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Hamburger Synchrotronstrahlungslabor
HASYLAB
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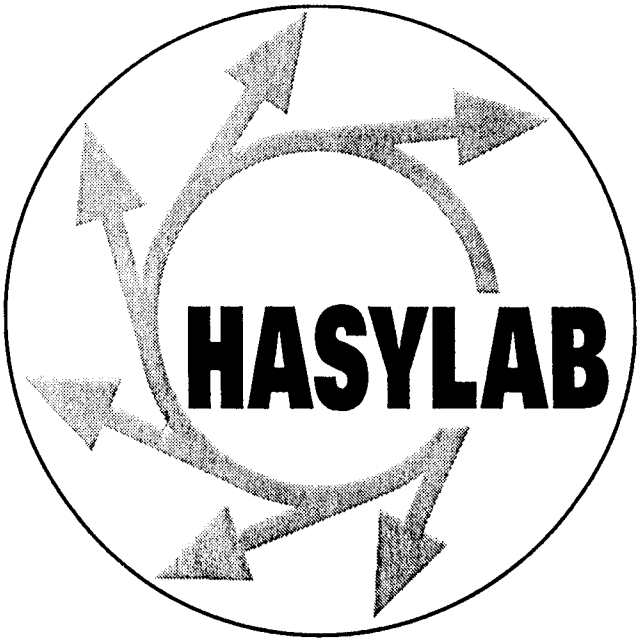
DESY

Contributions with the MPSD group :



Structural switch of ATP-synthase and F_1ATPase by Azide

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ATP-synthase is a large membrane protein complex of 500,000 mass, which plays a key role in energy storage of all known organisms. In the bioenergetic system of cells, ATP-synthase couples the proton-transport across a membrane with the chemical synthesis or hydrolysis of an energy rich compound: adenosine triphosphate, ATP [1]. ATP-synthase consists of two subcomplexes, the membrane intrinsic proton transporter F_O (100,000 mass) and the membrane peripheral F_1ATPase fragment (400,000 mass), which bears three catalytic and three non-catalytic nucleotide binding centers. Until now, only the structure of the F_1ATPase fragment has been solved after protease modification and inhibition of the enzyme [2]. The reaction and coupling mechanism is unknown because the structure of the intact complex and the structure dynamics of the working enzyme have only scarcely been investigated [3]. Possibly, ATP-synthase and further proton translocating membrane proteins, e.g. cytochrome oxidase, show general principles of structural energy storage and molecular regulation.
The three catalytic centers are coupled energetically in a highly cooperative manner. This coupling can be eliminated by the inhibitor azide, which switches over the enzyme from a highly active "multsite catalysis" to a low active unisite catalysis.
We have investigated the structure of purified ATP-synthase and F_1ATPase from *Micrococcus luteus* [4] in aqueous solution by X-ray small angle scattering at the JUSIFA camera at the beamline B1 at DESY / HASYLAB, Hamburg in the presence of various concentrations of sodium azide (0.03 - 10 mM). The solutions were irradiated at 4°C in a quartz flow-through capillary using a 0.7 x 1.1 mm^2 beam of 8 keV (1.5 Å) photons. Scattering profiles of protein solutions (4 and 10 g/l) and buffer were taken at 0.9 and 3.6 m distance from the sample using a 2D-detector (256 x 256 pixel) in 3 h for each specimen.. The ATP-synthase was subjected to the experiment as component of a weakly buffered detergent solution (5 mM taurodesoxycolate, TDOC, 50 mM Tris-buffer, pH7.5). The weak scattering signal of the small TDOC-micelles

[5], which were furthermore nearly matched in contrast by the glycerol content of the solvent (10% glycerol v/v) , was estimated with protein-free samples and subtracted. The experiental scattering profiles were interpreted by molecular modelling, which was performed with cube models according to the FVM procedure [6] (improved).

F_1ATPase and ATP-Synthase showed a shrinking of the proteins with very low azide concentration (0.03 mM). Between 0.1 an 1 mM an increase of R_g by 2.6% was observed, which was stable over a broad concentration range from 1 to 10 mM with ATP-synthase. In this region the inhibited enzyme catalyzes the unisite ATP hydrolysis even in the presence of high amounts of MgATP. At 37°C the reaction rate is only $k_{cat} = 1.08\ s^{-1}$, 2 % of the multisite reaction rate.

The result is consistent with the structural dynamics in reaction cycle [3] as it indicates a strong flexibilty of the hollow subunit assembly by reversible inhibition. In further experiments, the highly resolved results obtained at the JUSIFA instrument at HASYLAB are combined with time resolved experiments of azide-inhibited and native working ATP-synthase during catalysis at the ELETTRA and ESRF synchrotrons (concept of complementary instruments).

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2) Walker, J.E. (1994) Nature 370, 621-628
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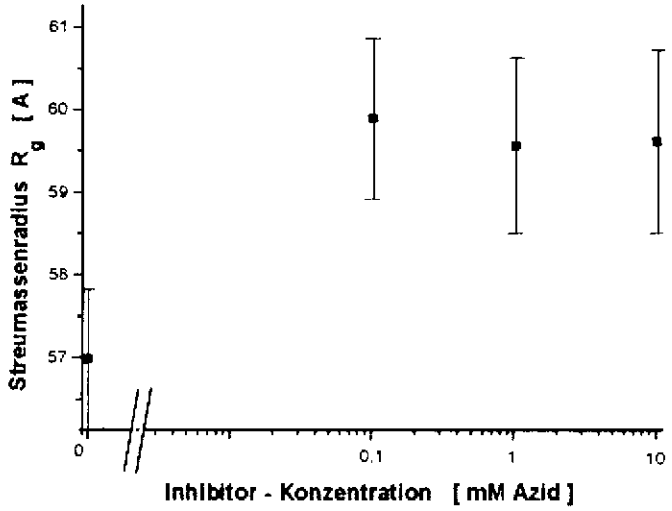
6) Nawroth, T. (1989) Physica B 156, 493-495



The cooperativity inhibitor azide yields a stable increase in size (plateau in R_g) of ATP-synthase from *Micrococcus luteus* in that range, where it switches the enzyme over from the cooperative "multisite" to the "unisite" catalysis (1 mM).