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DCCD-sensitive proton permeability of bacterial photosynthetic membranes. Cross-reconstitution studies with purified bovine heart F_o subunits

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Abstract

The DCCD-sensitive proton permeability of chromatophores, from a green strain of *Rhodobacter Capsulatus* is potentiometrically detected following the proton release induced by a transmembrane diffusion potential imposed by a valinomycin-mediated potassium influx with a procedure already used for bovine heart submitochondrial particles (ESMP) and vesicles from *Escherichia coli* (Zanotti et al. (1994) Eur. J. Biochem. 222, 733–741). In the photosynthetic system, addition of increasing amounts of DCCD inhibits, with a similar titre, both proton permeability and MgATP-dependent ATPase activity as detected in the dark. The titre for 50% inhibition coincides with that obtained measuring proton permeability and ATP hydrolysis in ESMP. Upon removal of F_1, the passive proton permeability is much less sensitive to DCCD in chromatophores than in USMP, suggesting that in chromatophores the F_1-F_o interaction shapes the DCCD-sensitive proton conducting pathway. Addition of the purified mitochondrial F_oI-PVP and oligomycin sensitivity-conferring (OSCP) proteins to the F_1 stripped chromatophores restored the sensitivity of proton permeability to DCCD detected in untreated chromatophores. Analysis of the binding of ^{14}C[DCCD] on F_1 stripped chromatophores shows that the increase of DCCD sensitivity of proton permeability, caused by addition of mitochondrial F_o proteins, is related to an increase of the binding of the inhibitor to subunit c of F_o sector of ATP synthase complex.

Keywords: N,N′-Dicyclohexylcarbodiimide; ATP synthase; F_oF_1 complex; Chromatophore

1. Introduction

The H^+-translocating sector (F_o) of the F_1F_o-ATP synthase of coupling membranes has changed its polypeptide composition during evolution [1]. *Escherichia coli* F_o is composed of three subunits: a, b, c [2]; in photosynthetic bacteria [3] and in chloroplasts [4] the F_o is composed of four different subunits; in yeast mitochondria of six subunits [5] and in mammalian mitochondria of nine different subunits [6]. All the F_o sectors contain the DCCD binding proteolipid (subunit c in *E. coli* [2]) which is essential for the proton conducting pathway of the enzyme [7]. The function of the additional polypeptides is not completely defined (see Ref. [8] for review); they could contribute to the formation of the transmembrane proton channel and/or to the coupling of proton translocation by F_o to the catalytic functions of F_1. Recently it has been shown that, in mitochondria, the F_o subunit of the highest molecular weight (denominated F_oI-PVP from the first N-terminal residues) is an essential component of the specific proton channel [9–13]. It has been suggested that this protein is analogous to bacterial subunit b on the basis of the distribution of hydrophobic and polar residues [14]. F_o from chloroplasts and from photosynthetic bacteria comprises two different subunits with a general distribution of hydrophobic and polar residues related to those of *E. coli* b subunit (see Ref. [15]). They are known as subunit b and b′

Abbreviations: DCCD, N,N′-dicyclohexylcarbodiimide; ESMP, submitochondrial particles prepared in the presence of EDTA; USMP, F_1-stripped submitochondrial particles; BChl, bacteriochlorophyll; OSCP, oligomycin-sensitivity conferring protein; F_oI-PVP, protein subunit of mitochondrial F_o; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; Val., valinomycin; Enzymes: H^+-ATPase (EC 3.6.1.34); Pyruvate kinase (E.C.2.7.1.40); Lactate dehydrogenase (E.C.1.1.1.27).

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in photosynthetic bacteria and as subunits II and IV in chloroplasts [16]. In a recent paper [17] we have shown that the combined addition of mitochondrial F_oI-PVP and OSCP to F_1 depleted *E. coli* membranes increased both the sensitivity of passive proton conduction by the membrane to DCCD and the affinity of subunit c for the binding of [^{14}C]DCCD. In this paper, we show that the removing of F_1 in photosynthetic membranes severely hampers the DCCD sensitivity of the proton permeability. This can be restored by addition of the mitochondrial F_o proteins (F_oI-PVP and OSCP) to F_1-depleted chromatophores. Considering the high homology existing between OSCP and the δ subunit of photosynthetic bacteria (and that F_oI-PVP is not homologous to any protein of the photosynthetic ATPase complex), our data suggest a possible role of the δ subunit in gating the proton conducting pathway in chromatophores.

2. Materials and methods

Acrylamide, N,N′-methylenebisacrylamide, sodium dodecyl sulfate (SDS) were from Bio Rad; valinomycin was from Sigma and [^{14}C]DCCD (50Ci/mol) was from Amersham. All other chemicals were of analytical grade.

