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Investigating the Oxygen Reactivity in Enzymes.
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Our presentation will summarize our efforts towards a more in-depth understanding of the ability of flavoenzymes to differentially react with oxygen [1]. We have investigated two different types of monooxygenases, which are capable of activating molecular oxygen through the stabilization of a flavin-(hydro)peroxide intermediate. These enzymes exhibit a properly shaped cavity in front of the C4a atom of the flavin that promotes intermediate stabilization [2,3]. Most remarkably, in flavin-containing monooxygenases flavin-hydroperoxide formation directly involves the $NADP^+$ ligand, which, therefore, appears to play the dual function of reducing the flavin and stabilizing a critical catalytic intermediate. We are using a combination of site directed mutagenesis and molecular dynamics to investigate the role of residues surrounding the flavin in tuning oxygen reactivity.

[1] Mattevi, A. (2006) *Trends Biochem. Sci. 31*, 276-283. [2] Alfieri, A., Ferini, F., Ruangchan, N., Prongjit, M., Chaiyen, P., Mattevi, A. (2007) *Proc. Natl. Acad. Sci. USA 104*, 1177-1182. [3] Alfieri, A., Malito, E., Orru, R. Fraaije, M.W., Mattevi, A. (2008) *Proc. Natl. Acad. Sci. USA* 195, 6572-6577. [4] Baron, R., Riley, C., Chenprakhon, P., Thotsaporn, K., Winter, R., Alfieri, A., Forneris, F., van Berkel, W., Chaiyen, P., Fraaije, M.W., Mattevi, A., McCammon, J.A. (2009) *Proc. Natl. Acad. Sci. USA* 106, 10603-10608. [5] Baron, R, McCammon, J.A., Mattevi, A. (2009) *Curr. Opin. Struct. Biol.* 19, 672-679.

Keywords: reactive oxygen species, binding, biocatalysis

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Photosystem II at 2.9 Å resolution - Quinones, lipids, channels and chloride ion.
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Photosystem II (PSII) is a homodimeric protein-cofactor complex acting as light-driven water:plastoquinone oxido-reductase and is located in the photosynthetic thylakoid membrane of plants, green algae and cyanobacteria. PSII oxidizes two water molecules at the unique Mn_4Ca cluster to molecular (atmospheric) oxygen, 4 protons and 4 electrons. The protons drive ATP synthetase, and the electrons reduce plastoquinone (Q_B) to plastoquinol (Q_BH_2) that is exported and delivers the electrons (through the cytochrome b_6f complex) to photosystem I. Here the electrons gain a high reducing potential and serve at NADP reductase to generate NADPH that together with ATP reduces CO_2 to carbohydrates in the Calvin cycle.
The crystal structure of PSII from *Thermosynecho-coccus elongatus* at 2.9-Å resolution allowed the unambiguous assignment of all 20 protein subunits and complete modeling of all 86 cofactors, among them 25 integral lipids, per PSII monomer [1]. The presence of a third plastoquinone Q_C and a second plastoquinone-transfer channel, which were not observed before, suggest mechanisms for plastoquinol-plasto-quinone exchange, and we calculated possible water or dioxygen and proton channels. Putative oxygen positions obtained from Xenon derivative crystals indicate a role for lipids in oxygen diffusion to the cytoplasmic side of PSII. The chloride position suggests a role in proton-transfer reactions because it is bound through a putative water molecule to the Mn_4Ca cluster and is close to two possible proton transfer channels.

[1] Guskov A., Gabdulkhakov A., Broser M., Zouni A., Saenger W. *Nature Struct. Mol. Biol.*, 2009, 16, 334.

Keywords: photosynthesis, membrane protein, cofactors

FA1-MS10-T03

Crystal Structure of of the Nitrogenase-like Dark Operative Protochlorophyllide Oxidoreductase Catalytic Complex,
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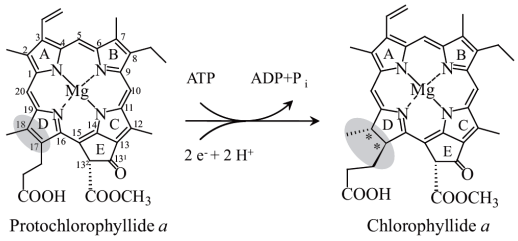

In the biosynthesis pathway of (bacterio)chlorophyll, two evolutionary distinct enzymes catalyze the two electron reduction of ring D of protochlorophyllide to chlorophyllide: In angiosperms monomeric, light-dependent protochloro-phyllide oxidoreductase (LPOR) catalyses the reaction, whereas anoxygenic, photosynthetic bacteria make use of an ATP-dependent process catalyzed by dark operative protochlorophyllide oxidoreductase (DPOR). DPOR is composed of three distinct subunits, ChlL, ChlN and ChlB. ChlL forms a homodimer $ChlL_2$ with an intersubunit [4Fe-4S] cluster. $ChlL_2$ is an ATP-dependent reductase transferring single electrons to the heterotetrameric complex of the other two proteins $(ChlN/ChlB)_2$. Each half of this tetramer bears an intersubunit [4Fe 4S]-cluster and has a protochlorophyllide binding site.

