THE PROTON CONDUCTING F₀-PART OF BACTERIAL ATP SYNTHASES

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Abbreviations: DCCD, N,N'-dicyclohexylcarbodiimide; PTH, phenylthiohydantoin; TID, 3 trifluoromethyl-m-(iodophenyl)diazirine; NCCD, N-(2,2,6,6-tetramethylpiperidyl-1-oxyl)-N'(cyclohexyl)carbodiimide.

I. Introduction

The membrane-integrated part F₀ of the ATP synthase translocates protons across the mem-
brane. In the whole ATP synthase (F}_{1}-F}_{0} the F}_{0}-mediated proton transport is tightly coupled to the synthesis or hydrolysis of ATP catalysed by the F}_{1}-ATPase part. By the removal of F}_{1}, the proton pathway in F}_{0} is opened, and can be studied independently. F}_{0} represents, therefore, an interesting and up to now unique system for analysis of (i) the structure and function of a proton conductor, and (ii) the coupling of the F}_{0}-mediated proton transport to the hydrolysis or synthesis of ATP occurring in the F}_{1}-ATPase part.

Several recent reviews covered the genetics of the ATP synthase [1,2], the nucleotide sequence of the ATP synthase genes of E. coli [Walker, J.E., Saraste, M. and Gay, N.J., unpublished results], the proton-ATPase from bacteria and mitochondrial [4], the structural basis of proton-translocating protein function [5], and the structure and genetics of the proteolipid subunit of the ATP synthase [6]. This review tries to collect new protein-chemical data on F}_{0} and to combine them with genetical and functional data in order to give the present-day knowledge on the structure-function relationships in this proton conductor. Although Saraste, M. and Gay, N.J., unpublished results, the structural basis of proton-translocating and up to now unique system for analysis of (i) the number of amino acid residues in the subunit polypeptide. Accordingly, both proton conductance and ATP-dependent proton translocation is restored. The data obtained with isolated F}_{0} part has been extensively evaluated by means of computer programs with respect to secondary structure predictions, polarity profiles and homologies with corresponding subunits from other organisms [3,4,28,29]. A summary of such evaluation is also shown in Figs. 12, 13 and 15 in the last section of this review.

The isolated F}_{0} part can be reinserted into artificial membranes, and proton conductance can be measured in response to a valinomycin-induced potassium diffusion potential [9-11] (Table I). The proton conductance is inhibited strongly by DCCD and by added F}_{1}, and, thereby, ATP-dependent proton translocation is restored. The data obtained with isolated F}_{0} are compiled in Table I together with corresponding data measured with F}_{0} in whole membranes. Accordingly, both proton conductance and functional rebinding of F}_{1} can be used to analyse F}_{0}.

The nucleotide sequence of the genes of all three F}_{0} subunits of E. coli was determined independently by three groups in 1981 [15,17,21]. Amino-terminal amino acid sequences of the isolated subunits a, b, and c were established [17] in order to prove the start of the individual subunit genes. The derived amino acid sequences of the three F}_{0} subunits are presented in Fig. 2. They have been extensively evaluated by means of computer programs with respect to secondary structure predictions, polarity profiles and homologies with corresponding subunits from other organisms [3,4,28,29]. A summary of such evaluation is also shown in Figs. 12, 13 and 15 in the last section of this review.

The established subunit composition and covalent structure of the E. coli F}_{0} reflect a major achievement in the characterization of the ATP synthase proton channel. However, for the elucidation of amino acid sequences of the isolated subunits a, b, and c was established [17] in order to prove the start of the individual subunit genes. The derived amino acid sequences of the three F}_{0} subunits are presented in Fig. 2. They have been extensively evaluated by means of computer programs with respect to secondary structure predictions, polarity profiles and homologies with corresponding subunits from other organisms [3,4,28,29]. A summary of such evaluation is also shown in Figs. 12, 13 and 15 in the last section of this review.

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functional amino acid residues and domains constituting the proton pathway and participating in the coupling of $F_0$ and $F_1$ functions.

A high resolution of the three-dimensional structure of $F_0$ will be achieved finally only by X-ray analysis after crystallization of the protein. With other integral membrane proteins, two-dimensional crystals [30-34] and, more recently, also three-dimensional crystals [35-37] have been produced. Crystals of the $F_1$ protein have not yet been obtained. Anyway, X-ray analysis of complex procrystallization of the protein.

the coupling of $F_0$ and $F_1$ functions.

access either only from the polar water phase or antibodies are valuable tools, not only for identification of domains in the membrane. The surface of $F_0$ accessible from the hydrophobic lipid phase has been studied with photoreactive membrane-permeating probes. Especially, carbene labels of broad specificity offer promising possibilities, since they should act like a razor blade and modify all accessible residues at the protein/lipid interface. Not only would this seeondary structures of the polypeptide chain be recovered from the analysis of $F_0$ mutants. Amino acid mutants might be involved in the binding of the inhibitors, and might therefore be located in proximity to each other at the surface of the membrane. Subunits from assembly-deficient mutants might be altered in residues involved in intermolecular or intramolecular protein contacts.

Several approaches were applied to identify the functional amino acid residues and domains in the $F_0$. Due to the ubiquitous occurrence of $F_0$, it is feasible to compare the amino acid sequences of $F_0$ subunits from very distantly related organisms. Based on the assumption that the mechanism of proton conductance remained unchanged during evolution, it can be expected that the group of invariant amino acids also comprises the functional residues.

Chemical modification may lead to the identification of functional residues, provided that the reaction of a defined residue can be correlated with an inhibition of $F_0$ functions.

Molecular genetics and genetic engineering offer further promising possibilities to study functional properties of $F_0$. Especially in E. coli, but also in yeast, a variety of mutants which contain a defective $F_0$ have been isolated. In some of these strains, $F_0$ appears to be normally assembled, and, therefore, functional residues might have been modified by the mutation. The altered residues might be identified by protein or DNA sequence analysis. Furthermore, the function of the different $F_0$ subunits has been studied by constructing strains where one or two of the $F_0$ subunits are missing.

III. Topographical studies

I1A. Studies with proteinases

The asymmetry of membrane proteins has been investigated in many instances by proteinase treatment of whole membranes and analysis of the cleavage product [38-41]. The sidedness of the C- and N-terminal segments as well as the location of some internal polar loops have been determined, for example, in the bacteriorhodopsin, by treatment of the purple membrane with papain, trypsin and chymotrypsin [42-44]. These studies, together with crystallographic data [30,31] and the primary structure [43,45], provided information on the folding and orientation of this polypeptide in the membrane [46,47].