2.1. Preparative procedures

Chromatophores were prepared from cells of *Rhodobacter Capsulatus*, strain ga, as described in [18]. F_1-deprived chromatophores were prepared by sonication in the cold for 90 s (three bursts of 30 s) of EDTA (4 mM) treated chromatophores (2–3 mM Bacteriochlorophyll) with a Branson sonifier B15, equipped with a microtip vibrating at maximal amplitude as reported in Ref. [19]. EDTA-treated submitochondrial particles were obtained by sonication of beef heart mitochondria in the presence of EDTA at pH 8.5 [20]. F_1-stripped everted submitochondrial particles (USMP) were obtained as described [21]. The bovine F_o subunits OSCP and F_oI-PVP protein were isolated by preparative gel electrophoresis [22]. After purification, the proteins were solubilized in 50 mM Tris-HCl (pH 8.8), 0.1% SDS, 50% glycerol at a protein concentration of approx. 0.1 μg/μl.

2.2. Reconstitution experiments

F_1 depleted chromatophores (1 mg membrane protein/ml) were incubated for 10 min in 0.25 M sucrose, 10 mM Tris-acetate (pH 7.5), 1 mM EDTA, 6 mM $MgCl_2$, with the purified F_o subunits F_oI-PVP (4 μg/mg membrane protein) and OSCP (4 μg/mg membrane protein). The incubation was stopped by centrifugation (20 min at 105 000 × *g*).

2.3. Electrophoresis and measurement of passive proton permeability

SDS/PAGE was carried out as described previously [21,22]. Proton permeability was analysed by following potentiometrically the H^+ release from the particles induced by diffusion potential (positive inside) imposed by valinomycin-mediated K^+ influx [22]. To minimize the electrode response time, a low-resistance and low-capacitance glass electrode connected to a MOS FET Electrometer Amplifier, model 604 Keithley, was used. With this system the pH in stirred suspension can be measured with resolutions of 0.01 pH unit and overall rise time (10–90% change) of less than 0.5 s [23].

2.4. Measurement of the [^{14}C]DCCD binding

F_1 depleted chromatophores at the concentration of 1 mg protein/ml, were incubated with [^{14}C]DCCD for 30 min at room temperature in the same mixture (pH 7.5) used for reconstitution experiments (see above). Separate aliquots were used to measure H^+ conduction, [^{14}C]DCCD binding and to carry out reconstitution experiments. For all the experiments, one batch of [^{14}C]DCCD was used. For measurement of [^{14}C]DCCD total binding to the membrane, 0.3 mg of membrane protein were incubated at 0°C for 20 min with 90% ice-cold acetone (v/v) to remove free [^{14}C]DCCD and [^{14}C]DCCD bound to membrane phospholipids. The acetone-washed membrane proteins were centrifuged at 20 000 × *g* for 20 min. Acetone-washed pellets, containing [^{14}C]DCCD-labelled membrane proteins, were suspended in 0.2 ml of 12% perchloric acid followed by sonication in a water-bath heat/ultrasonic system. The samples, boiled for 3 min, were left overnight at room temperature. After centrifugation at 20 000 × *g* for 20 min, the radioactivity in the supernatant was determined by liquid-scintillation counting. Binding of [^{14}C]DCCD to subunit c was measured as follows: 0.3 mg of membrane proteins, incubated with [^{14}C]DCCD as reported above, were treated with 90% ice-cold acetone (v/v) and incubated at 0°C for 20 min. After centrifugation at 20 000 × *g* for 20 min, acetone-washed pellets were dissolved in a lysis buffer containing 2.3% (w/v) SDS, 10 mM Tris/HCl, 10% (w/v) glycerol, 5% (v/v) 2-mercaptoethanol (pH 6.8). The samples were then boiled for 3 min and subjected to SDS/PAGE. SDS/PAGE (linear gradient from 14% to 20% of polyacrylamide) was performed on slab gels (1.5 × 140 × 140 mm) [9]. After electrophoresis, proteins were stained in a solution containing 0.25% Coomassie brillant blue R-250 in 45% methanol (v/v), 8.2% acetic acid (v/v). Each strip of the gel, containing electrophoresed membrane proteins, was sliced into pieces. Slices were treated with 1-ml aliquots of Beckman Tissue Solubilizer 450 at 60°C for 12 h and the radioactivity was determined by liquid-scintillation counting [13,22].

