Electron microscopy of beef heart mitochondrial F_1-ATPase

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The quaternary structure of isolated and membrane-bound F_1-ATPase (submitochondrial particles) has been studied by electron microscopy. A model of the molecule has been proposed: six protein masses are arranged in two layers approximately at the vertices of a triangular antiprism. Computer averaging of the images showed that the frontal view of the molecule can be approximately characterized by mirror plane symmetry.

F_1-ATPase *Enzyme structure* *Electron microscopy*

1. INTRODUCTION

F_1-ATPase (EC 3.6.1.3), the hydrophilic part of the membrane H^+-ATPase complex, catalyses the ATP synthesis, utilising the energy of the transmembrane electrochemical proton gradient. F_1-ATPases isolated from mitochondria and various aerobic bacteria are large oligomeric proteins, usually consisting of 5 types of subunits: α, β, γ, δ and ϵ. According to the numerous experimental data, the stoichiometry of the subunits in a molecule of F_1-ATPase is 3α, 3β, γ, δ, ϵ, while subunits α and β with molecular masses of approx. 50–60 kDa comprise the main part of the molecule (reviews [1–6]).

The spatial arrangement of the subunits of F_1-ATPase has been studied by various methods. Electron microscopy has been used to study molecules from a solution [7], two-dimensional crystals for which computer filtered images of particles in frontal projection ('in plane') were obtained [8], and three-dimensional crystals of F_1-ATPase [9]. It has been established that the frontal projection of the F_1-ATPase molecule is hexagonal, approx. 9 nm in diameter. Small-angle X-ray scattering was used to approximate the molecule of F_1-ATPase by an ellipsoid with axes of $12 \times 9 \times 7$ nm [10]. The crystals of F_1-ATPase were also studied by X-ray analysis [11].

It has been suggested that 3α and 3β subunits are located on a single plane [12,13]. On the other hand, there are some data contradicting this point of view [14,15].

Proceeding solely from the frontal view of the molecule of the F_1-ATPase observed on electron micrographs, two possible arrangements of its major subunits may also be suggested: all the α and β subunits are located on the same plane or on two planes, 3 subunits on each plane. When electron microscope studies of F_1-ATPase from the anaerobic bacteria *Lactobacillus casei* were carried out, additional projections of the molecule were obtained which allowed a choice to be made in favour of the bilayer molecule [16,17]. Recently [11], X-ray scattering data were obtained for mitochondrial F_1-ATPase. These data also support the bilayer model of ATPase molecule.

In this work the quaternary structure of mitochondrial F_1-ATPase was studied by electron microscopy. Four additional projections (beside the frontal one) were found on the micrographs. The results obtained left no doubt that the molecule of ATPase has two layers, 3 large subunits in each layer.

2. MATERIALS AND METHODS

Submitochondrial particles were obtained as in

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[18]. F_1-ATPase was isolated from beef heart mitochondria as in [1]. Solutions of desalinated F_1-ATPase [19] or submitochondrial particles (0.1 mg/ml) in 10 mM Tris buffer, containing 0.25 M sucrose (pH 7.5), were used for electron microscopy. In some cases, preparations of F_1-ATPase were treated with cross-linking reagents: dithiobissuccinimidylpropionate (the incubation mixture contained 2.5 mg/ml F_1-ATPase, 10 mM H_3BO_3–NaOH, 10% dimethyl sulphoxide, 300 μM dithiobissuccinimidylpropionate (pH 8.5); incubation, 15 min at room temperature) or dimethyl suberimidate (the incubation mixture contained 2.5 mg/ml F_1-ATPase, 100 mM triethanolamine–SO_4, 1% dimethyl suberimidate (pH 8.3); incubation, 60 min at room temperature). The cross-linking was controlled by SDS electrophoresis [20]. The preparations were negatively stained with 1% uranyl acetate. By way of control, some of the protein preparations were stained with 2% ammonium molybdate or 2% phosphotungstic acid solutions in water. The grids were examined in a Philips 400 electron microscope at an accelerating voltage of 80 kV.

Electron micrographs of small areas containing single particles were scanned into 160 × 160 arrays at (0.16 nm resolution). Each array was displayed on a computer-linked graphic display, and a mask with a radius of 50 units was applied to each image. The images were aligned by using the cross-correlation function, with one particle selected as a reference [21,22]. All the particles were low-pass filtered to a resolution of 1/2 nm^{-1} to eliminate high-resolution portions of the noise. For low-pass filtration, the Fourier transform was multiplied by the Gaussian filter function.

