The F_o Complex of the ATP Synthase of *Escherichia coli* Contains a Proton Pathway with Large Proton Polarizability Caused by Collective Proton Fluctuation

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ABSTRACT The F_o complex of the *Escherichia coli* ATP synthase embedded into cardiolipin liposomes was studied by FT-IR spectroscopy. For comparison, respective studies were performed with dried F_o liposomes and with F_o liposomes treated with *N,N'*-dicyclohexyl-carbodiimide (DCCD), which binds to Asp-61 of subunit *c*. Furthermore, the effect of $H_2O \rightarrow D_2O$ exchange on the infrared spectrum was investigated. With F_o liposomes an infrared continuum is observed beginning at about 3000 cm^{-1} and extending toward smaller wavenumbers. In the DCCD-treated sample, this continuum is no longer observed. It vanishes also with drying of the liposomes. After $H_2O \rightarrow D_2O$ exchange, this infrared continuum begins at about 2350 cm^{-1} and is less intense. All of these results demonstrate that a proton pathway in native F_o is present, in which the protons are shifted in a hydrogen-bonded chain with large proton polarizability due to collective proton tunneling. With the D_2O-hydrated system, deuteron polarizability due to collective deuteron motion is observed, but the polarizability due to collective deuteron motion is smaller. Such pathways are very efficient, because they conduct protons or deuterons within picoseconds. These pathways lose their polarizability if the F_o complex is blocked by DCCD or if the liposomes are dried. On the basis of our results on the proton polarizability of hydrogen bonds and hydrogen-bonded systems and on the basis of structural data from the literature, the nature of the proton pathway of the F_o complex of *E. coli* is discussed.

INTRODUCTION

For ATP synthesis via the F_oF_1 ATP synthase, the free enthalpy is supplied by proton and field gradients. The protons flow through F_o from the periplasmic to the cytoplasmic space of the cell. The F_o complex is embedded into the membrane and contains a proton pathway conducting the protons to F_1, in which the ATP synthesis reaction takes place. In the last years several review articles were published regarding this enzyme (Ovchinnikov et al., 1982; Hoppe and Sebald, 1984; Schneider and Altendorf, 1987; Senior, 1988; Futai et al., 1989; Fillingame, 1990; Senior, 1990; Fillingame, 1992; Deckers-Hebestreit and Altendorf, 1992; Boyer, 1993). *E. coli* F_o contains the subunits: *a*, 2 *b*, and 10 ± 1 *c* (Hoppe and Sebald, 1984; Foster and Fillingame, 1982; Hermolin and Fillingame, 1989). The subunits *a* and *c* are essential for the proton-conducting mechanism (Senior, 1988; Fillingame, 1990, 1992). If only one of the *c* subunits is labeled with DCCD, proton conduction through F_o is blocked (Fillingame, 1992; Hermolin and Fillingame, 1989).

Hydrogen bonds with double minimum proton potentials or broad flat proton potentials show polarizabilities that are 1–2 orders of magnitude larger than the usual polarizabilities due to distortion of electron systems. These proton polarizabilities arise because of shifts of the protons within these hydrogen bonds. All of these results were obtained by theoretical treatments (Weidemann and Zundel, 1970; Janoschek et al., 1973; Zundel, 1976; Eckert and Zundel, 1987; Borgis et al., 1992; Zundel and Eckert, 1989) based on and proven by a very large number of infrared (Zundel (1976), Zundel (1992a), and references therein) and Raman Danninger and Zundel (1980) studies. These proton polarizabilities are particularly large in the case of hydrogen-bonded chains because in such chains a collective proton-tunneling occurs (Zundel, 1976; Eckert and Zundel, 1988a, b; Zundel, 1992b; Zundel and Brzezinski, 1992). In the infrared spectra these hydrogen bonds or hydrogen-bonded chains cause continua because they interact very strongly with their environments because of their large proton polarizabilities. Vice versa hydrogen bonds and hydrogen-bonded chains with large proton polarizabilities are indicated by these infrared continua (Zundel, 1976; Danninger and Zundel, 1980; Eckert and Zundel, 1988a, b; Zundel, 1992b; Lindemann and Zundel, 1977a; Zundel et al., 1993; Zundel and Brzezinski, 1992).

Also, a large number of hydrogen bonds formed between side chains of proteins and hydrogen-bonded chains between side chains and phosphate groups of phospholipids show large proton polarizabilities due to collective proton-tunneling (Zundel (1992b), Zundel and Brzezinski (1992), Zundel (1988), and references therein). These proton fluctuations in hydrogen bonds occur faster than within picoseconds. Thus, such hydrogen-bonded chains are very effective proton pathways (Zundel and Brzezinski, 1992). They conduct protons within picoseconds.

Recently, we have shown that a proton pathway as described above is present in the L_{550} intermediate of the bacteriorhodopsin molecule (Olejnik et al., 1992). The presence of this pathway was proven by an infrared continuum observed in FT-IR difference spectra between the intermediates BR_{570} minus L_{550}. This continuum begins at 2800 cm^{-1} and

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extends toward smaller wavenumbers. Via this pathway the protons are conducted from the active center to the outside of the membrane in the step L_{550} to M_{412} (Olejnik et al., 1992).

In this paper, we investigate by FT-IR spectroscopy whether the proton pathway in F_0 of *E. coli* is also a proton pathway with the above-mentioned properties. It was previously proposed by Howitt et al. (1988) that His-254 *a*, Glu-219 *a*, Asp-61 *c*, and Arg-210 *a* are involved in such a pathway.

