Structure of the ATP-Synthase from Chloroplasts and Mitochondria Studied by Electron Microscopy

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The structure of the ATP-synthase, F_0F_1, from spinach chloroplasts and beef heart mitochondria has been investigated by electron microscopy with negatively stained specimens. The detergent-solubilized ATP-synthase forms string-like structures in which the F_0 parts are aggregated. In most cases, the F_1 parts are arranged at alternating sides along the string. The F_0 part has an approximate cylindrical shape with heights of 8.3 and 8.9 nm and diameters of 6.2 and 6.4 nm for the chloroplast and mitochondrial enzyme, respectively.

The F_1 parts are disk-like structures with a diameter of about 11.5 nm and a height of about 8.5 nm. The F_1 parts are attached to the strings, composed of F_0 parts, in most cases, with their smallest dimension parallel to the strings. The stalk connecting F_0 and F_1 has a length of 3.7 nm and 4.3 nm and a diameter of 2.7 nm and 4.3 nm for the chloroplast and mitochondrial enzyme, respectively.

Introduction

ATP synthesis/hydrolysis coupled with a transmembrane proton transport is catalyzed by a membrane-bound enzyme (ATP-synthase) in different types of organelles (chloroplasts, mitochondria, bacteria). It consists of a membrane-integrated part, F_0, which is supposed to act as a proton channel through the membrane and a hydrophilic part, F_1, which contains the nucleotide binding sites. The ATP-synthases from the different sources are very similar (for review see refs. [1–4]. The hydrophilic part of the ATP-synthase from chloroplasts (CF_1) and mitochondria (MF_1) is composed of five different subunits: α, β, γ, δ and ε [5, 6]. The stoichiometry of the MF_1 subunits is $\alpha_3\beta_3\gamma\delta\varepsilon$ [6]; however, the number of copies of δ and ε in CF_1 is uncertain [4].

The hydrophobic part of the ATP-synthase from chloroplasts (CF_0) is composed of four different subunits I (18 kDa), II (16 kDa), III (8 kDa) and IV (25 kDa) [7–9]. The stoichiometry of the subunits is presumably I, II_2, III_{12}, IV [10]. The hydrophobic MF_0 part contains six subunits: a, b, c and d and F_6 and A_6L [11] and is less well understood because of its complexity.

Structural studies have mainly concentrated on isolated F_1. X-ray diffraction has resulted in a low-resolution model showing six regions of approximately the same size [12]. On the basis of electron microscopy of single molecules and three-dimensional crystals, an arrangement of the larger subunits has been proposed [13–18]. The F_1 part is built up from two layers of the α- and β-subunits in the form of a flattened trigonal antiprism [14]. In the hexagonal projection, the small subunits γ, δ and ε appear as a seventh mass in the center of the large subunits. This central mass is slightly displaced to one α–β pair (in MF_1) [18].

Exact information on the shape and dimensions of the holoenzyme F_0F_1 is scarce. The F_1 part is connected by a stalk to the F_0 part [19–22]. Isolated CF_0CF_1 ATPase forms string-like aggregates in which the CF_0 parts stick together [20, 21]. In this work the ATP-synthetase from chloroplasts, CF_0F_1,

Abbreviations: F_0F_1, proton translocating ATP-synthase; F_0, membrane part of the ATP-synthase; F_1, hydrophilic part of the ATP-synthase.

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and mitochondria, MF_0F_1, has been investigated by electron microscopy of negatively stained samples.

Materials and Methods

CF_0CF_1 ATPase was isolated from spinach chloroplasts according to [23, 10]. It was finally dissolved in a concentration of 2–3 mg/ml in 30 mM Tris-succinate pH 6.5 containing 2 mg/ml Triton X–100, 0.2 mM ATP, 0.5 mM EDTA, 1 mg/ml asolectin and 900 mM sucrose. Reconstitution of CF_0CF_1 in asolectin liposomes was carried out as described in [24]. These preparations show rates of proton transport-coupled ATP synthesis practically identical with that observed in the natural membrane [24]. MF_0MF_1 was isolated according to [25] in a concentration of 33 mg/min 50 mM Tris-HCl pH 7.5, 250 mM sucrose, 1 mM dithiotreitol and 0.2 mM EDTA.