3. Results

The kinetics of the H^+ release, induced by transmembrane diffusion potential (positive inside) imposed on the membranes by valinomycin-mediated potassium influx in ESMP (trace a) and chromatophores (trace c), are shown in Fig. 1. In both membranes the process is inhibited, to a similar extent, by DCCD (traces b and d), a potent inhibitor of proton conduction in F_0. It is known that this molecule modifies, in bacterial and mitochondrial membranes, an acidic residue located in the carboxyl-terminal hydrophobic transmembrane α-helix of subunit c of the F_0 sector [24].

Increasing additions of DCCD to both membrane types promote inhibition of the ATP hydrolase activity and proton permeability with a very similar titre. This indicates that DCCD binding hampers F_1-F_0 functional interactions in ESMP and also in chromatophores.

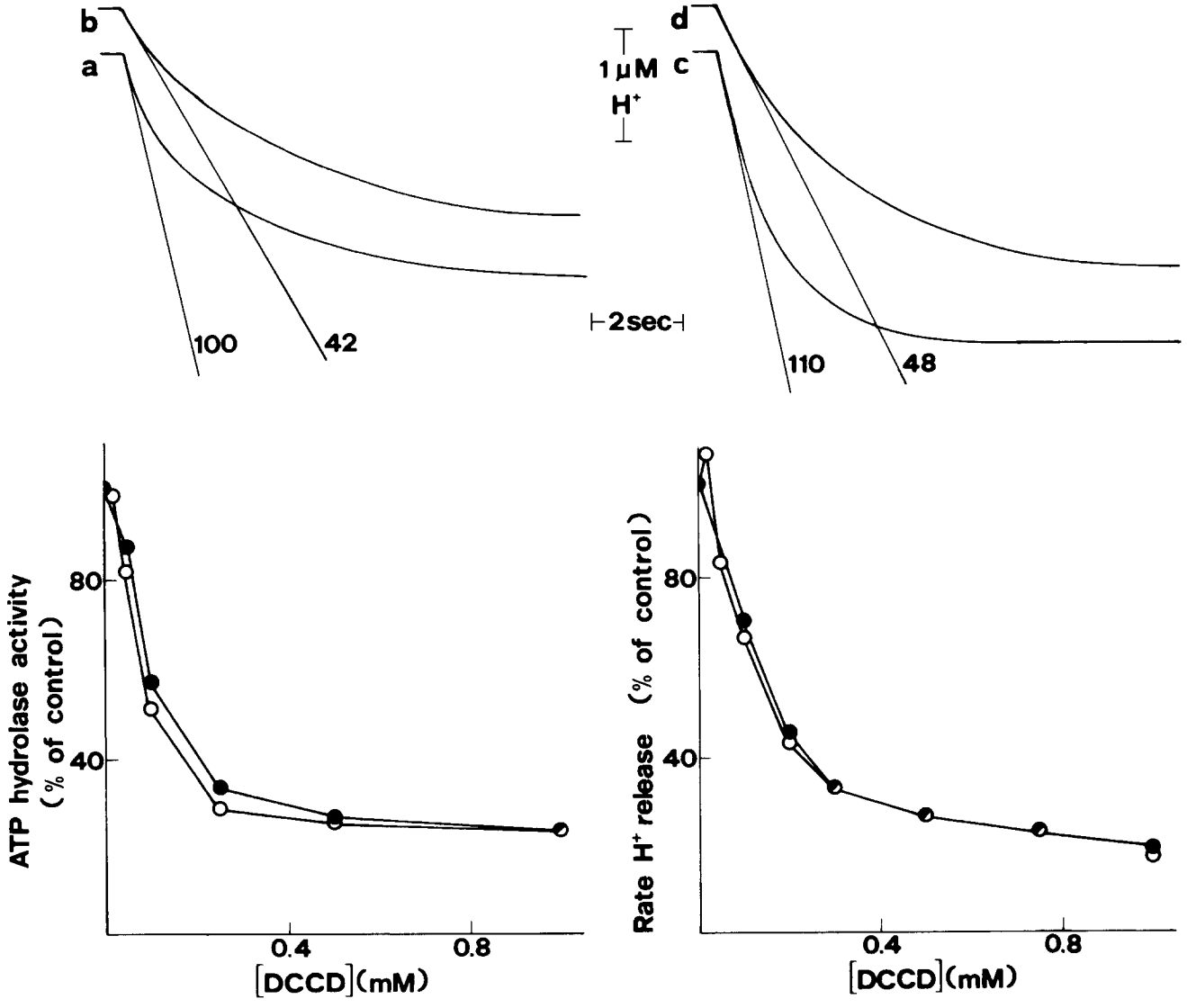

Fig. 1. H^+ permeability by ESMP and by chromatophores. Titration of DCCD inhibition of ATP hydrolase activity and H^+ permeability. ESMP (1 mg protein/ml) or chromatophores (1 mg protein/ml corresponding to about 0.1 μmol BChl/ml) were incubated for 2 min in 0.15 M KCl at 27°C, then 2 μg valinomycin/mg particle protein were added and proton release was followed potentiometrically as described under Section 2. Traces: (a and c) ESMP and chromatophores respectively; (b and d) ESMP and chromatophores respectively, incubated 30 min with 0.25 mM DCCD. Initial rates are expressed as ng ions H^+ min^{-1} mg particle $protein^{-1}$. The bottom of the figure shows the titration of the inhibitory effect of DCCD on ATP hydrolase activity (left panel) and H^+ permeability (right panel). ATP hydrolase activity was determined at 27°C in the presence of added pyruvate kinase, phosphoenolpyruvate and lactate dehydrogenase by following NADH oxidation spectrophotometrically as described in Refs. [41,42]. Symbols: open circles, ESMP; closed circles, chromatophores. The ATP hydrolase activity and the proton permeability are expressed as % of the control values which were: ATP hydrolase activity: 2.34 μmol ATP hydrolyzed min^{-1} mg ESMP $protein^{-1}$ and 0.7 μmol ATP hydrolyzed min^{-1} μmol $BChl^{-1}$ in chromatophores. H^+ permeability: 98.6 ng ions H^+ min^{-1} mg ESMP $protein^{-1}$ and 110 ng ions H^+ min^{-1} mg chromatophores. The results reported in the figure were the mean of four different experiments whose standard error was less than ± 10%.

