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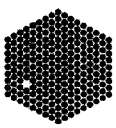

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Editorial Note (HASYLAB)
The authors of the individual scientific reports published in the HASYLAB annual report (part I and II) are fully responsible for the contents.
We kindly request your comprehension as pertains the adaption of the layout of contributions to our requirements (without changing the contents). Altough we tried to take care of any errors caused by the electronic submission of the contributions, we cannot fully exclude this possibility.

Contributions with the MPSD group :

Effect of DMSO and the inhibitors DCCD and Azide on ATP-synthase and F_1ATPase from Micrococcus luteus

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ATP-synthase, its catalytic head fragment F_1ATPase, the oligomeric oxygen transport proteins Hemocyanins and chaperonins as GroEL are large flexible multidomain proteins (≥ 400,000 mass). They exhibit a special form of structural dynamics, long range rearrangements of protein domains, which may be stable folding units inside a protein or subunits of a protein complex. For the investigation of those intramolecular movements we have established the open study group "structure dynamics of flexible protein domains" ("Strukturdynamik flexibler Proteindomänen") of presently five university and Max-Planck groups. As shown in figure1, the moleculare motions are studied using a concept of complementary structure investigation instruments. The choice of the method applied in parallel depends on the localization and course of the movement in space and time: If the structure dynamics of the whole protein is suffient, the study is done with synchrotron X-ray scattering, whereas a differentiation between domains of different scattering power requires neutron scattering with contrast variation, e.g. after selective deuteration. Transient states occuring during biological activity cycles are investigated by time resolved scattering, whereas the regulation of biological activity is studied by comparative static scattering of stable or frozen protein intermediates. The latter type of structure dynamics can be depicted as molecular switching or allosteric regulation, which may be a flip-flop mechanism according Lanyi [1].

ATP-synthase is a proton pumping membrane protein, which couples the energy dependent ion transfer to the synthesis of the energy rich compound ATP. It plays a key role in cellular energy metabolism (bioenergetics) as the terminal element in the protein chain of biological oxidation and photosynthesis . The membrane intrinsic F_0-part (15 subunits) pumps the protons, whereas the peripheral F_1-fragment (F_1ATPase; 80% of mass; 9 subunits) contains the three catalytic and three non-catalytic reaction centers in clefts at the outer surface of a flexible oligomeric protein cylinder (subunits: $\alpha_3\beta_3\delta\varepsilon$). Inside the cylinder a connective protein subunit (γ) couples the suggested movements of the two large subcomplexes by its rotational motion [2,3]. The structure of F_1ATPase is known with atomic resolution from X-ray crystallography [4]. Nevertheless the catalysis mechanism as well as its structural regulation is widely unknown, mainly because the molecular motions have been localized in time and space only scarcely [5-7]. This can be done by small angle scattering of protein solutions and interpretation by molecular modelling, which allows the direct inclusion of the results of crystallography, genetics and surface labeling or crosslinking in case of our FVM cube method [8,9]. A key technique is the static comparison of stable native and inhibited enzyme intermediates in parallel to time resolved studies.

We have investigated the structure of the ATP-synthase and its catalytic fragment F_1ATPase from the aerobic grampositive bacterium *Micrococcus luteus*, which are remarkable stable against aggregation and radiation demage. The study was done with native and inhibited modifications of the enzymes at the HASYLAB beamline B1 (JUSIFA). Highly precise results ($I/Io > 10^{-4}$) in a broad q-range (0.01 - 0.6 A^{-1}), as required for the comparison of protein modifications and

modelling, were obtained by irradiation of a quarz flow-through capillary with a small beam (0.7 x 0.9 mm; 10^8 ph/s). The protein solutions contained 10% (v/v) glycerol as radical scavenger, in case of complete ATP-synthase additionally as detergent the bile acid taurodesoxycholate (TDOC; 5 mM = 1.5 * cmc). The enzymes were inhibited by azide, which uncouples the three catalytic centers in the F_1-head reversibly in the concentration range c = 1-10 mM, and dicyclohexylcarbodiimide (DCCD), which blocks the proton transfer by reaction at c = 10^{-5} M covalently (irreversibly) with single carboxylic groups of the c-subunits inside the F_o-part. The hydrophobic DCCD has to be introduced by a solvent. First experiments showed that the usual solvent ethanol (1% v/v) yields a structural artefact additional to the inhibitor action. This was avoided by choosing DMSO (0.1-1.5% v/v), a polar but non-protic water soluble solvent suitable for ezyme kinetic studies. The water soluble inhibitor azide yielded a stepwise size increase, which was indicated by a jump of the radius of gyration during structural inhibitor titration (R_g-increase = 57.0 to 60.0 ± 0.8 Å for ATP-synthase, Δ= 4.6%; and R_g-increase = 45.09 ± 0.24 Å to 46,0 ± 0,2 Å for F_1ATPase, Δ= 2%). The results refer to the protein structures after equilibration with the inhibitors (t > 2 h at 20°C). The time course of the inhibitor reaction was investigated in parallel by time resolved small angle scattering at ELETTRA-SAXS using a stopped-flow device [6] and will be shown elsewere. As shown in fig.2, the size increase of the enzymes can be explained by a radial movement of the large subunits in the catalytic F1-head.

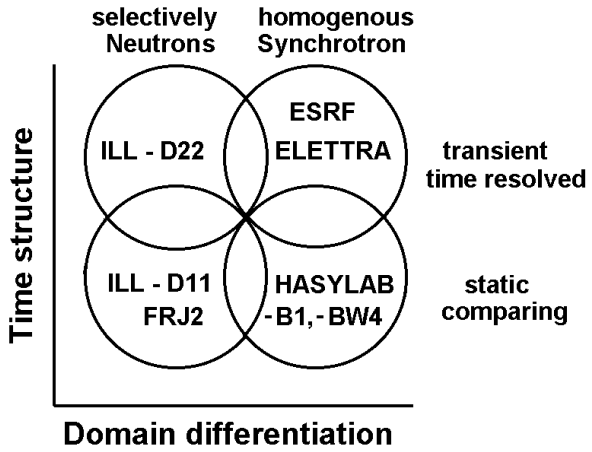

Figure 1: Concept of complementary instruments for protein dynamics research

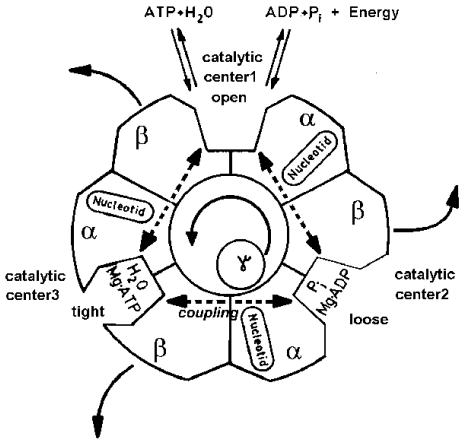

Figure 2: The size increase of ATP-synthase and F_1ATPase is explaind by a radial movement of large subunits.

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