Journal of Bioenergetics and Biomembranes, Vol. 14, No. 1, 1982

SHORT REVIEW
Structure and Function of the Membrane-Integral Components of the Mitochondrial H^+-ATPase[1]

J. Houštěk,[2] J. Kopecký,[2] P. Svoboda,[2] and Z. Drahota[2]

Received May 20, 1981

Key Words: H^+ ATPase; mitochondria; DCCD; binding protein.

Introduction

The main function of the mitochondrial proton-translocating ATPase (H^+-ATPase) is to utilize the transmembrane electrochemical gradient of protons for the synthesis of ATP (Boyer *et al.*, 1977). H^+-ATPase is, therefore, one of the key enzymes of the bioenergetic machinery and one of the most remarkable ion pumps (Kagawa, 1978) studied so far.

This article deals with the membrane-bound moiety of H^+-ATPase, the structure and function of which have been intensively studied during the last years (see Kagawa, 1978; Pedersen *et al.*, 1978; Senior, 1979a; and Fillingame, 1980, for reviews). The main attention is paid to the mitochondrial enzyme. However, the results obtained in other systems (bacteria, chloroplasts) will also be discussed in order to get a more generalized picture.

It is the intention of the authors to stress some of the latest results and ideas rather than to give a comprehensive review of this topic.

Structure of F_0

The membrane-bound moiety of H^+-ATPase (F_0) promotes the transmembrane flow of protons coupled to the activity of the catalytic part of the

[1]Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; SDS, sodium dodecyl sulfate.

[2]Institute of Physiology, Czechoslovak Academy of Sciences, Prague 4, Czechoslovakia.

0145-479X/82/0200-0001$03.00/0

enzyme (F_1). It is the site of action of certain agents such as DCCD, oligomycin, and organotins which inhibit the enzyme activity by blocking the H^+-translocation via F_0 (Linnett and Beechey, 1979).

F_0 is isolated by removing F_1 from the purified H^+-ATPase complex (Capaldi, 1973; Shchipakin *et al.*, 1976; Serrano *et al.*, 1976; Okamoto *et al.*, 1977; Berden and Voorn-Brouwer, 1978; Glaser *et al.*, 1980; Negrin *et al.*, 1980; Schneider and Altendorf, 1980; Sone *et al.*, 1978). Therefore, the composition of various preparations of F_0 also depends on the purity of the isolated H^+-ATPase.

The "purest" preparations of mitochondrial F_0 were resolved by SDS-polyacrylamide gel electrophoresis into six to seven protein subunits of molecular weight ranging from 5,000 to 31,000 (Capaldi, 1973; Serrano *et al.*, 1976; Berden and Voorn-Brouwer, 1978; Glaser *et al.*, 1980). In chloroplasts F_0 contained four subunits (Pick and Racker, 1979), and only three subunits were identified in bacterial F_0 (Fillingame, 1980; Okamoto *et al.*, 1977; Negrin *et al.*, 1980; Schneider and Altendorf, 1980). The simplest and functionally still active F_0 was prepared from thermophilic bacteria (Sone *et al.*, 1978). It consisted of only two subunits.

With respect to the different number of protein subunits reproducibly detected in F_0 preparations of different energy-transducing membranes, it appears most likely that the structure is more complex in mitochondria than in chloroplasts and bacteria. The precise structure of mitochondrial F_0, including the molecular weight, is, however, still not known with certainty. To solve this continuous point of contention, it is essential to establish the role of individual subunits in the two known functions of F_0, i.e., (a) the ability to bind F_1, and (b) the ability to translocate protons in the inhibitor-sensitive manner.

Binding of F_1

In mitochondria at least two protein subunits of F_0 are involved in the functional linkage and binding of F_1 (Glaser *et al.*, 1980; Vadineanu *et al.*, 1976). These include the oligomycin-sensitivity conferring protein (OSCP) and factor F_6 with molecular weights of 18,000–24,000 (Glaser *et al.*, 1980; Vadineanu *et al.*, 1976; Senior 1979b; Galante *et al.*, 1979) and 8,000–9,000 (Glaser *et al.*, 1980; Galante *et al.*, 1979; Racker 1979), respectively. Both OSCP and F_6 were purified to homogeneity (Senior, 1979b; Racker, 1979). F_1 binds to F_0 in the absence of OSCP but not in the absence of F_6. OSCP, however, increases the affinity of the binding and is essential for expression of the inhibitory effects of oligomycin and DCCD on catalytic activities of

F_1 (Glaser *et al.*, 1980; Vadineanu *et al.*, 1976). Furthermore, OSCP appears to be required (Glaser *et al.*, 1980) for the gate function of F_1.

In bacteria and chloroplasts, OSCP and F_6 were not found. In the former case the binding of F_1 is mediated by only one protein subunit of molecular weight 13,500 (Sone *et al.*, 1978). Similarly to OSCP and F_6, this subunit appears to be localized on the side of the membrane which faces F_1 (Sone *et al.*, 1978). The functional importance of a more complex F_0-F_1 interaction in mitochondria is not know. It is tempting to speculate that the two types of interaction might be a consequence of the opposite side membrane localization of F_1 with respect to the F_1-subunits-synthesizing machinery (Tzagoloff *et al.*, 1979; Downie *et al.*, 1979).

