Electron microscopy of two-dimensional crystals of mitochondrial ATP synthase

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Two-dimensional crystals of the mitochondrial ATP synthase up to 0.4 μm in size were obtained from the detergent-lipid-protein micelles by detergent dialysis. A projected map of the negatively stained crystal was calculated from electron microscopical images by the Fourier-filtering procedure at about 2.8 nm resolution. The unit cell (with not more than two ATP synthase molecules) has the following parameters: $a = 13.0$ nm, $b = 25.6$ nm and $\gamma = 86°$. Two alternative models for the crystal structural organization were suggested, viz. with one or two protein molecules per unit cell.

1. INTRODUCTION

ATP synthases carry out ATP synthesis (hydrolysis) coupled to transmembrane proton transport. The enzymes from different sources were found to be structurally similar. They consist of two main components: the integral membrane portion F_0 and the membrane-associated portion F_1 (F_1-ATPase). F_1 contains all catalytic and nucleotide-binding sites and F_0 apparently forms a proton channel (reviews in [1,2]). Mitochondrial ATP synthase consists of at least twelve subunits: α, β, γ, δ and ε in F_1 (in 3:3:1:1:1 stoichiometry) and a, b, c, d, OSCP (oligomycin sensitivity-conferring protein), F_6 and A_6L in F_0 [3,4].

According to electron microscopy [5,6] and X-ray analysis [7], the F_1-ATPase has a bilayer structure: 3α and 3β subunits are located on two planes and connected by 3-fold rotational symmetry, with the central space occupied by seventh protein mass (γ, δ and ε subunits).

The F_0F_1 spatial structure was not sufficiently studied. According to electron microscopy the F_1-ATPase is connected with the F_0 part by the stalk about 4.2 nm in length and 3–4 nm in width, actually being outside the membrane [8,9]. The dimensions of the hydrophobic F_0 component embedded in the membrane are 6–9 nm [8,9] to 6–12 nm [10], with its small part locating outside the lipid bilayer.

The structural organization of membrane proteins can be studied by electron microscopy of two-dimen-

sional crystals followed by the Fourier image processing [11]. The projected structure of ATP synthase crystals was determinated here by those methods at about 2.8 nm resolution.

2. MATERIALS AND METHODS

Bovine heart mitochondria were isolated according to Crane and Green [12]. Submitochondrial particles were prepared by the Racker and Fesseden method [13]. The ATP synthase was solubilized from the submitochondrial particles with sodium cholate, then extracted by precipitation with ammonium sulfate as described by Serrano et al. [14]. Protein concentrations were estimated by the Bradford method [15]. The ATPase activity was measured as described in [16]. The ATP synthase two-dimensional crystals were obtained from detergent-lipid-protein micelles by detergent dialysis as described in [17].

The preparations were negatively stained by 2% w/v uranyl acetate. The grids were examined in the Jeol EM 100CX-2 electron microscope at magnification of 31,000 and with the accelerating voltage of 80 kV. The magnification was calibrated with catalase crystals (Balzers).

Electron micrographs were preliminarily analysed with laser diffractometer as described in [18]. They were digitized on the Joyce-Loebl MDM-6 automatic microdensitometer, with a step size of 30 μm, that corresponded to about 1 nm in the images. The areas recorded consisted of 256 × 256 arrays of optical density values. Image analysis was carried out on the IBM/AT 286 computer, using programs described in [19].

3. RESULTS AND DISCUSSION

Earlier [17], we established that two-dimensional crystals of the ATP synthase were formed within the narrow pH range of 4.5–5.0. The optimum conditions for crystal obtaining should be: concentration of F_0F_1 3 mg/ml; lecithin from soybean/lauryldimethylamine *N*-oxide/F_0F_1 (1:1:1 by mass); composition of dialysis me-

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dium: 50 mM CH_3COONa, pH 4.5, 5 mM $MgCl_2$, 50 mM NaCl, 5 mM NaH_2PO_4; time of dialysis: 24 h; 20°C. The ATPase specific activity of the reconstituted ATP synthase was between 5 and 6 μmol of ATP hydro-lyzed/min/mg protein. The preparation exhibited 70–80% oligomycin sensitivity, which demonstrated intact-ness of the multisubunit enzyme complex.

Typical electron micrograph of the ATP synthase crystal is presented in Fig. 1. The maximum size of the crystals is about 0.4 μm. The optical diffraction and Fourier analysis of the crystal images showed the reso-lution to be about 2.8 nm. A unit cell has the following parameters: $a = 13.0$ nm, $b = 25.6$ nm, $\gamma = 86°$. Fig. 2 presents Fourier-filtered image of negatively stained two-dimensional crystals of ATP synthase (projection on the membrane plane). Note that stain-excluding re-gions corresponding to the protein molecules (negative staining of the protein) form typical bands in the direc-tion of vector $\vec{a}$. It was established that one of the projections of the F_1 molecule known as frontal was hexagonal, approx. 12 nm across [6,7] and the side pro-jections were in the shape of rectangle or parallelogram, about 7–7.4 nm in width [7,20]. The band thus consists of the aligned ATP synthase molecules with the F_1 parts presumable contacting to each other and with their hex-agonal profiles parallel to the membrane surface.

So, the ATP synthase molecule projection on the membrane plane corresponds to a circular region with a diameter of about 12 nm. It contains six maxima of the protein density around the stain-filled cavity, each of the two neighbouring ATP synthase molecules mak-ing its own contribution to the two maxima in-be-tween (see Fig. 2). In all probability, the absent of hexagonal symmetry is primarily a result of marked distortions in F_1 molecules packing on the membrane plane (it is these distortions that set a resolution limit equal to 2.8 nm). In addition, the frontal side of the F_1 may be slightly tilted relative to the membrane plane, resulting in the symmetry disruption.