After removal of F_1, the passive proton permeability of USMP, which still contain F_0-stalk proteins like OSCP and F_0I-PVP [25], is still sensitive to DCCD (Fig. 2). F_1 stripped chromatophores show a lower sensitivity of passive proton permeability to the inhibition by DCCD than USMP (Fig. 2). Addition of purified mitochondrial F_0I-PVP protein to F_1 stripped chromatophores causes some increase of the sensitivity of passive proton permeability to DCCD (Fig. 2). A sensitivity similar to that noticed in USMP is detected only after reconstitution of F_1-depleted chromatophores with F_0I-PVP plus OSCP. Addition of OSCP alone does not promote any significant increase of the DCCD sensitivity as compared to stripped chromatophores (data not shown), indicating that the interplay of the two mitochondrial proteins is essential to completely restore the DCCD inhibition of passive proton permeability of F_1-depleted chromatophores.

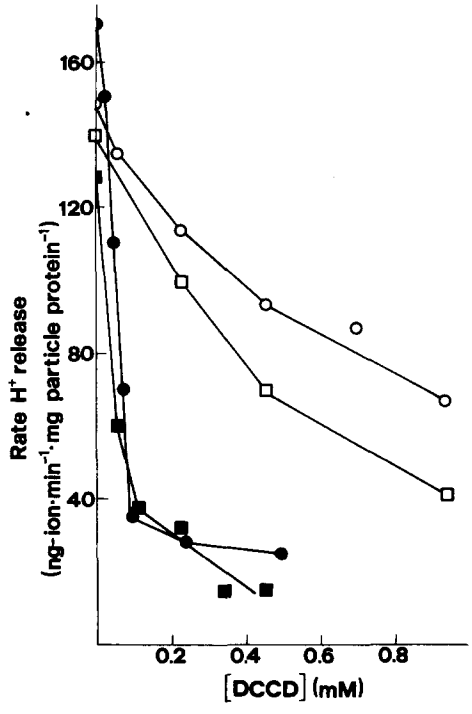

Fig. 2. Titration of the inhibitory effect of DCCD on the H^+ permeability in USMP and in F_1-deprived chromatophores. Incubation with DCCD (at the concentration reported in abscissa) was carried out for 30 min at room temperature in the same buffer used for reconstitution experiments (see Section 2). After centrifugation 1 mg protein/ml of USMP or of F_1-deprived chromatophores was used for measurement of proton conduction as described in the legend to Fig. 1. Symbols: (●) USMP; (○) F_1-deprived chromatophores; (□) F_1 deprived chromatophores preincubated with F_0I-PVP (4 μg/mg protein) and (■) F_1-deprived chromatophores preincubated with F_0I-PVP (4 μg/mg particle protein) + OSCP (4μg/mg particle protein) for 10 min at room temperature before the addition of DCCD at the concentrations reported in the figure. Separate controls showed that the addition of 1 mM DCCD to F_1-deprived chromatophores incubated in the same buffer used for reconstitution experiments containing 100 μl of the solution used for the electroelution of proteins (see Section 2), gave the same inhibition observed in the absence of the added solution (data not shown). For other experimental details see Section 2. The results reported in the figure were the mean of three different experiments whose standard error was less than ±10%.