First experiments on proteinase treatment of isolated $F_0$ from PS-3 were described by Sone et al. [48]. An $F_0$ subunit of $M_r$ 13,000 was readily digested with trypsin and nase, but the proton permeability of the $F_0$ was not affected by proteinases. $F_0$ binding and ATP-dependent $H^+$ translocation were lost after digestion, indicating a binding activity of the extramembranous part of the 13.5 kDa protein. Corresponding studies on the proteinase sensitivity of the $F_0$-subunits from E. coli [49] gave similar results. Experiments were carried out using $F_0$-depleted everted membrane vesicles from strain CM 2786, which overproduces ATP synthase about 5-fold (see also sections IA and B). Thus, the three $F_0$-subunits could be easily identified in whole membranes by SDS-polyacrylamide gel electrophoresis in combination with a silver stain method (Fig. 3). Subunit $c$ was not or only slightly affected by subunit $a$ to products with a $M_r$ of less than 15,000. The appearance of the polypeptide with a $M_r$ of 22,000 at higher subunit concentrations was thus indicative for a cleavage product of subunit $a$. Possibly, the quite polar N-terminal segment was attacked by the proteinase and, thus, might be located at the cytoplasmic side of the membrane.

Subunit $c$ was not or only slightly affected by any of the proteinase treatments in incubation times and high concentrations of proteinase did not lead to any extensive degradation (incubation time 5 days, at a ratio of proteinase to membrane protein of 1:5) [49]. Since subunit $c$, and especially the polar domain, is very resistant to proteolysis even in the denatured state, this result does not necessarily imply that the functional protein is completely inaccessible to proteinases.

![Fig. 3. Proteinolytic treatment of $F_0$ subunits in $F_1$-depleted membranes. $F_1$-depleted membranes of E. coli were prepared from an ATP synthase-overproducing strain [49]. Lane A shows separated $F_0$, $F_1$, and lane B, separated $F_0$-depleted membranes. The proteinase concentrations, increase in the samples from left to right for V8 (lanes 1-3), chymotrypsin (4-6), trypsin (7-9) and subtilisin (10-12). The star indicates the digestion product obtained with chymotrypsin. The arrow indicates the possible cleavage product from subunit $a$.](image)
III.B. Studies with antibodies

Lo and Bragg studied the effect of antiserum raised against subunit c on proton conductance and F1 binding in everted and right-side-out membrane vesicles of E. coli [51]. Antibodies added to everted membrane vesicles inhibited the proton conductance catalyzed by F0. There was no effect when these antibodies were added to right-side-out vesicles.

At present, the antigenic site(s) of subunit c are not determined. Computer analysis of the primary structure led to the prediction that the antigenic sites are located at the hydrophilic N-terminus and the hydrophobic segment in the middle of the polypeptide chain [52]. In principle, it should be possible to identify the accessible fragments of subunit c employing antibodies against defined peptide fragments.

III.C. Surface label

In recent years, the chemistry of photoreactive labels has rapidly advanced [for review see Refs. 53–56]. One type of these reagents is lipophilic compounds, which distribute in the membrane and upon illumination form a highly reactive nitrene or carbene group. Ideally, such a group should react unspecifically with a broad range of amino acid side-chains. This requires that the half-life of the reactive intermediate be shorter than its rotation correlation time [55]. Only if labelling occurs randomly will all amino acid residues at the protein/lipid interface be detected, thus allowing a detailed mapping of the protein surface. So far, none of the available photoreactive probes has been found to have an equal reactivity for all side-chains. Two types of hydrophobic label have been devised. The first group comprises phospholipid derivatives, bearing a phenylazidoprecursor, a phenylazidocyanine [62–67], or a trifluoromethylphenylisocyanide [68]. In these compounds, the photoreactive group is fixed and is expected to cross-link with defined regions of the protein. The ‘shallow label’, shown in Fig. 4, will only label residues which are in proximity to the headgroup of the lipids. There are a few examples with phospholipid analogues where the attached label has been traced back to individual amino acids. The ‘shallow label’ has been used by Bisson et al. [69] to label the membrane surface of the cytochrome c oxidase. The label has been traced back to amino acid residues, marking the beginning of one stretch of hydrophobic residues in subunit II of the complex. Ross et al. [70] described the labelling of the transmembrane domain of glycoporphin A with phospholipids, containing carbene precursors (aryldiazirines). They found that Glu-70 was the predominant site of labelling, with Cys-71 and Ser-72 receiving a label, and possibly Cys-74.

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**Fig. 4. Structure of the hydrophobic photoreactive labels used for the labelling of F0, INA, iodonaphthylazide.**

modified residues has not been reported so far.

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The identification of the nitrene or carbene precursor and the ‘shallow probe’ exhibited a high selectivity, both on the level of whole subunits and on the level of individual amino acid residues.

In the functional enzyme, solubilized in the detergent Aminoxid WS 35, (125I)iodonaphthylazide almost exclusively labelled subunit b, but not the other two hydrophobic subunits α and c (Fig. 5). With the subunits dissociated in sodium dodecyl sulfate, all subunits including the F0 subunits

were labelled with the exception of subunit c. Similarly, the ‘shallow probe’, also a precursor of a nitrene, reacted only with subunit b in the F1F0 complex reconstituted into proteoliposomes. Modified amino acid residues were identified by isolation of labelled peptides and amino acid sequencing. Both probes reacted only with the hydrophobic N-terminal segment of subunit b. The distribution of radioactive label among the sequenced amino acid residues is shown in Fig. 6. (125I)iodonaphthylazide labelled almost exclusively Cys-21. Also the ‘shallow probe’ reacted predominantly with this residue. In addition, Trp-26 which is close to Cys-21, received a label, and possibly the first three residues including Asp3 at the N-terminus were attacked. But with the latter, a clear interpretation was not possible due to the high radioactive background in the first step of Edman degradation. In conclusion, nitrene-generating hydrophobic precursors seem to be insufficient for a detailed map of the surface of membrane domains of proteins which are mainly composed of aliphatic residues.

The insufficiency may result from the rather long lifetime of the nitrene. Furthermore, aryl-nitrenes may undergo intramolecular rearrangements leading to long-lived electrophilic species [74]. Cysteines, as the most potent nucleophiles
among the amino acid residues, may act as a sink for such intermediates. The situation appears to be slightly better with the phospholipid, where diffusion of the reactive species is restricted.

Carbenes are much more reactive than nitrenes. They even insert into CH bonds of the aliphatic chain of phospholipids [67]. The aryldiazirines represent one type of carbene precursor. As a side-reaction of photolysis, these compounds undergo a rearrangement to the corresponding diazoisomer, which specifically will esterify carboxy groups. The problems with this type of precursor have been discussed recently [70].

