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SHORT REVIEW
Structure of F_1-ATPases

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Abstract

F_1-ATPases are large multimeric proteins that can be isolated from the membrane bound system that catalyzes the phosphorylation of ADP by inorganic phosphate in bacteria, plants, and mitochondria. They can be visualized in electron micrographs of the inner mitochondrial membranes where they appear as large protruding spheres 90 Å in diameter. The purified F_1-ATPases have a molecular weight of 320,000 to 400,000 daltons and are composed of five non-identical subunits (α, β, γ, δ and ϵ). The stoichiometry of these subunits in the complex is still unknown but compositions of the type $\alpha_3\beta_3\gamma\delta\epsilon$ and $\alpha_2\beta_2\gamma_2\delta_2\epsilon_2$ were found to be consistent with some of the available experimental data. This review discusses the recent data and the experimental approaches utilized for the structural characterization of F_1-ATPases.

Key Words: F_1-ATPases; subunits; stoichiometry; sedimentation equilibrium; electron microscopy; x-ray diffraction.

Introduction

The phosphorylation of ADP by P_i in mitochondria, chloroplast, and bacteria is one of the most fascinating biochemical pathways. These reactions involve the coupling of either the oxidation of substrates or the utilization of light quanta for the generation of ATP levels up to 10^8 times the concentration expected from the equilibrium ADP + $P_i \rightleftharpoons$ ATP + H_2O. This phosphoryl transfer reaction is catalyzed by a membrane-bound enzyme system that utilizes the energy provided by the electron transfer reactions. The energy is probably present in the form of an electrochemical proton gradient established across the membrane of the relevant organelles (Mitchell, 1974). Complete understanding of the mechanism of oxidative- and photophosphorylation will require the elucidation of the mechanism of the enzyme system

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involved in the last step of this process (i.e., the phosphorylation of ATP). In this paper we review the information about the structural properties of the soluble portion (F_1-ATPase) of this enzyme system.

Excellent reviews on F_1-ATPases have been published (Senior, 1978; Pedersen, 1975; Penefsky, 1974; Pedersen *et al.*, 1978; Kozlov and Skulachev, 1977; Kagawa, 1978) covering mammalian, bacterial, and plant systems. Therefore, we will discuss the latest new developments in the field and, in addition, several aspects that, although were previously reviewed, merit reexamination using recently published information.

Structural Aspects

F_1-ATPases have been isolated from a large number of biological systems ranging from beef heart and rat liver to *E. coli* and *Streptococcus faecalis*. In all cases the preparations involve the purification of a membrane-associated ATPase activity that can be isolated as a soluble protein. F_1-ATPases can, under appropriate conditions, restore the capacity to synthesize ATP to vesicles from which they have been depleted.

F_1-ATPases are large complex oligomeric proteins. Enzymes isolated from different systems seem to be structurally similar especially in regard to the molecular weight of the complete molecule and its subunits. However, the subunit stoichiometry of F_1-ATPases is still a matter of strong controversy. The different experimental methods used provided a profusion of seemingly contradictory results that eventually will have to be explained. It has to be realized that F_1-ATPases appear to be very large molecules with several subunits of different molecular weight. For a molecule of this kind many of the most widely used experimental methods give ambiguous results. For example, small errors associated with the determination of moelcular weights make the assignment of stoichiometry very unreliable when based solely on these data. Many other methods used rely on the molecular weight of the complete molecule for the normalization of measured parameters on a per molecule basis. These methods will also be affected by the errors in the determination of the molecular weight.

In the following sections we will discuss the published data on the determination of the molecular weights of F_1-ATPases and their subunits and discuss attempts to determine subunit stoichiometry. We will also comment on the determination of the shape and size of these molecules.

