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Structure of the ATP-synthase studied by electron microscopy and image processing

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The structure of ATP synthase from beef heart mitochondria has been studied by electron microscopy and image processing using negatively stained specimens of detergent-solubilized and membrane-bound molecules. The F_1-ATPase and the membrane-embedded F_0 sector of ATP synthase are found to be connected by a narrow stalk, 4–4.5 nm long and 3–3.5 nm wide, projecting about 4.2 nm from the membrane surface. The F_0 sector has a globular shape, 6–8 nm in diameter and, in part, extends from the membrane lipid bilayer.

ATP synthase; Enzyme structure; Electron microscopy

1. INTRODUCTION

Membrane-bound ATP synthase catalyzes ATP synthesis and hydrolysis coupled to vectorial transmembrane translocation of H^+. It includes a hydrophilic part, the F_1-ATPase and an integral membrane F_0 sector. The F_1-ATPase contains catalytic sites, while the F_0 sector is supposed to form a proton channel across the membrane (reviews [1,2]). The F_1-ATPases isolated from chloroplasts (CF_1), bacteria (BF_1) and mitochondria (MF_1) are very similar and consist of five types of subunits in a stoichiometry of 3α, 3β, γ, δ, ϵ [3]. The hydrophobic F_0 sector from mitochondria (MF_0) contains seven subunits: a, b, c, d, F6, A6L and OSCP [4]. X-ray diffraction analysis [5] and electron microscopy data [6,7] showed that in the F_1-ATPase six large subunits (3α and 3β) are arranged in two layers at the vertices of a triangular antiprism. According to immunoelectron microscopy with monoclonal antibodies, 3α and 3β subunits are located in different layers [8,9]. Six large subunits are clearly seen in the hexagonal (front view) projection of the MF_1 and the small subunits (γ, δ, ϵ) are interpreted in this projection as a seventh central mass displaced to one of the α-β pairs [10]: this is consistent with the non-equivalence of one of the subunits revealed by electron microscopic study on the location of SH groups in MF_1-ATPase with the aid of a ferritin label [11].

The structure of the entire F_0F_1-ATPase complex is less well known. The ATP synthase molecules, dissolved in the ionic detergent CHAPS, showed two globular parts and a stalk between them [12], but direct evidence for the existence of the stalk was obtained only by cryo-electron microscopy of unstained frozen-hydrated specimens of the F_0F_1 complex from *Escherichia coli* [13]. Detailed information about the shape and dimensions of the F_1-ATPase, the F_0 sector and the stalk was reported for ATP synthase from chloroplasts (CF_0F_1) and mitochondria (MF_0F_1) which were detergent-solubilized and formed string-like aggregates by binding with the hydrophobic parts, F_0 [14]. However, the position of F_1, F_0 and the stalk relative to the native membrane is not yet clear.

Here, the structure of the ATP synthase from

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bovine heart mitochondria was studied by electron microscopy and image processing with negatively stained specimens of detergent-solubilized and membrane-bound molecules in submitochondrial particles.

2. MATERIALS AND METHODS

Submitochondrial particles (SMP) from bovine heart mitochondria were obtained as described [15]. The ATP synthase was isolated using the detergent, sodium cholate, according to [16] with some modifications.

For electron microscopy, the preparations were negatively stained with a 5% solution of ammonium molybdate or 1% uranyl acetate. The grids were examined in a Philips EM 400 electron microscope at 80 kV with a magnification of 50 000.

The micrographs were digitized with a Perkin-Elmer PDS 1010A flatbed microdensitometer with a sampling corresponding to 0.5 nm on the object. A modified complex of programs [17] was used for correlation averaging of single particle images. Image processing was carried out by a NORD-100 minocomputer with a NORD-500 processor. The digitized image was displayed on a monitor where the coordinates of the particles were determined. At the first stage of processing the part of the image containing the particle was selected using a circular mask, the variance of its density was normalized and a Fourier spectrum was calculated. The next stage included band-pass filtering, alignment and summing of the images for their averaging. Band-pass filtering eliminated the high-frequency noise and the low-frequency variance of the background. For these purposes, the Fourier transform was multiplied by a function of a filter passing frequencies within a range of (1/10–1/2) nm^{-1}. The images to be averaged were aligned by a cross-correlation function. The quality of correlation was controlled by a correlation coefficient. Particles with a correlation coefficient higher than that of the given threshold were taken for averaging.