To circumvent this problem, trifluoromethyldiazirines have been introduced by Brunner [68]. The undesired diazoisomer is stabilized by the strong electron-withdrawing trifluoromethyl group, and esterification by the diazoisomer is thus negligible. Fig. 5 C and D shows that all three F₀ subunits in the isolated complex F₁F₀ are labelled by the carbene, roughly according to their protein mass. In F₁-depleted membranes, the same labelling pattern of subunits a and b is observed, the labelling of subunit c is obscured by the large amount of labelled phospholipids.

Labelling patterns were recorded with (i) isolated ATP synthase (F₁F₀), (ii) F₁-depleted membranes from strain CM 2786 which overproduces the ATP synthase, and (iii) subunits denatured in a 2% SDS-solution. [125I]TID labelled subunits b and c could be isolated and the modified residues could be determined. Sequence analysis was performed by automated solid phase Edman degradation. Released PTH amino acids were identified by thin-layer chromatography and associated [125I]-radioactivity was measured as shown in Fig. 7A-C. The reaction product with [125I]TID was further analyzed by autoradiography of the thin-layer plates. This allowed the identification of labelled amino acid residues even over a high background and an overlap from the preceding amino acid residue.

Proteinchemical analyses of TID-labelled subunit b by CNBr cleavage showed that more than 98% of the label was attached to the N-terminal segment. Fig. 7A, B depict the labelling of individual amino acid residues after reaction of [125I]TID with the isolated complex F₁F₀ or F₁-depleted membranes. In the N-terminus of subunit b, again Cys-21 received the bulk label. But other amino acids are significantly labelled. Most interestingly, Leu-3, Ile-7, Leu-8, Trp-26 and all residues from Phe-14 to Cys-21 were labelled as confirmed by thin-layer chromatography. A very similar labelling pattern of the N-terminal segment was obtained after reaction of [125I]TID with isolated subunit b in SDS-solution (Fig. 7C). Thus, most of the amino acid residues of the membrane-integrated N-terminus of subunit b are freely accessible from the lipid phase.

After reaction of [125I]TID with a functional F₀ (F₁-depleted or F₁F₀), only a few residues of subunit c were attacked (Fig. 8A–B) in contrast to the multiple labelling in the N-terminal segment of subunit b. Labelling is restricted to four to six residues in the N-terminal segment and to five to six residues in the C-terminal segment. No labelling occurred in the middle of the polypeptide chain from positions 20–52, despite the fact that the sequence from residues 20–33 is highly enriched in hydrophobic residues, and thus is a possible candidate for a membrane-embedded segment. The labelling pattern changes dramatically if [125I]TID is reacted with the SDS-solubilized isolated subunit c (Fig. 8C). Labelled residues are distributed over the whole length of the polypeptide, including the hydrophobic segment 20–30, which was not labelled in the functional subunit c. Apparently, these residues are shielded by other protein segments in functionally assembled subunit c.

In the functional subunit c, the [125I]TID modified residue at the N- and C-terminals exhibit a remarkable repeat. They occur at positions 4, 8, 10, 11, 15, 19 and 53, 57, 65, 73, 76 [74]. Such n + 3 or n + 4 patterns are typical for residues located on the same side of an α-helix.

IIID. Cross-linking experiments

The subunit contacts in the E. coli F₀ were studied with the isolated complex F₁F₀ by cross-linking experiments. Fluoromethylazidophenyl was used, which in the first step binds by a nucleophilic displacement reaction to the side-chains of cysteine, lysine, tyrosine or histidine. Subsequently,
the azido group can be converted into a reactive group, since the accuracy in the estimation of α-sheet content in the presence of a high fraction of α-helix is quite low. Therefore, the α-helical fraction seemed to be almost unaffected by the different environments. In the subunit c from E. coli, however, there was a more significant decrease in the α-helical content, especially in the length of the α-helical segments upon transition from organic solvents to the lipid phase. The major difference between the two sets of data was the calculated content of β-sheet. For the N. crassa protein incorporated into lipid vesicles, no β-sheet was calculated. In contrast to the E. coli subunit c, about 20% β-sheet was reported. It is questionable whether these differences are significant, since the accuracy in the estimation of β-sheet content in the presence of a high fraction of α-helix is quite low.

Most remarkably, the α-helical content in the large cyanogen bromide fragment from E. coli subunit c was considerably reduced. This suggests that non-helical segments of the protein reside in this fragment, which contains the conserved hydrophilic stretch, as well as the cluster of conserved glycine residues. It is uncertain at this time in how far the isolated proteolipid retains the secondary structure elements of the functional F1-F0-integrated protein. It has to be noted, however, that the secondary structure derived from CD measurements roughly compares with second structure predictions [6,77–80].

### IV. Genes of \( F_0 \)

#### IV.A. General aspects

The arrangement of the genes coding for the subunits of \( F_0 \) and \( F_1 \) is completely different for the enzyme from the plasma membrane of E. coli and from mitochondria or chloroplasts of eukaryotic cells. As described in section II (Fig. 1) and reviewed in detail elsewhere, the E. coli \( F_0 \) subunits and the sequence has been determined [81–84]. In yeast, subunit c (proteolipid subunit 9) is encoded on mitochondrial DNA [85], in other organisms in the nucleus [82–86]. The other genes have not been located, so far. With one possible exception they appear to be localized in the nucleus, as deduced from biosynthetic studies [89]. The chloroplastic enzyme is coded for to a large part on chloroplast DNA. Genes for \( F_1 \) subunits \( a, b, \gamma \) and \( \epsilon \), and for \( F_0 \) subunits \( a \) and \( c \) have been localized [87] in the organelle, and for some of them the sequence has been determined [90,91]. From biosynthetic studies, it can be concluded that only the genes for the \( F_1 \) subunit \( \delta \) and \( F_0 \) subunit \( b \) are located in the nucleus [92].

Mutants defective in ATP synthase can be detected only in facultative anaerobic organisms which in the absence of the ATP synthase, survive by substrate-level phosphorylation. For all the \( F_0 \) genes of the \( atp \) operon in E. coli mutations are known which result in a defective ATP synthase. This provides an excellent proof that the eight genes code for vital subunits of the enzyme. The genes have been designated \( unc \) A to \( unc \) H or \( atp \) A to \( atp \) H by different laboratories. In addition, inhibitor-resistant strains of E. coli are known which all are affected in the \( atp \) E (\( unc \) E) gene, coding for subunit c. In yeast, the mitochondrial...
genes encoding subunit a and c are covered by mutations leading to defective ATP synthase (Pho I, Pho II), or resistance towards inhibitors (Oli I, Oli II, Oli III, Oli IV) [93–96]. The corresponding nuclear subunit c gene in N. crassa can also be mutated to an allele leading to oligomycin resistance [97].