Norling et al. [26], using Western blotting technique, have calculated that about 20% of the total protein content of chromatophore membranes correspond to F_1F_0 complex with a molecular mass of 562 kDa. Using this value we can calculate a content of 0.36 nmol of F_1F_0 complex per mg of chromatophore proteins, which correspond to about $2 \cdot 10^{14}$ molecules of F_1F_0 per mg of chromatophore proteins. Our data show that complete recovering of DCCD sensitivity of F_1-depleted chromatophores, can be promoted by addition per mg of chromatophore proteins of 4 μg of F_0I-PVP subunit plus 4 μg of OSCP subunit which, on the basis of the molecular mass of 24670 Da and 20936 Da respectively [27], correspond to a number of mitochondrial subunit molecules of the same order of the content of F_1F_0 molecules in chromatophores.

Analysis of the binding of radioactive [^{14}C]DCCD to the F_1-stripped chromatophores showed that the addition of purified mitochondrial F_0I-PVP protein plus OSCP increased the affinity for the binding of [^{14}C]DCCD to chromatophores (not shown).

A typical experiment on the analysis of [^{14}C]DCCD binding to F_1-stripped chromatophore protein fractions separated by SDS/PAGE is shown in Fig. 3a. The binding profile is characterized by two peaks: the principal one is associated with a protein in the region of apparent molecular weight of 8000 Da and is likely to correspond to subunit c [3]; a small one is centered at a molecular weight of about 32000 Da and could represent a polymeric form of the DCCD binding protein, as observed in USMP [13,28]. Addition of mitochondrial F_0I-PVP protein and OSCP to the F_1-stripped chromatophores increased the affinity for the binding of [^{14}C]DCCD to the DCCD binding protein (subunit c) (Fig. 3b).

This effect of mitochondrial F_0 subunits is apparently associated to changes in the conformation of the proton channel as indicated by the fact that, in their presence, much less DCCD bound to subunit c is required for inhibition of the passive proton permeability (Fig. 3b).

4. Discussion

It is generally accepted (see Refs. [1,2,7] for review) that the hydrophobic DCCD binding subunit c of the ATP synthase complex is the main component of the proton conducting pathway in the membrane sector (F_0). In the bacterial enzyme from *E. coli* the presence of the other two F_0 subunits, a and b, is required for the proper assembly and function of F_0 [29].

Recently, comparative analysis between *E. coli* and bovine heart mitochondrial enzyme indicated that, in the eukaryotic enzyme, the passive proton conduction, through the F_0 sector, is much more sensitive to oligomycin and DCCD than in the bacterial system [17]. In the present

paper we show that in F_1 containing chromatophores from photosynthetic bacteria, proton permeability and hydrolytic activity in the dark are inhibited by DCCD with a titre similar to that necessary to obtain 50% inhibition of the same processes in ESMP, suggesting that a similar F_1F_o interaction is at the basis of the proton conducting pathway

