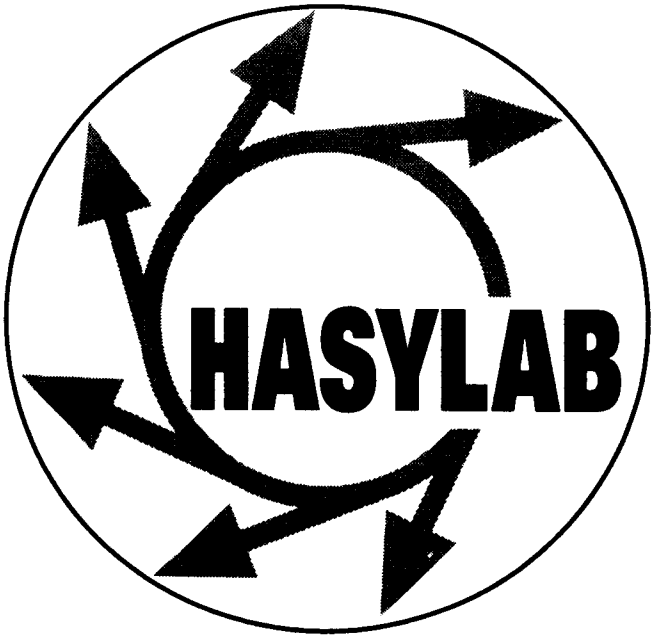
Jahresbericht 1993

Hamburger Synchrotronstrahlungslabor
HASYLAB
am Deutschen Elektronen-Synchrotron
DESY



X-ray small angle scattering of ATP-synthase from *Micrococcus luteus*

K. Zwicker[1]; G. Grüber[1]; G. Heinz[1]; P. Eßwein[1];
M. Hütsch[2]; H.B. Stuhrmann[2]; T. Nawroth[1]

1) Institut für Biochemie, AG Membranbiochemie, Becherweg 30, Gutenberg-Universität, 55099 MAINZ
2) HASYLAB/DESY, Notkestr 85, 22603 HAMBURG & GKSS Forschungszentrum, 21494 Geesthacht

ATP-synthase is the terminal element in the chain of membrane proteins of energy conservation in cells. This complex protein (M=500,000) constists of at least 8 types of subunits in up to 12 copies each [1]. As shown in fig.1 it couples the transport of protons with the synthesis of the energy-rich molecule ATP, which supplies energy to processes inside the cell. Despite a first, rough crystallographic analysis of the F_1ATPase subfragment [2], the structure of the complete enzyme is under discussion.

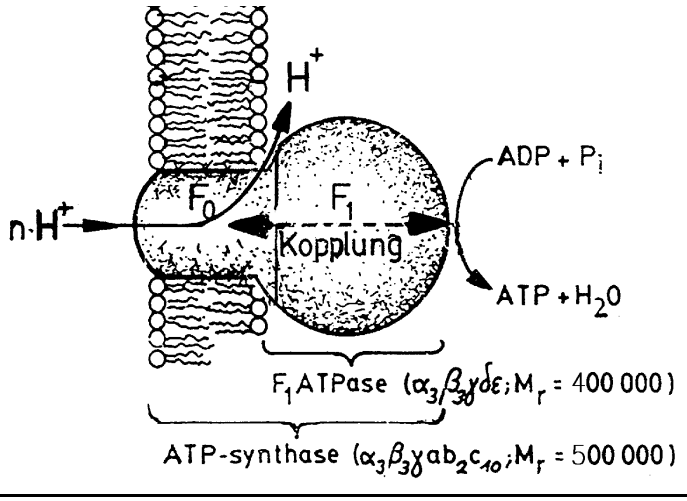


Fig.1: ATP-synthase is a large membrane protein (M=500.000), which couples the proton transport through the F_0-moiety to the synthesis or hydrolysis of ATP by the F_1-subcomplex (F_1ATPase).