Molecular Weight of Complete F_1

Many of the techniques available for the determination of molecular weights have been used for F_1-ATPases of different systems. They gave mostly values ranging from 320,000 to 400,000 daltons (Table I). As

Table I. Determination of the Molecular Weight of F_1-ATPases by Equilibrium Sedimentation (Selected Data)

Source	Name	$\bar{v}$ used (cm^3/g)	$\bar{v}$ (from amino acid composition)	Rotor speed (rpm)	MW	Buffer	Comments	Reference
Beef heart	F_1	0.74[a]	0.74	11,000	347,000	Sucrose, Trs-SO_4, EDTA, 4 mM ATP	—	Knowles and Pentsky, 1972
Chloroplast	CF_1	0.73(7)[b]	0.737	10,000				
				14,000	325,000			
				4,400	358,000	Phosphate ATP, eDTA	Authors consider that the low-speed run is in error due to aggregation	Farron, 1970
	CF_1	0.745[c]	0.737	15,000	325,000	Tris-HCl, KCl, EDTA, DTT		Paradies *et al.*, 1978
S. Faecalis	SF	0.742[d]	0.735	13,000	385,000	Na succinate, $MgCl_2$		Schnebli *et al.*, 1970
Thermophilic bacterium PS3	TF_1	0.75[d]	0.74	6,400	380,000	Tris/NaCl	Using $\bar{v}$ = 0.74 cm^3/g MW ≈ 365,000	Yoshida *et al.*, 1979
				12,000				
E. coli	ECF_1	0.742[c]	—	15,000	358,000	Tris/HCl, KCl mercaptoethanol		Paradis and Schmidt, 1979

[a]Measured using density gradients.
[b]Measured pycnometrically. Value used was 0.737 cm^3/g.
[c]Measured from the change of density with protein concentration.
[d]Measured from runs in water and D_2O.

mentioned before a precise value of the molecular weight is needed for this magnitude to be useful in the determination of subunit stoichiometry. For nonaggregating and nondissociating systems equilibrium sedimentation in the analytical ultracentrifuge is probably the most accurate method of molecular weight determination. In the case of F_1-ATPases both phenomena (aggregation and dissociation) have been invoked in order to explain the differences between the values obtained. Dissociation, if present, will affect more drastically determinations run at high speed while association will be a major factor in low-speed runs. In addition to the value of the $d \ln c/dr^2$ measured for each run the method requires the knowledge of the partial specific volume of the protein ($\bar{v}$). Small errors in this parameter can introduce significant errors in the determined molecular weights (Kawahara and Tanford, 1966).

Knowles and Penefsky (1972) used equilibrium sedimentation for the determination of the molecular weight of beef heart F_1-ATPase. The experiments were carried out using the meniscus depletion technique (Yphantis, 1964) (high speed). The variation of the protein concentration in the cell was measured using interference optics which is probably the most accurate available method. The method requires high protein concentrations and is therefore subject to errors due to association in the sample and to the variation of the activity coefficients throughout the cell. The problems can be overcome by making determinations at several protein concentrations. The molecular weight can be obtained by extrapolation to zero protein concentration. These corrections were apparently not used in the work of Knowles and Penefsky (1972). Equilibrium centrifugation runs at different speeds can be used to detect the occurrence of dissociation but were not performed in this work. The linearity of the plots of $\ln c$ vs r^2 (especially close to the meniscus) and the similarity between the weight-average and the number-average molecular weights were considered sufficient indication of the absence of dissociation. The buffer used in the runs included EDTA and 4 mM ATP. The presence of ATP probably prevented dissociation of the subunits of the F_1-ATPase. The value of $\bar{v}$ (0.74 cm^3/g) was determined using density gradients (Penefsky and Warner, 1965). This value is identical to the one that can be calculated from the amino acid composition by the method of Cohn and Edsall (1943). The authors estimated the molecular weight to be 347,000 daltons. Farron (1970) had previously determined the molecular weight of F_1-ATPase from chloroplast (CF_1) using a similar protocol. The buffers used also contained EDTA and 4 mM ATP. From high-speed runs carried out with the meniscus depletion technique they obtained a value of 325,000 daltons for the molecular weight of CF_1. However, low-speed runs gave a value of 358,000 daltons. The authors account for this discrepancy by possible aggregation. They support this explanation with the observation that samples stored for one or two days show high molecular weight aggregates when run in gels. However, the possibility that the lower value obtained in high-speed