We present the crystal structure of the catalytic (ChlN/ChlB)2 complex of DPOR from the cyanobacterium *Thermo¬synecho¬coccus elongatus* at 2.4 Å resolution. Subunits ChlN and ChlB are structurally related to each other as well as to the subunits NifD and NifK of the MoFe-protein of nitrogenase.
The intersubunit [4Fe-4S] cluster of DPOR is coordinated by 3 cysteines from ChlN, while the fourth ligand is an aspartate residue of ChlB. This coordination destabilizes the cluster dramatically making it extremely sensitive to oxygen. Although aspartatic acid residues have been known to function in iron-sulfur cluster coordination for many years, this is the first crystal structure actually demonstrating this unusual coordination.

Keywords: nitrogenase-like metalloprotein, intersubunit [4Fe-4S] cluster, aspartate ligation of cluster

FA1-MS10-T04

Redox regulation and substrate specificity in plant peroxisomal β-oxidation. Anette Henriksen, Caspar E. Christensen, Valerie E. Pye. *The Protein Chemistry Group, Carlsberg Laboratory, Valby, Denmark.*
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Fatty acids are essential biomolecules used to create cell membranes, to store energy, for intracellular signalling and for cell-to-cell communication. In plants, all fatty acids are catabolised in the peroxisomes by a process known as β-oxidation [1]. The break-down of fatty acids is required for mobilisation of storage fats to power germination and for the synthesis of growth and stress response hormones. Four enzyme activities residing on three proteins are involved in the core of the process, namely acyl-CoA oxidase, dehydrogenase, hydratase and thiolase activities.
Crystal structures have been determined of MFP2 (hydratase, dehydrogenase) and KAT2 (thiolase) from *Arabidopsis thaliana*. One of the by-products generated by peroxisomal β-oxidation is H_2O_2 and it is in itself an important signalling molecule. Based on disulfide bond reduction analysis, antibody pull-downs and enzyme activity measurements three aspects of peroxisomal β-oxidation are discussed: the role of KAT2 in regulating fatty acid metabolism and H_2O_2 level, the very limited activity of the known multifunctional enzymes with long chain substrates and the role of protein-protein interactions.

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Keywords: thiolase, multifunctional protein, fatty acid metabolism

FA1-MS10-T05

Monomeric Isocitrate Dehydrogenase in an Open-Conformation Complex with $NADP^+$. Navdeep S. Sidhu[a], Sanjukta Aich[b], George M. Sheldrick[a], Louis T.J. Delbaere[b]. [a]*Department of Structural Chemistry, University of Goettingen, Germany.* [b]*Department of Biochemistry, University of Saskatchewan, Canada.*
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The Krebs cycle is a central biochemical pathway in aerobic pro- and eukaryotes. A crucial regulatory step is the oxidative decarboxylation of 2R,3S-isocitrate to form α-ketoglutarate and CO_2, which is released into the environment, with the simultaneous reduction of $NAD(P)^+$ to NAD(P)H, catalyzed by the enzyme isocitrate dehydrogenase (IDH). Most IDHs are hetero-oligomeric or homodimeric; more recently, monomeric IDHs have been described exclusively in some bacteria. The 80-kDa monomeric IDH from *Corynebacterium glutamicum* (CgIDH) has, to our knowledge, the highest described coenzyme specificity of all dehydrogenases, preferring $NADP^+$ over NAD^+ by a factor of 5×10^4 [1]. It also represents an alternative model for IDH regulation in the Krebs cycle as compared with the more widely studied model organisms *Bacillus subtilis* and *Escherichia coli* [2]. A 3.2 Å crystal structure of a monomeric IDH holoenzyme, from *Azotobacter vinelandii*, has been solved previously [3]. However, it displayed the enzyme in a closed conformation in which the substrate does not have access to the active site. The 1.75 Å apoenzyme structure of CgIDH has also been solved, displaying an open conformation [4]. In the present study, we report the 1.9 Å crystal structure of CgIDH with two molecules in the asymmetric unit, one of them in the apoenzyme, and the other in the $NADP^+$-bound holoenzyme form. Both forms display a similar, open conformation, which allows the substrate isocitrate access to the active site. $NADP^+$ binds to the protein through interactions of its 2'-phosphoadenosine and diphosphate moieties while the nicotinamide nucleoside moiety is disordered, which is consistent with the prediction made by Chen and Yang, based on their substrate specificity study and on a crystallographic study of dimeric IDH from *E. coli* (EcIDH) by Stoddard *et al.* [5], that the binding site for the nicotinamide ring of $NADP^+$ is formed by the γ-carboxylate of bound isocitrate in CgIDH as in EcIDH [1]. The holoenzyme displays a significantly lower average B factor than the apoenzyme in the asymmetric unit, suggesting that $NADP^+$-binding reduces the conformational freedom of the enzyme.

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Keywords: Krebs cycle, monomeric isocitrate dehydrogenase, NADP binding