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COMPARISON OF NATIVE AND δ-SUBUNIT DEPLEATED F_1ATPase FROM MICROCOCCUS LUTEUS BY X-RAY SMALL ANGLE SCATTERING

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ATP-Synthase is a large membrane protein (M = 480 000), which is present in nearly all known organisms. It has a central function in the energy conservation of cells and consists of a membrane integral F_o-part and a large subcomplex, the F_1ATPase, which contains the active centers of the protein. The F_1ATPase consists of 5 types of protein subunits ($\alpha, \beta, \gamma, \delta, \varepsilon$), which are present in up to 3 copies. We have shown, that the elimination of the δ-subunit from F_1ATPase from Micrococcus luteus ATCC4698 [1] yields an enhancement of the enzymatic activity, which is proposed to be caused by conformational changes of the enzyme [2-6]. Possibly this subunit dissociation is involved in the regulation of the biological activity of the ATP-synthase. We have thus compared the structure of native and δ-subunit depleated F_1ATPase from Micrococcus luteus by X-ray small angle scattering.

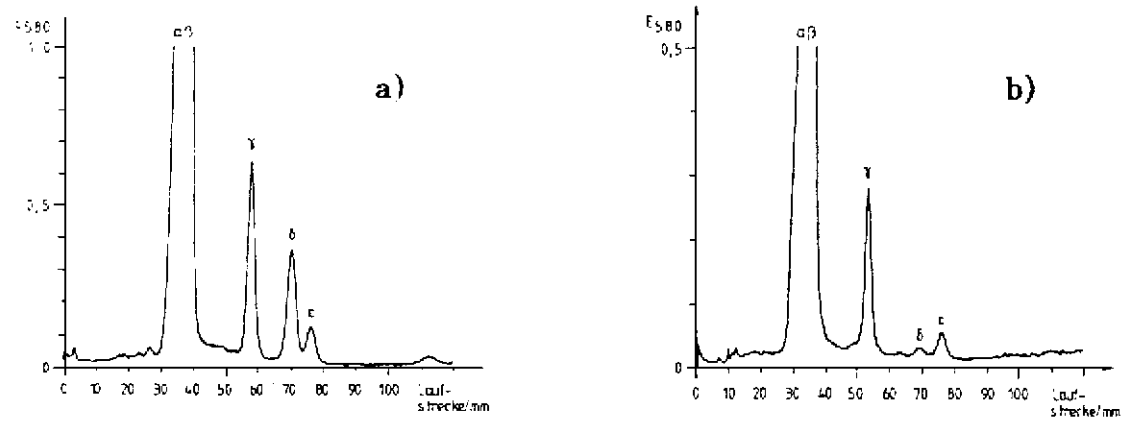

Fig. 1: The electrophoretic analysis of native (a) and δ-subunit depleated (b) F_1ATPase indicates that the elemination of the δ-subunit is nearly perfect.

In Fig.1 the chemical (electrophoretic) analysis of native (a) and δ-subunit depleated (b) F_1ATPase is shown. The analysis indicates, that the latter protein, prepared by a novel procedure [3], is nearly δ-subunit free. As the δ-subunit takes only 5% of the molecular weight of F_1ATPase (M = 360 000), proposed changes in the X-ray small angle scattering of the enzyme should be due to conformational changes of the protein complex. The X-ray small angle scattering of native (a) and δ-subunit depleated (b) F_1ATPase solutions is shown in Fig.2.

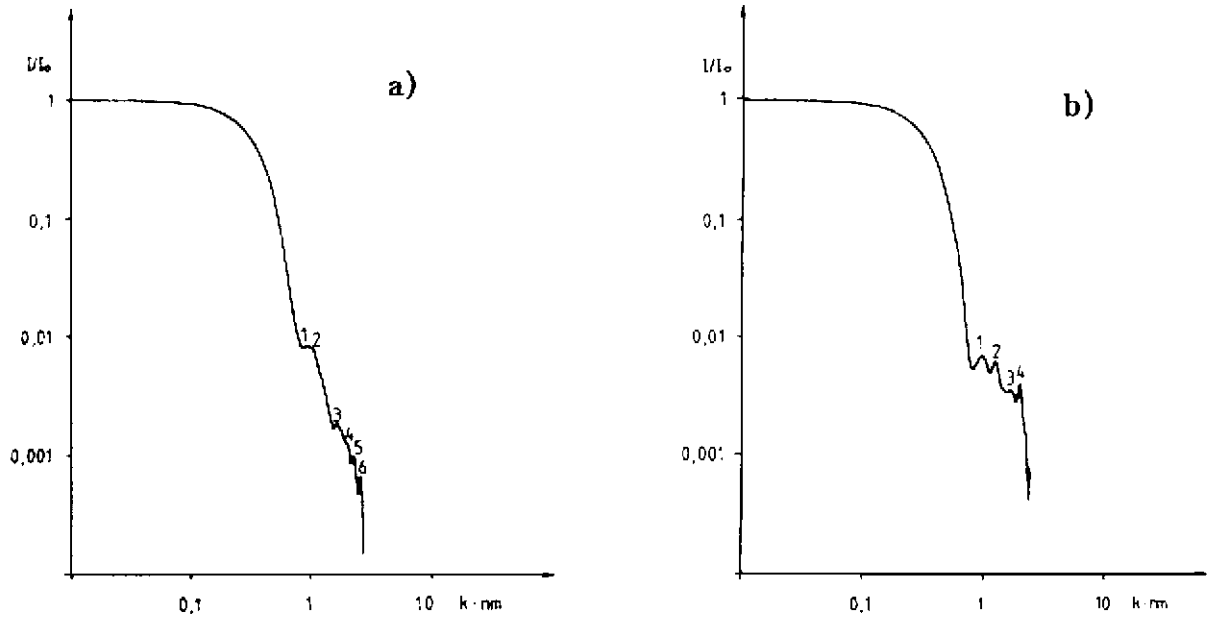

Fig. 2: The X-ray small angle scattering of native (a) and δ-subunit depleated (b) F_1ATPase solutions indicates structural rearrangements of the protein complex.

The elemination of the δ-subunit yields only a small increase in the radius of gyration of the enzyme (R_g = 4.71 $\pm$ 0.09 nm and 4.80 $\pm$ 0.2 nm at 35°C). In contrast to these slight differences, the positions and amplitudes of the side maxima in the scattering profiles (1-4) are altered significantly. As model calculations suggest, these side maxima are caused by the distances of the subunits within the protein complex. Thus the variation in the scattering profile by the dissociation of the δ-subunit indicates structural rearrangements of the protein complex.

The scattering experiments were carried out with a new instrument designed for anomalous X-ray scattering. The very high quality and resolution of the obtained scattering profiles indicates, that this is an excellent instrument for conventional X-ray scattering also.

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