runs is the one in error due to dissociation cannot be ruled out. The value of $\bar{v}$ was determined pycnometrically (0.737 cm^3/g) and calculated from the amino acid composition (0.737 cm^3/g). The molecular weights of CF_1 was also determined by Paradies *et al.* (1978) using sedimentation equilibrium. The value of $\bar{v}$ used (0.745 cm^3/g) was determined by measuring the density of solutions of CF_1 in solvents of varying densities. They obtained a value of 325,000 daltons, in good agreement with the value reported by Farron (1970).

The molecular weight of the F_1-ATPase from *S. Faecalis* (SF_1) was determined by a similar method (Schnebli *et al.*, 1970) and gave a value of 385,000 daltons. The value of $\bar{v}$ (0.742 cm^3/g) was determined by equilibrium sedimentations in D_2O and water following the method of Edelstein and Schachman (1967). The value of $\bar{v}$ calculated from the amino acid composition if 0.735 cm^3/g. Had this value been used in the calculations the value of the molecular weight would have been 375,000 daltons. It has to be realized that the method of Edelstein and Schachman is subject to errors of this magnitude. When proposing the method the authors state that an error of 1% in the slope of the ln c vs r^2 plots will produce an error of 2% in the value of $\bar{v}$ (Edelstein and Schachman, 1967).

Recently, Yoshida *et al.* (1979) determined the molecular weight of the F_1-ATPase of the thermophilic bacterium PS3 (TF_1). Low- and high-speed runs were carried out and found to give a value of 380,000 daltons for the molecular weight. The authors point out that due to the stability of TF_1 this value should be considered as the best estimation of the molecular weight of F_1. There are a few points, however, about this work that merit comment. It is unfortunate that such a careful determination was not performed using the techniques recommended for very accurate determinations. For example, equilibrium sedimentation in D_2O and H_2O were used to determine the value 0.75 cm^3/g for the partial specific volume. This value is very important since, despite the authors' statements to the effect that dissociation is the cause of the lower values obtained in previous molecular weight determinations, most of the differences can be accounted for by the values of $\bar{v}$ used. The amino acid composition of TF_1 presented in the same paper can be used to calculate $\bar{v}$ by the method of Cohn and Edsall (1943). This calculation (not reported by the authors) gives a value of 0.74_0 cm^3/g. This value is very similar to the one used in the other determinations. Using this value the authors would have obtained a molecular weight of 365,000 daltons, indicating that differences in $\bar{v}$ can account for most of the discrepancy between this paper and, for example, that of Knowles and Penefsky (1972). It is unfortunate that the authors did not use a more accurate method for the determination of $\bar{v}$ especially considering that the protein is available in the milligram amounts needed. In any case it is difficult to understand why the value of $\bar{v}$ based on the determined amino acid composition was neither used nor discussed.

Yoshida *et al.* (1979) also determined the molecular weight of F_1 and CF_1. They used 10% methanol to prevent dissociation. This very high concentration of methanol could introduce serious complications in the interpretation of sedimentation equilibrium runs and therefore we will not discuss these results. Paradies and Schmidt (1979) determined the molecular weight of the F_1-ATPase from *E. coli* using equilibrium sedimentation. With a $\bar{v}$ value of 0.742 cm^3/g they obtained a molecular weight of 358,000 daltons.