Protonophoric Activity of F_0

The ability of F_0 to translocate protons is very well demonstrated by reconstitution experiments. After incorporation of the purified F_0 into liposomes, H^+-conductance was increased proportionally to the amount of F_0 incorporated (Okamoto *et al.*, 1977; Glaser *et al.*, 1980; Negrin *et al.*, 1980), since it is sensitive to DCCD (Okamoto *et al.*, 1977; Glaser *et al.*, 1980, Negrin *et al.*, 1980) and oligomycin (Schipakin *et al.*, 1976; Glaser *et al.*, 1980). In agreement with the gate function of F_1 (Kagawa, 1978; Fillingame, 1980) H^+-conductance was also blocked as a consequence of F_1 binding to F_0 (Okamoto *et al.*, 1977). The rate of transport increased with increasing external H^+ concentration, indicating that H^+ rather than OH^- was the ion translocated (Okamota *et al.*, 1977).

The kinetics of the H^+-translocation via mitochondrial F_0 was further characterized using F_1-depleted submitochondrial particles (Pansini *et al.*, 1975, 1978). A monophasic, pseudomonomolecular character was found. A slow phase of proton diffusion appeared as a consequence of the binding of F_1 and was inhibited by ligands of F_1, including the natural protein inhibitor. On the other hand, DCCD and oligomycin blocked both phases of proton diffusion, indicating cooperativity between F_0 and F_1. The initial rate of oligomycin-sensitive H^+-flow in F_1-depleted particles was comparable to the rate of oxidative phosphorylation (Pansini *et al.*, 1978).

In conclusion, the passive transport of protons through mitochondrial F_0 is quite well characterized and the properties of the channel are consistent with the chemiosmotic principles (Boyer, 1977) of H^+-ATPase function. Nevertheless, precise kinetic measurements of H^+-flow coupled to the catalytic turnover of F_1 are still missing and have to be performed (Fillingame, 1980).

DCCD-Binding Protein

A small hydrophobic protein which is directly involved in H^+-translocation represents the best characterized subunit of mitochondrial, chloroplast, and bacterial F_0. This applies to its function and particularly to its structure. Because of its high reactivity to DCCD, it is usually referred to as DCCD-binding protein.

Identification and Isolation

DCCD proved to be a potent tool in the identification and characterization of the most hydrophobic subunit of F_0. The apolar DCCD penetrates into the membrane and, due to its preferential reactivity with carboxyl groups (Kurzer and Douraghi-Zadeh, 1967), it modifies several proteins (Linnett *et al.*, 1979), some of them involved in H^+-translocation (Linnett *et al.*, 1979; Pougeois *et al.*, 1980; Casey *et al.*, 1980; Houštěk *et al.*, 1981a).

In 1967 Beechey and co-workers discovered the irreversible inhibitory effect of low concentrations of DCCD on H^+-ATPase functions) Beechey *et al.*, 1967). By means of ^{14}C-DCCD the target site of the inhibitor action in bovine heart mitochondria was identified as a low-molecular-weight protein of F_0 (Knight *et al.*, 1968; Cattell *et al.*, 1971; Stekhoven *et al.*, 1972). Because of its solubility in certain organic solvents it was called a proteolipid. Later, DCCD-binding protein was also found in chloroplasts and bacteria and it was isolated and purified to homogeneity (Fillingame, 1980).

The isolation of the DCCD-binding protein is based on its solubility in chloroform–methanol (2:1, v/v; Cattell *et al.*, 1971; Stekhoven *et al.*, 1972). This protein is extracted quantitatively together with several other hydrophobic proteins and phospholipids. In some mitochondria, the extraction becomes rather specific, if membranes are prewashed with methanol or chloroform–methanol–diethyl ether (Sebald *et al.*, 1979a; Kužela *et al.*, 1980; Aroskar and Avadhani, 1979). In order to extract DCCD-binding protein from chloroplasts, the original procedure was replaced by extraction with *n*-butanol (Sigrist *et al;* 1977). In most cases, the final purification is achieved by repeated precipitation with diethyl ether followed by adsorption chromatography (Cattell *et al.*, 1971; Fillingame, 1976; Graf and Sebald, 1978; Sigrist-Nelson and Azzi, 1980), thin-layer chromatography (Sierra and Tzagoloff, 1973; Dianoux *et al.*, 1978), or reversed-phase high-pressure liquid chromatography (Blondin, 1979). Homogeneous preparations of the protein are thus obtained in both the free and DCCD-modified form.

The purified protein has a slightly different molecular weight in mitochondria, chloroplasts, and bacteria which ranges for 6,500 to 8,000 (Sebald *et al.*, 1979a; Sone *et al.*, 1979a; Sigrist-Nelson *et al.*, 1978). It consists of

76–81 amino acids, mostly exhibiting low polarity. Small amino acids, glycine and alanine, comprise one-quarter of the total residues. The percentage of the hydrophilic side chains is extremely low (16–25%), and generally no tryptophan and histidine, and only few arginine residues are present. Cysteine, serine, and lysine were found only in some preparations (Blondin, 1979; Sone *et al.*, 1979a; Sigrist-Nelson *et al.*, 1978; Sebald *et al.*, 1979b; Schmid *et al.*, 1981).

