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DIFFERENTIATION OF DICYCLOHEXYLCARBODIIMIDE REACTIVE SITES OF THE ATPase COMPLEX IN BOVINE HEART MITOCHONDRIA

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Summary

1. In isolated bovine heart mitochondria, the ^{14}C-labelled dicyclohexylcarbodiimide (DCCD) induced inhibition of the ATPase activity is accompanied by labelling of three polypeptides of M_r 9000, 16 000 and 33 000. Of these, only the 9000 polypeptide reacts with [^{14}C]DCCD proportionally to the inhibitory effect, being saturated when the enzyme is maximally inhibited.

2. The 9000 and 16 000 polypeptides are extracted by neutral chloroform/methanol (2 : 1, v/v) while the 33 000 polypeptide remains in the non-extractable residue. No disaggregation of the polypeptides takes place during the extraction.

3. In the ATPase complex immunoprecipitated with antibody against F_1, the 9000 and 16 000 polypeptides are present, but the 33 000 polypeptide is absent.

4. The results obtained indicate that the 33 000 polypeptide is not a component of the ATPase complex. As far as F_0 is concerned, two types of the binding sites for DCCD were demonstrated, corresponding to the 9000 and 16 000 polypeptides. Their existence is explained by a non-random arrangement among individual monomers of the DCCD-binding protein.

Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; F_1, catalytic part of mitochondrial ATPase; F_0, membrane-bound moiety of mitochondrial ATPase; EDTA, ethylenediamine tetraacetic acid; SDS, sodium dodecyl sulphate; PPO, 2,5-diphenyloxazole.

Introduction

Dicyclohexylcarbodiimide (DCCD) irreversibly inhibits both the synthetic and hydrolytic functions of the mitochondrial ATPase [1]. Similarly to oligomycin it blocks the H^+ translocation across the enzyme [2,3]. At low concentrations DCCD reacts preferentially with the membrane sector (F_0) of ATPase [4—6] and this specific binding can be distinguished from the nonspecific binding to other membrane proteins and phospholipids [5—9]. The DCCD-binding moiety of ATPase was isolated and the purified protein was designated as the DCCD-binding protein [5,6], identical with the subunit 9 of the ATPase complex [10,11]. It was found that in a great variety of energy transducing membranes it exhibits rather homologous properties, i.e. unusual hydrophobicity, M_r of approximately 8000 and an analogous amino acid composition (for review see Ref. 12). Finally, it was shown that the isolated protein enhances the proton conductance of phospholipid membranes, indicating that the protein itself can form a H^+-channel [13,14].

The experimental data on the interaction of DCCD with mitochondrial ATPase are, however, still contraversial. It was demonstrated that in the isolated ATPase complex either a single or two polypeptides are covalently labelled with [^{14}C]DCCD [6,8,15—19]. In the mitochondrial membrane [^{14}C]DCCD reacted, in parallel to the inhibition of ATPase, with up to three polypeptides of M_r of 7000—45 000 [5,16,20,21]. In accordance with the stoichiometry of several monomers of the DCCD-binding protein per F_0 [13,20,22,23], the multiple DCCD-reactive polypeptides were regarded as oligomers of the DCCD-binding protein [16,17,20]. Their existence in the presence of SDS was awarded to the account of strong hydrophobic interactions [20]. Finally, the above multiple DCCD-reactive polypeptides were shown to possess a different reactivity towards the inhibitor [16], however, their specific function is still not clear.

It was the aim of the present study to distinguish between individual DCCD-reactive polypeptides of the mitochondrial membrane of bovine heart in order to specify their respective role in inhibition of the catalytic part of ATPase.

Materials and Methods

Preparation of mitochondria. Bovine heart mitochondria were isolated according to procedure 3 of Smith [24] and rat liver mitochondria according to Schneider and Hageboom [25]. Prior to further use, bovine heart mitochondria were frozen-thawed three times and sedimented by centrifugation for 15 min at 30 000 × *g*.

Incubation with radioactive DCCD. Mitochondria (1—2.5 mg protein/ml) were incubated with [^{14}C]DCCD in a medium containing 0.25 M sucrose, 10 mM Tris-HCl (pH 7.4), 1.0 mM EDTA (medium A) for 4 h at 28°C. To measure the binding, 0.1-ml aliquots were mixed with 0.9 ml acetone and centrifuged for 1 min at 12 000 × *g*. The pellet was resuspended in 0.9 ml of 2.5% trichloroacetic acid and centrifuged for 1 min at 12 000 × *g*. Radioactivity of the pellets solubilised by 0.05 ml Soluene (Packard) was determined by liquid scintillation.

