Friday Morning Session III - September 25

L9

STRUCTURAL BACKGROUND OF NON-ANTIBIOTIC AFFINITY OF MINOCYCLINE TO SECRETORY PHOSPHOLIPASE A_2

D. Dalm, G. J. Palm, W. Hinrichs

Institute for Biochemistry, Ernst-Moritz-Arndt-University of Greifswald, Germany
daniela.dalm@uni-greifswald.de

Apart from their antibiotic capacity tetracyclines like other antibiotics, have been found to have various non-antibiotic properties [1]. Inhibitory activities of tetracyclines have been reported for rheumatoid synovium, cornea, inflamed gingiva, osteoarthritic cartilage, allergen induced inflammation and cancer cells [2].

Phospholipase A_2 (PLA_2) catalyzes the hydrolysis of the fatty acid ester in the *sn*-2 position of membrane phospholipids. The liberation of arachidonate is the rate-limiting step for the biosynthesis of eicosanoids, which act as proinflammatory mediators [2].

The secretory phospholipase A_2 ($sPLA_2$) which is implicated in inflammatory diseases was shown to be inhibited by the lipophilic tetracyclines minocycline and doxycycline [3].

X-ray crystal structure analysis at 1.65 Å resolution demonstrates that the minocycline (minoTC) molecule makes a number of van der Waals contacts in the hydrophobic environment of the substrate binding site. The minocycline shields the entrance for substrates to the active site resulting in the inhibition of the phospholipase A_2.

The location of the inhibiting minocycline molecule was confirmed by well defined electron density for minoTc rings B, C and D. In comparison to the inhibitor free structure (PDB entry 1PSH), conformational changes upon tetracycline binding have been observed in the calcium binding loop.

The affinity of minocycline to PLA_2 was measured by surface plasmon resonance. The resulting dissociation constant K_D was $1.76 * 10^{-4}$ M.

These results provide the structural background for the usage of tetracycline derivatives in the therapy of inflammatory processes where the secretory PLA_2 plays a crucial role. Lipophilic tetracyclines might be a new type of lead compound for the design of specific inhibitors of phospholipase A_2.

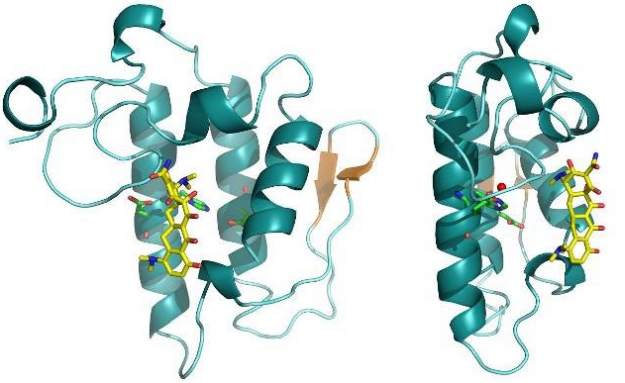

Figure 1. This figure shows two overall views of the PLA_2 with a bound minocycline molecule (yellow). The active site residues are highlighted in green. The figure was created with PyMOL.

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L10

STABILIZING PROTEINS FOR CRYSTALLIZATION – HOW THERMOFLUOR CAN HELP

Linda Schuldt and Manfred. S. Weiss

EMBL Hamburg Outstation, c/o DESY, Notkestr. 85, D-22603 Hamburg, Germany

One of the major limiting steps in macromolecular structure determination is the crystallization of the target molecules into well-ordered three-dimensional crystals. Key prerequisites for successful crystallization of biological macromolecules are the homogeneity, stability and solubility of the molecule to be investigated [1]. Consequently, it appears to be obvious that the optimization of these sample properties improves the success rate of crystallization. One potential approach to perform this analysis is Thermofluor®, which is a method to monitor ligand effects on tem-

perature-dependent protein unfolding [2]. With the Thermofluor® method stabilizing buffer compositions as well as potential ligands can be identified, which turned out to be beneficial for sample purification and crystallization. The fundamentals of the Thermofluor method will be presented, which will be supplemented with results obtained in our laboratory.

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L11

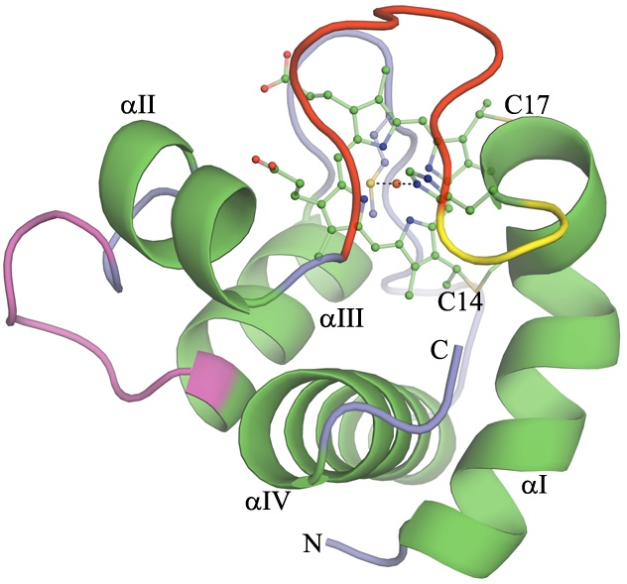
ATOMIC-RESOLUTION STRUCTURE OF REDUCED AND OXIDIZED CYTOCHROME c_6

W. Bialek[1], S. Krzywda[2], A. Szczepaniak[1], M. Jaskolski[2,3]

[1]Department of Biophysics, Faculty of Biotechnology, University of Wroclaw, Poland
[2]Department of Crystallography, Faculty of Chemistry, A. Mickiewicz University, Poznan, Poland
[3]Center for Biocrystallographic Research, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland
bialekw@uwosh.edu