Water molecules are highly ordered because of entropic reasons if they are in a hydrophobic environment (Zundel and Brzezinski, 1992). Therefore, they build up a particularly stable pathway. In this discussion, it must be taken into account that water molecules can be inserted between groups, forming a polarizable hydrogen-bonded chain without disturbing the proton polarizability (Eckert and Zundel, 1987, 1988a; Kristof and Zundel, 1980a).

The fact that monomeric molecules can replace a proton side chain of the pathway must be taken into account considering genetic engineering experiments. If the modified group is smaller than the respective group in the wild type, a monomere can replace the modified group in the pathway. These monomeres must have hydrogen bond donor and acceptor properties being comparable with the groups that were modified. Then the pathway remains active after modification.

MATERIALS AND METHODS

Purification of F_0

E. coli strain KY7485, which carries a λ prophage containing the complete *atp* operon and which overproduces the ATP synthase several times after heat induction, was grown as described in Deckers-Hebestreit et al. (1992). The F_0 complex of the ATP synthase was prepared according to the procedure described in Schneider and Altendorf (1984).

Reconstitution

The reconstitution of F_0 of the ATP synthase into cardiolipin liposomes was carried out by suspending 3 mg of cardiolipin in 300 μl of buffer (Tricin-NaOH pH 8.0) + 2.5 mM $MgSO_4$ + 0.2 mM EDTA pH 8.0 + 0.2 mM DTT (dithiotreithol). The mixture was shaken until clear. 300 μg of the purified F_0-subunit were then added, and the reconstitution was performed by passing the sample through a 27 cm long G-50 sephadex column (diameter 1.5 cm) at a flow rate of 2 ml/min. This procedure is described in Goormaghtigh et al. (1979) and references therein.

DCCD treatment and activity

The inhibition of the activity by DCCD (250 μM) was achieved by suspending the F_0 containing liposomes in 2 ml of 50 mM Tricin-NaOH pH 8.0, 2 mM $MgCl_2$ for 30 min at 35°C (Steffens et al., 1984). The activity of the F_0 containing and of the DCCD-treated liposomes before and after the measurement was checked by the procedure given in Schneider and Altendorf (1982).

Infrared spectroscopy

The spectra were recorded with a Bruker IFS 113v FT-IR spectrometer with a resolution of 2 cm^{-1}. As an internal reflection element, a germanium crystal (52×18×2 mm) with an aperture angle of 45° was used. 100 μl of the F_0-containing and of the DCCD-treated liposomes were spread and dried on this internal reflection element under a stream of nitrogen and then hydrated in a desiccator with H_2O-saturated N_2 for 6 h. The sample was deuterated with D_2O-saturated N_2 for 7 h.

RESULTS

Fig. 1 shows infrared spectra of F_0 prepared from *E. coli* ATP synthase embedded in cardiolipin liposomes. In Fig. 1 *A* the infrared spectra of the hydrated samples, and in Fig. 1 *B* the spectra of the dried samples, are shown. In both cases, the infrared spectra of liposomes with the native F_0 complex (*solid line*) are compared with liposomes, in which the F_0 complex is blocked by the covalent reaction with DCCD (*dashed line*) (Hoppe and Sebald, 1984; Fillingame, 1990; Hermolin and Fillingame, 1989).

Fig. 1 *A* shows that with the native F_0 complex (*solid line*) an infrared continuum is observed beginning at about 3000 cm^{-1} and extending toward smaller wavenumbers over the whole region studied. This infrared continuum vanishes completely if F_0 is blocked by DCCD (Fig. 1 *A*, *dashed line*). Fig. 1 *B* shows that this infrared continuum also vanishes completely if the liposomes are dehydrated. The observed infrared continuum demonstrates that in the native hydrated F_0 complex a proton pathway with large proton polarizability due to collective proton-tunneling in a hydrogen-bonded

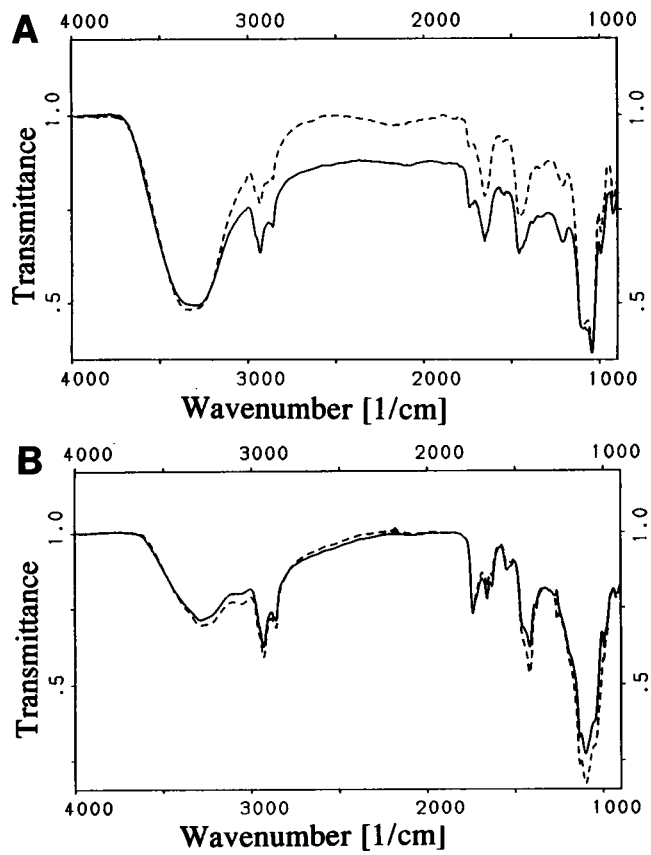

FIGURE 1 Fourier transform spectra in the region 4000–800 cm^{-1} of films of the F_0 complex embedded into cardiolipin liposomes. (*A*) (——) Native, hydrated; (– – – –) DCCD-blocked, hydrated. (*B*) (——) Native, dried; (– – – –) DCCD-blocked, dried.

chain is present that conducts protons within picoseconds. Hydrated vesicles of cardolipin without F_0-subunit cause no continuous absorption.