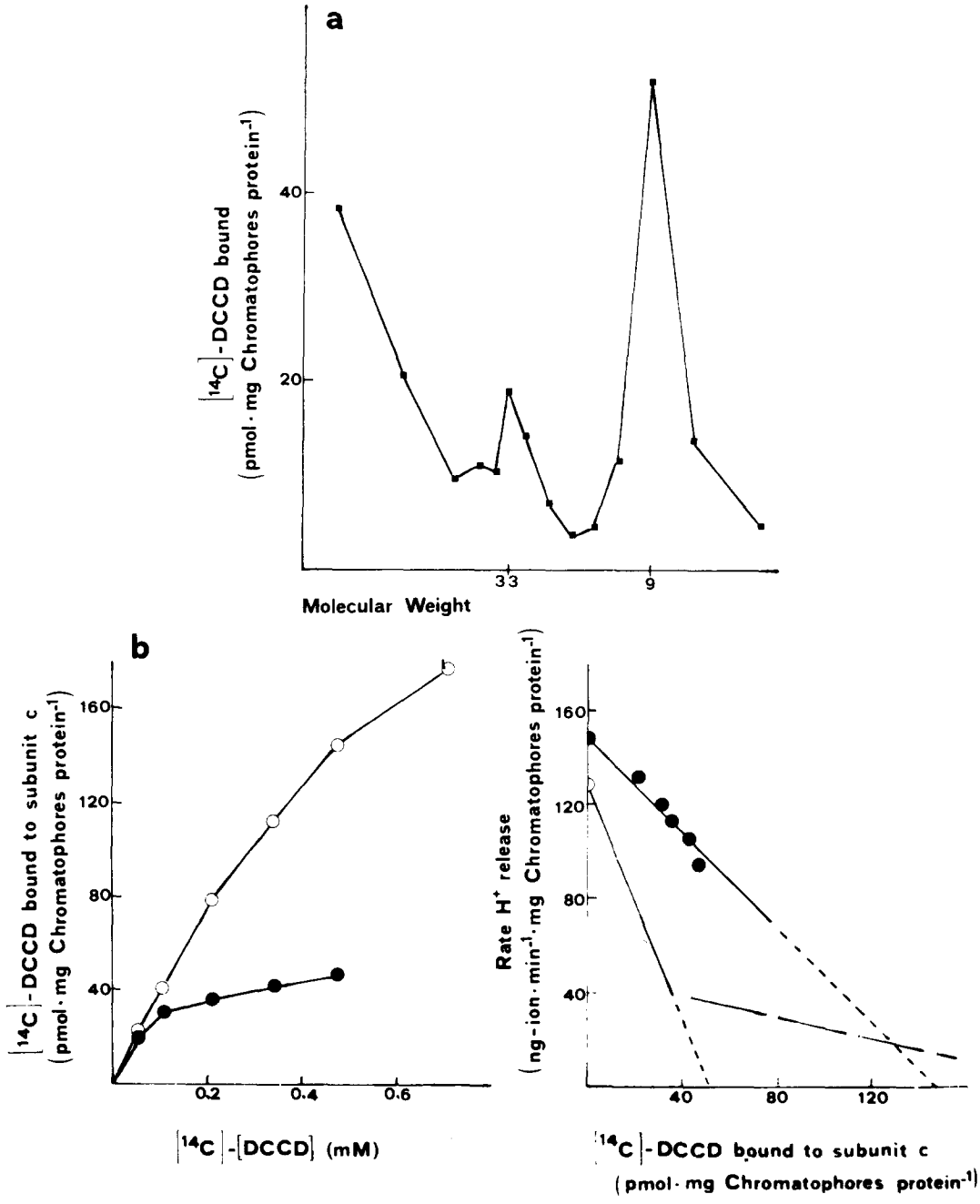

Fig. 3. Binding of [^{14}C]DCCD to subunit c and relationship with the inhibition of H^+ permeability. Effect of F_oI-PVP plus OSCP. (a) Binding of [^{14}C]DCCD on SDS/PAGE of F_1-deprived chromatophores incubated 30 min at room temperature in the same buffer used for reconstitution experiments (see Section 2), with 0.5 mM [^{14}C]DCCD. After incubation, 300 μg of particle protein were subjected to SDS/PAGE and the radioactivity, measured on the slices of the gel containing the proteins bands identified by Coomassie Brilliant Blue R 250, was converted to pmol DCCD/mg particle protein. (b) Titration of binding of [^{14}C]DCCD on F_o subunit c in F_1-deprived chromatophores (left panel) and relationship with the inhibition of H^+ permeability (right panel). The radioactivity measured on subunit c band was referred to 1 mg of F_1-deprived chromatophores protein. Symbols: (●) F_1-deprived chromatophores; (○) F_1-deprived chromatophores incubated for 10 min with F_oI-PVP (4 μg/mg particle protein) + OSCP (4 μg/mg particle protein) before treatment with [^{14}C]DCCD. The results reported in the figure were the mean of three different experiments whose standard error was less than ±10%.

in the two systems. After removal of F_1 the DCCD sensitivity of proton permeability of chromatophores is severely hampered.

Previous observations based on proteolytic cleavage and reconstitution experiments showed that, in the eukaryotic enzyme, the mitochondrial F_oI-PVP protein [9–13] is involved in proton conduction and in the sensitivity to oligomycin [9–11] and DCCD [12,13]. Mutations in the hydrophobic domain of subunit 4 (which is homologous to the *E. coli* b subunit [30] and to F_oI-PVP protein of beef heart mitochondria [31]) of yeast mitochondria caused oligomycin resistance of proton conduction by F_o [32]. These observations (cf. Ref. [17]) suggest that the mitochondrial F_oI-PVP protein [33–35] and its homologous subunit 4 of yeast F_o [32] contribute to the organization of oligomycin and DCCD sensitive proton channel in F_o.

In the present paper, addition to F_1-deprived chromatophores of F_oI-PVP protein alone partially restored the sensitivity of passive H^+ permeability to DCCD. Total restoration of the DCCD sensitivity was observed only when OSCP was added together with F_oI-PVP protein to the F_1-deprived chromatophores. These observations indicate that either F_1-F_o interaction or interaction between the bovine binary complex (F_oI-PVP + OSCP) and the bacterial F_o promotes a proton conducting pathway that can be modulated by DCCD binding with the same titre as F_1 containing chromatophores.