3. RESULTS AND DISCUSSION

General views of negatively stained preparations of SMP and detergent-solubilized ATP synthase are shown in fig.1a and b, respectively. The F_1-ATPase is bound on the matrix side of the membrane with the membrane-embedded F_0 sector by a narrow stalk normal to the surface of SMP, flattened onto the grid (fig.1a,c). The length of the stalk projecting over the membrane surface was found to be 4.2 ± 0.2 nm and its width 3–3.5 nm (100 individual particles measured). The single ATP synthase molecules isolated in the detergent (fig.1b,d) consist of two globular domains, corresponding to F_1 and F_0, linked by a stalk 3.7–4.3 nm long and 3–3.5 nm wide (30 particles measured). These parameters are very close to those of the stalk portion protruding from the membrane surface in SMP. This means that the whole stalk or most of it is outside the lipid bilayer. The F_0 sector (fig.1d) is 6–8 nm in diameter.

For a more detailed analysis of the ATPase complex structure, the membrane-associated ATP synthase molecules in SMP preparations were computer averaged by the correlation method (see section 2). First, 120 single ATP synthase particles similar to those in fig.1c were averaged and this average (after high-pass filtration) was used as reference image for the next averaging. Then 60 particles out of the 120 having the best correlation coefficients with the reference image obtained were correlation aligned and summed. The final image (fig.2) shows three morphologically distinct regions of protein distribution: one extrinsic to the membrane F_1, the stalk and the membrane F_0 sector. The stalk is 4–4.5 nm long and about 3.5 nm wide. These dimensions accord with the data of other electron microscopic works on ATP synthetase from *E. coli* (4.5 × 2 nm [13]), mitochondria (4.3 × 3.7 nm) and chloroplasts (4.3 × 2.7 [14]). Fig.2 confirms the stalk location preferentially outside the membrane as found earlier by a comparison of individual molecular images of ATP synthase in SMP preparations (fig.1a,c) and detergent-solubilized samples (fig.1b,d). The F_0 sector (membrane stain exclusion region) is also resolved (fig.2). It has a circular shape 6–8 nm in diameter, that coincides with the measurements for the solubilized MF_0F_1 complex (fig.1b,d). This value differs somewhat from the 6–12 nm diameter of F_0 in MF_0F_1-ATPase treated with CHAPS [12], but is very close to the data of Boekema et al. [14], where F_0 was approximated by a cylinder 8.9 nm high and 6.4 nm in diameter. Since the length of the F_0 sector is larger than the 5–6 nm thickness of the membrane lipid bilayer, it partially extends from this bilayer.

One can see that the hexagonal-view (front view – fig.1c, upper row) and side-view projections of membrane-bound F_1-ATPase in SMP preparations often show the smaller dimension perpendicular to the membrane surface (fig.1c, lower row). Similar views of the F_1-ATPase were also observed in string-like aggregates of solubilized MF_0F_1 and CF_0F_1 molecules [14]. Side-view projections of single MF_1 particles are rarely seen on the support film [10]. This indicates that in intact membranes the front view of F_1-ATPase is faced parallel to the