3. RESULTS AND DISCUSSION

Several types of particle images (fig.1,2) were observed in micrographs. The particles of the first type have a form close to a right hexagon 10 ± 1 nm in size (fig.2a). This type is found more frequently than others and most probably corresponds to the stable position of the molecule on the support film. However, more thorough studies showed that these particles have no 6-fold or 3-fold symmetry. This conclusion is supported by the averaged computer data (see section 2). Thirty images were studied of which 10 (with the best correlation coefficient) were used. The results of this averaging are shown in fig.3. The resulting image may be better characterised by a mirror plane symmetry than by 3- or 6-fold symmetry.

In fig.2b the second type of particles, about 10 ± 1 nm in length and 6.5 ± 0.5 width in size, parallelogram-shaped, is shown. The third type (fig.2c) is rectangular particles. The images shown in fig.2d are related to the fourth type. They possess a square configuration. In the fifth type (fig.2e) cross-like particles can be observed. When comparing the images of the molecule obtained, it may be suggested that the first type is a frontal projection, and the second and third types are lateral ones. An analysis of all the projections allowed us to suggest a simplified model for the molecule: 6 protein masses are arranged approximately at the vertices of a triangular antiprism so that the molecule has a bilayer structure.

The projections of the model based on the data obtained (not taking into consideration the differences in protein masses) are shown on the right side of fig.2. When the model is viewed along the two axes intersecting one another at an angle of about 30°, it is reminiscent of the second and third type of images (fig.2b,c). Based on the model suggested, we can explain the variants of the images of the fourth and fifth type (fig.2d,e), which could not be observed in the case of the subunits arranged on a single plane. Although the projections of the molecule differing from the frontal one, are found relatively seldom, their form and size, and also the fact that similar projections are observed using staining of F_1-ATPase with ammonium molybdate and phosphotungstic acid, give sufficient grounds for considering that they are indeed a projection of F_1-ATPase and that the molecule has a bilayer structure. This conclusion is also supported by the data obtained by studying membrane-bound F_1-ATPase (submitochondrial particles). When analysing the micrographs of negatively stained submitochondrial particles (fig.2f), we found all the projections of F_1-ATPase which were discovered on the micrographs of isolated F_1-ATPase.

Authors in [23] proposed a bilayer model of the F_1-ATPase molecule. These authors suggested that 3α and 3β subunits are located on different planes and are connected by a 3-fold rotational symmetry