Sedimentation velocity runs for F_1-ATPases gave s values ranging from 12.2S to 13.8S. Since s is a directly determined magnitude, it is difficult to explain the differences found. Recently Paradies (1979) reported that the sedimentation coefficient of CF_1 changes from 12.8S to 13.5S when 4 mM ATP is added. This finding is in agreement with the results of small-angle X-ray scattering that suggested a reduction in the radius of gyration of CF_1 upon addition of ATP. This correlation seems to also apply to the determinations in other systems; that is, large s values (13.5–13.8) are usually obtained in the presence of ATP while small values (12.2–12.8) are usually measured in its absence. The molecular weight of rat liver F_1-ATPase was also determined from single-crystal X-ray diffraction data. This work will be discussed in a later section.

Molecular Weight of the Subunits of F_1-ATPases

Preparations of F_1-ATPases give, in general, five protein bands when subjected to electrophoresis in the presence of dissociating agents (see, for example, Mitchell, 1974; Senior, 1978; Pedersen, 1975; Penefsky, 1974). These five subunits are usually labeled α, β, γ, δ, and ϵ (A, B, C, D, and E). The molecular weights of the subunits were determined by electrophoresis in sodium dodecylsulfate and by sedimentation equilibrium in dissociating agents. The values obtained by electrophoresis in the presence of sodium dodecylsulfate fall in general within the following ranges: α from 53,000 to 62,500; β from 50,000 to 57,000; γ from 28,000 to 43,000; δ from 12,000 to 21,000; ϵ from 7,500 to 14,000. These differences can probably be attributed to intrinsic uncertainties in the method. However, it is important to point out that clear differences seem to exist between different preparations. For example, the α subunit of beef heart F_1-ATPase seems to run slightly faster than the catalase subunit (Knowles and Penefsky, 1972) while that of the rat liver enzyme has definitively a slower mobility (Catterall and Pedersen, 1971).

Molecular weights obtained by gel filtration in the presence of 6 M guanidinium hydrochloride give similar results. However, this method gives more consistent values for the α (54,000 daltons) and the β (50,000–51,000 daltons) subunits.

Determination of the molecular weights of the purified subunits was also

carried out using equilibrium centrifugation in the presence of urea or guanidinium hydrochloride. The values reported from two laboratories for TF_1 (Yoshida *et al.*, 1979) and beef heart F_1 (Knowles and Penefsky, 1972) are in good agreement; α 54,000 daltons in both reports, β 50,000 and 51,000 daltons, and δ 17,300 and 21,000. Both groups used similar values (0.74 cm^3/g) for the partial specific volume. Knowles and Penefsky (1972) obtained theirs from the amino acid composition. Yoshida *et al.* (1979) subtracted 0.01 from the value 0.75 cm^3/g (previously discussed) as suggested by Kielley and Harrington (1960) for runs performed in 6 M guanidinium hydrochloride. However, the justification of this correction is weak since, as pointed out by Tanford (Kawahara and Tanford, 1966), Reithel and Skura (1963) find that good values of molecular weights are obtained without this correction. The value used (0.74 cm^3/g) is also the one that can be obtained from the amino acid composition of the subunits of TF_1. In any case, if this value (0.74 cm^3/g) is used for the determination of the molecular weight of both the intact TF_1 and its subunits, the point stressed by the authors about the exact agreement between the observed molecular weight of TF_1 and the one predicted from the stoichiometry they propose becomes significantly weakened. The work of Yoshida *et al.* (1979) gives also an indication of the existence of other possible problems. Low- and high-speed runs were performed. The molecular weights measured at high speed for the α and β subunits were 5000 to 6000 daltons lower than those obtained at low speed while that of the γ subunit was 3000 daltons higher. The authors state that the low values obtained for α and β in high-speed runs are probably due to contamination by small peptides. However, other explanations (and therefore different estimations of the molecular weights) cannot be ruled out. Further information could have been obtained by measuring the concentration dependence of the molecular weight.

The molecular weights of the small subunits (δ and ϵ) are more uncertain than those of the larger subunits. In this case the fact that these peptides could be different in the different preparations remains a distinct possibility.

Subunit Stoichiometry

Irrespective of what the subunit stoichiometry of F_1-ATPases turns out to be, there will be a large amount of seemingly contradictory data that will have to be explained.