Extraction of mitochondria with organic solvents. [^{14}C]DCCD-labelled mitochondria were washed with the medium A. 100 mg of mitochondria were extracted with 35 ml $CHCl_3/CH_3OH$ (2 : 1, v/v), pH 7.4 for 24 h at 0°C according to Folch et al. [26]. Phospholipids and hydrophobic proteins, present in the crude chloroform-methanol extract were separated by thin-layer chromatography on Silica gel Merck G, ($CHCl_3/CH_3OH/H_2O$, 65 : 24 : 4, v/v) as described previously [21].

Isolation of the ATPase complex. The ATPase complex was purified from Triton X-100 solubilised mitochondria by immunoprecipitation with antibody against F_1, essentially as described by Ludwig and Capaldi [27]; for immunisation [19] F_1 was purified from the '$CHCl_3$-released ATPase' [28]. To obtain uniform labelling of subunits of the ATPase complex, immunoprecipitation was also performed with mitochondria isolated from the liver of the rat injected with a mixture of ^{14}C-labelled amino acids [19].

Polyacrylamide gel electrophoresis. Electrophoresis was performed in the presence of 0.1% SDS on slabs of linear gradient polyacrylamide (12—20%) gel as described by O'Farrell [29]. Unless otherwise stated, samples were dissociated in 2.3% SDS, 5% 2-mercaptoethanol (3 min, 100°C). Radioactivity was detected after staining (Coomassie Brilliant Blue R 250) and destaining in acidic methanol ($CH_3OH/CH_3COOH/H_2O$, 5 : 1 : 5, v/v) by fluorography [30] with the aid of Kodak X-OMAT R films.

Analytical methods. ATPase activity was measured as P_i release [31] in the presence of the ATP-regenerating system [32]. Protein was determined according to Lowry et al. [33].

Materials. [^{14}C]DCCD was obtained from CEA, France; ^{14}C-labelled amino acids from Amersham, England. Other chemicals were of analytical grade.

Results

Identification of the [^{14}C]DCCD-reacting polypeptides in bovine heart mitochondria. Correlation with the inhibition of ATPase

Bovine heart mitochondria were incubated with [^{14}C]DCCD within a concentration range of 0.02—15.0 nmol/mg protein. Binding of the inhibitor and ATPase activity were determined simultaneously. In agreement with previous data [9,16], the binding of [^{14}C]DCCD was biphasic and only the initial steep phase of the binding curve corresponded to the inhibition of ATPase. Maximum inhibition (90%) was obtained at 0.5 nmol [^{14}C]DCCD bound/mg of mitochondrial protein.

Analysis of the labelled mitochondria by SDS-polyacrylamide gel electrophoresis and fluorography revealed that the binding detected at the low DCCD concentrations is a result of labelling of three polypeptides with M_r 9000, 16 000 and 33 000 (Fig. 1). At higher concentrations of the inhibitor six to ten other polypeptides began to interact with [^{14}C]DCCD while the labelling of the 9000, 16 000 and 33 000 polypeptides did not significantly change (Fig. 1, V—VII). The label, detected in the front of the gel was completely removed by extraction of the mitochondria with aqueous acetone (FIg. 1, VII, VIII) and apparently represented the binding to phospholipids [8,9]. The extraction, however, did not influence the labelling of polypeptides.