During the past few years, a considerable attention was devoted to the properties of DCCD-binding protein when assembled in the intact mitochondrial membrane. It was shown that at concentrations that are inhibitory for H^+ATPase, ^{14}C-DCCD modifies three proteins of mammalian mitochondria. Their molecular weights were determined by various systems of SDS-polyacrylamide gel electrophoresis and ranged from 5,000 to 14,000, from 16,000 to 18,000, and from 33,000 to 45,000 (Sebald *et al.*, 1979a; Kužela *et al.*, 1980; Glaser *et al.*, 1981a; Houštěk *et al.*, 1981a). They will be further referred to as 8,000-M_r, 16,000-M_r, and 33,000-M_r proteins.

The two proteins with the higher molecular weight were originally considered as aggregates of the 8,000-M_r protein, thus representing the same DCCD-reactive component of F_0 (Sebald *et al.*, 1979a; Glaser *et al.*, 1981a). However, it was later demonstrated that this does not hold for the 33,000-M_r protein. In contrast to the other two proteins, the 33,000-M_r protein was neither soluble in chloroform–methanol nor was it detected in isolated H^+-ATPase (Kužela *et al.*, 1980; Glaser *et al.*, 1981a; Houštěk *et al.*, 1981a; Kiehl and Hatefi, 1980) and, in addition, its labeling with ^{14}C-DCCD was not influenced by oligomycin (Drahota *et al.*, 1981). It was identified and isolated as the *N*-ethylmaleimide-reactive protein, most probably the phosphate translocator (Houštěk *et al.*, 1981a).

Thus, in mammalian mitochondria, the binding of DCCD to F_0 is associated with modification of two electrophoretically different forms of DCCD-binding protein: the 8,000-M_r form and the 16,000-M_r form. The 16,000-M_r form is an aggregate in which the 8,000-M_r form is involved. It can be dissociated under appropriate conditions, especially in the presence of urea (Berden and Voorn-Brouwer, 1978) or performic acid (Glaser *et al.*, 1981a). The electrophoretic resolution of the two forms decreases in the presence of phospholipids and nonionic detergents (Dianoux *et al.*, 1978). Due probably to these reasons only one of the two forms was sometimes detected in isolated H^+-ATPase (Stekhoven *et al.*, 1972; Sebald *et al.*, 1979a; Dianoux *et al.*, 1978; Kiehl and Hatefi, 1980). In yeast mitochondria, DCCD-binding protein was present as a component with a molecular weight of 45,000–50,000 which splits into the 8,000-M_r form when extracted with chloroform–methanol (Sierra and Tzagoloff, 1973; Tzagoloff and Akai, 1972; Partis *et al.*, 1976; Stephanson *et al.*, 1980) or at alkaline pH (Tzagoloff and Akai,

1972; Partis *et al.*, 1976). On the contrary, in bovine heart mitochondria both the 8,000-M_r and 16,000-M_r forms were present in the crude chloroform–methanol extract (Knight *et al.*, 1968; Glaser *et al.*, 1981a; Houštěk *et al.*, 1981a) and were not influenced by alkaline pH (J. Kopecký, unpublished).

As the isolation techniques always yielded only the monomeric DCCD-binding protein, it appears very likely that the 16,000-M_r form is quantitatively dissociated into the monomeric 8,000-M_r form during the isolation. It will be shown in the following sections that the two forms do not result from a random aggregation of DCCD-binding protein but mirror the properties of the protein when assembled in the intact membrane.

The Role of DCCD-Binding Protein in H^+-Translocation

Since the DCCD-binding protein was isolated, attempts were made to verify its role in H^+-translocation via F_0. The purified protein of mitochondria and chloroplasts was incorporated into liposomes (Sigrist-Nelson and Azzi, 1980; Cellis, 1980; Konishi *et al.*, 1979) or lipid-impregnated Millipore filters (Criddle *et al.*, 1977). An increased H^+-conductivity sensitive to DCCD (Sigrist-Nelson and Azzi, 1980) and/or oligomycin (Cellis, 1980; Konishi *et al.*, 1979; Criddle *et al.*, 1977) was demonstrated. The sensitivity to oligomycin was lost when the protein of an oligomycin-resistant mutant was used (Konishi *et al.*, 1979; Criddle *et al.*, 1977). It was concluded that the sole DCCD-binding protein is capable of conducting protons in the reconstituted system. In the native membrane, however, other subunits of F_0 might also be involved in H^+-translocation (see below).

Recent studies on the chemical structure of DCCD-binding protein, most extensively performed by Sebald and co-workers (Sebald *et al.*, 1979b) yielded basic data for understanding of the molecular mechanism of H^+-translocation. It is now well established that the amino acid composition and particularly the primary structure of DCCD-binding protein are highly homologous in different organisms (Sebald *et al.*, 1979b). Numerous hydrophobic residues are localized in the two segments, 20–25 amino acids each, which are separated by a short polar chain. In a short segment at the N-terminus, polar residues are also accumulated, but very few of them are found in the hydrophobic segments (Tzagoloff *et al.*, 1979; Sebald *et al.*, 1979b; Hoppe and Sebald, 1980).