In E. coli, plasmids and phages have been isolated containing all F_{o}F_{1} subunit genes, i.e., the whole atp operon [98–101]. This allows, for example, the construction of strains where the level of F_{o}F_{1} in the membrane is increased several-fold [48]. The membranes of such overproducing strains offer advantages for structural studies. Furthermore, plasmids are now available containing only segments of the atp operon [101,102]. Thus, a subset of subunits or in certain cases even a single subunit can be expressed at an increased level in the cell (Fig. 10). The latter plasmids are useful, especially when introduced into mutant strains where large parts of the atp operon are deleted or not expressed.

In principle, a mutation in a subunit gene can affect the properties and function of F_{o} at several levels. (i) The mutation may prevent the biosynthesis of a subunit or its integration into the membrane. (ii) A mutation may affect the assembly of F_{o}, even if the mutated subunit is integrated into the membrane. (iii) A mutation may affect the function of F_{o}, even if the altered subunit is assembled into F_{o}.

In the following, mutant strains will be described, where both the genotypes and the phenotypic alterations have been studied in some detail.

**IVB. Deletion mutants and plasmids**

The deletion mutants of E. coli, compiled in Fig. 10, allow the study of membranes in which selected F_{o} subunits are missing. Strain CM 2080, characterized in some detail, has a deletion of about 500 basepairs at the start of the subunit a gene [102]. Membranes of this deletion mutant contain F_{o} subunits b and c, but only if isolated from exponentially growing cells. If membranes are prepared from mutant cells in the stationary growth phase, only subunit c is detected. Most likely, subunit b is proteolytically degraded in stationary phase cells [49]. As described in subsection IIIA, the large polar domain of subunit b is easily attacked by proteinases in vitro [49]. Another deletion mutant CM 1470 has a large deletion including genes of subunits a, c, b, d, and part of a [13]. The membrane of this strain is devoid of all F_{o} subunits.

In several mutants, a Mu phage has been inserted at various points of the atp operon. The inserted phage DNA prevents the efficient transcription of all genes located downstream. These polar mutations, therefore, result in membranes which are devoid of certain subunits. One example is the strain AS 12 (Table IV) [103,104], where the Mu phage is inserted in the atp a gene coding for F_{o} subunit a. Accordingly, F_{o} subunits a, γ, β, and c are not expressed. In addition, F_{o} subunit b is not present in the membrane. Possibly, the defect in F_{o} prevents the assembly of subunit b or facilitates a proteolytic degradation.

Finally, several segments of the atp operon have been cloned on plasmids. One plasmid, pOM11, contains the intact coding sequence for subunit a [13]. A functional subunit a is expressed from the cloned gene, since it complements the deletion of the subunit a gene in strain CM 2080. Another plasmid, pRPG 51 [101], contains the sequences coding for subunits b and δ. The cloned genes are expressed. The plasmids have been introduced into the deletion mutant CM 1470, where all F_{o} genes are missing [13]. The strains obtained contain in the membrane either only F_{o} subunit a or only subunit b.

**TABLE III**

<table>
<thead>
<tr>
<th>Strain</th>
<th>F_{o} subunits</th>
<th>H^{+} conduct.</th>
<th>F_{1} binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>a b c + +</td>
<td>+</td>
<td>F_{1}</td>
</tr>
<tr>
<td>CM 10/6</td>
<td>a b c +</td>
<td>+</td>
<td>F_{1}</td>
</tr>
<tr>
<td>AM 12</td>
<td>a c + +</td>
<td>+</td>
<td>F_{1}</td>
</tr>
<tr>
<td>CM 1470+pOM 11-1</td>
<td>a +</td>
<td>+</td>
<td>F_{1}</td>
</tr>
<tr>
<td>CM 1470</td>
<td>a + + +</td>
<td>+</td>
<td>F_{1}</td>
</tr>
</tbody>
</table>

The membranes of all these strains, in which single or multiple F_{o} subunits are genetically deleted, have been analysed for their capability to rebind F_{1} and to conduct H^{+} [102] (Table III). Also included in these studies was a mutant membrane which is devoid of subunit c due to a point mutation in the atp E (unc E) gene (see below).

The results are summarized in Table III. The membranes of these strains comprise all possible subsets of F_{o} subunits. None of the F_{o} subunits alone and none of the combinations of two F_{o} subunits allows a H^{+} conductance across the membrane. Subunit a, as well as b, each present individually in the membrane, promote the binding of F_{1}, while subunit c alone does not. Accordingly, each pairwise combination of two F_{o} subunits allows a reactivation of F_{1} to the membrane. The observed physical binding of F_{1} to incompletely F_{o} must be different from the binding to intact F_{o}.

The rebound F_{1}, ATPase is active, although the proton conductance is abolished in the incomplete F_{o}.

**IVC. Point mutations**

IVC-1. Mutants with defective F_{o}

All mutant strains compiled in Table IV reverts...
readily, and consequently originated from single point mutations [12,105-112]. The mutational
events in genes for subunit a and b have not yet been identified. Several amino acid substitution in
the subunit c gene have been determined by amino
acid sequence analysis of the mutated gene [107-111]. Remarkably, most of the subunit c
mutations are dominant over the wild-type allele [106,113]. A few subunit c mutations, and all
analysed mutations in genes for subunit a and b, are recessive [12]. The dominant character of many
subunit c mutations is explained most probably by
negative complementation [113]. F6 contains 6-10
subunit c polypeptides which function in an inter-
dependent way. Thus, in a diploid situation a
mutated subunit c, which is still assembled into F6,
will inactivate most of the available wild type
subunit c. Accordingly, such dominant mutations in
subunit c do not interfere with the assembly
process, whereas the recessive mutations ap-
parently are defective in assembly. Negative com-
plementation should occur either not at all or to a
less extent with mutations in genes for subunits a
and b, which exist in F6 as single or dimeric
copies, respectively.

