Macromolecular Organization of F₁-ATPase Isolated from Clostridium thermoceticum as Revealed by Electron Microscopy

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Membrane vesicles and the F₁-ATPase from Clostridium thermoceticum were examined by electron microscopy. F₁-ATPase particles projecting from the vesicles have a diameter of 10 to 12 nm. The F₁-ATPase has an αβγδε structure. The α and β subunits are most likely arranged in an alternating sequence around a central protein mass consisting of the γ and δ subunits.

Clostridium thermoceticum is an obligate anaerobic thermophile that carries out homoacetogenic fermentation of sugars (10). It also grows autotrophically (8) by using a novel pathway for the incorporation of CO or CO₂ into acetate (10, 15). The ability of this bacterium to grow autotrophically, along with other findings (2, 5) has led to the suggestion that C. thermoceticum can couple electron transport with ATP synthesis via an H⁺-translocating ATPase. In a previous report (6), we described the purification of the F₁-ATPase from C. thermoceticum and showed that it is an oligomer with an Mr of about 370,000 consisting of four different subunits in the apparent molar ratio of αβγδ. The quaternary structure of this enzyme appears to be similar to that of the F₁-ATPases isolated from several respiratory organisms, (4, 13, 14), but different from that of the enzymes isolated from two nonrespiratory bacteria (3, 17). In this report, we describe an electron microscopic study of the three-dimensional structure of the F₁-ATPase from C. thermoceticum.

The preparation of membrane vesicles and purified F₁-ATPase from C. thermoceticum was as described earlier (6), except that ATP (1 mM) was included in all buffers used for purification. Also, the final DEAE step was replaced by high-performance liquid chromatography with a TSK DEAE .5PW column (8 by 75 mm; LKB Instruments, Inc., Rockville, Md.). F₁-ATPase was eluted with a linear gradient from 0 to 1 M KCl in a buffer solution containing 50 mM Tris hydrochloride (pH 7.6), 1 mM MgCl₂, 1 mM ATP, and 10% (vol/vol) glycerol. The specific activity of the purified enzyme was 12.4 μmol of P₃ released min⁻¹ mg⁻¹ at pH 8.5 and 38°C.

Membrane vesicles with attached F₁-ATPase particles (100 μg of protein ml⁻¹) and purified F₁-ATPase particles (10 μg ml⁻¹) were negatively stained with an aqueous uranyl acetate solution (3.5%, [wt/vol], pH 4.8) by the diffusion technique of Valentine et al. (18). Deep stain conditions were applied as specified by Johannsen et al. (7). Samples were observed, tilt series were performed, and micrographs were taken with a Philips EM 400 ST electron microscope operated at 80 keV in the conventional transmission mode. Calibration of instrument magnifications was done as described previously (12). Micrographs were taken at magnifications ranging from 46,000× to 60,000×. Tilt series were done at 46,000×. Measurements of particle sizes were made from prints at calibrated magnifications ranging from 143,000× to 200,000×.

Membrane vesicles with attached F₁-ATPase particles are shown in Fig. 1. The number of F₁-ATPase particles per vesicle is relatively low, often not exceeding three for a vesicle of 50-nm diameter. Frames 3 to 5 of Fig. 1 clearly demonstrate a stalk connecting the F₁-ATPase particle with the vesicle. The attached F₁-ATPase particles have diameters between 10 and 12 nm, which is similar to the diameter obtained for F₁-ATPase from mitochondria (16). The projections representing F₁-ATPase particles are not seen in vesicles stripped of F₁-ATPase (6), and re-binding of F₁-ATPase to stripped vesicle results in the reappearance of the projections.

Micrographs of purified F₁-ATPase are shown in Fig. 2 through 4. Figure 2 is an overview of a negatively stained sample, whereas Fig. 3 and 4 depict results of tilting experiments. Figure 2 shows that the F₁-ATPase sample used for the electron microscopic analysis was homogeneous. The diameter of the particle types 1 to 4, assumed to be face views of F₁-ATPase, is 11 ± 1.5 nm. Type 6 particle projections are approximately 11 nm in the direction parallel to the long axis and 8 to 9 nm perpendicular to that axis and are interpreted to be projections of F₁-ATPase particles attached to the support film in a tilted fashion. Type 5 projections are also interpreted to be views of attached F₁-ATPase particles slightly tilted with respect to the most commonly seen face-view.

Type 1 projections in Fig. 2 reveal six intensity maxima arranged in a circle at average intervals of approximately 60°. The center of these projections appears to be white, indicating the presence of a proteinaceous mass therein. The difference between type 1 and type 2 projections is that type 2 projections exhibit negative stain trapped in the center of the particles, causing a dark central dot. Type 3 is very similar to types 1 and 2; however, the six intensity maxima arranged in a circle in types 1 and 2 appear to be arranged into two groups, each group being made up of three intensity maxima arranged in a plane at 120° intervals. One plane appears to be rotated 60° with respect to the other, thus giving rise to a threefold rotational symmetry. The existence of that type of symmetry is confirmed by the existence of type 4 projections, clearly indicating the subdivision of the particle mass into three submasses arranged at angles of 120° (Fig. 2).

