. Protein and DNA adduct residues are determined by assay of enzymatic hydrolysates of protein and DNA extracts prepared using cocktails of proteases and nucleases, respectively. Protein damage markers (13 glycation adducts, 3 oxidation adduct and 3-nitrotyrosine) and DNA damage markers (2 glycation adducts and one oxidation adduct) are quantified using 25 μg protein, 10 μg DNA or 25 μl physiological fluid. Levels of markers of protein and DNA damage increase tissue and blood cell protein extracts, plasma, urine and other body fluids in ageing and disease, reflecting the state of disease development and therapeutic intervention. Examples of marker profile changes in diabetes, renal failure, cirrhosis, Alzheimer's disease, cancer chemotherapy and aging will be described.

Electrochemistry – mass spectrometry: A tool to study oxidative modifications in drugs and proteins

Rainer Bischoff

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Electrochemistry (EC) followed by mass spectrometry (MS) is a versatile approach to study oxidative modifications of pharmaceuticals, proteins and other molecules that are susceptible to oxidative modifications, for example due to electrochemically-generated reactive oxygen species (ROS). We have shown that EC can be used to generate a wide range of drug metabolites that are also observed *in vivo* due to the activity of Cytochrome P450 (Cyp450) enzymes (1-3). By coupling a mass spectrometer on-line with the EC cells even short-lived reaction products may be analyzed or potentially captured prior to analysis. We have shown that it is possible to target different reaction pathways by modulating the EC reaction conditions (e.g. electrode material, square-wave pulses). We are currently extending this work by performing a systematic optimization of EC conditions using a Design of Experiment (DoE) approach.

Electrochemical reactions can also be used to oxidize peptides and to cleave the peptide bond C-terminal to tyrosine or tryptophan residues (4-6). This is a novel approach that does not depend on enzymes such as trypsin and may be developed into a fully instrumental protein digestion method. We are currently optimizing the cleavage conditions and notably exploring electrode surfaces that show less protein and peptide adsorption than for example the commonly used glassy carbon electrodes. Our current work aims at combining EC-mediated peptide bond cleavage with the enrichment of the cleavage products through reversible, covalent chemistry.

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A biomimetic model for the study of the free radical-induced lipid modification processes

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The interest in the study of lipid peroxidation processes in model systems has been motivated by aspects of the adverse consequences associated with peroxidation of unsaturated lipids in different natural and biological systems. In an attempt to elucidate the complex process responsible for lipid peroxidation in these systems, various simple model systems including those with surfactants were used. Surfactant supramolecules (micelles, vesicles, lyotropic mesophases) generally serve as models mimicking complex biological systems.[1] An additional advantage of the surfactant supramolecules used as a model system is the possibility to investigate lipid peroxidation under acidic conditions. Low fatty acid solubility under acidic conditions can be bypassed by lipid solubilization into surfactant supramolecules using them as nanoreactors. We have chosen a nonionic surfactant, TWEEN®-20, whose stability and relative non-toxicity allows for its use as a suitable model system for biological media.[2] This model system allows lipid reactivity to be studied in the context of the organization of the lipid molecules within the system, as well as their possible interactions with other types of molecules in their immediate vicinity, which can influence the lipid processes.[3]

A simple system with mixed nonionic surfactants TWEEN®-20/LA micelles as the model for the PUFA oxidation susceptibility assay, also as a very useful tool for studying thiyl radical-catalyzed *cis-trans* isomerization of unsaturated lipids will be described.

References:

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Uncoupling proteins: the mystery about their function(s)

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The uncoupling proteins (UCPs) are a subfamily in a family of mitochondrial membrane anion carriers. The accepted function of the best studied uncoupling protein, UCP1 (earlier thermogenin), is to dissipate the proton gradient of the inner mitochondrial membrane as heat. UCP2, UCP3, UCP4 and UCP5 were discovered in 1997-1999 based on the homology to UCP1. Until now, no function for these proteins has been reliably demonstrated despite over 25 years of intensive research. UCP2-UCP5 are thought to be involved in ROS regulation due to their involvement in proton transport through the inner mitochondrial membrane, thereby diminishing the transmembrane potential. The more recent hypothesis proposes that some UCPs' (i.e. UCP2) are involved in cell proliferation or/and cell metabolism. Here, the latest data from my laboratory concerning UCPs distribution, regulation and evidence about those putative functions will be discussed.