In some of the compiled mutant strains, the
affected subunit is not present in the membrane.
No direct information is available on the subunit a
mutant. This extremely hydrophobic protein could
not be analyzed by two-dimensional gel electro-
phoresis, and antibodies are not yet available.
Effects in subunit b mutants are difficult to inter-
pret at the moment. The membrane of ATP F
mutant DG 25/9 contains subunit b, as de-
termined by immunological techniques (Friedl, P.,
unpublished data). This mutant subunit c is integrated
into the membrane. The mutation is recessive, thus
the mutant subunit c is apparently not assembled
into F6 (see above). In another mutant, the subunit c,
exhibiting an exchange Pro-64 by leucine is present
in the membrane [2]. The mutant allele shows a partial dominance over the wild-type allele.
This suggests that this mutation does allow the assembly into F6 [106,111].

Mutants DG 7/1 and DG 18/3 are both af-
fected in the invariant DCCD-reactive aspartic
residue 61 [107-110]. These mutations are domi-
ant, and both mutant proteolipids are present in
the membrane [113]. Thus, these alterations allow
an assembly of the mutant protein into F6.

The membranes of all F6 mutants compiled in
Table IV are tight for protons with the exception of
unc F mutant DG 25/9. Thus, these mutations inter-
ference with the H+ conduction catalysed by F6.
This applies for mutants where the assembly of F6
is defective. The phenotype of these point mutants
Corresponds to that of deletion mutants, which
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table IV are tight for protons with the exception of
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This applies for mutants where the assembly of F6
is defective. The phenotype of these point mutants
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have been discussed in detail above (Table III).

But it applies also to those mutants where the
mutant subunit apparently is assembled into F6.
Of particular interest is the question of whether
the binding of the F6-ATPase is influenced by
mutations in F6 subunits. The binding affinity of
F6, N. crassa revealed that two proteins may be involved in the inhibitor binding. Besides the subunit a (proteo-
lipid) [97], in yeast subunit 6, a 259-residue protein
has been found to determine oligomycin sensitivity
of the ATP synthase complex [81] (Fig. 12). This
protein is homologous to subunit a of E. coli
ATP synthase. Interestingly, the mutations in sub-
unit 6 are located close to the small conserved
region of the protein.

Four different amino acid exchanges leading to
oligomycin resistance have been described for the
yeast and three for N. crassa (proteolipid) [97,116].
All amino acid exchanges reside in the C-terminal
hydrophobic stretch and are clustered around the
invariant DCCD reactive glutamic (aspartic) re-
sidue. It is a distinct possibility, that the amino
acid residues altered in the resistant mutants are
involved in the binding of oligomycin. Thus, these
residues might be located at the lipid/protein in-
terphase. Some of them result in a hypersensitivity
towards DCCD. Both facts suggest that the bind-
ing sites for DCCD and oligomycin are overlap-
ning.

V. Functional aspects

VA. Evolutionary aspects

In recent years, numerous proteolipid subunits from
various organisms have been sequenced, as
described elsewhere [6]. The proteins contain
70-82 residues and all may be purified by
chloroform/methanol extraction. They are ex-
tremely hydrophobic, containing only 16-25%

Long hydrophobic stretches of about 25 amino
acid residues are found both in the N-terminal
as well as in the C-terminal part of the sequence.
This is reminiscent of the most thoroughly investigated
membrane protein, the bacteriorhodopsin from
Halobacterium halobium, where stretches of 25 hy-
drophobic amino acids are found to traverse the
lipid bilayer most probably in an α-helical confor-
mation (Fig. 11). A comparison of the sequences of the homolo-
gous proteolipid subunits revealed numerous con-
served amino acid residues. These residues might
be important for the maintenance of a certain structure of the protein.
residues are conserved (Gly-27, Gly-29, Gly-31, and Gly-33). Gly-42, Arg-45, Asn/Qln-46 and Pro-47 localized in the middle hydrophilic segment were also found to be invariant. In this segment the single conserved basic amino acid residue (Arg-45) occurs. The single invariant acidic amino acid residue is observed in the middle of the C-terminal hydrophobic stretch (position 65). With one exception (aspartic acid in *E. coli*), this amino acid is a glutamic acid. This acidic residue is flanked by two conserved alanine residues (positions 62 and 66). At the C-terminus (position 80), a conserved phenylalanine was found.

There seem to be some specifically conserved residues either for the mitochondrial type or for the bacterial type of proteolipid. Lys-14 is typical for the mitochondrial proteins plus *Rhodospirillum rubrum*. For the same family also the following positions are occupied by an identical isofunctional residue: Leu-16, Gly-17, Gly-19, Gly-24, Leu-29, Arg/Lys-50, Gly-60, Phe-61, Gly/Ala-68, Leu-69, Phe-70, Met/Val-73, Val-74 and Ala/Ser-75. For the bacterial family the following residues are invariant or isofunctional: Ala-16, Ala-17, Ala/Gly-18, Gly-22, Leu-23, Ala/Gly-24, Ala/Ser-25, Lys-38, Glu-41, Glu-48, Phe-58, Ile/Leu-59, Val/Met-61, Leu-63, Val-64, Pro-68 and Ile/Leu-70.

The two analyzed 'chloroplast' proteins resemble more the bacterial ones. It is noteworthy that the proteolipids from spinach and wheat chloroplasts have the same amino acid sequence [90,91].

These clear differences between the 'oligomycin-sensitive' proteolipids and the 'bacterial' proteolipids could indicate a different environment and different interactions of the protein in the assembled F$_{1}$F$_{0}$. Reports on the higher complexity in the subunit composition of F$_{0}$ in mitochondria support this view [4,123].

Amino acid sequences of proteins from other organisms homologous to subunit b from *E. coli* ATP synthase have not been reported so far.

For subunit a, homologous proteins have been sequenced. DNA sequences of the respective gene have been described for *S. cerevisiae*, human, bovine and mouse [81–84]. Conserved residues are indicated in Fig. 12.

### VB. Chemical modification

Group specific reagents are versatile tools for detecting essential residues in enzymes. Ideally, these reagents modify single amino residues.

The interpretation and validity of chemical modification studies thus heavily depend on the determination of the modified amino acid(s). Furthermore, direct correlation between the extent of the modification of a specific amino acid residue with function must be established.

The binding of [14C]DCCD to subunit c (proteolipid) in all analyzed organisms was rather specific and occurred at low concentration (1–10 nmol/mg membrane protein) [6,118]. Maximum inhibition was observed when only a fraction of the subunits was labelled, indicating a cooperative interaction of the subunits [26,27,118–123]. For the beef-heart ATP synthase, the existence of a dimer of two proteolipids binding [14C]DCCD with a different affinity [123,124] compared with the monomeric subunits has been demonstrated.

Sequence analyses revealed an acidic residue (glutamic acid, with the exception of *E. coli* where aspartic acid was found) as the target of DCCD. This residue is located in a homologous position in all ten analyzed proteolipids [110,125].