The spectrum of the F_0 cardiolipin liposomes after $H_2O \rightarrow D_2O$ exchange is shown in Fig. 2 *A* (*dotted line*). For comparison, the spectra of the native H_2O-hydrated liposomes (*solid line*), and of F_0 liposomes blocked with DCCD (*dashed line*), are given. The exchange of H_2O against D_2O in the samples is proven by a strong decrease of the ν(OH) stretching vibration of H_2O in the region 3500–3200 cm^{-1} and a strong increase of the respective ν(OD) stretching vibration of D_2O at 2490 cm^{-1}. At the same time, the masked bending vibration of H_2O δ(OH) at 1645 cm^{-1} vanishes and the respective δ(OD) bending vibration arises at 1206 cm^{-1}. These changes of bands mask the changes of the continuum in these regions. Fig. 2 *B* shows that the infrared continuum also vanishes if the deuterated sample is treated with DCCD. All results taken together show that after $H_2O \rightarrow D_2O$ exchange the continuum begins at 2280 cm^{-1} and extends toward smaller wavenumbers. Its intensity decreases to about 50% with deuteration. From experimental results as well as theoretical treatments (Zundel et al., 1993; Zundel, 1969; Janoschek, et al., 1978), it is well known that the infrared continua caused by polarizable deuterium bonds begin at the

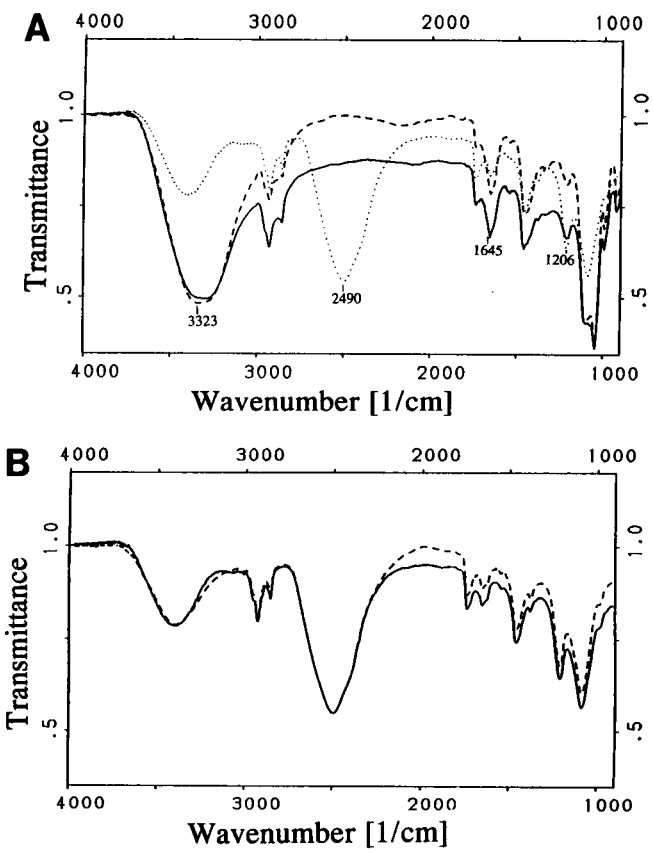

FIGURE 2 Fourier transform spectra in the region 4000–800 cm^{-1} of films of the F_0 complex embedded into cardiolipin liposomes. (*A*) (——) Native, hydrated; (· · · ·) native, after $H_2O \rightarrow D_2O$ exchange; (– – – –) DCCD-blocked, hydrated. (*B*) (——) Native, after $H_2O \rightarrow D_2O$ exchange; (– – – –) DCCD-blocked, after $H_2O \rightarrow D_2O$ exchange.

ν(OD) band and their intensity is less than that of polarizable hydrogen bonds. Thus, the observed infrared continuum demonstrates that in the pathway H^+ is exchanged against D^+. The deuterium bonds of this pathway show deuterium polarizability due to collective deuteron fluctuation. The deuteron polarizability is, however, lower compared with the proton polarizability.

DISCUSSION

Our FT-IR experiments demonstrate that the proton pathway in F_0 is a hydrogen-bonded chain with large proton polarizability due to collective proton tunneling, which conducts protons within picoseconds (Eckert and Zundel, 1988a, b; Zundel, 1992b; Zundel and Brzezinski, 1992; Zundel, 1988). The proton polarizability of hydrogen-bonded chains vanishes if the F_0 complex is blocked by labeling of Asp-61 of subunit *c* with DCCD. Furthermore, it vanishes if the F_0 liposomes are dried. Hence, the conductivity vanishes as well. In the D_2O-hydrated F_0, a deuteron-bonded chain with large deuteron polarizability is observed due to collective deuteron motion. The deuteron polarizability vanishes, also, if F_0 is blocked by DCCD.