Similarly to bacteriorhodopsin (Konishi and Packer, 1978) the existence of polar residues is essential for the protonophoric function (Fillingame, 1980, Pansini *et al.*, 1975; Sone *et al.*, 1979b). Using F_1-depleted membranes (Pansini *et al.*, 1975; Ho and Wang, 1980) and liposomes reconstituted with F_0 (Sone *et al.*, 1979b) or with the purified DCCD-binding protein (Sigrist-Nelson and Azzi, 1980), it was demonstrated that the protonophoric activity

is blocked when amino (Sigrist-Nelson and Azzi, 1980) and carboxyl groups (Ho and Wang, 1980) in general or specifically tyrosine (Pansini *et al.*, 1975, Sigrist-Nelson and Azzi, 1980; Sone *et al.*, 1979b), arginine (Pansini *et al.*, 1975; Sigrist-Nelson and Azzi, 1980; Sone *et al.*, 1979b), and glutamic or aspartic acid (Fillingame, 1976; Sebald *et al.*, 1979b) are chemically modified. It is implicative that one basic position (Arg^{45}; for numbering see Sebald *et al.*, 1979b), one acidic position (Glu/Asp^{65}), and one polar uncharged position (Asp/Gln^{46}) remained conserved during evolution (Sebald *et al.*, 1979b). The involvement in H^+-translocation is most pronounced in the case of Glu/Asp^{65} (position 61 in bacteria), which is localized in the center of the second hydrophobic domain representing the only DCCD-reactive residue in the molecule. In mutants of *E. coli* its replacement with Gly abolished both the binding of DCCD and H^+-translocation (Wachter *et al.*, 1980a; Hoppe *et al.*, 1980a).

According to the genetic evidence (Tzagoloff *et al.*, 1979), the amino acids localized in the close vicinity of the DCCD-reactive residue are involved in oligomycin and venturicidin binding. This is in agreement with the oligomycin-sensitive labeling of the protein with ^3H-borohydride in yeast (Enns and Criddle, 1977) and with reciprocal competitive binding of DCCD, oligomycin, and venturicidin (Glaser *et al.*, 1981a; Kiehl and Hatefi, 1980; Enns and Criddle, 1977). Irrespective of the differences in the mechanism of their action (Linnett *et al.*, 1975; Glaser *et al.*, 1981b) and the fact that the inhibitory effects of oligomycin and DCCD on H^+-ATPase activity are not additive (Glaser *et al.*, 1981b), the three inhibitors might inhibit H^+-translocation through the same acidic residue.

Using DCCD-resistant mutants of *E. coli,* it was demonstrated that replacement of Ile^{28} with Val or Thr prevents the binding of DCCD to Asp^{61} without abolishing the protonophoric activity (Wachter *et al.*, 1980a; Hoppe *et al.*, 1980). Although these amino acids are 33 residues apart, they would be brought together if the "hair pin"structure, similar to that of bacteriorhodopsin (Stoeckenius *et al.*, 1979), existed here. Remarkably, such an arrangement is inherent with the calculated secondary structure (Hoppe *et al.*, 1980a) which offers a possibility that the two α-helical hydrophobic segments span the membrane, whereas the β-helical hydrophilic central loop is in contact with the aqueous phase or with some other subunit of the enzyme. The "hairpin" structure of DCCD-binding protein would also agree with the accessibility of carboxyl groups of the polar region to hydrophilic carbodiimides (Wachter *et al.*, 1980b).

Oligomeric Structure

Given that DCCD-binding protein promotes H^+-translocation via F_0, several important question arise. How is the H^+-channel constructed? How

many molecules of DCCD-binding protein does it contain? Are all of them necessary for H^+-translocation?

In 1976 Fillingame proposed the oligomeric structure of DCCD-binding protein in bacteria (Fillingame, 1976). As concluded by Sebald et al. (1979a), the content of DCCD-binding protein in mitochondria exceeds six to seven times that of H^+-ATPase. This conclusion is based on (a) distribution of radioactivity among DCCD-binding protein and other subunits of H^+-ATPase which was uniformly labeled with ^{3}H-leucine, (b) comparison of specific radioactivity of ^{14}C-DCCD present in intact mitochondria and in isolated protein, and (c) identification of the 45,000-M_r DCCD-reactive component in yeast mitochondria as a hexamer of the DCCD-binding protein. These calculations might not always be accurate. When the approach (a) is used in bacteria, four to six copies of DCCD-binding protein per enzyme are obtained (Sone *et al.*, 1979a). Using the approach (b), the value is overestimated because under the conditions of labeling used, up to 30% of the bound label are recovered in the 33,000-M_r protein which is not a component of H^+-ATPase (Houštěk *et al.*, 1981a). Nevertheless, irrespective of what the exact stoichiometry turns out to be, it is clear that one molecule of H^+-ATPase contains several molecules of DCCD-binding protein.

