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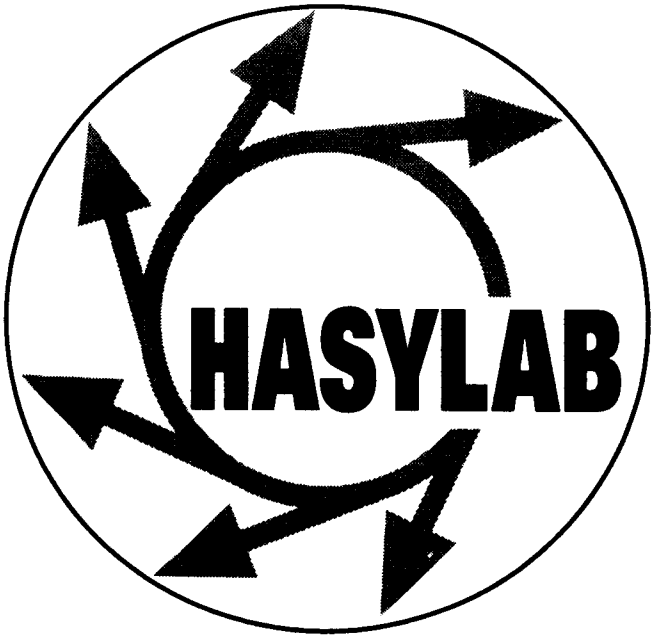

X-ray small angle scattering of Cytochrome-c Oxidase (cytochrome-aa_3) and Cytochrome-c Reductase (cytochrome-bc_1) from beef heart mitochondria

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Cytochrome-c reductase (cytochrome-bc1 complex) and cytochrome-c oxidase (cytochrome-aa3 complex) are large membrane protein complexes of energy conservating systems in many organisms, e.g. man [1]. These enzymes couple a redox reaction to the transport of protons accross the membrane and thus the biological oxidation to the formation of an electrochemical proton potential difference of the membrane. These biological redox-catalysts consist of a functional core, which is present also in bacterial proteins, and additional protein subunits which are found in cytochrome proteins of higher organisms only.

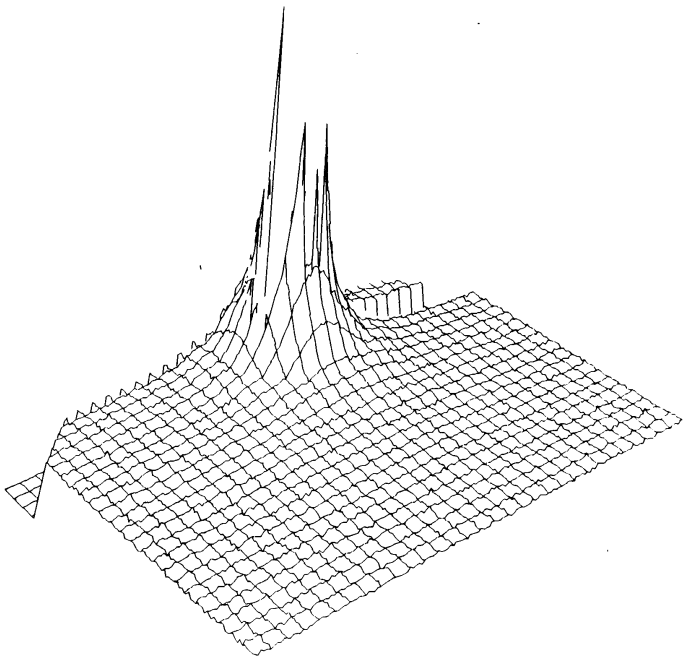

Fig.1: The X-ray small angle scattering of cytochrome-c reductase (cytochrome-bc_1 complex) shows a weak side maximum, which is visible also in the crude data. It results from the H-type structure of the membrane protein.

We have isolated, purified and characterized cytochrome-c reductase and cytochrome-c oxidase from beef heart mitochondria [2,3]. By detergent solubilization studies and analytical ultracentrifugation we found that functional cytochrome-c reductase is always a dimer,

whereas the dimeric cytochrome-c oxidase can be split into monomeric native enzyme entities. The structure of the enzymes was investigated at the A1 beamline at HASYLAB/DESY using radiation of 0.15 nm wavelength. The detector-sample distance was 1.9 m. The detergent contribution to the over all scattering of cytochrome-c reductase was small because of the small surfactant concentration (0.1% w/v laurylmaltoside) and the nearly contrast-matching buffer. In case of cytochrome-c oxidase we solubilized the enzyme by 5 mM taurodeoxycholate (TDOC), which was earlier shown to yield only negligible scattering because of its very small mizelle size [4]. The residual micelle scattering was eliminated by subtracting the scattering of a protein-free detergent buffer.

The X-ray small angle scattering of cytochrome-c reductase showed a side maximum, that results of the H-type structure of the dimeric enzyme (fig.1) [5]. The small angle scattering of monomeric cytochrome-c oxidase in solution of 5 mM TDOC (fig.2) showed only a less pronounced shoulder. This corresponds to the proposed structure of a long flat asymmetric 'y'. The radius of gyration of this enzyme was R_g= 3.73 nm. The scattering of both enzymes is now interpreted by molecular models consisting of cubes according to the FVM-method [6].

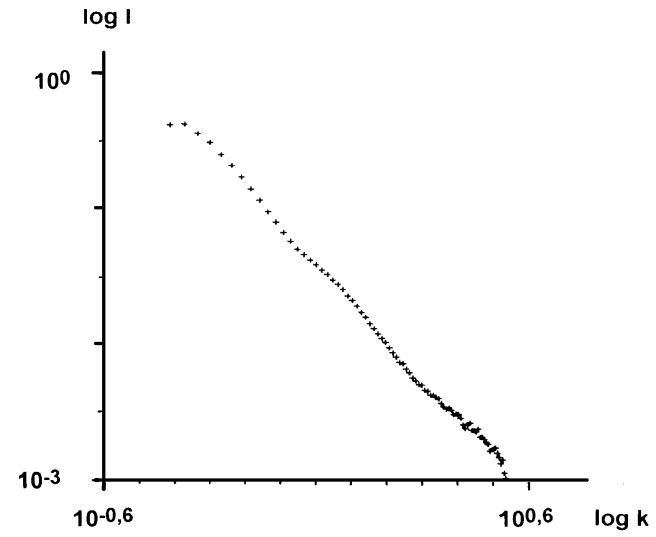

Fig.2: X-ray small angle scattering (radial averaged) of monomeric cytochrome-c oxidase from beef heart mitochondria in presence of 5 mM TDOC at 4°C.

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