The detected increase of DCCD binding to subunit c, in the presence of the bovine binary complex, suggests a possible structural interaction between the mitochondrial proteins and the c subunit of F_o from photosynthetic bacteria.

A search for homology using multiple sequence alignment (with the HSSP program [36] available at the EMBL server in Heidelberg) indicates that F_oI-PVP is not homologous to any protein of the ATPase complex in photosynthetic bacteria, in particular the alignment of the sequence of bovine F_oI-PVP protein with those of subunit b and b′ of *Rhodobacter Rubrum* showed an homology less than 20%. OSCP is highly homologous to the δ subunit of chloroplasts (see also Ref. [37]) and of photosynthetic bacteria.

Based on the notion that a high degree of sequential homology corresponds to a high degree of structural homology [38], it may be suggested that the folding of OSCP (as present in the binary complex with F_oI-PVP) mimic the folding of the δ subunit essential to interact with the membrane sector. It was suggested before that in chloroplasts the δ subunit, located at the interfaces between F_1 and F_o, plays a key role in the coupling mechanism between the reaction cycle of the ATPase and the transmembrane proton flow in chloroplasts [39].

In addition it has been recently observed that the interaction between δ subunit and F_o in *E. coli* during synthesis and assembly produces a significant change in the proton permeability of the F_o proton channel [40].

Mitochondrial OSCP [13,16,17] and F_oI-PVP protein [13,16,17,25,33,34] are both involved in the formation of the 'stalk' of the mitochondrial enzyme. A direct interaction of OSCP and F_oI-PVP protein has been demonstrated by Walker and Collison [16] who describe the formation in vitro of a binary complex between the carboxyl terminal region of the F_oI-PVP protein (amino acids from 79-214) and OSCP. On the other hand it has been shown that in the mitochondrial F_o, the DCCD-sensitive proton conduction depends on the interaction of the F_oI-PVP protein with subunit c [13].

On the basis of the present experimental results, we conclude that the dimer OSCP/F_oI-PVP interacts with subunit c of the photosynthetic bacterial F_o in order to mimic the native functional F_1F_o interaction in chromatophores.

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References

[1] Cox, G.B., Devenish, R.J., Gibson, F., Howett, S.M. and Nagley, P. (1992) in Molecular Mechanisms in Bioenergetics (L. Ernster, ed.), Elsevier, Amsterdam, pp. 283–315.
[2] Fillingame, R. (1990) in The Bacteria: A Treatise on Structure and Function (T.A. Krulwich, ed.), Academic Press, New York, Vol. 12, pp. 345–391.
[3] Gabellini, N., Gao, Z. Eckerskorn, C., Lattspeich, F. and Oesterhelt, D. (1988) Biochim. Biophys. Acta 934, 227–234.
[4] Boekema, E.J., Fromme, P. and Graber, P. (1988) Ber. Bunsenges Phys. Chem. 92, 1031–1036.
[5] Grandier-Vazeille, X., Ouhabi, R. and Guerin, M. (1994) Eur. J. Biochem. 223, 521–528.
[6] Collinson, I.R., Runswick, M.J., Buckanam, S.K., Fearnley, I.M., Skehel, J.M., Van Raaij, M.J., Griffiths, D.E. and Walker, J.E. (1994) Biochemistry 33, 7971–7978.
[7] Hoppe, J. and Sebald, W. (1984) Biochim. Biophys. Acta 768, 1–27.
[8] Papa, S., Guerrieri, F., Zanotti, F., Altendorf, K. and Deckers-Hebestreit, G. (1992) in Adenine Nucleotides in Cellular Energy Transfer and Signal Transduction (S. Papa, A. Azzi and J.M. Tager, eds.), Birkhauser, Basel, pp. 13–32.
[9] Houstek, J. Kopecky, J., Zanotti, F., Guerrieri, F., Jirillo, E., Capozza, G. and Papa, S. (1988) Eur. J. Biochem. 173, 1–8.
[10] Zanotti, F., Guerrieri, F., Capozza, G., Houstek, J., Ronchi, S. and Papa, S. (1988) FEBS Lett. 237, 9–14.
[11] Papa, S., Guerrieri, F., Zanotti, F., Houstek, J., Capozza, G. and Ronchi, S. (1989) FEBS Lett. 249, 62–66.
[12] Guerrieri, F., Capozza, G., Houstek, J., Zanotti, F., Colaianni, G., Jirillo, E. and Papa, S. (1989) FEBS Lett. 250, 60–66.
[13] Guerrieri, F., Zanotti, F., Capozza, G., Colaianni, G., Ronchi, S. and Papa, S. (1991) Biochim. Biophys. Acta 1059, 348–354.
[14] Walker, J.E., Lutter, R., Dupuis, A. and Runswick, M.J. (1991) Biochemistry 30, 5369–5378.
[15] Van Walraven, H.S., Lutter, R. and Walker, J.E. (1993) Biochem. J. 294, 239–251.
[16] Walker, J.E. and Collison, I.R. (1994) FEBS Lett. 346, 39–43.