The arrangement of the oligomer can be deduced from the correlations between the ^{14}C-DCCD labeling and resulting inhibition of H^+-ATPase activity (Fillingame, 1976; Graf and Sebald, 1978; Sigrist-Nelson *et al.*, 1978; Kopecký *et al.*, 1981) or H^+-conductivity of F_0 (Sone *et al.*, 1979a; Kopecký *et al.*, 1981). In mitochondria (Graf and Sebald, 1978; Kopecký *et al.*, 1981), chloroplasts (Sigrist-Nelson *et al.*, 1978), and bacteria (Fillingame, 1976; Sone *et al.*, 1979a), at the maximal inhibition not more than one-sixth to one-third of DCCD-binding protein present was modified by the inhibitor. The value might be underestimated if noncovalently bound DCCD is inhibitory, and/or if condensation of the DCCD-activated carboxyl occurs (Sebald *et al.*, 1979a; Kiehl and Hatefi, 1980; Drahota *et al.*, 1981). However, in mitochondria and in chloroplasts, the full inhibition of ATP synthesis and hydrolysis corresponds roughly to the binding of 1 mol of DCCD per 1 mol of H^+-ATPase (Graf and Sebald, 1978; Sigrist-Nelson *et al.*, 1978; Kopecký *et al.*, 1981). A similar estimate was also made when the inhibition of H^+-conductivity of F_1-depleted submitochondrial particles by oligomycin was measured (Glaser *et al.*, 1981b). Therefore, those molecules of DCCD-binding protein which are involved in the inhibition react preferentially with DCCD. A possible explanation would be a negative cooperativity of DCCD-binding or an asymmetrical arrangement of the oligomer. However, the former possibility does not agree with the kinetics of DCCD-induced inhibition of H^+-conductivity of the bacterial F_0 (Sone *et al.*, 1979a), as well as of synthesis and hydrolysis of ATP in mitochondria (Drahota *et al.*, 1981).

In all these cases the inhibition followed pseudo-first-order kinetics, where 1 mol of DCCD per 1 mol of the enzyme (F_0) eliminates the activity, attacking the DCCD-binding site without cooperativity.

The most direct evidence for the oligomeric and asymmetrical arrangement of DCCD-binding protein was obtained in experiments with a spin label analog of DCCD, NCCD (Sigrist-Nelson and Azzi, 1979). It was shown that in the chloroplast membrane, at least some of the monomers are localized at the maximal distance of 15–20 Å from each other. When only one-third of DCCD-binding protein present was blocked by DCCD (the molecules of DCCD-binding protein with higher affinity) the spin–spin interaction was abolished.

In mammalian mitochondria, the asymmetrical arrangement of the oligomer might be reflected by the two electrophoretic forms of DCCD-binding protein (8,000-M_r and 16,000-M_r forms, see above). The 8,000-M_r form reacted with ^{14}C-DCCD proportionally to the inhibition, and the saturation of its binding capacity coincided with the full inhibition (Glaser *et al.*, 1981a; Houštěk *et al.*, 1981a). On the other hand, the binding of ^{14}C-DCCD to the remaining copies of DCCD-binding protein, represented by the 16,000-M_r form, began at higher inhibitor concentrations when H^+-ATPase activity had already been inhibited by 50% (Houštěk *et al.*, 1981a). The binding capacity of the 16,000-M_r form was several times higher than that of the 8,000-M_r form (Glaser *et al.*, 1981a; Houstek *et al.*, 1981). Hence the inhibition by DCCD seems to be mediated by the 8,000-M_r form only, whereas the other form is not directly involved. This line of evidence is again in favor of a nonrandom organization of the oligomer.

The oligomeric structure of DCCD-binding protein of F_0 resembles some other translocators of biomembranes the function of which can be ascribed to allosteric conformational changes (Kagawa, 1978; Fillingame, 1980), e.g., the sliding of some subunits, one along the other (Okamoto *et al.*, 1977). The evidence for the involvement of all DCCD-binding protein molecules in H^+-translocation via F_0 is still missing. Nevertheless, the effect of temperature on H^+-translocation via bacterial F_0 (Okamoto *et al.*, 1977), as well as the effect on F_1 on the sensitivity of H^+-translocation in mitochondrial F_0 to DCCD (Kopecký *et al.*, 1981), imply the relevance of the conformational changes within the H^+-channel. In addition, the conformational changes of F_0 were also deduced on the basis of the labeling of F_0 subunits in chloroplasts with a membrane-nonpenetrating agent (Ellenson *et al.*, 1978; Prochaska and Dolley, 1978), or on the basis of the sensitivity of the mitochondrial H^+-ATPase to oligomycin (Bertina *et al.*, 1974), both effects being dependent on the energetic state of the membrane.

As in other membrane-bound enzymes (Lenaz, 1979), the activity of the H^+ channel of F_0 is affected by membrane phospholipids (Okamoto *et al.*,

1977), (Sigrist-Nelson and Azzi, 1980; Pitotti *et al.*, 1980). Their physical state influences both the H^+-translocation and the inhibitory effects of oligomycin and DCCD (Linnett *et al.*, 1975; Pitotti *et al.*, 1980; Parenti-Castelli *et al.*, 1979). Therefore, the fluid bilayer structure around F_0 might be essential for the conformational changes of its subunits and namely for the function of the H^+-channel.