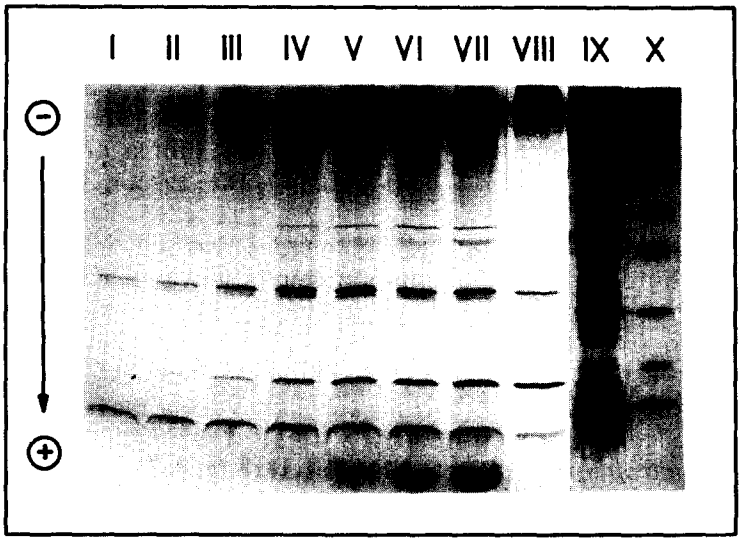

Fig. 1. Distribution of radioactivity due to [^{14}C]DCCD in mitochondria as analysed by SDS-polyacrylamide gel electrophoresis. Mitochondria were incubated with [^{14}]DCCD for 4 h at 28°C and samples (always 0.2 mg protein) were analysed by SDS-polyacrylamide gel electrophoresis. The stained-destained gel was impregnated with PPO and fluorography was performed for 21 days at −60°C. (I—VII) are fluorograms of the mitochondria incubated with 0.29, 0.51, 1.13, 1.88, 3.68, 6.95 and 14.8 nmol [^{14}C]DCCD/mg of protein. Lane VIII is the fluorogram of the mitochondria (VII) that were washed with 10% water in acetone, lane IX is the stained pattern of analysed mitochondria and lane X shows the stained pattern of the following protein standards: phosphorylase *b* (94 000), bovine serum albumin (67 000), ovalbumin (43 000), carbonic anhydrase (30 000), trypsin inhibitor (20 000) and lactalbumin (14 000).

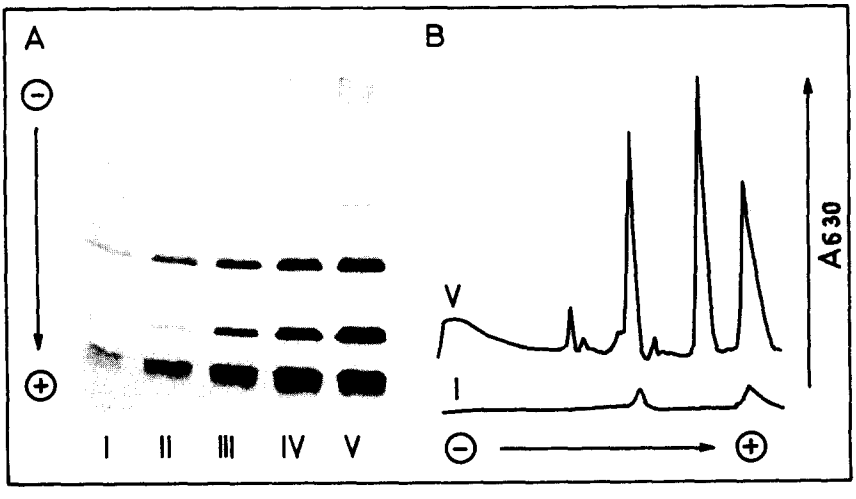

Fig. 2. Binding of [^{14}C]DCCD to the polypeptides of M_r of 9000, 16 000 and 33 000. Mitochondria were incubated with [^{14}C]DCCD (0.14—1.88 nmol/mg of mitochondrial protein), washed with 10% water in acetone and analysed by SDS-polyacrylamide gel electrophoresis as in Fig. 1. In (A) fluorograms (60 days) of mitochondria incubated with 0.14 (I), 0.29 (II), 0.51 (III), 1.13 (IV) and 1.88 (V) nmol [^{14}C]DCCD/mg of mitochondrial protein are shown. In (B) the respective densitometric scans of the samples (I) and (V) (Beckman Acta III spectrophotometer, 630 nm) are shown.

As the 9000, 16 000 and 33 000 polypeptides appeared to be related with the inhibition of the enzyme activity, mitochondria were further incubated only with low, inhibitory concentrations of [^{14}C]DCCD (0.14—1.88 nmol/mg of protein) and extracted with acetone. Mitochondria were analysed by SDS-polyacrylamide gel electrophoresis and fluorograms were scanned and quantified (Fig. 2). The integrated peak areas were plotted against the total bound radioactivity and a linear relationship was obtained. The binding to individual polypeptides was expressed in nanomole of the inhibitor/mg protein of the mitochondrial membrane.

