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Hamburger Synchrotronstrahlungslabor
HASYLAB
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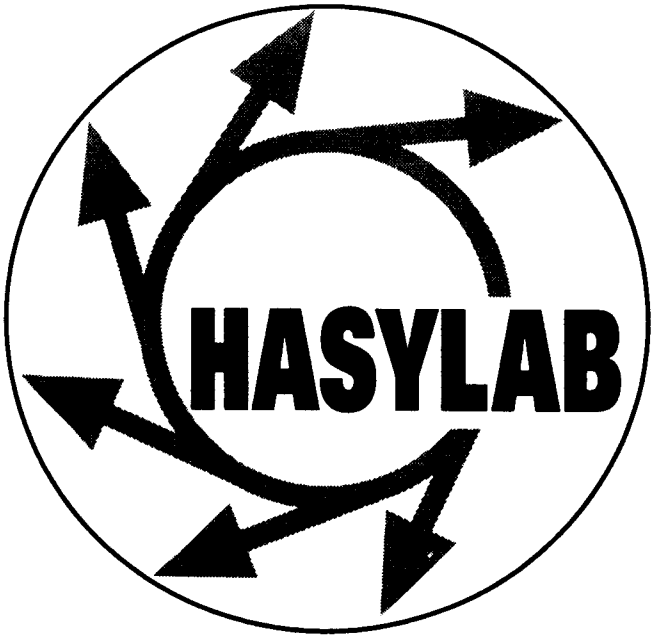

Oxidized and reduced Cytochrome-c containing terminal Oxidase (QOX) from *Micrococcus luteus* differs in structure in detergent solution

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Cytochrome containing terminal oxidases a large membrane proteins (M >= 120,000 as monomeric unit), that play a key role in the energy conservation of many organisms, e.g. man or aerobic bacteria as *Micrococcus luteus* [1]. They catalyze the electron transfer from the substrate, (ubi)quinol or cytochrome-c, to molecular oxygen and thus the final step in biological oxidation of food. The redox-reaction is coupled to a proton transport accross the membrane and thus to the activity of other membran proteins. The enzymes from bacteria are more simple in comparison to that of higher organisms and well objects for studying the structure-function relation and the proposed structure-dynamics during regulation.

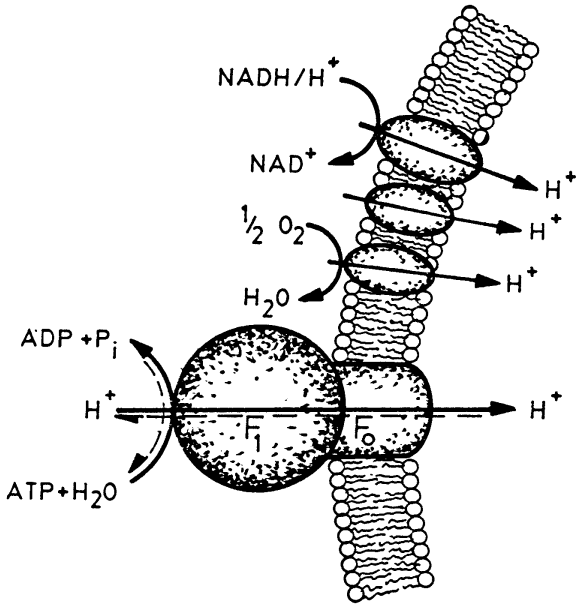

Fig.1: The energy conservation during biological oxidation of *Micrococcus luteus* is performed by a network of membrane proteins which couple a chemical reaction with a proton transport accross membranes: NADH-dehydrogenase, cytochrome-c reductase, cytochrome-c oxidase (COX) or cytochrome-c containing terminal quinol oxidase (QOX) and ATP-synthase.