The hydrophobicity of DCCD indicates that the acidic residue should be located in the lipid bilayer. This observation was confirmed by the use of the paramagnetic carbodiimide NCCD (N-2,2,6,6-tetramethylpiperidyl-1-oxyl-N'-cydohexyl carbodiimide) [126]. Ascorbate, a water-soluble agent, did not reduce the signal-generating nitroxide group. From spin-spin interactions between the NCCD molecules bound to the protein, it was suggested that the proteins exist in a polymeric form, in which some of the monomers are located at a maximal distance of 15–20 Å from each other. Bound NCCD was firmly immobilized as rotation correlation of 3–4 μs where determined. Reconstitution of the purified protein yielded a high mobility of 2–3 ns. Apparently, in this experiment the polymeric structure was not reconstituted.

The high immobilization of NCCD in native membrane may be caused by a rather tight fitting of the DCCD into a 'binding site' at the proteo-
lipid subunit. To obtain further information on the steric requirements, a small set of DCCD analogues was synthesized (Table V) (Hoppe, J., unpublished data). Apparently, it is difficult to assign the different effect of these reagents exclusively to the size of the ligand, as the reactivity of the carbodiimide might be different. But, interestingly, the grade of substitution at the carbon atom was not an inhibitor. Further hints for a tight fitting of the shape of DCCD to the surface of the proteolipid came from the analyses of DCCD-resistant mutants from E. coli [115].

There are data on the modification of tyrosine with tetratrimethylethandio and iodine and arginines with phenylglyoxal in a T4F preparation from PS-3 [127,128]. Modification of either of these residues inhibited the proton conductance. Though modification of these residues has been demonstrated in the proteolipid, it is not possible to correlate the modification of these residues with the inhibition of functions, since the fate of corresponding residues in the other membrane subunits has not been studied.

VC. H+ conductance in reconstituted subunits

So far, reconstitution experiments with isolated subunits have only been performed with the proteolipid subunit (subunit c). For the proteolipids extracted with butanol from lettuce chloroplasts or yeast mitochondria, a proton permeability of reconstituted lipid-proteolipid bilayers has been demonstrated [129–132].

Recently, single-channel conductance has been measured after reconstitution of the proteolipid from yeast into planar lipid bilayers [133]. Thus, in contrast to earlier studies, proton conductivities could be measured at a high-time resolution as a function of voltage, protein concentration, H+ concentration and lipid composition. At the high H+ concentration of about 10 mM (corresponding to a pH of 2.2), single-channel conductivity has been established.

Discrete current steps were measured, indicating the opening and closing of single channels. The observed ion path represents the operation of a single type of pathway with a mean conductance of 40 ps and a lifetime of 4 ms. The single channels operate independently, since their occurrence obeys a Poisson distribution. About 105 protons pass each channel per s at 100 mV potential and pH 2.2. At pH 7 and 100 mV, about 100 protons/s are transferred through the channel. The channel was highly selective for protons as the conductivity for potassium, sodium and chloride ions was at least 1000-fold lower.

Interestingly, most of the proteolipid molecules were not in an active state, explaining the low overall activity measured in previous systems [129–132]. The dependence of proton conductance on proteolipid concentration at pH 2.2 had a molecularity of about 2. The formation of channels from a large pool of proteolipid molecules thus involved a bimolecular reaction. In a most simple assumption, the associating species might be the monomeric proteolipid. In principle, however, the reacting species might already be a dimer or trimer.

Between the bimolecular dependence of the proton conductance of the proteolipid concentration at pH 2.2 and the linear dependence at pH 5.5, there was a transition point at pH 4.5 where the molecularity were much larger than 2. This suggests that the stabilization of the proton pathway at higher pH values involves the formation of structures larger than dimers. These associates were considered as oligomimered dimers.

But it could not be excluded that the increased conductivity at higher pH results from an increased proton permeability of a single channel.

In the reported experiments, the pH induced conductance transition was only found in the presence of cholesterol and sufficiently high proteolipid concentrations. The interaction between proteolipid subunits therefore may not be sufficiently strong to stabilize proton-conducting proteolipid oligomers in a biological membrane.

VI. Summary and Models

If the sequence of 271 amino acid residues of subunit a from E. coli is analyzed, seven segments can be discriminated where predominantly lipophilic residues are clustered (Fig. 12). In segments 1, 3, 4, 6 and 7, the hydrophobic character is most pronounced. With the possible exception of segment 1, no charged residues are present. The hydrophobic character of segments 2 and 5 is less pronounced. Charged residues occur just in the middle of the segments. The hydrophobic profile of subunit a is similar to the respective profiles of the mitochondrial proteins [3] if a deletion from residue 125 to residue 150 in the E. coli protein is assumed. It is noteworthy that the homology between the E. coli and the mitochondrial subunits is restricted to a short segment near the C-terminus corresponding to residues 189–219 of subunit a from E. coli, whereas the rest of the polypeptide chains are completely unrelated. The similarity in the polarity profile suggests, however, that the general folding of the subunit has been conserved.

The length of subunit a as well as the occurrence of six or perhaps seven lipophilic segments are reminiscent to the amino acid sequence of bacteriorhodopsin. It has to be mentioned that in bacteriorhodopsin, segments 3, 4, 6, 7 contain several basic and acidic residues and exhibit only a weak hydrophobicity [43,46]. Thus, in the F0 subunit a, segment 2, despite its relatively high polarity, might traverse the membrane. In yeast subunit 6, two amino acid substitutions leading to oligomycin resistance have been identified [81].

The mutated residues would correspond to positions 195 and 256 in subunit a of E. coli, located in the middle of the hydrophobic segments 5 and 7. These residues might be directly involved in the binding of oligomycin, and thus be located in proximity to each other at the lipid phase. But this straightforward conclusion is not possible if these mutated residues affect allosterically the oligomycin binding. This alternative will be discussed in detail with subunit c.

Subunit a is heavily labelled by the membrane-soluble carbene-generating label [123]ITID [73] (cf. Fig. 5). Unfortunately, the modified amino acid residues could not yet be identified in this large hydrophobic protein. A rough quantitative evaluation of [123]ITID radioactivity bound to each of the three F0 subunits (see Fig. 5) indicates that subunit a is labelled twice as much as subunit b (Hoppe, J., Brunner, J. and Jørgensen, B.B. (1983), unpublished data). Accordingly, large parts of subunit a are accessible from the lipid phase.