As shown in Fig. 3A, the labelling of the three polypeptides as a function of the total [^{14}C]DCCD binding to the mitochondrial membrane was different. The 9000 polypeptide was labelled beginning with the lowest concentration of the inhibitor and the labelling was saturable. The maximum value of 0.18 nmol of bound [^{14}C]DCCD was recovered in this polypeptide. In the 16 000 polypeptide no significant radioactivity was found below 0.2 nmol of [^{14}C]DCCD bound/mg of mitochondrial membrane. Above this concentration, a nonsaturable labelling was observed. The 33 000 polypeptide was labelled beginning with the lowest concentrations of the inhibitor and the labelling was again nonsaturable within this concentration range.

To correlate directly the labelling of individual polypeptides with the inhibi-

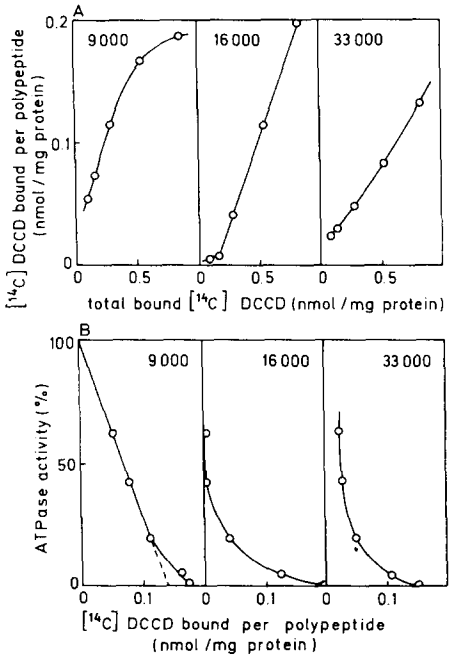

Fig. 3. Relationship between the inhibition of ATPase and the binding of [^{14}C]DCCD to polypeptides of M_r 9000, 16 000 and 33 000. Samples from Fig. 2 were analysed for the ATPase activity (specific activity of the control was 0.7 μmol P_i released/min per mg protein at 30°C) and for the [^{14}C]DCCD binding. Binding of [^{14}C]DCCD to individual polypeptides was evaluated from Fig. 2. (A) represents the binding to individual polypeptides as a function of the total inhibitor found, (B) represents the ATPase activity as a function of [^{14}C]DCCD binding to individual polypeptides. In this calculation the residual ATPase activity (7%) which was not inhibited by the highest concentrations of DCCD was subtracted.

tory effect of the bound [^{14}C]DCCD, data from Fig. 3A were plotted against the ATPase activity. As demonstrated in Fig. 3B, a linear relationship was found only for the 9000 polypeptide. The intercept with abscissa revealed the value of 0.15 nmol which corresponded well to the saturation level at 0.18 nmol, demonstrated on the binding curve in Fig. 3A. In both the 16 000 and 33 000 polypeptides a nonlinear relationship was obtained.

Therefore, as far as the inhibition of the ATPase activity is concerned, only the interaction of the inhibitor with the 9000 polypeptide appears to be directly involved. In order to analyse, whether the nonequivalent interaction of DCCD with the 9000, 16 000 and 33 000 polypeptides was accompanied by other differences among these polypeptides, extraction with organic solvents and isolation of the ATPase complex from [^{14}C]DCCD-labelled mitochondria were performed.

Extraction of [^{14}C]DCCD-labelled polypeptides with organic solvents

Hydrophobic nature of the DCCD-binding protein of the ATPase complex permits extraction with organic solvents [5—7]. To test properties of the individual DCCD-reacting polypeptides, [^{14}C]DCCD-labelled mitochondria (4.0 nmol/mg protein) were extracted with neutral chloroform/methanol (2 : 1, v/v). 15% of the bound [^{14}C]DCCD was present in the non-extractable residue (hydrophilic proteins) and 85% was present in the crude chloroform-methanol extract (hydrophobic proteins and phospholipids). Thin-layer chromatography further revealed the distribution of radioactivity among hydrophobic proteins and phospholipids to be 40% and 60%, respectively. As shown in Fig. 4A, the 9000 and 16 000 polypeptides were recovered in the chloroform-methanol extract but the 33 000 polypeptide remained in the non-extractable residue. Efficiency of the extraction, or rather separation, is demonstrated by a complete absence of the 9000 and 16 000 polypeptides in the precipitated material and, on the contrary, by the absence of the 33 000 polypeptide in the extract. The proportion between the 9000 and 16 000 polypeptides was not significantly changed during the extraction. Also the 33 000 polypeptide did not apparently disintegrate to low molecular weight components. Hence, whereas both the 9000 and 16 000 polypeptides are of hydrophobic nature typical of the DCCD-binding subunit of F_0, the 33 000 polypeptide is neither hydrophobic, nor does it behave as an aggregate of the latter two polypeptides.