We were successful in isolating and purifying two cytochrome-c containing oxidases from *Micrococcus luteus* ATCC4698 by solubilization and chromatography in the presence of detergents (laurylmaltoside) [2,3]. The purified proteins showed typical cytochrome-aa_3 spectra, oxidase activity and inhibitor reactions that were in good aggreement with the properties of cytochrome-c oxidases from other organisms. The estimated molecular mass was 120.000 g/mol for the terminal

oxidase QOX reported here. The cytochrome-c containing oxidases showed significant alterations in the optical spectra when oxidized or reduced under physiological conditions (by air or ascorbate). Thus we proposed structural changes of the enzyme during redox-reactions, e.g. in the regulation of the biological activity.

The structure of cytochrome-c containing terminal oxidase QOX from *Micrococcus luteus* was investigated by X-ray small angle scattering of the enzyme in solution of the detergent laurylmatoside at the A1-beamline of HASYLAB and the EMBL beamline. With both cameras radiation of 0.15 nm wavelength was used. The detector-sample distance was 1.9 and 3.7 m. The detergent contribution to the over all scattering was small because of the small surfactant concentration (0.02% w/v) and the nearly contrast-matching buffer. The residual micelle scattering was eliminated by subtracting the scattering of a protein-free detergent buffer. The radial averaged scattering profiles were plotted as Guinier representation, which did not give evidence for any protein aggregates. The radius of gyration of the air-oxidized cytochrome-c containing terminal oxidase at 4°C was R_g= 4.38 nm. The comparison with the ascorbate-reduced enzyme (see figure) yielded an expansion to an 0.8% larger protein modification.

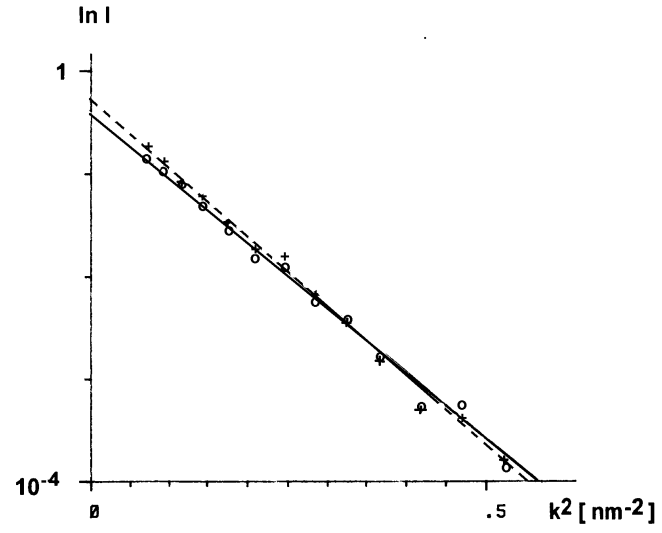

Fig.2: The Guinier-representation of the X-ray small angle scattering of oxidized (o) and reduced (+) cytochrome-c containing terminal quinol oxidase QOX of the aerobic bacterium *Micrococcus luteus* shows a structural change during the redox reaction. The ascorbate-reduced enzyme exhibits a 0.8% larger Radius of gyration.

1) Saraste, M. (1990) Quart.Rev.Biophys. 23, 331

2) Heinz, G.; Nawroth, T.; Dose, K. (1993) Biol.Chem.Hoppe Seyler 374, 740

3) Heinz, G. (1992) Diploma thesis, Mainz

Addendum in proof: After finishing the original HASYLAB report it was shown that both terminal oxidases of *Micrococcus luteus*, the cytochrome-c oxidase COX and the cytochrome-c containing terminal quinol oxidase QOX, contain the internal cytochrome-groups **a**, $\mathbf{a_3}$ and **c**, as is the case with the terminal $\mathbf{caa_3}$ oxidase from *Bacillus subtilis*. The identity of the terminal oxidase QOX reported here was published in: Heinz G.; Dose, K.; Nawroth, T. (1994) FEMS Microbiol. Lett. 124, 173-178. Thus the report was slightly revised to avoid confusion between COX and QOX.