Specimens were prepared by the droplet method or the Valentine method [26] using a 1% uranyl acetate solution as negative stain. Electron microscopy was carried out on Philips EM 300 and EM 400 microscopes at 60–70,000 magnification. We used relatively small defocus values (mostly less than 200 nm) which enhances the direct visualization of small details, since phase-contrast noise in the images is suppressed.

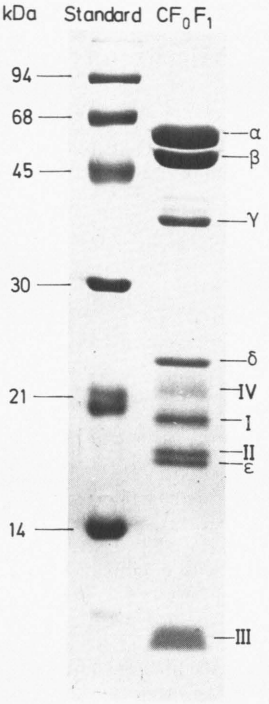


Fig. 1. SDS-gel of CF_0F_1; left lane protein standards, right lane CF_0F_1. CF_0F_1 was incubated in a buffer containing 62.5 mM Tris-Cl pH = 7.5, 2% (w/v) SDS, 5% (v/v) glycerol and 10% mercaptoethanol for 5 min at 95 °C. Then 25 µg of the preincubated CF_0F_1 was used for gel electrophoresis. Stacking gel contains 3.75% acrylamide, the separation gel 15%.

Results

Fig. 1 shows an SDS-gel of the CF_0F_1 preparation used in this work. The preparation contains only the nine different subunits of CF_0F_1, *i.e.*, it is practically free of any impurity. An SDS-gel of a MF_0F_1 preparation, similar in composition to the one used in this work, is shown in ref. [27].

Fig. 2 shows a part of an original micrograph of isolated CF_0F_1. Single CF_0F_1 molecules and different aggregates of CF_0F_1 can be seen. Additionally, CF_1 can be seen which is evidently disconnected from CF_0. When CF_0F_1 from this preparation is reconstituted into asolectin liposomes, half of the maximal rate of ATP synthesis (200 s^{-1}) as in thylakoid membranes is observed [28]. We conclude from this result that the isolated CF_0F_1 is essentially intact and that the disconnection of $CF_1$from CF_0 occurs during the dilution and staining procedure for electron microscopy. The best specimens for electron microscopy were obtained by diluting the sample about 30 times with 10 mM Tris-HC_1 pH 7.5. Electron micrographs from MF_0F_1 show similar results. In this case optimal results were obtained by diluting the sample about 250 times with 10 mM Tris-HC_1 pH 7.5, containing 0.15% octylglucoside. Omission of the detergent results in big aggregates of randomly clustered protein material.

Fig. 3 A shows a gallery of F_0F_1 strings. The upper three rows show CF_0F_1, the lower two MF_0F_1. Fig. 3 B shows some single molecules and strings with our schematic interpretation on the left side of the corresponding electron micrograph(s). The F_0 part (dashed area) is strongly hydrophobic and therefore has the tendency to aggregate. The F_0 part is smaller than the F_1 part. This has consequences for the preparation of the strings for electron microscopy. Upon drying, the F_1 parts will become attached to the carbon support film. Since they are so big, their position will mostly alternate on the strings, avoiding overlap or friction. It can be seen from Fig. 3 that on most