Most preparations when subjected to SDS-gel electrophoresis stain with intensities compatible with a stoichiometry $\alpha_3\beta_3\gamma\delta\epsilon$ (see Mitchell, 1974; Senior, 1978; Pedersen, 1979). The fraction of the stain intensity contained in the small subunits δ and ϵ is very small and variable, and the subunits are sometimes observed only in overloaded gels. Even though in some cases (Catterall and Pedersen, 1973) great care was taken to calibrate stain

intensity per unit mass, the method should not be considered very reliable. Support for this stoichiometry, however, was obtained by a large number of experimental approaches. For example, when *E. coli* (Bragg and Hou, 1975), *S. typhimurium* (Bragg and Hou, 1975), and thermophilic bacterium PS3 (Kagawa *et al.*, 1976) were grown in the presence of uniformly labeled ^{14}C amino acids (U-^{14}C), the separated subunits had radioactivities compatible with $\alpha_3\beta_3\gamma\delta\epsilon$. However, results obtained with CF_1 purified from pea plants grown in $^{14}CO_2$ indicated a stoichiometry of $\alpha_2\beta_2\gamma\delta\epsilon_{1-2}$. These methods, however, are not to be considered reliable either since they are subject to problems arising from pool equilibration. One way of avoiding some of the problems of these experiments would be to use several bacterial auxotrophs for amino acids and grow them in the presence of labeled required amino acid. Using several auxotrophs and carefully determined amino acid compositions of the subunits, we can expect invaluable information regarding subunit stoichiometry to be obtained.

Chemical labeling of cystine (cysteine) residues was attempted on F_1-ATPases from several systems. Both beef heart F_1 (Senior, 1975) and CF_1 (Farron and Racker, 1970) were found to have 12 total SH groups and eight free SH groups per F_1 molecule. The beef heart enzyme seemed to contain four SH groups per α subunit, one per γ subunit, and one per ϵ subunit. These values seem to indicate that F_1-ATPase contains two of each α, γ and ϵ subunits. Similar experiments carried out on TF_1 (Yoshida *et al.*, 1979) seemed to indicate that there are three SH groups per TF_1 molecule and that only the α subunits has an SH group. This experiment suggests that there are three α subunits per TF_1 molecule, clearly at variance with the results on beef heart F_1. Other labeling experiments as well as cross-linking (Baird and Hammes, 1976, 1977) and reconstitution (Vogel and Steinhart, 1976) experiments, failed to give an unequivocal answer to the stoichiometry of F_1-ATPases. Single-crystal X-ray crystallographic studies on the F_1-ATPase from rat liver indicated that the molecule behaves as a crystallographic dimer putting strong constraints on the possible stoichiometries. These results will be described in a following section.

Size and Shape of F_1-ATPases

F_1-ATPases can be visualized in negatively stained mitochondria or submitochondrial particles. They appear in the electron micrographs as spheres of 90 Å diameter protruding from the inner membrane. Purified preparations of F_1-ATPases have also been visualized by electron microscopy. The stained particles are 90 Å in diameter and seem to show some internal structure (Howell and Moudrianakis, 1976; Kagawa and Racker, 1966). However, no consistent pattern can be observed in the published micrographs. The conclusions about subunit organization based on these photographs

should therefore be considered tentative. CF_1 was also visualized in preparations of the thylakoid membrane (Howell and Moudrianakis, 1976) and appeared to be a dimer of stain excluding regions separated by a central depression. The size of the particles was estimated to be 100 Å.