Uncoupling proteins and reactive aldehydes

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Excessive production of reactive oxygen species (ROS) during mitochondrial respiration leads to oxidative stress and thereby mitochondrial dysfunction, which has been associated with degenerative diseases and aging. Under oxidative stress conditions, ROS causes peroxidation of membrane lipids and production of reactive aldehydes. Generation of ROS is strongly regulated by proton leak across the inner mitochondrial membrane. Uncoupling proteins (UCPs), a subfamily of mitochondrial membrane proteins, are proposed to mediate the proton transport across inner mitochondrial membranes thereby dissipating the proton gradient and reducing generation of ROS. In our current work, we are studying the effect of reactive aldehydic products of lipid peroxidation (malondialdehyde, 4-hydroxy-2-nonenal, 4-oxo-2-nonenal, and 4-hydroxy-2-hexenal) on UCPs. For this we measured proton conductance G, across bilayers of various composition reconstituted with recombinant UCP11. We found that 4-oxo-2-nonenal and 4-hydroxy-2-hexenal significantly increases G only in the presence of the activated uncoupling protein. In contrast, 4-hydroxy-2-nonenal and malondialdehyde influences the conductance of the membranes both with and without protein, but in the presence of FA. We propose that both of the following mechanisms, (i) the binding to the positively charged UCP amino acid residues with the subsequent protein conformational change and (ii) the modification of phosphatidylethanolamine (PE) are responsible for the measured increase in G in the membrane.

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A quantitative study on the pro-oxidant behavior of metal nanoparticles

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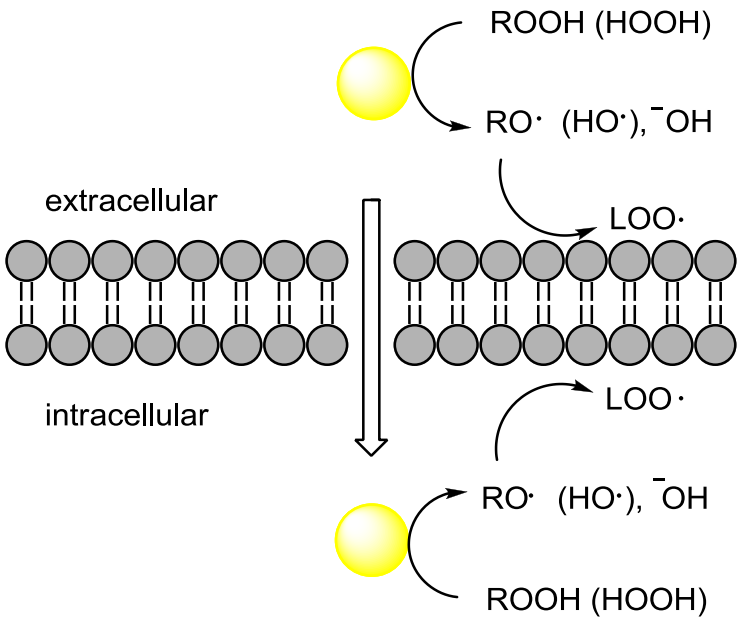
Metal nanoparticles have gained extraordinary popularity in recent years for their potential application in medicine, among other fields. Their potential role in human health is however controversial as most investigations in biological systems agree on outlining their toxicity [1], which has generally been attributed to induction of oxidative stress or pro-oxidant activity [2, 3]. While this specific ability could be potentially exploited for therapeutic use, e.g. as antibacterial and anti-carcinogenic agents, it also requires further understanding, since very little is actually known of the radical surface chemistry of metal nanoparticles [4]. Herein we present an explorative work to clarify the kinetics and mechanism of pro-oxidant activity of metal nanoparticles (gold and cobalt) and we discuss potential strategies to counteract the pro-oxidant behavior by surface functionalization.



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Selenenic acids as chain-breaking antioxidants. Kinetics, thermodynamics and mechanisms