We have isolated, purified and characterized the ATP-synthase from the aerobic bacterium *Micrococcus luteus*. This enzyme is capable of all reactions of a native ATP-synthase: ATP-hydrolysis, proton-transport, and ATP-synthesis. For the F_1ATPase subcomplex of this enzyme we have found large structural changes during the reaction cycle using a stopped-flow device [3]. Now we have investigated the structure of complete ATP-synthase in presence of the detergent taurodeoxycholate (TDOC) by X-ray small angle scattering at the A1-beamline at HASYLAB/DESY. For the experiments radiation of 0.15 nm wavelength was used. The detector-sample distance was 1.9 m. In earlier studies we have shown that TDOC is sufficient for scattering experiments with ATP-synthases [4] because of its very small micelle size [5]. The residual micelle scattering was eliminated by subtracting the scattering of a protein-free detergent buffer with nearly contrast-matching glycerol content.

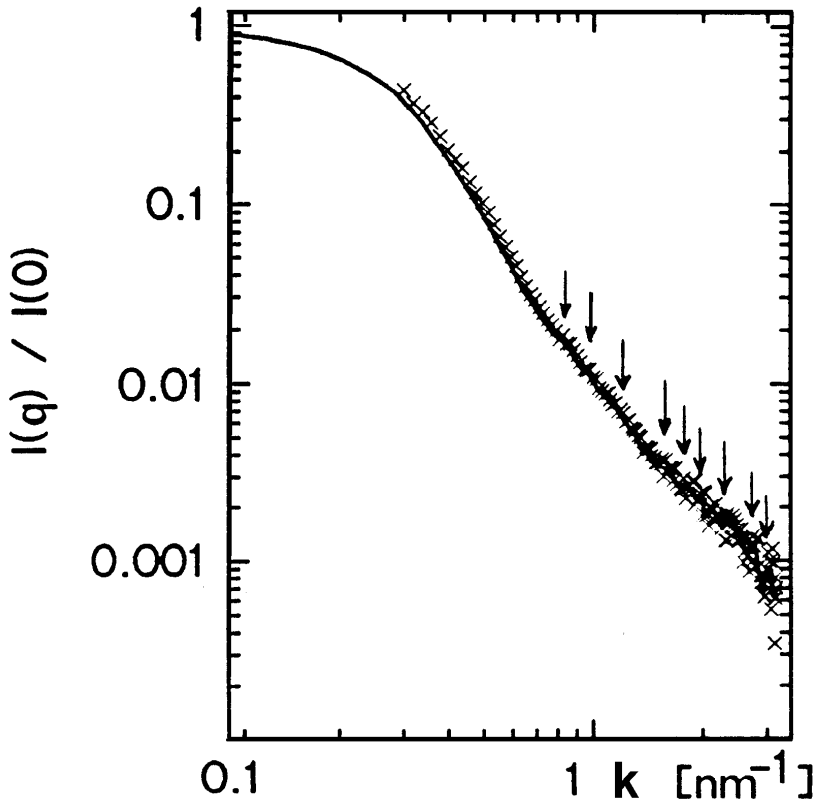


Fig.2: X-ray small angle scattering of ATP-synthase from *Micrococcus luteus* in presence of 5 mM TDOC. The shoulders in the profile correspond to spacings between protein-subunits.

The scattering profile (fig.2) shows a series of shoulders additional to the central signal. The scattering vectors ($k= (4\pi/\lambda) \sin(\theta)$) of 0.85; 1.0; 1.1; 1.5 and 2.6 nm^{-1} correspond to mass distances of d= 7.4; 6.3; 5.7; 4.9 and 2.4 nm. These were interpreted as the overall spacings between the protein subunits, which are known to be well separated from electron microscopy studies. The Guinier extrapolation of the profile yielded a radius of gyration of R_g= 5.81 ± 0.08 nm; the Fourier transformation yielded a maximal molecule dimension of r_{max}= 17.5 nm. The scattering is now interpreted by molecular models consisting of cubes according to the FVM-method [6].

1) Penefski, H.S.; Cross, R.L. (1991) Adv. Enzymol. 64, 173-215
2) Bianchet, M.; Ysern, X.; Hulihen, J.; Pedersen, P.L.; Amzel, M. (1991) J. Biol. Chem. 266, 21197-21201
3) Neidhardt, A.; Nawroth, T.; Dose, K. (1991) FEBS Lett. 280, 179-182
4) Nawroth, T.; Conrad, H.; Vienken, J.; Dose, K. (1983) Hoppe Seyler's Z.Physiol.Chem. 364, 923-931
5) Conrad, H.; Dose, K.; Nawroth, T. (1989) Physica 156 B, 474-476
6) Nawroth, T. (1989) Physica 156 B, 493-495