Further information about the size and shape of F_1-ATPases and of some of the subunits was obtained by small-angle X-ray scattering. The experiments performed with F_1-ATPases from spinach chloroplasts (Paradies *et al.*, 1978) and *E. coli* (Paradies and Schmidt, 1979) were complemented with the measurement of hydrodynamic parameters. The methods used provided values of the radius of gyration, the molecular volume, the degree of hydration, the diffusion coefficient, etc. It is important to realize, however, that the molecular information obtained from small-angle X-ray diffraction experiments is spherically averaged. Furthermore, because phase information is not available, the experiments cannot provide a molecular structure. Instead, they provide some molecular parameters, and they can also be used to compare possible molecular models by testing their ability to "predict" the observed experimental scattering curve. When these techniques were used, the radii of gyration obtained for CF_1 and ECF_1 were 42.3 and 41.98 Å. If the molecules are assumed to be spherical, these values correspond to molecular radii of 55.20 and 54.50 Å, in good agreement with the Stokes radius obtained for CF_1 from gel filtration (59.0 Å) and from sedimentation velocity (57.3 Å) experiments. These radii are much larger than those that can be calculated for the dry protein based on the molecular weight and the partial specific volume (~48 Å), suggesting a high degree of hydration (~0.6 ml of water per gram of dry weight of protein). From small-angle X-ray diffraction data the maximum dimensions of CF_1 and ECF_1 were estimated to be approximately 120 Å. The detailed experimental diffraction curves were also used to test possible models for the shapes of CF_1 and ECF_1. Even though the "best" fit was described as based on very different models (Paradies *et al.*, 1978; Paradies, 1979), the experimental curves of both enzymes are very similar, suggesting that the molecules probably have very similar shapes. No simple geometrical figure gave an accurate representation of the diffraction data, indicating (not surprisingly) that the shape of the molecules (even at very low resolution) is more complicated than a sphere or an ellipsoid of revolution.

The δ and ϵ subunits (Schmidt and Paradies, 1977a, 1977b) of CF_1 were studied using the same techniques. The δ subunit (Schmidt and Paradies, 1977a) gave a radius of gyration of 21.80 Å, and the diffraction data seemed to fit that of an ellipsoid with axis $2a = 25$ Å, $2b = 28$ Å, and $2c = 90$ Å. If this description turns out to be correct, it could have important implications. The length of this model would be comparable to the dimensions of CF_1, suggesting that the δ subunits could span the whole F_1-ATPase molecule.

The ϵ subunit (Schmidt and Paradies, 1977b) was found to have a radius

of gyration of 11.80 Å, and the X-ray scattering curve was best explained by an ellipsoid of revolution with axis $2a = 2b = 25.4$ Å and $2c = 50.8$ Å.

Information about the structure of TF_1 was obtained using electron microscopy. TF_1 can be precipitated with ammonium sulfate with the formation of ordered two-dimensional arrays (Kagawa *et al.*, 1976). Micrographs of negatively stained samples showed a hexagonal array with a 90-Å spacing. The published optical diffraction diagrams show spots up to a resolution of approximately 30 Å (Wakabayashi *et al.*, 1977). The diagrams have only $p\bar{1}$ symmetry even though the first- and second-order reflections (45 Å resolution) appear to have hexagonal symmetry. These low-order reflections probably reflect more the symmetry of the packing than that of molecules. Digital image reconstruction was performed on these micrographs (Wakabayashi *et al.*, 1977). The images obtained seemed to indicate that the TF_1 molecules excluded stain from a circular area of approximately 90 Å in diameter. A small area at the center of the molecule seemed to be consistently stained more heavily than the rest of the molecule. The authors propose that the large subunits (α and β) can be correlated with some regions of the image. However, the poor crystallinity of the sample and the method used for the reconstruction (elliptical filtering holes) would caution against overinterpretation of these images.