Nature of this pathway

The question arises which side chains in F_0 are able to build up a hydrogen-bonded chain with such properties. From mutation studies (Senoir (1988), Fillingame (1990), and references therein), it is well known that in subunit *a*, His-245, Glu-219, and Arg-210 are essential for H^+ conduction. Residues of subunit *b* are not directly involved in the conducting pathway (Senior, 1988; Fillingame, 1990; Cox et al., 1986; Setffens et al., 1987). In subunit *c*, Asp-61 is involved in proton conduction as shown by DCCD blocking (Hermolin and Fillingame, 1989) and genetic means (Wachter et al., 1980; Hoppe et al., 1982). Furthermore, it was shown by chemical modification (Moody et al., 1987) that at least one tyrosine residue is necessary for the proton-conducting mechanism.

NMR studies (Moody et al., 1987; Norwood et al., 1992) have proven that the pK_a value of Asp-61 is increased. The same is true for Glu-2, Asp-7, and the carboxyl group of the C-terminal Ala-79. An increase of the pK_a value indicates that these groups are involved in intra- or intermolecular hydrogen bonds (Brzezinski et al., 1992) or at least stabilized by a hydrophobic environment. In addition, in Fraga and Fillingame (1991) and Hatch et al. (1993) it is demonstrated that Arg-41, which is strictly conserved in all *c* subunits studied so far, is absolutely necessary for the function of the ATP synthase. However, because of incomplete assembly of F_1 and an affected assembly of subunit *b* into the F_0 complex it remained unclear whether this basic residue is involved in the proton-conducting pathway. Also, the C-terminal carboxylic group of subunit *c* seems to be involved in proton translocation (Lötscher et al., 1984). In Deckers-Hebestreit and Altendorf (1992), it is shown that in subunit *c* Tyr-10,

Lys-34, Arg-41, Asp-61, and Ala-79 with the C-terminal carboxylic group are conserved. Recently, it was shown that the substitution of Lys-43, the only lysine residue of subunit *c*, has no effect on the function of the ATP synthase (Norris et al., 1992). As already mentioned in the introduction, a monomeric molecule may substitute a modified residue in the chain. According to Burget and Zundel (1986) a Lys residue present in the pathway may be substituted by a dihydrogen phosphate because the hydrogen bond donor and acceptor properties are similar to those of the lysine.

Furthermore, a class of subunit *a* mutants exists, where substitutions reduce but do not abolish proton conductance activity, such as Asp-44, Asp-119, Pro-190, Glu-196, Ser-206, and Asn-214 (Howitt et al., 1988; Vik et al., 1988; Cain and Simoni, 1989; Paule and Fillingame, 1989). However, in the latter cases a conformational change affecting the function of F_0 due to the substitution or modification is a highly probable explanation.

All of these results taken together suggest the following pathway: $-CO_2^-$ of Ala-79 *c*, Tyr-10 *c*, Glu-219 *a*, His-245 *a*, Asp-61 *c*, Lys-34 *c*, Arg-210 *a*, Arg-41 *c*.

This chain starts with the carboxylate group of the C-terminal Ala-79, the phenolic group of Tyr-10, and the carboxylic acid group of Glu-219. We already studied such a chain corresponding to the first section of the pathway by an intramolecular model system (Zundel, 1992b; Brzezinski et al., 1987), using infrared spectroscopy. This hydrogen-bonded chain shows large proton polarizability due to collective proton fluctuation.

The next two hydrogen bonds of the pathway are carboxylic acid-histidine bonds. From infrared spectroscopic studies with model systems, it is well known that these bonds show large proton polarizability due to proton tunneling. The protons can easily be shifted within these bonds (Lindemann and Zundel, 1977b; Rastogi et al., 1981). In addition, Lys-Arg hydrogen bonds and the system $N^+\cdots OH_2\cdots N \rightleftharpoons N\cdots OH_2\cdots {}^+N$ shows also large proton polarizability (B. Vogt, B. Brzezinski, and G. Zundel, unpublished data). In carboxylic acid-lysine bonds, a double-minimum proton potential is present with a much deeper well at the lysine (Kristof and Zundel 1980b). Thus, in this step a particularly large amount of energy becomes available that may be converted into conformational energy and may cause the conformational change or provide the energy necessary for rotation of the 10 ± 1 copies of subunit *c* along subunit *a* as discussed in the literature (Schneider and Altendorf, 1987; Fillingame, 1990; Fillingame, 1992; Cox et al., 1986; Hoppe and Sebald, 1986).

From cryomicroscopic measurements, it is known that the F_0 complex is about 60 Å long (Gogol et al., 1987). The number of hydrogen bonds discussed above is not sufficient to build up a proton pathway of this length. It is easily possible, however, that structural water is included in this chain of hydrogen bonds without loss of the proton polarizability (Eckert and Zundel, 1988a, b). We have demonstrated that carboxylic acid-carboxylate hydrogen bonds show large proton polarizability because they cause intense infrared continua (Kristof and Zundel, 1980a; Rastogi and Zundel, 1981). Furthermore, we have shown by theoretical treatments (Eckert and Zundel, 1988a) that if between the carboxylic acid and the carboxylate groups one or two water molecules are inserted, the large proton polarizability does not vanish, because large proton polarizability arises due to the strongly coupled, collective tunneling of the protons (Eckert and Zundel, 1988a). Herewith the polarizability increases with increasing chain length (Eckert and Zundel, 1988a, b).