About 20 amino acid residues of subunit a can be removed proteolytically from the cytoplasmic side with subtilisin (see Fig. 3) [49]. Since the C-terminus is very hydrophobic, it is most likely that the N-terminal end is susceptible to pro-
teinases, and thus located at the cytoplasmic surface [49]. Taken that seven transmembrane segments exist, this would indicate that the C-terminal end would be located on the periplasmic side. The proteolytic breakdown products of subunit $a$ are difficult to analyze, even with membranes from an ATP synthase overproducing strain, due to the presence of many additional proteins. But at raised concentrations of proteinases the amount intact of subunit $a$ in the membrane decreases, indicating that other parts of the polypeptide chain are accessible to proteinases. It is conceivable that polar segment domains of subunits exposed at the cytoplasmic surface of the membrane are involved in the binding of $F_1$, as was observed in mutant strains containing only this $F_0$ subunit.

**V1B. Subunit $b$**

The polarity profile of the sequence of 151 residues of subunit $b$ is striking in that about 30 hydrophobic amino acids are clustered at the N-terminus (Fig. 13), whereas the rest of the polypeptide chain is very polar similar to a water-soluble protein. This immediately suggests that the N-terminus of subunit $b$ is integrated in the membrane. In fact, all applied hydrophobic photoactive probes reacted exclusively with this hydrophobic segment (Refs. 71 and 72, see also Hoppe, J., Brunner, J. and Jorgensen, B.B. (1983), unpublished data).

Labelling with the freely mobile carbene-generating probe $^{[125]}$TID started very closely to the N-terminus at Leu-3 and ceased at Trp-26. With a nitrene-generating probe fixed to the polar headgroups of a phospholipid (‘shallow probe’). Fig. 14, residues Asn-2 as well as Cys-21 and Trp-26 were modified. Thus, the entire N-terminus up to Trp-26 is embedded in the membrane and the boundaries are defined by the residues affected by the ‘shallow probe’. Most likely, the N-terminal segment traverses the whole phospholipid bilayer in an $\alpha$-helical conformation [134,135]. But the other possibility, that the chain folds back and that Asn-2 and Trp-26 are located at the same boundary, is not excluded by the available experimental results [136]. At positions 27 and 28, two proline residues are located which will not fit into an $\alpha$-helix, and, consequently, their peptide bonds can generate a rather large hydrophilic surface area. It is thus reasonable that the polypeptide chain leaves the membrane at this point.

Surprisingly, most of the N-terminal residues were accessible to the small diffusible probe $^{[125]}$TID. This demonstrates that this segment is not buried in a core of $F_0$ but rather is located at the periphery. As two subunits $b$ exist in one $F_0$, it remains uncertain whether the observed $^{[125]}$TID labelling pattern represents the same modification of each of the subunits or the sum of two different labelling patterns. Several of the residues not attacked by $^{[125]}$TID have hydrogen-bonding capacity (Asn-2, Thr-6, Gln-10, Lys-23, Tyr-24). These residues might be involved in contacts with other subunits. But it could well be that a microenvironment created by these side-chains does not allow the accumulation of $^{[125]}$TID at these positions and thus prevents an efficient modification.

The large polar domain of subunit $b$ is clearly exposed at the cytoplasmic side of the membrane, since it can be completely degraded with proteinases [49]. The two molecules of subunit $b$ in $F_0$ can be efficiently cross-linked (see Fig. 9). They exist, therefore, as a dimer. Most likely, the contact is formed by the large polar domain. This large polar domain binds $F_1$ as demonstrated in mutant membrane where the other $F_0$ subunits $a$ and $c$ are deleted [102]. This is not the only contact site between $F_0$ and $F_1$. Since – as already mentioned – subunit $a$ also is involved in the binding of $F_1$ [101] in cross-linking experiment, dimers of subunit $b$ with $F_1$ subunits $a$ and $\beta$ are observed. A close interaction of the $F_0$ subunits $a$ and $\beta$ with subunit $b$ is also indicated by the observation that in certain mutant strains subunit $b$ is protected against proteolytic degradation in vivo by the $F_1$ subunits $\alpha$ and/or $\beta$, even in the absence of the subunits $\gamma$, $\delta$, and $k$ [49]. Interestingly enough, the polar domain of subunit $b$ contains internal repeats [29]. Residues 53–82 are clearly homologous with residues 85–105. Furthermore, a shorter repeat is found, residues 84–98 are homologous to residues 101–113 [29]. Thus it can be visualized that the subunit $b$ dimer contains four roughly equivalent domains which might interact with the multiple copies of subunits $\alpha$ and $\beta$ present in $F_1$. Point mutations in subunit $b$ (see Table III) [105,112] result in a weakened binding of $F_1$. Unfortunately, the mutated amino acid residue has not yet been identified.

**V1C. Subunit $c$**

The polarity profile of the amino acid sequence of the $E$. coli subunit $c$ (see Fig. 15), i.e., two hydrophobic segments interrupted by a hydrophilic segment, is found to be conserved in the homologous subunits from other bacteria, as well as from mitochondria and chloroplasts. This clustering of hydrophobic and hydrophilic residues immediately suggested that the protein might traverse the membrane twice in a hairpin-like structure. Indeed, labelling experiments with $^{[125]}$TID indicate both hydrophobic segments being located in the lipid bilayer. Especially intriguing is the location of the residue Asp-60 in the membrane, as demonstrated by its reactivity towards only hydrophobic carbodiimides. Remarkably, an acidic group is not tolerated in the N-terminal segment at position 23 of the $E$. coli protein, as seen with subunit $c$ from a mutant unc E 429, (see Table IV) which is not integrated into the membrane. Only distinct residues of the subunit $c$ are accessible to the label $^{[125]}$TID in functional $F_0$. In certain special cases, the nonlabelling by TID might be explained by a low reactivity of the side chain (e.g., alanine; see Hoppe, J., Brunner, J. and Jorgensen, B.B. (1983), unpublished data) or by the instability of the reaction product (e.g., aspartyl ester). But certainly, in most cases residues are not labelled, since they are buried in the interior of the quaternary structure of $F_0$. It may be relevant in this connection that a mutant proteolipid from $E$. coli, which is unable to assemble (see Table IV), has an amino acid substitution...
of Ala-21 by valine in a segment not accessible to TID.

Possibly, the small side-chain of the alanine at position 21 is part of a contact site in the tertiary structure of the proteolipid or the quarterly structure of the F0 might be different. It is conspicuous, however, that at the N-terminal segment the labelled residues would mark one side of a continuous a-helix. Also in the C-terminal part the labelled residues could be located on a-helical segments.