F_1-ATPase from rat liver mitochondria has been crystallized by ammonium sulfate precipitation in buffers containing ATP and phosphate (Amzel and Pedersen, 1978, 1979). The crystals obtained are suitable for X-ray diffraction studies and they appear to diffract to 3.5 Å resolution. The crystals belong to the space group $R32$. The molecular weight of the asymmetric unit of the crystals was estimated using the cell dimensions, the measured density, and the partial specific volume of the protein (0.74 cm^3/g, calculated from the amino acid composition). A value of 190,000 daltons was obtained. Considering that the molecular weight of F_1 is around 384,000 daltons, the data suggest that rat liver F_1-ATPase has a molecular two-fold axis of symmetry; in other words, the molecules of F_1-ATPase behave as crystallographic dimers. The simplest but not the only way to interpret this information is to take it as indicating that F_1-ATPase has an even number of all five subunits. If such were the case, the only stoichiometry compatible with the molecular weights of F_1-ATPase and its subunits would be $\alpha_2\beta_2\gamma_2\delta_2\epsilon_2$. This stoichiometry is supported by other experimental data (*vide supra*) and should be considered as a plausible model. On the other hand, as we discussed previously, evidence supporting other stoichiometries have been reported (*vide supra*). However, the results of these reports and the crystallographic data need not be contradictory. The crystallographic evidence only indicates that the crystal can be described as being formed by masses of 190,000 daltons repeated through a diad and triplicated through a triad. Molecular

weight considerations indicate, therefore, that the molecule is a dimer. Amzel and Pedersen (1978) avoid suggesting a subunit stoichiometry based on these data because in a system of this complexity the obvious interpretation ($\alpha_1\beta_2\gamma_2\delta_2\epsilon_2$) need not necessarily be the correct one. At least two other interpretations can be suggested. The first is that some of the subunits or their combinations form domains that are structurally so similar to other subunits that the crystallographic dimer reflects these similarities. This will require that these quasi-identical domains take each other's place in the crystal such that the resulting effect would be some disorder but the preservation of the observed two-fold symmetry. Unlikely as it may seem, this has to be considered as a real possibility. The second possible explanation would be that all subunits behave crystallographically as dimers. Two such cases have recently been reported for crystals of α-mannosidase and α-amylase (Fitzgerald *et al.*, 1979). In these cases the polypeptide chains are internally perfect sequence repeats and they behave as almost perfect crystallographic dimers. In the case of F_1-ATPase, not all subunits need to behave as dimers since the fractional mass of the small subunits is so small their apparent symmetry could be due to orientational disorder around the two-fold axes. This disorder would only slightly affect the overall quality of the diffraction pattern. Disorders of this kind have previously been observed in protein crystals diffracting to 3.5 Å resolution (Matsushima *et al.*, 1977).

These different possibilities can probably be resolved experimentally even before the determination of the primary, tertiary, and quaternary structure of the molecule. For example, they predict different distributions and number of tryptic peptides. While the first possibility does not introduce any restrictions, the second will predict common peptides between different subunits, and the third would predict that each subunit will have approximately half the number of different peptides expected from the arginine and lysine content.

The overall dimensions of the F_1-ATPase molecules in the crystals were obtained from packing considerations. It was determined that the molecules can be described as ellipsoids of approximate maximum dimensions 90 × 90 × 120 Å (Amzel and Pedersen, unpublished results).

Cantley and Hammes (1975, 1976) have performed measurements of fluorescence energy transfer on CF_1 and obtained rather interesting estimates of the distances between several groups specifically bound to CF_1. However, the description and interpretation of the results are based on estimations of the number of binding sites of CF_1 for different compounds and their affinities. Because this subject is still quite controversial, we choose not to discuss it in this review. However, it should be noted that when the structure of F_1-ATPase is determined, the distances between putative binding sites can be obtained and compared to the values of energy transfer experiments.

Conclusions

F_1-ATPases are large complex multimeric enzymes. As isolated they are obtained by disrupting an even larger membrane-bound multisubunit complex. The determination of their structure is (as expected) fraught with difficulties. Even though structural information is accumulating steadily, most of the reported structural parameters still have to be considered tentative. Some of the outstanding questions can probably be answered with classical biochemical approaches. Determination of the primary structure (through protein or DNA sequencing) and of the tertiary and quaternary structures will eventually provide a satisfactory answer. With this information at hand the study of the mechanisms of ATP synthesis and ATP hydrolysis should proceed on a firmer basis.

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