Thus, the above-mentioned residues, together with structural water may form a very effective proton pathway, which conducts protons within picoseconds due to collective proton-tunneling. This pathway is very sensitive to electrical fields caused by its large proton polarizability. If the proton is present at Lys-34 *c*, the positive charge could be conducted via Arg-41 *c*, water, and Arg-210 *a* to the F_1 complex, because hydrogen bonds between such residues also show large proton polarizability. The chemical potential of a proton at an Arg residue is, however, relatively low. Furthermore, it was already mentioned that it is questionable whether Arg-41 *c* is present within this pathway. Therefore, the following mechanism has to be considered: the positive charge could be shifted directly from Lys-34 *c* via hydrogen bonds to the F_1 complex because this lysine residue is in the neighborhood of F_1 (Deckers-Hebestreit and Altendorf, 1992). During the presence of the positive charge at the lysine residue, the proton in a $N^+\cdots OH_2\cdots N \rightleftharpoons N\cdots OH_2\cdots {}^+N$ hydrogen-bonded system formed between Arg-41 *c*, water, and Arg-210 *a* could be shifted to subunit *a* because this structurally symmetrical hydrogen-bonded system shows very large proton polarizability. Thus, if the proton transfers from Asp-61 to Lys-34 or to a dihydrogen phosphate the energy gained could be used for the translocation of the subunits *c* along the subunit *a*. Such a translocation has been postulated in the models discussed in Schneider and Altendorf (1987), Deckers-Hebestreit and Altendorf (1992), and Henderson et al. (1990). Of course, such translocations are necessary because only one subunit *a* exists and 10 ± 1 subunits *c* are present in F_0 of *E. coli*. Hence, it becomes understandable that, as shown by Hermolin and Fillingame (1989), the blocking of only one subunit *c* of F_0 is sufficient to block the whole mechanism.

Finally, two view points have to be discussed. 1) The enzyme can function in opposite direction depending on the physiological conditions (Maloney, 1982). 2) The hydrogen-bonded chain is asymmetrical because in the Asp-61—Lys-34 hydrogen bond an asymmetrical double-minimum with deeper well at the lysine residue is present and, hence, no infrared continuum should be observed. In the case of hydrogen bonds with large proton polarizability a double-minimum potential with deeper well at the acceptor can easily be changed by local fields to a hydrogen bond with the deeper well at the donor (Zundel and Eckert, 1989). Thus, if enough protons are present in the cytoplasmic space of the

liposomes, their local field or specific interactions may reverse the shape of the proton potential discussed above. This also explains that the enzyme may work in opposite direction.

In the last years, a F_oF_1-type ATP synthase has been isolated and characterized from the strictly anaerobic bacterium *Propionigenium modestum*, where the synthesis of ATP is driven by an electrochemical gradient of Na^+ ions (Laubinger and Dimroth, 1987, 1988; Dmitriev et al., 1993). Because of the high degree of homology in primary and secondary structure, it has to be favored that the mechanism of ion translocation is the same in H^+-pumping as well as in Na^+-pumping systems (Laubinger et al., 1990) and, furthermore, the F_oF_1-ATPase of *P. modestum* can switch from a Na^+- to a H^+-translocating mode in the presence of low Na^+ concentrations ($\leq$1 mM) (Laubinger and Dimroth, 1989c). In addition, in the presence of Na^+ ions the ATP synthase is specifically protected from inactivation by DCCD, and a characterization of the inactivation kinetics showed that the DCCD-binding carboxylic acid residue of subunit *c* (Asp-61 in *E. coli*, Glu-65 in *P. modestum*) is the binding site for the coupling ions (Kluge and Dimroth, 1993a; Kluge and Dimroth, 1993b). In this context, it is particularly noticeable that also Li^+ and Na^+ bonds with double-minimum potential may show large cation polarizability (Zundel et al., 1993). Recently, it was shown (Brzezinski and Zundel, 1994) that also channels of such bonds may show large cation polarizabilities due to collective cation-tunneling and, hence, they may conduct these cations.

CONCLUSIONS

Infrared continua observed with the F_o complex of *E. coli* embedded into cardiolipin liposomes demonstrate that the proton pathway in F_o is a hydrogen-bonded chain. This chain shows large proton polarizability due to collective tunneling of the fluctuating protons. The conduction of protons in such chains proceeds within picoseconds. Thus, such chains are very effective proton pathways. On the basis of our results and structural data from the literature, a proton pathway with large proton polarizability due to collective fluctuation is proposed in which side chains of subunits *a* and *c* as well as structural water and perhaps also dihydrogenphosphates are involved. Because of the large proton polarizability of this chain, it can easily be regulated by local electrical fields.

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REFERENCES

Borgis, D., G. Tarjus, and H. Azzouz. 1992. An adiabatic dynamical simulation study of the Zundel polarization of strongly H-bonded complexes in solution. *J. Chem. Phys.* 97:1390–1400.

Boyer, P. D. 1993. The binding change mechanism for ATP synthase—some probabilities and possibilities. *Biochim. Biophys. Acta.* 1140: 215–250.

Brzezinski, B., G. Schroeder, G., Zundel, and Th. Keil. 1992. Complex formation and proton transfer in the polarizable hydrogen bonds of two N,N′-dioxides + substituted phenol systems as a function of the pK_a of the phenols. An FTIR study. *J. Chem. Soc. Perkin Trans.* 2:819–823.