Comparison of the unit cell dimensions (13 × 25.6 nm) with those of the frontal projection of F_1 (12 × 12 nm) led to the conclusion that there was one or two ATP
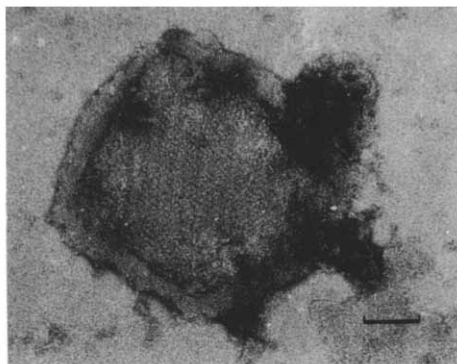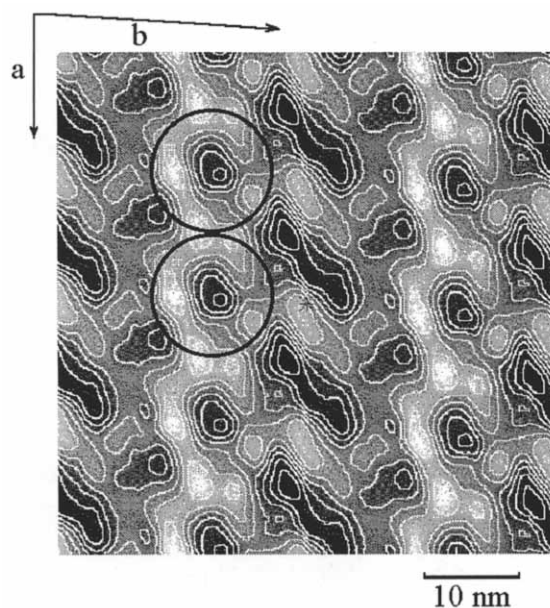

Fig. 2. Fourier-filtered image of negatively stained two-dimensional crystals of ATP synthase (projection on the membrane plane). Light regions are stain-excluding regions (protein), dark regions are stain-filled regions (lipid). Encircled regions show the ATP synthase mole-cules as seen from the F_1 parts. The basic vectors of the unit cell, a and b are depicted by the arrows.

synthase molecules per unit cell. In the first case, the stain-excluding region, interposed between neighbour-ing protein rows, corresponds to lipid surroundings. In the second case, it corresponds to the integral mem-brane part F_0 of the other ATP synthase molecule with the opposite orientation in the membrane. Therefore, two alternative models for the crystal structural organi-zation may be proposed: with one or two protein mole-cules per unit cell (see Fig. 3). The first model offers the asymmetric incorporation of the protein molecules into the lipid bilayer: extramembranous portions F_1 are lo-cated on one side of the crystal membrane plane. Ac-cording to the second model, the incorporation occurs on each side of the lipid bilayer: there are two ATP synthase molecules with the relative opposite orienta-tion per unit cell.

Noteworthy, according to the first model (see Fig. 3A), the following two types of the projected images are to exist: a projection of the crystal membrane from the F_1 parts shown in Fig. 2 and a rear view of the crystal membrane is rather different. Since the images of the latter were not found, the second model should be pref-erable. Besides, only this model suggests that a regular arrangement of the protein molecules in the crystal is provided by their interactions with each other within a protein row (by way of the F_1 parts) and between them (by way of the F_0 parts). The protein interaction be-tween the rows occurs only in the second model charac-terized by the interrow space of about 12.8 nm, twice less as compared with the first model. Such space corre-

Fig. 1. Electron micrograph of negatively stained two-dimensional crystals of ATP synthase. Scale bar = 0.1 μm.
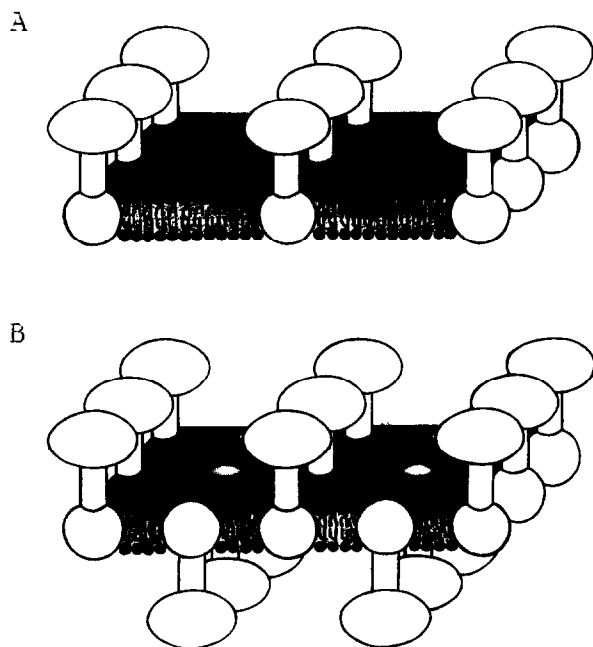
A

B

Fig. 3. Two alternative models of the two-dimensional crystal of ATP synthase: with one (A) or with two (B) protein molecules per unit cell.

sponds to the maximum longitudinal size of the F_0 (12 nm; see [10]). As for the first model, it suggests that the protein rows interact due to the lipid surroundings, but that is improbable.

The three-dimensional reconstruction is necessary for the final structural determination of the two-dimensional crystals of ATP synthase, that is the aim of our further studies.

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