Other F_0 Subunits

As discussed in previous sections, three of the subunits of mitochondrial F_0, i.e., OSCP, F_6, and DCCD-binding protein, have already been demonstrated. Their functional equivalents are also found in the four-subunit preparation of chloroplast F_0 (Pick and Racker, 1979), as well as in the preparation of bacterial F_0 which contains three subunits (Linnett and Beechey, 1979; Okamoto *et al.*, 1977; Negrin *et al.*, 1980; Schneider and Attendorf, 1980) all of them genetically confirmed. However, mitochondrial F_0 is more complex and at least four other components are likely candidates to be the true subunit of F_0. Their authenticity, however, is still unclear.

In preparations of mitochondrial F_0 and H^+-ATPase, 21,000–25,000-M_r protein was repeatedly observed (Serrano *et al.*, 1976; Glaser *et al.*, 1980; Galante *et al.*, 1979; Kužela *et al.*, 1980; De Jong *et al.*, 1980). This protein, distinct from OSCP, is coded for by mitochondrial DNA (Kužela *et al.*, 1980; De Jong *et al.*, 1980) and is sensitive to trypsin (Glaser *et al.*, 1980). Its function is not known. In bacteria, a protein of a similar molecular weight (24,000) might be involved in H^+-translocation (Downie *et al.*, 1981).

The second component, often found in mitochondrial F_0, is a trypsin-sensitive protein (Glaser *et al.*, 1980) with molecular weight of about 10,000 (Glaser *et al.*, 1980; Galante *et al.*, 1979; Kužela *et al.*, 1980). It might be identical with factor B, which is a component involved in energy transduction (Joshi and Sanadi, 1979).

The next likely subunit of mitochondrial F_0 is another low-molecular-weight protein (9,000–10,000) which is hydrophobic and can be extracted with chloroform–methanol (Kužela *et al.*, 1980). This protein is also synthesized in mitochondria (Kužela *et al.*, 1980; De Jong *et al.*, 1980). It might be equivalent to the protein isolated from heart (Blondin, 1979) and yeast mitochondria (Velours *et al.*, 1980) which exhibits high affinity to phosphate.

The last component to be mentioned is a protein with molecular weight of about 30,000 (Serrano *et al.*, 1976; Berden and Voorn-Brouwer, 1978; Galante *et al.*, 1979), also identified as an uncoupler-binding protein (Galante *et al.*, 1979). In some preparations of mitochondrial H^+-ATPase the

presence of this protein was connected with preservation of sensitivity to oligomycin and DCCD (Serrano *et al.*, 1976; Berden and Voorn-Brouwer, 1978; Pitotti *et al.*, 1980). In various types of mitochondria the content of specific uncoupler-binding sites (Cyboron and Dryer, 1977), however, did not correlate with the content of H^+-ATPase (Svoboda *et al.*, 1981), and recently it was shown that this protein can be removed from H^+-ATPase without diminishing its protonophoric activity (Berden and Henneke, 1981).

Conclusions

Based on our present knowledge about the composition of mitochondrial F_0, it is evident that its mode of interaction with F_1 is more complex in comparison with bacteria and chloroplasts. As far as the H^+-channel is concerned, no definite conclusion about the involvement of other subunits besides the DCCD-binding protein can be drawn at present. This holds for mitochondria as well as for chloroplasts and bacteria. Experimental evidence is accumulating in favor of the oligomeric and asymmetrical arrangement of the H^+-channel. Extraction of its few polar amino acid residues by specific agents reveals the fundamental functional importance of these residues in the path of protons across the membrane. In particular, the use of DCCD was of primary importance for elucidation of the structural features underlying the protonophoric activity. It may be hoped that application of similar new approaches in combination with studies of the intact phosphorylating assembly will help us to clarify the molecular mechanism of ATP synthesis.

References

Aroskar, V. A. and Avadhani, N. G. (1979). *Biochem. Biophys. Res. Commun.*, **91,** 17.
Beechey, R. B., Roberton, A. M., Holloway, C. T., and Knight, I. G. (1967). *Biochemistry* **6,** 3867.
Berden, J. A. and Voorn-Brouwer, M. M. (1978). *Biochim. Biophys. Acta* **501,** 424.
Berden, J. A. and Henneke, M. A. C. (1981). *FEBS Lett.* **126,** 211.
Bertina, R. M., Steenstra, J. A., and Slater, E. C. (1974). *Biochim. Biophys. Acta* **368,** 279.
Blondin, G. A. (1979). *Biochem. Biophys. Res. Commun.* **87,** 1087.
Boyer, P. D., Chance, B., Ernster, L., Mitchell, P., Racker, E., and Slater E. C. (1977). *Ann. Rev. Biochem.* **46,** 955.
Capaldi, R. A. (1973). *Biochem. Biophys. Res. Commun.* **53,** 1331.
Casey, R. P., Thelen, M., and Azzi, A. (1980). *J. Biol. Chem.* **255,** 3994.
Cattell, K. J., Lindop, C. R., Knight, I. G., and Beechey, R. B. (1971). *Biochem. J.* **125,** 169.
Cellis, H. (1980). *Biochim. Biophys. Res. Commun.* **92,** 26.
Criddle, R. S., Packer, L., and Shieh, P. (1977). *Proc. Natl. Acad. Sci. USA* **74,** 4306.
Cyboron, G. W. and Dryer, R. L. (1977). *Arch. Biochem. Biophys.* **179,** 141.
De Jong, L., Holtrop, M., and Kroon, A. M. (1980). *Biochim. Biophys. Acta* **606,** 331.
Dianoux, A.-CH, Bof, M., and Vignais, P.V. (1978). *Eur. J. Biochem.* **88,** 69.