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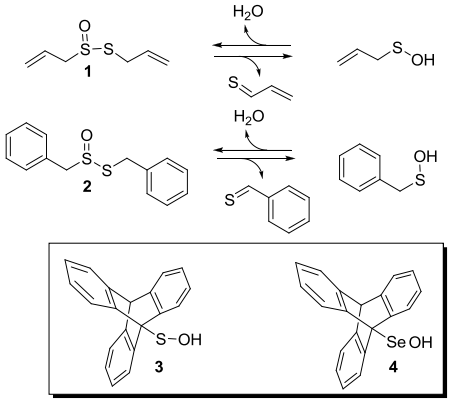
Thiols and derived organusulfur compounds such as disulfides and thiosulfinates have been recognized a pivotal role in cellular redox homeostasis and it is suggested that also the antioxidant and related biological properties of garlic and other *Allium* plants are due to release of similar volatile organosulfur metabolites.[1] The original suggestion that the antioxidant activity of naturally occurring allyl and benzyl disulfides, and their thiosulfinate oxidation products allicin **1** and petivericin **2**, would arise from their fast reaction with peroxyl radicals has been disproven,[2] demonstrating that it comes instead from generation of a sulfenic acid,[3],[4] which undergoes extremely fast reactions with peroxyl radicals in homogenous organic solution.[3],[4] Optimization of the synthetic route has recently brought to the efficient preparation of persistent 9-triptycenesulfenic acid **3**,[5] opening the way to in depth investigation of the thermodynamic and kinetic aspects of its homolytic reactivity. Stimulated by the interplay of sulfur and selenium in biological systems, we also aimed at extending such knowledge to homologue selenenic acids, whose role in cellular antioxidant defense has been suggested for long time, *e.g.* as intermediates in the redox cycle of enzyme glutathione peroxidase (GPx). Synthesis of 9-triptyceneselenenic acid **4** has allowed for the first time direct experimental comparison of the different redox properties of the two homologous functional groups, which will be discussed with a focus on their biological implications.



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Preventive effects of red wine polyphenols on oxidative stress and metabolic syndrom

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High fat and high sugar diets and lack of physical activity are believed to contribute to the increasing rates of obesity in wealthy societies. This trend is associated with a parallel increase in the prevalence of insulin resistance (IR) and non-alcoholic fatty liver disease (NAFLD). Recent studies demonstrated that some dietary polyphenols as resveratrol may prevent oxidative stress, modulate lipid metabolism and thus prevent against hepatic steatosis and/or IR. The present study was designed to evaluate the preventive effect of a red wine polyphenol (PP) extract on liver and skeletal muscle redox status (mainly mitochondrial respiratory chain and the NADPH oxidase system), lipid content and on the lipid metabolism signaling pathway in rats fed a high fat-high sucrose diet. This study showed an induction of mitochondrial dysfunction by high fat high sucrose diet and the development of oxidative stress in liver while PP extract was shown to partially prevent oxidative stress in liver. In skeletal muscle, the NADPH oxidase system seems to be mainly involved in oxidative stress.

Moreover, our results showed clearly that high fat high sucrose diets and polyphenols modulate differently lipid metabolism in tissues. In liver, PP prevent lipid accumulation and hepatic steatosis by activating fatty acid oxidation. In skeletal muscle, PP regulate membrane fatty acids composition and fatty acid and glucose transporters expression, thus preventing lipid accumulation and enhancing glucose transport. These modifications may prevent IR in skeletal muscle and metabolic syndrome.

4-Hydroxynonenal-histidine adducts: Major bioactive marker of oxidative stress, lipid peroxidation and oxidative homeostasis

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While oxidative stress implies fast chemical reactions of reactive oxygen species with major biomolecules, lipid peroxidation cannot be considered just as an integral part of oxidative stress, but as fundamental pathophysiological process of various disorders, mediated through reactive aldehydes, the end-products of lipid peroxidation.

Findings on growth regulating activities of the end-product of lipid peroxidation 4-hydroxy-2-nonenal (HNE), which acts as a "second messenger of free radicals", overlapped with the development of antibodies specific for the aldehyde-protein adducts. These led to qualitative immunochemical determinations of the HNE presence in various pathophysiological processes and to the change of consideration of the aldehyde's bioactivities from toxicity into cell signalling

Moreover, immunohistochemical findings of the HNE-protein adduct in various organs under physiological circumstances support the concept of "oxidative homeostasis", which implies that oxidative stress and lipid peroxidation are not only pathological but also physiological processes. Reactive aldehydes, at least HNE, could play important role in oxidative homeostasis, while complementary research approaches might reveal the relevance of the aldehydic-protein adducts as major biomarkers of oxidative stress, lipid peroxidation and oxidative homeostasis.

Through networking projects of the European Cooperation in Science and Technology (COST) and the International HNE-Club validation of the methods for lipid peroxidation and further developed the genuine 4-HNE-His ELISA founding quantitative and qualitative methods for detection of 4-HNE-His adducts as valuable tool to study oxidative stress and lipid peroxidation in cell cultures, various organs and tissues and eventually for human plasma and serum analyses.

Notes