It has been discussed previously that certain residues of the proteolipid altered in oligomycin-resistant mutants from yeast and Neurospora are accessible from the lipid phase. It was therefore interesting to examine whether these residues correspond to the TID accessible residues in the E. coli proteolipid. Three amino acids altered in the proteolipid from inhibitor-resistant mutants occur at positions coinciding or juxtaposed to TID-labelled residues from the E. coli proteolipid (Fig. 16). In two instances, however, the mutated residue is located two positions apart.

Unfortunately, [125I]TID labelling experiments have not been performed with the mitochondrial proteolipids. Thus, it is uncertain whether corresponding positions in the bacterial or mitochondrial proteolipids are equally accessible from the lipid phase. It has to be stated, however, that in an a-helical confirmation, some mutated residues would point in opposite directions.

The various positions determining the oligomycin resistance all are located in the vicinity of the DCCD-reactive acidic residue in the C-terminal segment [97]. In contrast, only one position determining DCCD resistance in the E. coli proteolipid, i.e., Ile-28, was found in the N-terminal segment [115]. In a hairpin-like structure of the proteolipid, this residue would be located in proximity to the carbodiimide reactive aminoisobutyric residue. It is likely that Ile-28 is involved in noncovalent binding of e.g., the cyclohexyl moiety of DCCD. Surprisingly enough, Ile-28 is not accessible to TID in the functional F0. Differences in steric factors between TID and DCCD are difficult to evaluate, but are not a likely explanation for the different accessibility of Ile-28 for both compounds. It is a distinct possibility that either DCCD is bound via an 'induced fit' or that DCCD binds only to a certain conformation of the F0 occurring with a low probability.

The experimental data discussed above are relevant with respect to the structural properties of the proteolipid. In order to elucidate the mechanism of the proton conduction, it is also important to identify functional residues – i.e., residues directly involved in proton conductance. It has been suggested that Asp-61 in the E. coli proteolipid is such a functional residue. (All structural evidence indicates this residue being located in the interior of the membrane.) Its selective modification with DCCD abolishes proton conductance. The importance of this residue was further indicated by the observation that this acidic residue is strictly conserved in the proteolipids from mitochondria, bacterial and chloroplasts. Furthermore, proton conduction is lost if this residue is genetically altered.

Some observations, however, are difficult to reconcile with a purely functional role of this acidic residue. This residue is accessible from the lipid phase and is therefore most likely not located in the interior of a pore. A minimal genetic alteration of this residue (Asp → Asn) leads to impaired binding of F1, (see chapter IVC-1) and the F0 subunit b. Apparently, large conformational changes are induced in the whole F0 by this mutation.

VTD. Models for proton conductance

All available evidence points to the proteolipid subunit as the protonophoric entity of the F0. Several lines of evidence argue against a direct participation of subunits a and b in transmembrane protontranslocation. The large hydrophilic part of subunit b might be involved in the coupling between the proton translocation across the membrane and ATP synthesis/hydrolysis on the F1, but it is unlikely, that the short uncharged NH2-terminal segment, which has only close contact with the other subunits, provides residues participating in the mechanism of H+ conduction. If subunit a contains the proton pathway, then only the few conserved residues may be considered which are clustered in a short segment. For subunit c, Schindler and Nelson [133] provided convincing evidence that the isolated protein functions as a protonophore, when reconstituted in black lipid membranes. These in vitro experiments seem to contradict results obtained with mutant membranes from E. coli, which contain only the F0- subunit c and which are unable to conduct protons [102]. This discrepancy might be resolved by the observation of Schindler and Nelson that the active proton channel formed in black lipid membranes is at least a dimer and probably a higher oligomer of the subunit c. In vivo this functional oligomer might be stabilized by the F0 subunit a and/or b as originally proposed by Nelson and Schatz [92].

Therefore, at present any discussion on the mechanism of proton conductance will concentrate on a subunit c oligomer. However, based on the presently available structural data, the mechanism of proton conductance is completely obscure. The membrane spanning segments of subunit c contain only a few polar residues and only one charged residue – the invariant DCCD-reactive aspartic acid. Therefore, it appears difficult to construct a network of hydrogen bonds across the membrane from amino acid side-chains of Ala-21 by valine in a segment not accessible to TID.
However, the participation of this acide residue in the formation of a water cluster inside a pore is difficult to reconcile with its reactivity to DCCD.

The central question concerning all studies directed to structure and function is whether $F_0$ can exist in different conformations. In the F$_1$-ATPase, extensive conformational changes occur upon energization of the membrane and during ATP hydrolysis or synthesis [4]. It could well be that conformational changes in $F_1$ are accompanied or even coupled to conformational changes in $F_0$. Papadopoulos and co-workers [140] provided experimental evidence for two different conformation states of the proton conductor in dependency of the applied $\Delta \phi_m$, in beef-heart mitochondria. Also, the inhibitory action of oligomycin can be readily explained if this macroscopic conductance induces or stabilizes one structural state. The same inhibitory mechanism may apply for DCCD. Pleiotropic effects of amino acid substitutions in submit $e$ (see subsection IV) might be caused by allosteric interaction. A mechanism of proton translocation by a reorientating basic or acidic group has been developed by Boyer [141]. Obviously, such a mechanism requires conformational changes in the $F_0$. It should be possible with the techniques now available to define these putative conformational states more precisely and to correlate these states with the function of the enzyme.

VII Perspectives

ATP synthase and the proton conducting component $F_0$ from the bacterial PS-3 [48] and E. coli [9–11] have been purified only a few years ago. Since then, our understanding of structure and function of $F_0$ has rapidly advanced, as detailed in this review. For the studies on $F_0$, the experimental possibilities now available have not been fully exploited. The availability of isolated subunit $F_0$ gene will allow the construction of defined mutants by site-directed mutagenesis. Hopefully, mutants will be obtained which exhibit specific defects, e.g., only in proton conductance or only in $F_1$-binding. Furthermore, the assembly of $F_0$ might now be studied, and an in vivo reconstitution of $F_0$ seems to be feasible after recombination of the isolated subunit genes with appropriate expression vectors.

The topology of all three $F_0$-subunits might be elucidated in more detail by means of antibodies, directed against defined polar segments of the polypeptide chains. The amino acid sequences will furthermore give hint for possible chemical modification studies directed towards identification of functional residues or towards the accessibility of certain segments.

Conformational changes might be defined by membrane permeating photoactive probes and by the covalent attachment of reporter groups, i.e., spin label or fluorescence label. Finally, it might be possible to obtain $F_0$-crystals sufficient for high resolution X-ray analysis. Hopefully, the results obtained by proteinchemical, physicochemical and genetic methods then can be integrated into a consistent picture.

References

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