Brzezinski, B., and G. Zundel. 1994. Collective H^+, Li^+ and Na^+ motions and cation polarizabilities of the cation-bonded systems with 1, 11, 12, 13, 14-pentahydroxypentacene salts—a FTIR study *J. Phys. Chem.* 98:2271–2274.

Brzezinski, B., G. Zundel, and R. Krämer. 1987. Proton polarizability caused by collective proton motion in intramolecular chains formed by two and three hydrogen bonds—implications for the charge conduction in bacteriorhodopsin. *J. Phys. Chem.* 91:3077–3080.

Burget, U., and G. Zundel. 1986. Lysine—phosphate hydrogen bonds and hydrogen-bonded chains with large proton polarizability and charge shifts due to local electrical fields: IR investigations. *J. Mol. Struct.* 145: 93–109.

Cain, B. D., and R. D. Simoni. 1989. Proton translocation by the F_1F_oATPase of *Escherichia coli*. *J. Biol. Chem.* 264:3292–3300.

Cox, G. B., A. L. Fimmel, F. Gibson, and L. Hatch. 1986. The mechanism of ATP synthase: a reassessment of the functions of the b and a subunits. *Biochim. Biophys. Acta.* 849:62–69.

Danninger, W., and G. Zundel. 1980. Intense depolarized rayleigh scattering in raman spectra of acids caused by large proton polarizabilities of hydrogen bonds. *J. Chem. Phys.* 74:2769–2777.

Deckers-Hebestreit, G., and K. Altendorf. 1992. The F_o complex of the proton translocating F-Type ATPase of Escherichia Coli. *J. Exp. Biol.* 172:451–459.

Deckers-Hebestreit, G., R. H. Simoni, and K. Altendorf. 1992. Influence of subunit-specific antibodies on the activity of the F_o complex of the ATP synthase of Escherichia coli. *J. Biol. Chem.* 267:12364–12369.

Dmitriev, O., G. Deckers-Hebestreit, and K. Altendorf. 1993. ATP synthesis energized by ΔpNa and $\Delta\phi$ in proteoliposomes containing the F_o1-ATPase from *Propionigenium modestum*. *J. Biol. Chem.* 268: 14776–14780.

Eckert, M., and Zundel, G. 1987. Proton polarizability, dipole moment and proton transitions of an $AH\cdots B \rightleftharpoons A^-\cdots H^+B$ proton transfer hydrogen bond as a function of an external electrical field: an ab initio SCF treatment. *J. Phys. Chem.* 91:5170–5177.

Eckert, M., and G. Zundel. 1988a. Energy surfaces and proton polarizability of hydrogen bonded Chains: an ab initio treatment with respect to the charge conduction in biological systems. *J. Phys. Chem.* 92:7016–7023.

Eckert, M., and G. Zundel. 1988b. Motion of one excess proton between various acceptors: theoretical treatment of the proton polarizability of such systems. *J. Mol. Struct.* 181:141–148.

Fillingame, R. H. 1990. Molecular mechanics of ATP synthesis by F_1F_o-type H^+ transporting ATP synthases. *In* The Bacteria: A Treatise on Structure and Function, Vol. 12. T. A. Krulwich, editor. Academic Press, New York. 345–391.

Fillingame, R. H. 1992. Subunit c of F_1F_o ATP synthase: structure and role in transmembrane energy transduction. *Biochim. Biophys. Acta.* 1101: 240–243.

Foster, D. L., and R. H. Fillingame. 1982. Stoichiometry of subunits in the H^+-ATPase Complex of *Escherichia coli*. *J. Biol. Chem.* 257: 2004–2015.

Fraga, D., and R. H. Fillingame. 1991. Essential residues in the polar loop region of subunit c of *Escherichia coli* F_1F_o ATP synthase defined by random oligonucleotide-primed mutagenesis. *J. Bacteriol.* 173:2639–2643.

Futai, M., T. Noumi, and M. Maeda. 1989. ATP Synthase (H^+-ATPase) Results by combined biochemical and molecular biological approaches. *Annu. Rev. Biochem.* 58:111–136.

Gogol, E. P., U. Lücken, and R. A. Capaldi. 1987. The stalk connecting the F_1 and F_o domains of ATP synthase visualized by electron microscopy of unstained specimens. *FEBS Lett.* 219:274–278.

Goormaghtigh, E., J. M. Ruyschaart, and G. H. Scarborough. 1979. High yield incorporation of the Neurospora Plasma membrane H^+ATPase into proteoliposomes: lipid requirement and secondary structure of the enzyme by IR spectroscopy. *In* The Ion Pumps: Structure, Function and Regulation. W. D. Stein, editor. Alan R. Liss, New York.

Hatch, L., A. L. Fimmel, and F. Gibson. 1993. The role of arginine in the conserved polar loop of the c-subunit of the *Escherichia coli* H^+-ATPase. *Biochim. Biophys. Acta.* 1141:183–189.

Henderson, R., J. M. Baldwin, T. A. Ceska, F. Zemlin, E. Beckmann, and K. H. Downing. 1990. Model for the structure of bacteriorhodopsin based on high-resolution electron cryomicroscopy. *J. Mol. Biol.* 213: 2639–2643.

Hermolin, J., and R. H. Fillingame. 1989. H^+-ATPase activity of *Escherichia coli* F_1F_0 is blocked after reaction of dicyclohexylcarbodiimide with a single proteolipid (Subunit c) of the F_0 complex. *J. Biol. Chem.* 264:3896–3903.