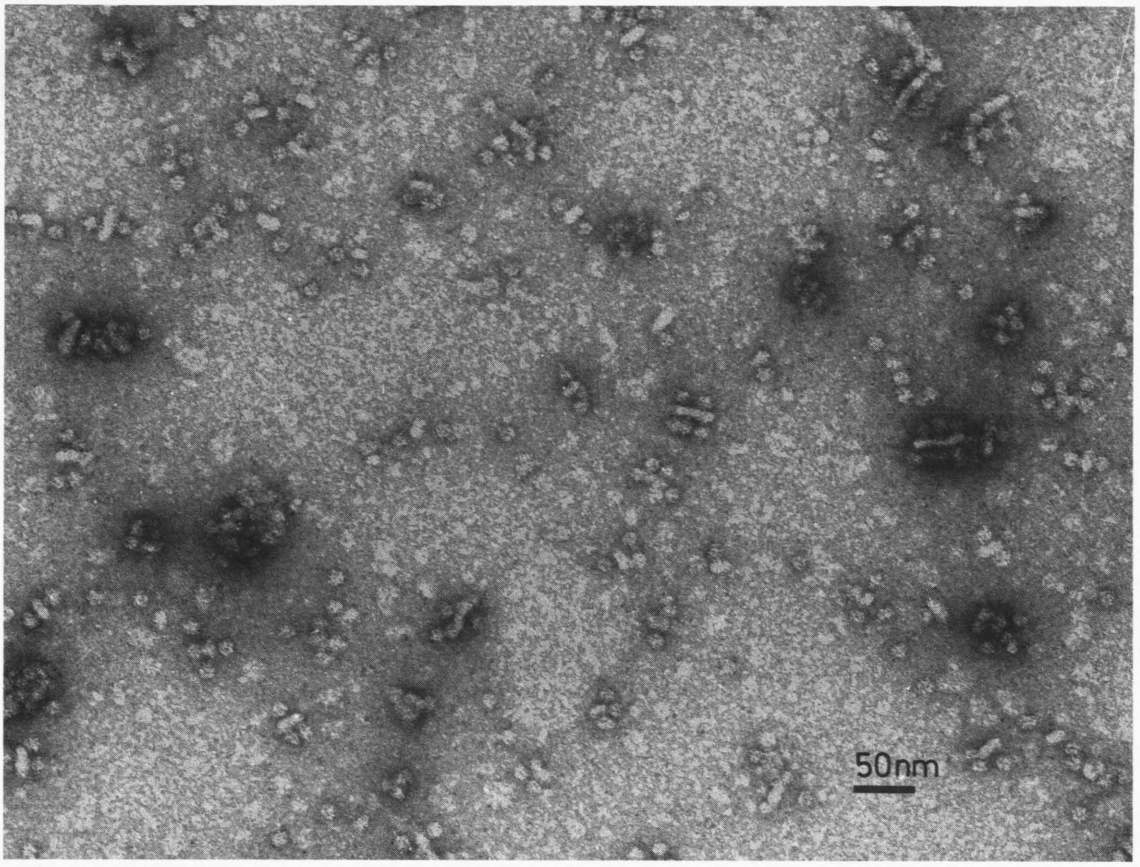


Fig. 2. Part of an original electron micrograph showing CF_0F_1. The specimen was stained negative with 1% uranyl acetate. The electron optical magnification was 70,000.

strings adjacent F_1-parts are alternating and not neighboring.