Downie, W. I. A., Gibson, F., and Cox, G. B. (1979). *Ann Rev. Biochem.* **48,** 103.
Downie, J. A., Cox, G. B., Lanfman, L., Ash, G., Becker, M. and Gibson, F. (1981). *J. Bacteriol.* **145,** 200.
Drahota, Z., Kopecký, J., Svoboda, P., Pavelka, S., and Houštěk, J. (1981). In *Vectorial Reactions in Electron and Ion Transport in Mitochondria and Bacteria* (Palmieri, F. ed.), Elsevier, North-Holland, Amsterdam, p. 223.
Ellenson, J. L., Pheasant, D. J., Levine, R. P. (1978). *Biochim. Biophys. Acta* **504,** 123.
Enns, R. K., and Criddle, R. S. (1977). *Arch. Biochem. Biophys.* **182,** 587.
Fillingame, R. H., (1976). *J. Biol. Chem.* **251,** 6630.
Fillingame, R.H. (1980). *Ann. Rev. Biochem.* **49,** 1079.
Foster, D. L., Mosher, M. E., Futai, M., and Fillingame, R. H. (1980). *J. Biol. Chem.* **255,** 12037.
Galante, Y. M., Wong, S. Y., and Hatefi, Y. (1979). *J. Biol. Chem.* **254,** 12372.
Glaser, E., Norling, B., and Ernster, L. (1980). *Eur. J. Biochem.* **110,** 225.
Glaser, E., Norling, B., and Ernster, L. (1981a). *Eur. J. Biochem.* **115,** 189.
Glaser, E., Norling, B., Kopecký, J., and Ernster, L. (1981b). *Eur. J. Biochem.,* in press.
Graf, Th., and Sebald, W. (1978). *FEBS Lett.* **94,** 218.
Ho, Y.-K., and Wang, J. H. (1980). *Biochemistry* **19,** 2650.
Hoppe, J., and Sebald, W. (1980). *Eur. J. Biochem.* **107,** 57.
Hoppe, J., Schaiver, H. U., and Sebald, W. (1980a). *FEBS Lett.* **109,** 107.
Hoppe, J., Schairer, H. U., and Sebald, W. (1980). *Eur. J. Biochem.* **12,** 17.
Houštěk, J., Pavelka, S., Kopecký, J., Drahota, Z., and Palmieri, F. (1981a). FEBS Lett., **103,** 137.
Houštěk, J., Svoboda, P., Kopecký, J., Kužela, Š., and Drahota, Z. (1981a). *Biochim. Biophys. Acta* **634,** 331.
Joshi, S., and Sanadi, R. R. (1979). *Methods in Enzymology,* Vol LV, (Fleischer, S., and Packer, L., eds.), Academic Press, New York, p. 384.
Kagawa, Y. (1978). *Biochim. Biophys. Acta* **505,** 45.
Kiehl, R., and Hatefi, Y. (1980). *Biochemistry* **19,** 541.
Knight, I. G., Holloway, C. T., Roberton, A. M., and Beechey, R. B. (1968). *Biochem. J.* **109,** 27.
Konishi, T., and Packer, L. (1978). *FEBS Lett.* **92,** 1.
Konishi, T., Packer, L., and Criddle, R. (1979). *Methods Enzymol.* **55,** 414.
Kopecký, J., Norling, B., Glaser, E., and Ernster, L. (1981). *FEBS Lett.,* **131,** 208.
Kurzer, F., and Douraghi-Zadeh, K. (1967). *Chem. Rev.* **67,** 107.
Kužela, Š., Luciaková, K., and Lakota, J. (1980). *FEBS Lett.* **114,** 197.
Lenaz, G. (1979). In *Subcellular Biochemistry,* Vol. 6 (Roodyn, D. B. ed.), Plenum, New York, p. 233.
Linnett, P. E., and Beechey, R. B. (1979). *Methods in Enzymology,* Vol. LV, (Fleischer, S., and Packer, L., eds.), Academic Press, New York, p. 472.
Linnett, P. E., Mitchell, A. D., and Beechey, R. B. (1975). *FEBS Lett.* **53,** 180.
Negrin, R. S., Foster, D. L., and Fillingame, R. H. (1980). *J. Biol. Chem.* **255,** 5643.
Okamoto, H., Sone, N., Hirata, H., Yoshida, M., and Kagawa, Y. (1977). *J. Biol. Chem.* **252,** 6125.
Pansini, A., Guerrieri, F., and Papa, S. (1978). *Eur. J. Biochem.,* 92, 545.
Pansini, A., Gurerrieri, F., and Papa, S. (1975). In *Membrane Bioenergetics* (Lee, C. P., *et al.* eds.), Addison-Wesley, Reading, Massachusetts, p. 413.
Parenti-Castelli, G., Sechi, A. M., Iandi, L., Cabrini, L., Mascarello, S., and Lenaz, G. (1979) *Biochim. Biophys. Acta* **547,** 161.
Partis, M. D., Bertoli, E., Mascavello, S., and Griffiths, D. E. (1976). *Biochem. Soc. Trans.* **4,** 88.
Pedersen, P. L., Amzel, L. M., Soper, J. W., Cintrón, N., and Hullihen, J. (1978). In *Energy Conservation in Biological Membranes* (Schäfer, G., and Klingenberg, M., eds.), Springer-Verlag, Berlin, Heidelberg, p. 159.
Pick, U., and Racker, E. (1979). *J. Biol. Chem.* **254,** 2793.