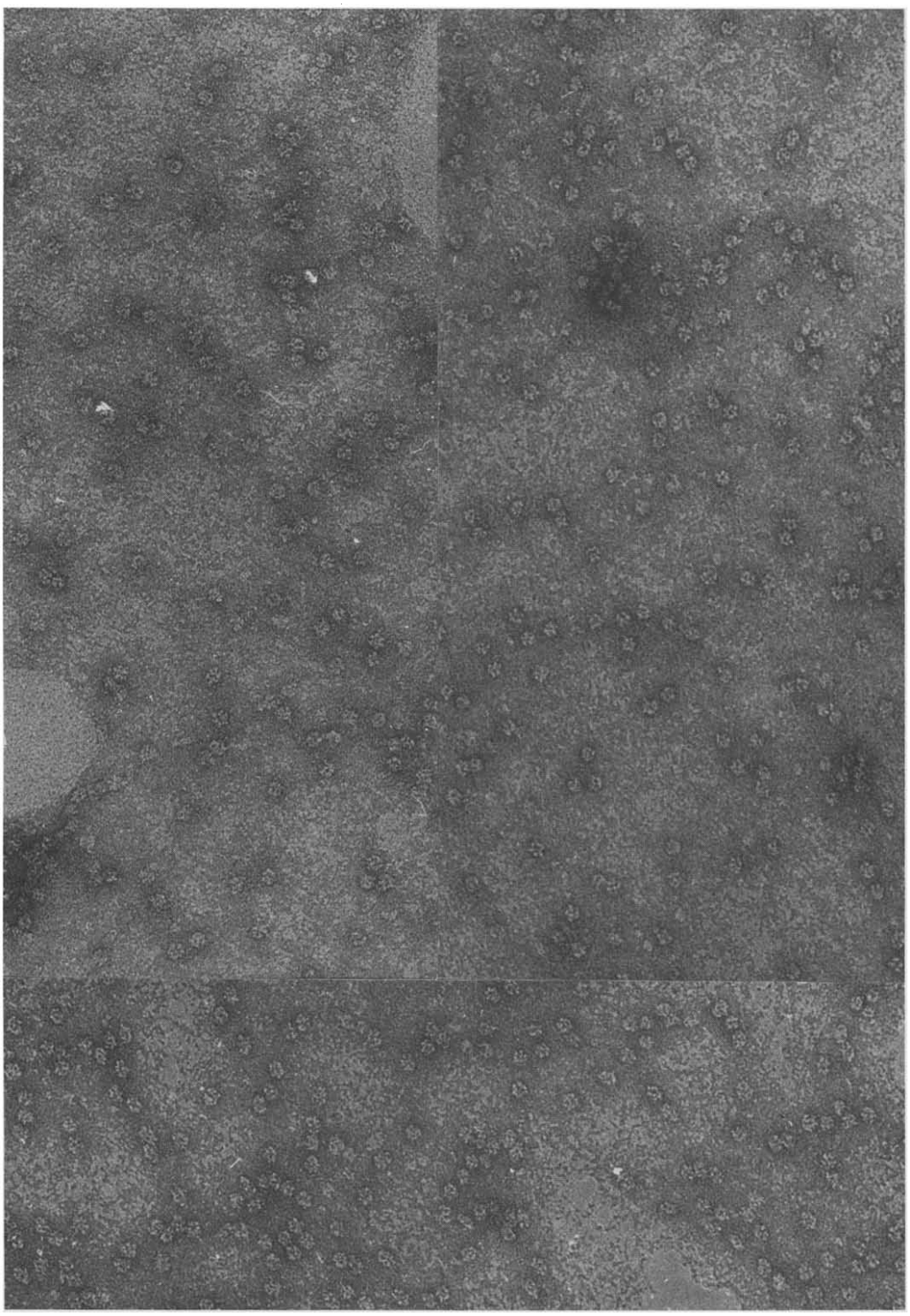

Fig.1. A general view of the F_1-ATPase preparations from beef heart mitochondria negatively stained with uranyl acetate. Magnification × 250000.