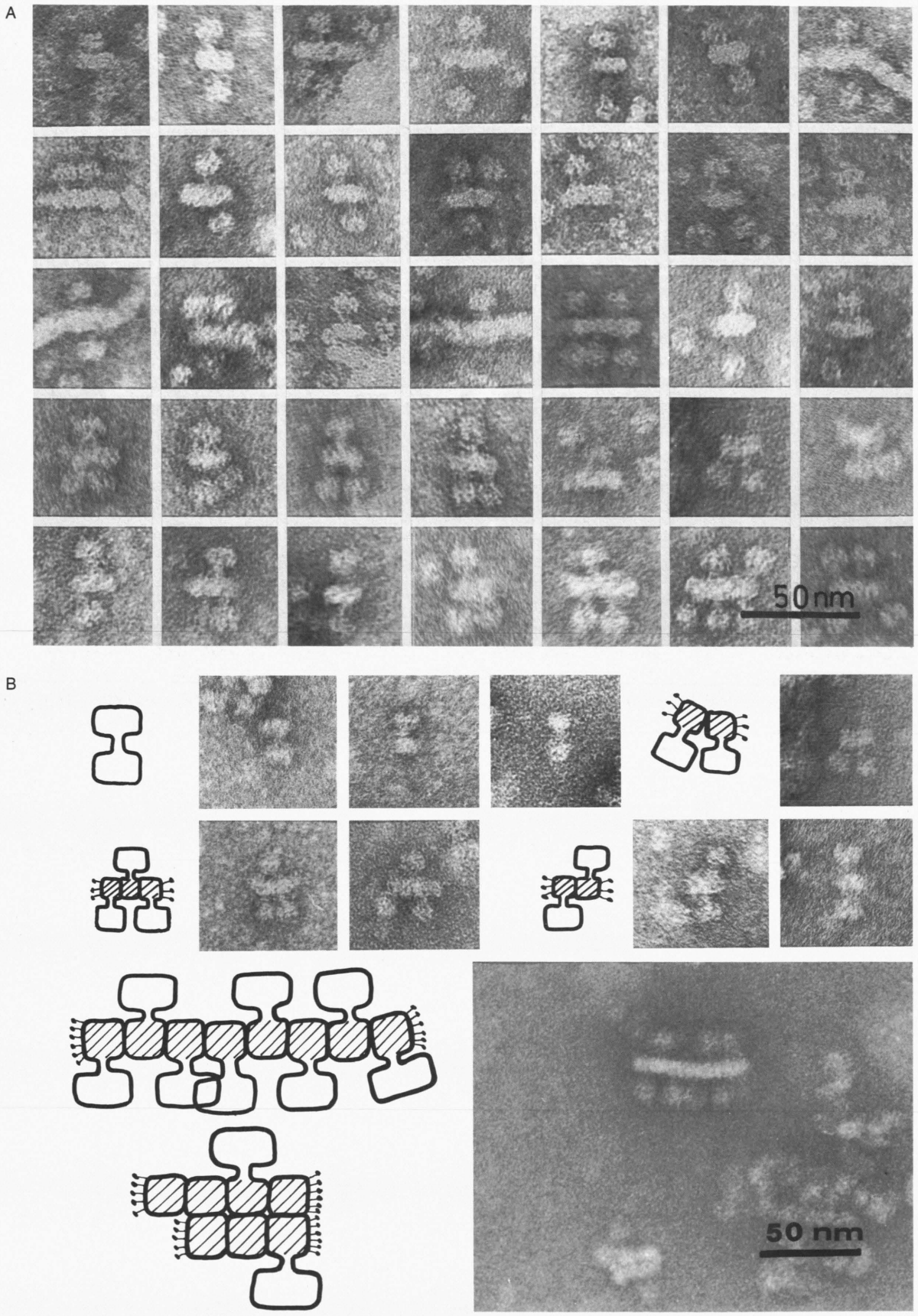
The contours of the F_1 parts on the strings can be seen clearly. In some cases, the length of F_1 parallel to the string was considerably longer than that vertical to the string (*e.g.*, Fig. 3A, the first micrographs in rows 1–3). In other cases, the dimensions parallel and vertical to the string were quite similar (see *e.g.* micrograph 7, row 1). On the average, for CF_1 11 nm resulted parallel to the string and 8.3 nm vertical to the string; for MF_1 11.7 nm and 9.0 nm, resp., resulted (see Table I).

The F_1 parts are connected to the strings by a stalk. For CF_1 the length of the stalk is 3.7 nm, its diameter 2.7 nm (see Table I). If CF_0CF_1 is reconstituted into asolectin liposomes, the length of the stalk is found to be 3.2 ± 0.8 nm (number of measurements: 24). The diameter of the stalk could not be measured with accuracy in the reconstituted system. For MF_1 the length of the stalk is 4.3 nm, its diameter is also 4.3 nm (see Table I).

The thickness of the strings can be easily measured. Values of 8.3 and 8.9 nm were found for CF_0 and MF_0, respectively (Table I). A phospholipid

Table I. Dimensions of the isolated CF_0F_1 and MF_0F_1. The measured parameters are defined in Fig. 5. Additionally, the standard deviation and number of measurements (in brackets) are given.

Length [nm]	MF_0F_1	CF_0F_1
a	11.7 ± 0.9 (50)	11.0 ± 1.1 (36)
b	9.0 ± 0.8 (50)	8.3 ± 1.0 (36)
c	4.3 ± 1.0 (42)	2.7 ± 0.6 (36)
d	4.3 ± 0.6 (42)	3.7 ± 0.7 (36)
e	8.9 ± 0.8 (68)	8.3 ± 0.8 (83)
f	6.4 ± 0.7 (72)	6.2 ± 0.4 (24)


A
50 nm
B
50 nm

bilayer has a thickness of about 5 nm [29], and, in principle, even thinner proteins can span the lipid membrane, like bacteriorhodopsin which has a thickness of about 4 nm [30]. In theory, it could be that the strings are built from a double row of F_0 parts having a thickness of about 4.3 nm. But this is unlikely since in no case single strings of that size were observed. Moreover, single MF_0F_1 molecules never show such a small dimension. Therefore we conclude that the strings must be formed from rows of F_0 parts having a thickness of about 8 nm. The interpretation is illustrated by examples in Fig. 3B.