[17] Zanotti, F., Guerrieri, F., Deckers-Hebestreit, G., Fiermonte, M., Altendorf, K. and Papa, S. (1994) Eur. J. Biochem. 222, 733–741.
[18] Baccarini-Melandri, A. and Melandri, B.A. (1971) Methods in Enzymol. 23, 556–561.
[19] Casadio, R. and Wagner, R. (1985) Biochim. Biophys. Acta 809, 215–227.
[20] Lee, C.P. and Ernster, L. (1968) Eur J. Biochem. 3, 391–400.
[21] Guerrieri, F., Kopecky, J. and Zanotti, F. (1989) in Organelles of Eukaryotic Cells: Molecular Structure and Interactions (J.M. Tager, F. Guerrieri, A. Azzi and S. Papa, eds.), Plenum, New York, pp. 197–208.
[22] Zanotti, F., Guerrieri, F., Che, Y.W., Scarfò, R. and Papa, S. (1987) Eur. J. Biochem. 164, 517–523.
[23] Papa, S., Guerrieri, F. and Rossi, L. (1978) in Methods in Enzymology, vol. LV, pp. 614–627.
[24] Linnet, P.E. and Beechey, R.B. (1979) Methods in Enzymol. 55, 472–518.
[25] Papa, S., Guerrieri, F., Zanotti, F., Fiermonte, M., Capozza, G. and Jirillo, E. (1990) FEBS Lett. 272, 117–120.
[26] Norling, B., Strid, A., Tourikas, C. and Nyren, P. (1989) Eur. J. Biochem. 186, 333–337.
[27] Collinson, I.R., Fearnley, I.M., Skehel, J.M., Runswick, M.J. and Walker, J.E. (1994) Biochem. J. 303, 639–645.
[28] Kopecky, J., Guerrieri, F. and Papa, S. (1983) Eur. J. Biochem. 131, 17–24.
[29] Schneider, E. and Altendorf, K. (1985) EMBO J. 4, 515–518.
[30] Velours, J., Durrens, P., Aigle, M. and Guerin, B. (1988) Eur. J. Biochem. 170, 637–642.
[31] Walker, J.E., Runswick, M.J. and Poulter, L. (1987) J. Mol. Biochem. 197, 89–100.
[32] Razaka-Jolly, D., Rigoulet, M., Guerin, B. and Velours, J. (1994) Biochemistry 33, 9684–9691.
[33] Guerrieri, F., Zanotti, F., Capozza, G., Colaianni, G., Houstek, J. and Papa, S. (1990) in Structure, Function and Biogenesis of Energy Transfer Systems (E. Quagliariello, S. Papa, F. Palmieri and C. Saccone, eds.), Elsevier, Amsterdam, pp. 77–82.
[34] Papa, S. (1989) in Organelles of Eukaryotic Cells: Molecular Structure and Interactions (J.M. Tager, A. Azzi, S. Papa and F. Guerrieri, eds.), pp. 9–26, Plenum Press, New York.
[35] Guerrieri, F., Zanotti, F., Capozza, G. and Papa, S. (1995) in Progress in Cell Research – Thirty Years of Progress in Mitochondrial Bioenergetics and Molecular Biology (F. Palmieri, S. Papa, C. Saccone and M.N. Gadaleta, eds.) Elsevier, Amsterdam, pp. 31–38.
[36] Sander, C. and Schneider, R. (1991) Proteins 9, 56–68.
[37] Engelbrecht, S., Reed, J., Penin, F., Gautheron, D.C. and Junge, W. (1991) Z. Naturforsch. 46c, 759–764.
[38] May, A.C.W. and Blundell, T.L. (1994) Curr. Opin. Biotechnol. 5, 355–360.
[39] Engelbrecht, S. and Junge, W. (1990) Biochim. Biophys. Acta 1015, 379–390.
[40] Monticello, R.A. and Brusilow, W.S.A. (1994) J. Bacteriol. 176, 1383–1389.
[41] Pansini, A., Guerrieri, F. and Papa, S. (1978) Eur. J. Biochem. 92, 545–551.
[42] Fregni, V. and Casadio, R. (1993) Biochim. Biophys. Acta 1143, 215–222.