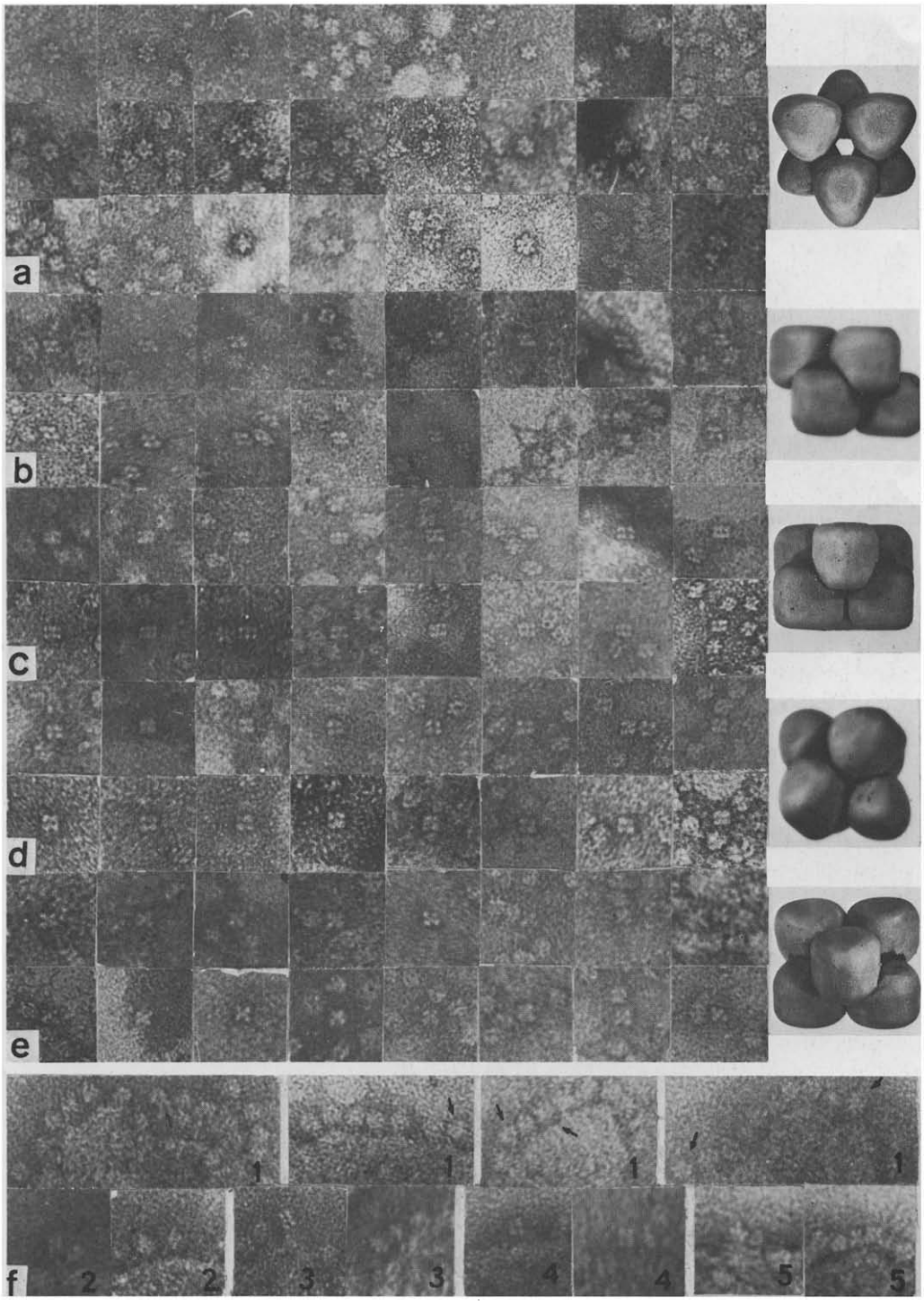

Fig.2. Negatively stained molecules of F_1-ATPase. Magnification × 300000. (a–e) Selected particles of isolated molecules of F_1-ATPase of the first–fifth types, respectively. (f) Micrographs of membrane-bound F_1 molecules having projections similar to those of the particles observed in the samples of isolated molecules. The numbers show types of the particles in accordance with the system accepted for isolated F_1-ATPase (a–e). Different views of the model are shown on the right. For simplicity's sake, all the 6 protein masses are shown to be the same.

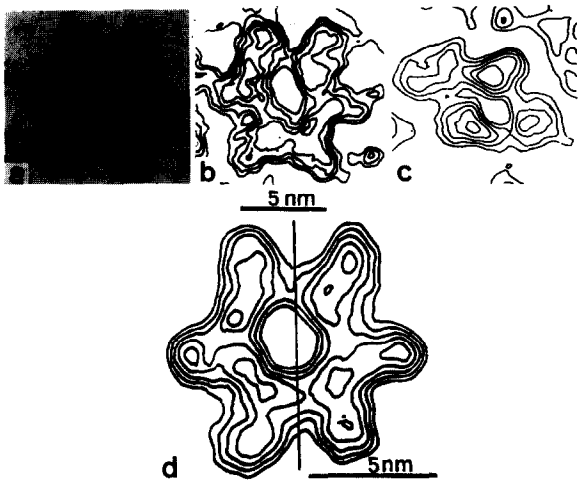

Fig.3. Computer averaging of F_1-ATPase images. (a) A typical digitised single particle (frontal projection) of F_1-ATPase. (b) Low-pass Fourier filtered image of the particle, shown in (a). (c) Low-pass Fourier filtered image of a side projection of F_1-ATPase (second type). (d) The computer averaging of 10 aligned images of the frontal projection displayed as a contour map. The resulting image can be approximately characterized by a mirror plane symmetry (solid line).

with the central space occupied by a seventh protein mass. In that case, each of the 3α subunits and each of the 3β subunits forms an identical set of bonds with the neighbouring subunits.

On the other hand, authors in [11], using an X-ray analysis of rat liver F_1-ATPase, did not find a 3-fold rotational symmetry for the major subunits. In the model proposed in [11], subunits of a single type are located in different layers and are not equivalent in binding to their neighbours.

According to the models proposed in [23] or in [11], a frontal projection of the F_1-ATPase molecule should exhibit a 3-fold rotational or mirror plane symmetry, respectively. Our results (fig.3) are in better agreement with the model proposed in [11].

The bilayer model for mitochondrial F_1-ATPase obtained here is very similar to the model for F_1-ATPase from *Lactobacillus casei* published by us earlier [16]. Thus, the results of this work and [11,16,17] testify to the fact that a bilayer structure is a common feature of different H^+-ATPases.

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