The dimensions of the F_0 parts parallel to the strings are difficult to measure directly from single units since these parts usually stick together forming smaller or longer beads, their length depending on the detergent concentration. By measuring the full length of well preserved strings and by counting the number of attached F_1 parts on both sides, there is a way to estimate the length of F_0 along the strings. We assume that the F_0 parts do not overlap significantly, since the strings are evenly stained. We measured 126 of these strings being composed of 2–11 F_0F_1 complexes. Fig. 4 shows the length of the string as a function of the number of F_1 parts. The slope of this curve is the increase in length of the string per F_1. A length of 6.2 and 6.4 nm resulted for CF_1 and MF_1, respectively (Fig. 4 and Table I).

In our interpretation this length is the smallest dimension of the F_0 part along the string. The straight curves in Fig. 4A and 4B imply that all strings are packed in a similar way. It is noteworthy that the curves, if extrapolated, are not going through the origin. They extrapolate to 3.6 nm and this length can be interpreted as the length of the detergent which must be present at both ends of the string. The length of the detergent octylglucoside is 1.8 nm, which is in surprisingly good agreement with the extrapolated value.

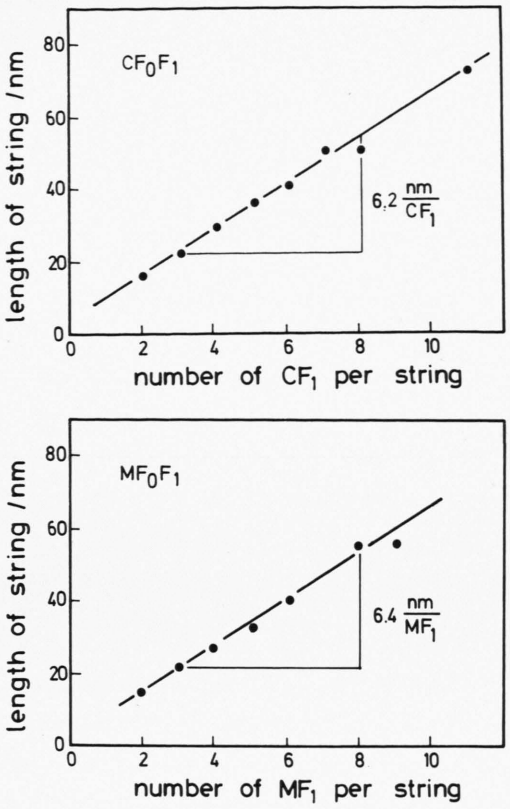


Fig. 4. String length as a function of the number of attached F_1-parts. The slope gives the string length per F_1, *i.e.*, the maximal dimensions of F_0 in the direction of the string. Top: CF_0F_1; bottom: MF_0F_1.

Discussion

These results and considerations lead to a model of the F_0F_1 ATP-synthase shown in Fig. 5. The hydrophilic F_1 part is connected by a stalk to the F_0 part, the F_0 part is embedded in the membrane and the thickness of the membrane is smaller than the length of F_0. Additionally, in Fig. 5 the dimensions are difined which are listed in Table I. The dimensions of the F_1 part found in our work are, within error limits, in accordance with the data from X-ray diffraction where dimensions of $12 \times 11 \times 8$ nm have been found [12] and also with earlier electron microscopy data [13–17]. The dimensions of the stalk reported here are also in accordance with earlier electronmicroscopic work: for MF_0F_1 a stalk of 4.0×3 nm has been reported [31], for *E. coli* F_0F_1, EF_0F_1, a stalk of 4.5×2 nm was found [32], and for CF_0F_1 the distance of the CF_1 part from the membrane has been estimated from fluorescence energy transfer data to be

Fig. 3. A: A gallery of isolated ATP-synthases. Upper three rows show CF_0F_1, the lower two rows show MF_0F_1. Total magnification is 420,000.

B: Selected parts of micrographs illustrating the packing of isolated F_0F_1 molecules. On the left side of each micrograph our interpretation of the observed structure is given. The dashed area shows the aggregated F_0 parts.