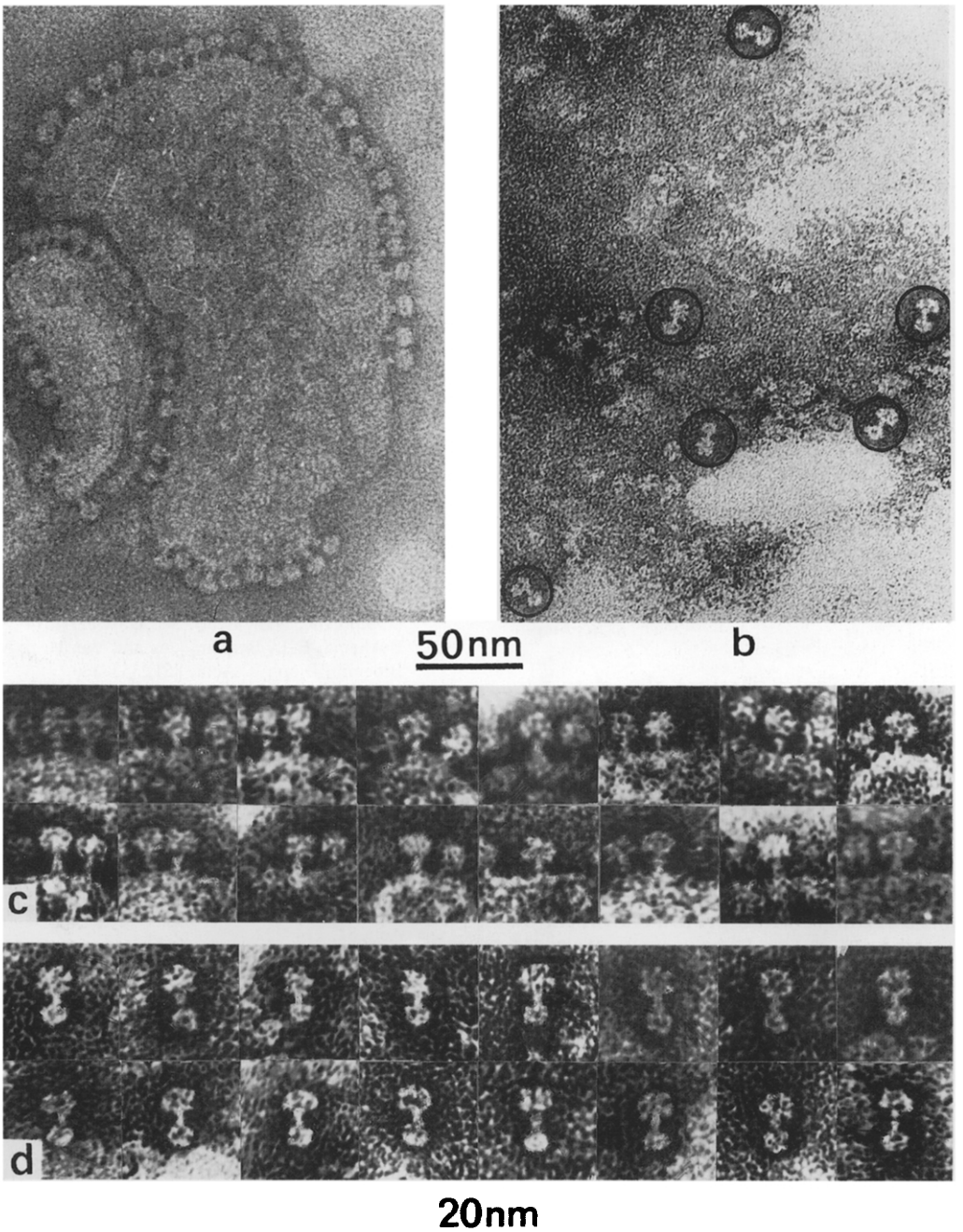

Fig.1. Micrographs of negatively stained preparations of submitochondrial particles (SMP) (a) and isolated ATP synthase (b). In (b) some ATP-synthase molecules are encircled. A gallery of ATP synthase molecules membrane-bound in SMP (c) and solubilized in sodium cholate (d).

membrane surface [14]. This conclusion is consistent with the results of computer averaging (fig.2). which show that the F_1 dimension (about 10 nm) along the membrane surface is longer than the dimension (about 8.5 nm) in the vertical direction. We postulate that the bond between the F_1-ATPase

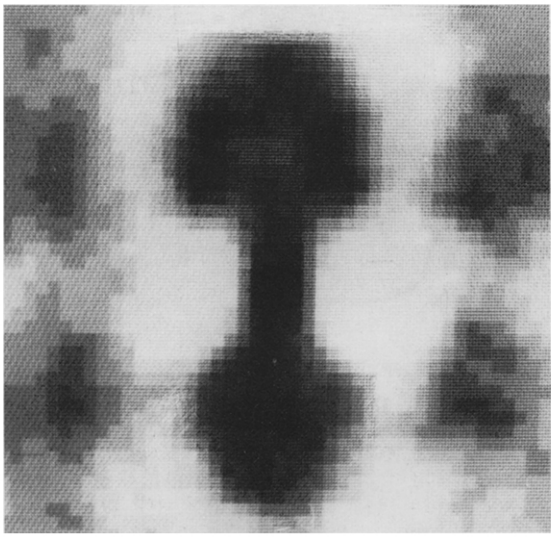

Fig.2. Computer-averaged image of membrane-bound ATP synthase molecule in SMP represented as a half-tone picture.

and the stalk is not rigid. Therefore, the relative arrangement of these structures may change during specimen preparation. Thus, F_1-ATPase tends to occupy the most stable front-view position on the support. The obvious difference in the F_1-ATPase orientation in the upper and lower rows in fig.1d could be explained by this very tendency.

Since the overall length of F_0 plus stalk is more than 10 nm, it is unlikely that proton translocation occurs over such a distance through the stalk, 3–3.5 nm thick [13].

We suggest, as an alternative, that proton transport via the membrane-embedded F_0 sector induces a conformational change in the stalk which in turn alters the catalytic center of the F_1-ATPase.

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