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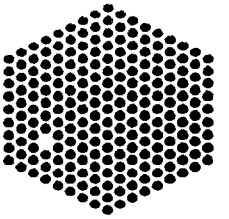

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Molekularbiologie

Structure and dynamics of bacterial ATP-synthase and F_1ATPase

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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.
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 [1]. 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 [2]. Possibly, ATP-synthase and further proton translocating membrane proteins, e.g. cytochrome oxidase, show general principles of structural energy storage and molecular regulation.
We have investigated the structure of purified ATP-synthase and F_1ATPase from *Micrococcus luteus* [3] in aqueous solution by X-ray small angle scattering at the JUSIFA camera at the beamline B1 at DESY / HASYLAB, Hamburg. The solutions were irradiated at 4°C in a quartz flow-through capillary using a 0.9 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. Additional experiments were performed at the RöFo camera at the beamline A1 at HASYLAB with the same cell at 1.85 m distance from a 2D-detector (1.54 Å). 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 [4], 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 X-ray scattering of F_1ATPase solution consisted of a central signal and a series of sharp side maxima, namely at q_S = 1.08, 1.65 and 2.0 nm^{-1}. These correspond to average subunit spacings of d_S = 6.8, 3.8 and 3.1 nm according to Bragg's law ($d_S = 2\pi / q_S$). The scattering profile of ATP-synthase showed only a series of smoothed shoulders at q_S = 0.85, 1.0, 1.1, and 1.5 nm^{-1}. The evaluation yielded a radius of gyration of R_g=4.3 nm for F_1ATPase and 5.9 nm for ATP-synthase. The experiental scattering profiles were interpreted by molecular modelling, which was performed with cube models according to the FVM procedure [5] (improved). The present model of ATP-synthase shown in the figure fits the experimental data at $0.1 < q < 2.5\ m^{-1}$.
By incubation with sodium azide (0.1 to 26 mM) we found a stepwise shrinking of the F_1ATPase molecule, which was indicated by a decrease of the radius of gyration to R_g = 3.7 nm. This result is consistent with the structural dynamics in reaction cycle [2] as it indicates a strong flexibilty of the hollow subunit assembly by reversible inhibition.

1) Walker, J.E. (1994) Nature 370, 621-628
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3) Freisleben, H.-J.; Zwicker, K.; Jezek, P.; John, G.; Bettin-Bogutzki, A.; Ring, K.; Nawroth, T. (1996) Chem. Phys. Lipids 78, 137-147
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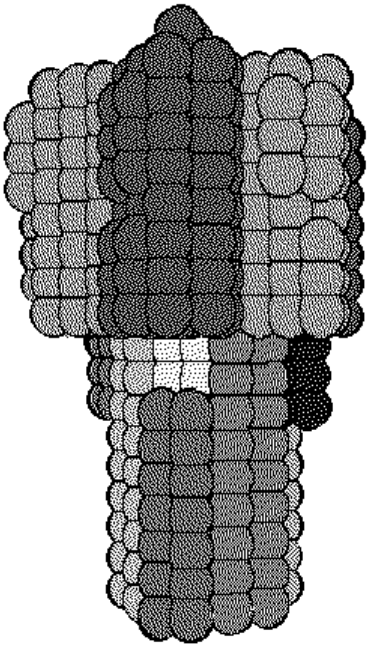

The cube model of ATP-synthase from *Micrococcus luteus* fits the experimental scattering profile in central signal (size) and the side maxima ($q < 2.5\ nm^{-1}$).