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

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Z. Naturforsch. 43c, 219—225 (1988); received December 1, 1987

ATP-Synthase, Enzyme Structure, Electron Microscopy

The structure of the ATP-synthase, F₀F₁, 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 F₀ parts are aggregated. In most cases, the F₁ parts are arranged at alternating sides along the string. The F₀ 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 F₁ parts are disk-like structures with a diameter of about 11.5 nm and a height of about 8.5 nm. The F₁ parts are attached to the strings, composed of F₀ parts, in most cases, with their smallest dimension parallel to the strings. The stalk connecting F₀ and F₁ 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, F₀, which is supposed to act as a proton channel through the membrane and a hydrophilic part, F₁, 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 (CF₁) and mitochondria (MF₁) is composed of five different subunits: α, β, γ, δ and ε [5, 6]. The stoichiometry of the MF₁ subunits is α₂β₂γδε [6]; however, the number of copies of δ and ε in CF₁ is uncertain [4].

The hydrophobic part of the ATP-synthase from chloroplasts (CF₀) 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, II₂, III₁₂, IV [10]. The hydrophobic MF₀ part contains six subunits: α, β, c and d and F₀ and A₀L [11] and is less well understood because of its complexity.

Structural studies have mainly concentrated on isolated F₁. 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 F₁ 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 MF₁) [18].

Exact information on the shape and dimensions of the holoenzyme F₀F₁ is scarce. The F₁ part is connected by a stalk to the F₀ part [19—22]. Isolated CF₀CF₁ ATPase forms string-like aggregates in which the CF₀ parts stick together [20, 21]. In this work the ATP-synthetase from chloroplasts, CF₀F₁,
and mitochondria, MF$_0$F$_i$, has been investigated by electron microscopy of negatively stained samples.

**Materials and Methods**

CF$_0$CF$_1$ 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$_0$CF$_1$ 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$_0$MF$_i$ 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.

**Results**

Fig. 1 shows an SDS-gel of the CF$_0$F$_i$ preparation used in this work. The preparation contains only the nine different subunits of CF$_0$F$_i$, i.e., it is practically free of any impurity. An SDS-gel of a MF$_0$F$_i$ 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$_0$F$_i$. Single CF$_0$F$_i$ molecules and different aggregates of CF$_0$F$_i$ can be seen. Additionally, CF$_i$ can be seen which is evidently disconnected from CF$_0$. When CF$_0$F$_i$ 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$_0$F$_i$ is essentially intact and that the disconnection of CF$_i$ 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$_0$F$_i$ 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. 3A shows a gallery of F$_0$F$_i$ strings. The upper three rows show CF$_0$F$_i$, the lower two MF$_0$F$_i$. Fig. 3B 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$_i$ part. This has consequences for the preparation of the strings for electron microscopy. Upon drying, the F$_i$ 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
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. 3 A, 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$_0$CF$_1$ 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 molecule is located in the interface of each string (Fig. 4 A) and the relative positions of the four phospholipids in each string are somewhat different (Fig. 4 B). Table I. Dimensions of the isolated CF$_0$F$_1$ and MF$_0$F$_1$. The measured parameters are defined in Fig. 5. Additionally, the standard deviation and number of measurements (in brackets) are given.

<table>
<thead>
<tr>
<th>Length [nm]</th>
<th>MF$_0$F$_1$</th>
<th>CF$_0$F$_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>11.7 ± 0.9 (50)</td>
<td>11.0 ± 1.1 (36)</td>
</tr>
<tr>
<td>b</td>
<td>9.0 ± 0.8 (50)</td>
<td>8.3 ± 1.0 (36)</td>
</tr>
<tr>
<td>c</td>
<td>4.3 ± 1.0 (42)</td>
<td>2.7 ± 0.6 (36)</td>
</tr>
<tr>
<td>d</td>
<td>4.3 ± 0.6 (42)</td>
<td>3.7 ± 0.7 (36)</td>
</tr>
<tr>
<td>e</td>
<td>8.9 ± 0.8 (68)</td>
<td>8.3 ± 0.8 (83)</td>
</tr>
<tr>
<td>f</td>
<td>6.4 ± 0.7 (72)</td>
<td>6.2 ± 0.4 (24)</td>
</tr>
</tbody>
</table>
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 F₀ 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 MF₀F₁ molecules never show such a small dimension. Therefore we conclude that the strings must be formed from rows of F₀ parts having a thickness of about 8 nm. The interpretation is illustrated by examples in Fig. 3B.

The dimensions of the F₀ 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 F₁ parts on both sides, there is a way to estimate the length of F₀ along the strings. We assume that the F₀ parts do not overlap significantly, since the strings are evenly stained. We measured 126 of these strings being composed of 2–11 F₀F₁ complexes. Fig. 4 shows the length of the string as a function of the number of F₁ parts. The slope of this curve is the increase in length of the string per F₁. A length of 6.2 and 6.4 nm resulted for CF₁ and MF₁, respectively (Fig. 4 and Table I).

In our interpretation this length is the smallest dimension of the F₀ 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.

**Discussion**

These results and considerations lead to a model of the F₀F₁ ATP-synthase shown in Fig. 5. The hydrophilic F₁ part is connected by a stalk to the F₀ part, the F₀ part is embedded in the membrane and the thickness of the membrane is smaller than the length of F₀. Additionally, in Fig. 5 the dimensions are defined which are listed in Table I. The dimensions of the F₁ part found in our work are, within error limits, in accordance with the data from X-ray diffraction where dimensions of 12×11×8 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 MF₀F₁ a stalk of 4.0×3 nm has been reported [31], for *E. coli* F₀F₁, EF₀F₁, a stalk of 4.5×2 nm was found [32], and for CF₀F₁ the distance of the CF₁ part from the membrane has been estimated from fluorescence energy transfer data to be
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$_0$F$_1$.

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$_0$F$_1$ 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$_0$F$_1$ 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$_0$F$_1$ 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$_0$F$_1$ and MF$_0$F$_1$ 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$_0$F$_1$ and CF$_0$F$_1$ 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$_0$F$_1$ molecules arranged in this position.

**Acknowledgements**

We thank Prof. B. Rumberg for stimulating this work, Prof. E. Zeitler for continuous support, P. Fromme for the SDS-gel of CF$_0$F$_1$ and Matina Gerdsmeyer for excellent technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (Sfb 312).