Pitotti, A., Dabbeni-Sala, F., and Bruni, A. (1980). *Biochim. Biophys. Acta* **600,** 79.
Prochaska, L. J., and Dolley, R. A. (1978). *Biochim. Biophys. Res. Commun.* **83,** 664.
Pougeois, R., Satre, M., and Vignais, P. V. (1980). *FEBS Lett.* **117,** 344.
Racker, E. (1979). *Methods in Enzymology,* Vol. LV (Fleischer, S., and Packer, R. L., eds.), Academic Press, New York, 398.
Schmid, R., Kiltz, H. H., Schneider, E., and Altendorf, K. (1981). *FEBS Lett* **125,** 97.
Schneider, E., and Altendorf, K. (1980). *FEBS Lett.* **116,** 173.
Sebald, W., Hope, J., and Wachter, E. (1979b). *Function and Molecular Aspects of Biomembrane Transport* (Quagliariello, E., Palmieri, F., Papa, S., and Klingenberg, M., eds.), Elsevier, North Holland, Amsterdam.
Sebald, W., Graf, Th. and Lukins, H. B. (1979a). *Eur. J. Biochem.* **93,** 587.
Senior, A. E. (1979a), In *Membrane Proteins in Energy Transduction* (Capaldi, R. A., ed.), Marcel Dekker, New York, p. 233.
Senior, A. E. (1979b). *Methods in Enzymology,* Vol. LV (Fleischer, S., and Packer, L., eds.), Academic Press, New York, p. 391.
Serrano, R., Kanner, B. J., and Racker, E. (1976). *J. Biol. Chem.* **251,** 2453.
Shchipakin, V., Chuchlova, E., and Evtodienko, Y. (1976). *Biochem. Biophys. Res. Commun.* **69,** 123.
Sierra, M. F., and Tzagoloff, A. (1973). *Proc. Natl. Acad. Sci. USA* **70,** 3155.
Sigrist, H., Sigrist-Nelson, K., and Gitler, C. (1977). *Biochem. Biophys. Res. Commun.* **74,** 178.
Sigrist-Nelson, K., and Azzi, A. (1979). *J. Biol. Chem.* **254,** 4470.
Sigrist-Nelson, K., and Azzi, A. (1980). *J. Biol. Chem.* **255,** 10638.
Sigrist-Nelson, K., Sigrist, H., and Azzi, A. (1978). *Eur. J. Biochem.* **92,** 9.
Sone, N., Yoshida, M., Hirata, H., and Kagawa, Y. (1978). *Proc. Natl. Acad. Sci. USA* **75,** 4219.
Sone, N., Yoshida, M., Hirata, H., and Kagawa, Y. (1979a). *J. Biochem.* **85,** 503.
Sone, N., Ikeba, K., and Kagawa, Y. (1979b). *FEBS Lett.* **97,** 61.
Stekhoven, F. S., Waitkus, R. F., and van Moerkerk, H. Th. B. (1972). *Biochemistry* **11,** 1144.
Stephanson, G., Marzuki, S., and Linnane, A. W. (1980). *Biochim. Biophys. Acta* **609,** 329.
Stoeckenius, W., Lozier, R. H., and Pogomolni, R. A. (1979). *Biochim. Biophys. Acta* **505,** 215.
Svoboda, P., Houštěk, J., Kopecký, J., and Drahota, Z. (1981). *Biochim. Biophys. Acta* **634,** 321.
Tzagoloff, A., and Akai, A. (1972). *J. Biol. Chem.* **247,** 6517.
Tzagoloff, A., Maccino, G., and Sebald, W. (1979). *Ann. Rev. Biochem.* **48,** 419.
Vadineanu, A., Berden, J. A., and Slater, E. C. (1976). *Biochim. Biophys. Acta* **449,** 468.
Velours, J., Guerin, M., and Guerin, B. (1980). *Arch. Biochem. Biophys.* **201,** 615.
Wachter, E., Schmid, R., Deckers, G., and Altendorf, K. (1980a). *FEBS Lett.* **113,** 265.
Wachter, E., Schmid, R., Deckers, G., and Altendorf, K. (1980b). Abstract from the First European Bioenergetics Conference, Bari, p. 173.