Hoppe, J., H. U. Schairer, P. Friedl, and W. Sebald. 1982. An Asp-Asn substitution in the channel. *FEBS Lett.* 145:21–24.

Hoppe, J., and W. Sebald. 1984. The proton conducting F_0-Part of bacterial ATP Synthases. *Biochim. Biophys. Acta.* 768:1–27.

Hoppe, J., and W. Sebald. 1986. Topological studies suggest that the pathway of the protons through F_0 is provided by amino acid residues accessible from the lipid phase. *Biochimie.* 68:427–434.

Howitt, S. M., F. Gibson, and G. B. Cox. 1988. The proton pore of the F_0F_1-ATPase of Escherichia coli: Ser-206 is not required for proton translocation. *Biochim. Biophys. Acta.* 936:74–80.

Janoschek, R., E. G. Weidemann, A. Hayd, M. Leuchs, and G. Zundel. 1978. Calculated and observed isotope effects with easily polarizable hydrogen and deuterium Bonds. *J. Chem. Soc. Faraday Trans. II.* 74:1238–1245.

Janoschek, R., E. G. Weidemann, and G. Zundel. 1973. Calculated frequencies and intensities associated with coupling of the proton motion with the hydrogen bond stretching vibrations in a double minimum potential surface. *Chem. Soc. Faraday Trans. II.* 69:505–520.

Kluge, C., and P. Dimroth. 1993a. Specific protection by Na^+ or Li^+ of the F_1F_0-ATPase of Propionigenium modestum from the reaction with dicyclohexylcarbodiimide. *J. Biol. Chem.* 268:14557–14560.

Kluge, C., and P. Dimroth. 1993b. Kinetics of inactivation of the F_1F_0 ATPase of Propionigenium modestum by dicyclohexylcarbodiimide in relationship to H^+ and Na^+ concentration: probing the binding site for the coupling ions. *Biochemistry.* 32:10378–10380.

Kristof, W., and G. Zundel. 1980a. Structurally symmetrical, easily polarizable hydrogen bonds between side chains in proteins and proton conducting mechanisms. *Biopolymers.* 19:1753–1769.

Kristof, W., and G. Zundel. 1980b. Proton transfer and polarizability of hydrogen bonds in proteins, tyrosine-lysine and glutamic acid-lysine hydrogen bonds—IR investigations. *Biophys. Struct. Mech.* 6:205–225.

Laubinger, W., G. Deckers-Hebestreit, K. Altendorf, and P. Dimroth. 1990. A hybrid adenosinetriphosphatase composed of F_1 of *Escherichia coli* and F_0 of *Propionigenium modestum* is a functional sodium ion pump. *Biochemistry.* 29:5458–5463.

Laubinger, W., and P. Dimroth. 1987a. Characterization of the Na^+-stimulated ATPase of Propionigenium modestum as an enzyme of the F_1F_0 type. *Eur. J. Biochem.* 168:475–480.

Laubinger, W., and P. Dimroth. 1988b. Characterization of the ATP synthase of Propionigenium modestum as a primary sodium pump. *Biochemistry.* 27:7531–7537.

Laubinger, W., and P. Dimroth. 1989c. The sodium ion translocating adenosinetriphosphatase of Propionigenium modestum pumps protons at low sodium ion concentrations. *Biochemistry.* 28:7194–7198.

Lindemann, R., and G. Zundel. 1977a. Polarizability, proton transfer and symmetry of energy surfaces of carboxylic acid-N base hydrogen bonds. *J. Chem. Soc. Faraday Trans. II.* 73:788–803.

Lindemann, R., and G. Zundel. 1977b. Proton transfer in and polarizability of hydrogen bonds coupled with conformational changes in proteins. Infrared Investigation of polyglutamic acid with various N-bases. *Biopolymers.* 16:2407–2418.

Lötscher, H. R., C. de Jong, and R. A. Capaldi. 1984. Modification of the F_0 portion of the H^+ translocating adenosinetriphosphatase complex of *Escherichia coli* by the water-soluble carbodiimide 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide and effect on the proton channeling function. *Biochemistry.* 23:4128–4134.

Maloney, P. C. 1982. 17. Coupling to an energized membrane: role of ion-motive gradients in the transduction of metabolic energy. *In* *Escherichia coli* and *Salmonella typiumrium*: Cellular and Molecular Biology. F. C. Neidhardt, editor. American Society of Microbiology, Washington, D.C. 1:222–243.

Moody, M. F., P. T. Jones, J. A. Carvar, J. Boyd, and I. D. Campbell. 1987. ^1H nuclear magnetic resonance studies of an integral membrane protein: subunit c of the F_1F_0 ATP synthase *J. Mol. Biol.* 193: 759–774.

Norwood, T. J., D. A. Crawford, M. E. Steventon, P. C. Driscoll, and I. D. Campbell. 1992. Heteronuclear ^1H-^{15}N nuclear magnetic resonance studies of the c subunit of the *Escherichia coli* F_1F_0 ATP synthase: assignment and secondary Structure. *Biochemistry.* 31:6285–6290.

Norris, U., P. E. Karp, and A. L. Fimmel. 1992. Mutational analysis of the glycine-rich region of the c subunit of the *Escherichia coli* F_0F_1 ATPase. *J. Bacteriol.* 174:4496–4499.