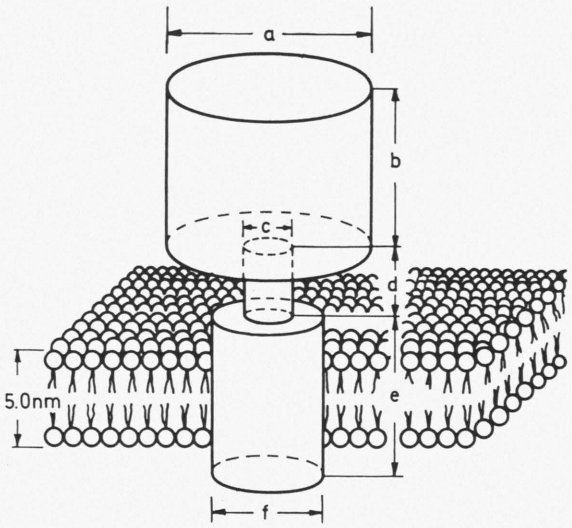


Fig. 5. A model for the shape of the ATP-synthase in a lipid bilayer. The different measured parameters are defined in this figure. The corresponding numbers are given in Table I. The figure is drawn in scale for CF_0F_1.

between 3–4.5 nm [33]. However, the stalk dimensions of several systems do not need to be the same since the number of subunits differs. The ATP-synthase from *E. coli* has the smallest stalk [32], and is the simplest system with only 8 subunits, whereas MF_0F_1 has 5 subunits more, located in the stalk and F_0 part [11]. For the F_0 part, a thickness of the string of 8.3 nm (or 8.9 nm) was found and we assume that this represents the length of F_0 through the membrane. The length of the F_0 part is surprisingly long. Since a phospholipid bilayer has a thickness of only 5 nm [29], this means that a large part of F_0 is outside the membrane.

The length of the string per F_1 is 6.2 nm (or 6.4 nm) and in our interpretation this represents the diameter of the F_0 part, in case the F_0 part has a circular shape. The length of 6.2 nm has to be considered as the maximal value: if there were detergent molecules located between neighboring F_0 parts, this would be included in this type of measurement and the F_0 part alone would be smaller. It is not clear from our results whether the F_0 part has indeed a circular shape, *e.g.* an ellipsoid shape could also be realized. In the latter case, one has to assume that along the string axis the smaller dimension is found and that the dimension perpendicular to the string axis should be bigger since this would result in a bigger area of contact between the hydrophobic F_0 parts with a corresponding lowering of the free energy of the system. However, presently there is no evidence for this, and for the sake of simplicity we assume a circular shape of the F_0 part.

Micrographs of single ATP-synthase molecules, purified from rat liver mitochondria with deoxycholate have been presented [19]. From these images it was concluded that F_0 has a diameter of 10–12 nm. Since it is impossible that such F_0F_1 particles remain monodispersed without attached detergents, the real diameter is much smaller. According to Fig. 4 the (extrapolated) diameter for one F_0 is about 10 nm, however, 3.6 nm are due to the detergent. Electron micrographs from reconstituted CF_0F_1 obtained with the freeze-fracture technique have shown particles with a diameter of 8.3 nm [21, 34]. If the size overestimation due to the platinum replication technique is corrected, a diameter of about 6 nm can be estimated; in good agreement with our results.

However, the arrangement of the F_1 part relative to the F_0 part is controversial. Our data indicate that in most cases the longer dimension of F_1 is parallel to the string axis and, therefore, we assume that it is also parallel to the membrane surface. Electron micrographs from reconstituted EF_0F_1 and MF_0F_1 indicate that the longer dimension is vertical to the membrane surface [31, 32]. It is not yet clear whether this difference reflects actual differences (isolated versus reconstituted) or whether it represents an artifact of the specimen preparation, *i.e.*, upon staining and drying on the carbon film the position of the F_1 parts relative to the F_0 parts is changed. Single MF_1 and CF_1 molecules, if prepared under the same conditions as the MF_0F_1 and CF_0F_1 molecules, will seldom lay on their side (less than 1% out of 4000 molecules ([18] and unpublished), since the molecules have a strong tendency to stick with their hexagonal (top) side to the carbon support. Despite the fact that the side view position for F_1 molecules is apparently very unfavourable, we still find most F_0F_1 molecules arranged in this position.

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