The four tilt series presented in Fig. 3 demonstrate that the different views of F₁-ATPase depicted in Fig. 2 can be obtained by tilting. These micrographs also demonstrate that the enzyme seen in projections close to side views (frames 1,
FIG. 1. F$_1$-ATPase particles (arrowheads) attached by stalks (arrows) to membrane vesicles. Negative staining was with uranyl acetate.

4, 7, 9, 10, and 12 of Fig. 3) exhibits a smaller diameter than that measured from face views (frames 2, 5, 8, and 11 of Fig. 3). The central mass of the F$_1$-ATPase is clearly visible in most face views shown in Fig. 3 (frames 2, 5, 6, 7, 8, 9, 11, and 12). This indicates that the failure to see the central mass observed in several cases (types 2 and 3 in Fig. 2) is probably not caused by the absence of such a central mass, but rather by a superimposition of trapped negative-staining salt in the central area of the F$_1$-ATPase. Thus, the imaging of a central protein mass is hindered.

In the tilt series in Fig. 4 (frames A to C) the central mass visible in C moves to the periphery of the projection when the particle is tilted. This indicates that the central mass is located in a plane different from that of the surrounding major masses. The latter are considered to represent the three $\alpha$ and three $\beta$ subunits, whereas the central mass may represent the $\gamma$ and $\delta$ subunits that may form a stalklike structure. Frames D to G of Fig. 4 show that the central mass

FIG. 2. F$_1$-ATPase particles depicted after negative staining with uranyl acetate. Several types of projections (numbered 1 through 6) can be seen. Types 1 and 2 exhibit, within the limit of resolution, a sixfold rotational symmetry. Type 2 shows negative staining salt trapped in the particle center. Type 3 and, even more explicitly, type 4 particles show a threefold rotational symmetry. Types 5 and 6 represent particles attached to the support film in positions tilted at different degrees relative to the electron beam. Types 1 to 4 are interpreted to be face views of F$_1$-ATPase particles.

FIG. 3. Four examples of tilt series (tilting axis, Ti, and tilting angles, $+45^\circ$ and $-35^\circ$, as indicated). Projections arising from particles positioned approximately edge on to the support film (frames 1, 4, 6, 7, 10, 11, and 12) exhibit a smaller size in one direction as compared with projections interpreted to be face views (frames 2, 5, 8, 11, and 12). In almost all particles depicted in face views, a solid central mass can be seen. Negative staining was with uranyl acetate.
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FIG. 4. Higher magnifications and drawings of projections from Fig. 3. Bar, 20 nm. Frames A to C constitute the tilt series of one projection (lower left corner) of frames 10 to 12 from Fig. 3, and D to G are selected projections from frames 2, 5, 7, and 8 from Fig. 3.

appears to be connected to the peripheral masses with spokes. It is likely that each α and β subunit is so connected with the central mass, since spokes are visibly connecting neighboring peripheral masses. According to Lünsdorf et al. (11), the neighboring subunits of the peripheral structure of F1-ATPase of Escherichia coli are never the same. Thus one α subunit is flanked by two β subunits and vice versa.

The results presented here, when compared with previous findings obtained with F1-ATPase isolated from mitochondria (16), chloroplasts (1), and E. coli (1, 11), strongly suggest that the quaternary structure of the C. thermoeacetica enzyme is of the form α3β2γ8, with the α and β subunits arranged in alternating sequence around the periphery of the particle. In addition, the angles between the three α subunits, as in the mitochondrial F1-ATPase (16), seem to be 120°. The three α subunits are located in one layer. In a second layer the 3 β subunits, also separated from one another by 120°, are each offset relative to each α subunit by 60°. This appears to fit with the model of Tiedge et al. (16) for the mitochondrial F1-ATPase. The central mass usually seen in face views is assumed to represent the γ and 6 subunits.

The structural similarity between the F1-ATPase isolated from C. thermoeacetica and those isolated from mitochondria, chloroplasts, and E. coli implies a functional similarity as well. We feel that the enzyme from C. thermoeacetica, like its counterparts in respiratory organisms, can synthesize ATP by using an electrochemical gradient of protons as the driving force. The mechanism by which this bacterium produces an electrochemical gradient is unknown, but probably involves the membrane-bound carriers cytochrome b and menaquinone (5) and perhaps one or several unknown additional electron acceptors that carry electrons to the pathway of the synthesis of acetate from CO2 (9).

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