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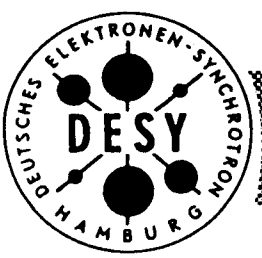

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STRUCTURAL DYNAMICS OF F_1ATPase DURING ONE REACTION CYCLE

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ATP, the universal currency of free energy in biological systems is synthesized by an enzyme complex consisting of a proton-conducting F_o unit and a catalytic F_1 moiety. Models on the reaction mechanism postulate regular conformational changes within the F_1 unit on synthesizing or hydrolizing ATP [1]. When detaching the F_1 moiety (F_1ATPase) from the complex only the ATP hydrolyzing direction is catalyzed [2]. F_1ATPases from different organisms all have the same subunit composition and a comparable molecular mass around 380000. There are three catalytic active sites within one molecule. It is not clear whether all three or only two of the sites contribute mainly to the ATP hydrolizing reaction [3]. Our kinetic experiments with F_1ATPase from Micrococcus luteus favour the two site model [4]. With two sites of the enzyme involved in hydrolysis one reaction cycle i. e. once the transition through all the hypothetical intermediate conformational states would be completed after 20 seconds at 13°C [5]. This time range is suitable to investigate structural parameters of the transition process by time resolved X-ray small angle scattering. The X-ray scattering was performed at HASYLAB's windowless beamline A1 with double focussing mirror using 1.3A photons. Scattered photons were counted in 1.9m distance of the sample by a position sensitive detector (Ar/CO_2 filled multi-wire proportional chamber) with a sensitive area of $290X290mm^2$. So a Q range between 0.018 and $0.62A^{-1}$ was covered ($Q=(4\pi\sin\Theta)/\lambda$, 2Θ being the scattering angle). The fluid sample was irradiated in a flow through quartz capillary (0.8mm inner diameter) connected to a stopped flow device. The observed count rate of scattered photons from the sample was about 10000cps on the whole detector. Data aquisitation was started after triggering the enzymatic reaction by mixing with ATP (220ms mixing time) at 13°C. From one single enzyme/substrate mixture 20 datasets in subsequent one second time intervals were generated and stored in histogramming memories as arrays of 256X256. To improve the statistical error the time resolved scattering

data from 5 F_1ATPase/ATP mixtures were accumulated and reduced to one-dimensional scattering profiles by radial averaging. Fig.1 shows the dynamics of the molecular shape of the F_1ATPase occuring during one reaction cycle. The radius of gyration calculated from the innermost part of the scattering spectra increases after 9 seconds from starting the enzymatic reaction to 46.6±0.7A. This expanded state in the middle of the reaction cycle is kept for 4 seconds and clearly deviates from the radius of gyration (43.8±0.5A) found at the beginning and the end of the reaction cycle.

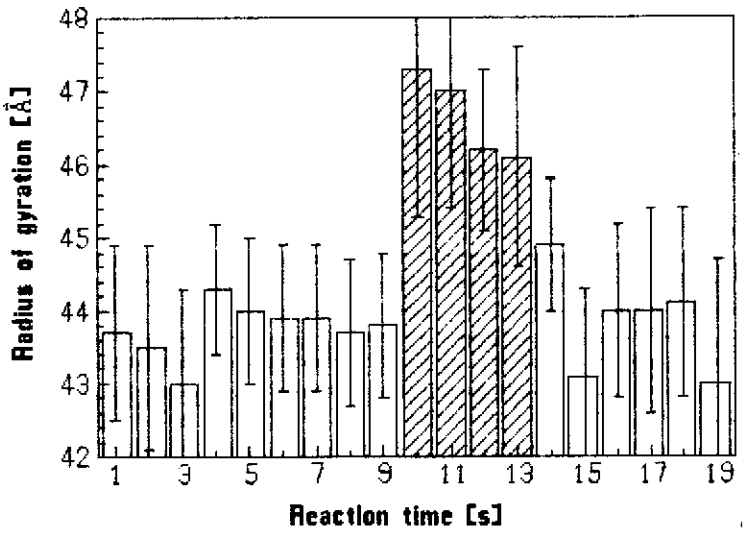

Fig. 1: Dynamics of the molecular expansion of F_1ATPase detected during the first reaction cycle of the hydrolytic catalysis.

Such a large change in the molecular structure could be due to a subunit rearrangement during catalysis. So the F_1 molecule passes at least two structural distingusiable intermediate states upon hydrolizing ATP (Fig.2) were the R- and S-phase are probably not identical.

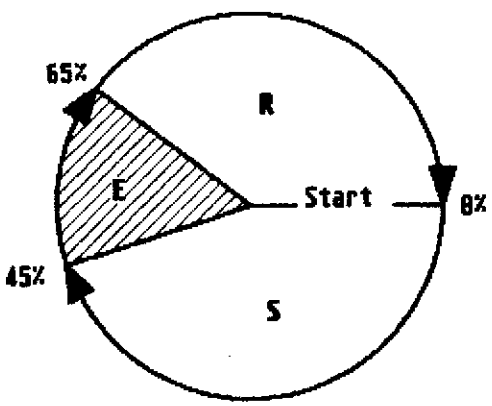

Fig. 2: F_1ATPase passes possibly three intermediate conformations during one (100%) reaction cycle of ATP cleavage: Starting (0%) with a compact molecular shape (S-phase) the enzyme passes a short expanded E-phase (45-65%) and returns to the initial state through a further compact conformation (R-phase).

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