Olejnik, J., B. Brzezinski, and G. Zundel. 1992. A proton pathway with large proton polarizability and the proton pumping mechanism in bacteriorhodopsin-Fourier Transform Infrared difference spectra of photoproducts of bacteriorhodopsin and of its pentadimethyl analogue. *J. Mol. Struct.* 271:157–173.

Ovchinnikov, G. A., A. Abdulaev, and N. N. Madganov. 1982. Structural basis of proton translocating protein function. *Annu. Rev. Biophys. Bioeng.* 11:445–463.

Paule, C. R., and R. H. Fillingame. 1989. Mutations in three of the putative transmembrane helices of subunits a of the *Escherichia coli* F_1F_0-ATPase disrupt ATP-driven proton translocation. *Arch. Biochem. Biophys.* 274: 270–284.

Rastogi, P. P., W. Kristof, and G. Zundel. 1981. Easily polarizable proton transfer hydrogen bonds between the side chains of histidine and carboxylic groups of glutamic and aspartic acid residues in proteins. *Int. J. Biol. Macromol.* 3:154–158.

Rastogi, P. P., and G. Zundel. 1981. Aspartic acid-aspartate and glutamic acid-glutamate hydrogen bonds having great proton polarizability—IR investigations. *Z. Naturforschg.* 36c:961–963.

Schneider, E., and K. H. Altendorf. 1982. ATP synthetase (F_1F_0) of *Escherichia coli* K12. *Eur. J. Biochem.* 126:149–153

Schneider, E., and K. Altendorf. 1984. Subunit b of the membrane moiety (F_0) of ATP synthase (F_1F_0) from *Escherichia coli* is indispensable for H^+ translocation and binding of the water soluble F_1 moiety. *Proc. Natl. Acad. Sci. USA.* 81:7279–7283.

Schneider, E., and K. Altendorf 1987. Bacterial adenosine 5′-triphosphate synthase (F_1F_0): purification and reconstitution of F_0 complexes and biochemical and functional characterization of their subunits. *Microbiol. Rev.* 51:447–497.

Senior, A. E. 1988. ATP synthesis by oxidative phosphorylation. *Physiol. Rev.* 68:177–231.

Senior, A. E. 1990. The proton-translocating ATPase of *Escherichia coli*. *Annu. Rev. Biophys. Biophys. Chem.* 19:7–41.

Steffens, K., E. Schneider, G. Deckers-Hebestreit, and K. Altendorf. 1987. F_0 portion of *Escherichia coli* ATP synthase. *J. Biol. Chem.* 262: 5866–5869.

Steffens, K., E. Schneider, B. Herkenhoff, R. Schmid, and K. Altendorf. 1984. Chemical modification of the F_0 part of the ATP synthase (F_1F_0) from *Escherichia coli*. *Eur. J. Biochem.* 138:617–622.

Vik, S. B., B. D. Cain, K. T. Chun, and R. D. Simoni. 1988. Mutagenesis of the a subunit of the F_1F_0-ATPase from *Escherichia coli*. *J. Biol. Chem.* 263:6599–6605.

Wachter, E., R. Schmid, G. Deckers, G., and K. Altendorf. 1980. Amino acid replacement in dicyclohexylcarbodiimide-reactive proteins from mutant strains of *Escherichia coli* defective in the energy-transducing ATPase complex. *FEBS Lett.* 113:265–270.

Weidemann, E. G., and G. Zundel. 1970. Field-dependent mechanism of anomalous proton conductivity and the polarizability of hydrogen bonds with tunneling protons. *Z. Naturforschg.* 25a:627–637.

Zundel, G. 1969. Monography. Hydration and intermolecular interaction. Academic Press, New York.

Zundel, G. 1976. Easily polarizable hydrogen bonds—their interactions with the environment IR continuum and anomalous large proton conductivity. *In* The Hydrogen Bond—Recent Developments in Theory and Experiments, Vol. 2. P. Schuster, G. Zundel, and C. Sandorfy, editors. North Holland, Amsterdam. 686–766.

Zundel, G. 1988. Hydrogen-bonded systems as proton wires formed by side chains of proteins and by side chains and phosphates. *In* Transport through Membranes, Carriers, Channels and Pumps. A. Pullman,

A. Jortner, and B. Pullman, editors. Kluver Academic Publishing, Dortrecht. 409–420.

Zundel, G. 1992a. Proton polarizability and proton transfer processes in hydrogen bonds and cation polarizabilities of other cation bonds—their importance to understand molecular processes in electrochemistry and biology. *In* Trends in Physical Chemistry, Vol. 3. J. Menon, editor. Publ. Research Trends, Trivandrum. 129–156.

Zundel, G. 1992b. Hydrogen-bonded systems with large proton polarizability due to collective proton motion as pathways of protons in biological systems. *In* Electron and Proton Transfer in Chemistry and Biology. A. Müller, H. Ratajczak, W. Junge, and E. Diemann, editors. Elsevier, Amsterdam. 313–327.

Zundel, G., and B. Brzezinski. 1992. Proton polarizability of hydrogen-bonded systems due to collective proton motion—with a remark to the proton pathways in bacteriorhodopsin. *In* Proton Transfer in Hydrogen-Bonded Systems. T. Bountis, editor. Plenum Press, New York. 153–166.

Zundel, G., B. Brzezinski, and J. Olejnik. 1993. On hydrogen and deuterium bonds as well as on Li^+, Na^+ and Be^{2+} bonds—IR-continua and cation polarizabilities. *J. Mol. Struct.* 300:573–592.-