During photosynthesis, electron transfer between two membrane-bound complexes, cytochrome (cyt) b_6f and photosystem I (PSI), can be accomplished by the copper-containing protein, plastocyanin (PC), or the heme protein, cytochrome c_6. PC is found as a unique electron carrier in higher plants. Some algae and cyanobacteria express either PC or cyt c_6, whereas other are able to produce both proteins depending on copper availability. Cytochromes c_6 are water-soluble, low-spin heme-containing proteins involved in the high-potential (340-390 mV) electron transport chain. They are characterized by low molecular mass (80-90 residues) and have a heme group covalently bound *via* two Cys residues. The c_6 protein from the mesophilic cyanobacterium *Synechococcus* sp. PCC7002 has an unusual amino acid composition, with the two smallest residues, Gly and Ala, accounting for 36% of its sequence. Furthermore, the protein contains an unusual heptapeptide $K_{44}DGSKSL_{50}$ insertion that has not been detected in other cytochromes c_6.

The 3D structure of this unusual cytochrome c_6 has been determined at atomic resolution by X-ray crystallography for the reduced (1.22 Å) and oxidized (0.83 Å) forms of the protein. The structures have been refined to *R*-factors of, respectively, 0.107 and 0.095. Despite the presence of the unusual insertion, the overall fold is similar to that of other class I cytochromes *c*. The insertion is located between helix II and III, which is the most variable region of the protein, as assessed by comparison with crystal structures of other c_6 molecules. The first six residues of the insertion form a loop exposed to the solvent, whereas Leu50 is the first residue of helix III. Several specific noncovalent interactions are found inside the insertion, as well as between the insertion and the rest of the protein. A comparison of the two structures reveals only slight conformational changes of the insertion. Some changes are observed at residues located inside the heme pocket, namely Lys29, Gln57 and Gln62. The most conspicuous difference between the reduced and oxidized protein is a distortion along the axis between the heme iron and its sixth axial ligand, Met65.



The structure of the reduced form of cytochrome c_6 from *Synechococcus* 7002 determined at 1.22 Å resolution. The 3_{10} helix (yellow) is followed by the -loop (red). The specific insertion is shown in magenta. The N and C termini as well as the two Cys residues with covalent links to the heme group are marked by labels. The heme group is shown in ball-and-stick representation.

L12

SUBDOMAINS OF THE AMYLOID PRECURSOR PROTEIN (APP) FALL INTO PLACE: X-RAY STRUCTURE OF ITS N-TERMINAL HEPARIN BINDING DOMAIN E1

Sven O. Dahms, Sandra Hoefgen, Dirk Roeser and Manuel E. Than

Leibniz Institute for Age Research (FLI), Protein Crystallography Group, Beutenbergstr. 11, 07745 Jena, Germany, than@fli-leibniz.de

Alzheimer's disease (AD) is the most frequent dementia worldwide occurring predominantly in the elderly population. The amyloid precursor protein (APP) is the key player in Alzheimer's disease pathology hence abnormal proteolytic processing by the -, and -secretases leads to excessive overproduction of neurotoxic A peptide species [1]. On the other hand transmembrane APP and its analogues are essential for neuronal development and cell homeostasis in mammals. We have extensively investigated structural and biochemical properties of a recombinant protein corresponding to the N-terminal 190 amino acids. Our X ray structure at 2.7 Å resolution shows for the first time how the growth-factor like [2] and the copper binding domain [3] of APP interact together forming one closed conformational and functional entity, the heparin binding domain E1. The rigidity of the inter-domain association proves pH dependent in limited proteolysis and the resulting interaction interface includes evolutionary highly conserved residues. In addition, heparin derived dodecasaccharides induced in an entothermic and pH dependent process heparin-bridged APP-E1-dimers characterized by low micromolar binding constants. Limited proteolysis experiments in presence and absence of heparin enabled us to model the heparin $[APP\text{-}E1]_2$ complex based on a dimer contact observed in our crystals. These results shed new light on the function of APP in cell signaling and cell-surface interactions, arguing that APP might fulfill different functions depending on its (sub)cellular localization and its oligomerization state.

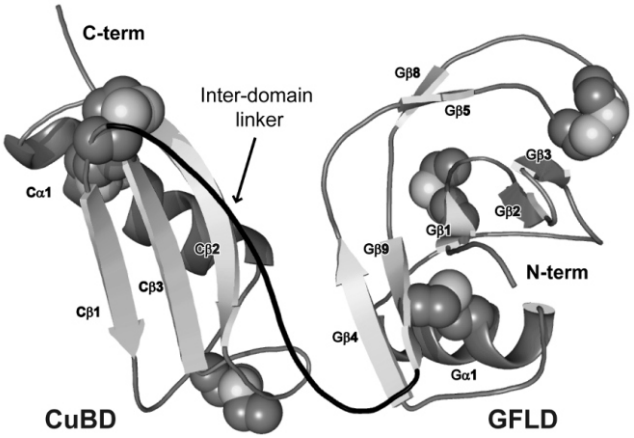


Figure 1 Structure of the APP-E1 domain.

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