Isolation of the ATPase complex from [^{14}C]DCCD-labelled mitochondria

A direct evidence of the presence of individual DCCD-reactive polypeptides in the F_0 part of ATPase can be achieved by analysing the ATPase complex devoid of other membrane constituents. Immunoprecipitation of the ATPase complex from Triton X-100 solubilised mitochondria with antibody against F_1 yielded an ATPase complex of a purity [27] comparable with that obtained by other isolation procedures.

Subunit composition was first tested with the ATPase complex, immunoprecipitated from rat liver mitochondria uniformally labelled 'in vivo' with ^{14}C-labelled amino acids. As shown in Fig. 4B, both the Coomassie Blue staining and the radioactivity profile of the polypeptides of the complex exhibited a typical subunit pattern. With the exception of the antibody, only minor im-

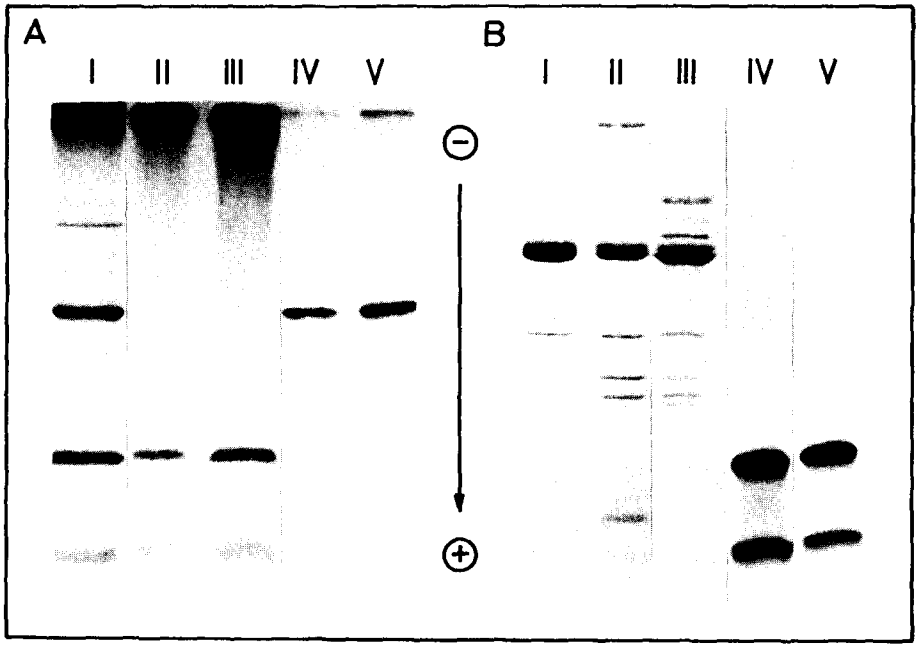

Fig. 4. Extraction of [^{14}C]DCCD-labelled mitochondria with chloroform-methanol (A). Isolation of the ATPase complex (B). (A) Mitochondria were labelled with 4.0 nmol of [^{14}C]DCCD and extracted with chloroform/methanol (2 : 1, v/v). (I) represents original mitochondrial (18 000 dpm), (II, III) extracted material (20 000, 40 000 dpm) and (IV, V) non-extracted residue (2500, 5000 dpm) analysed by SDS-polyacrylamide gel electrophoresis. Fluorography was performed for 30 days. For further details see Methods. (B) The ATPase complex was immunoprecipitated from Triton X-100 solubilised mitochondria with antibody against F_1. Samples were analysed by SDS-polyacrylamide gel electrophoresis and fluorography (60 days). (I) represents the stained pattern of F_1, (II, III) the stained pattern and fluorogram of the ATPase complex prepared from rat liver mitochondria that were labelled in vivo with ^{14}C-labelled amino acids, (IV, V) fluorograms of the ATPase complex prepared from rat liver and bovine heart mitochondria labelled with 2.0 nmol [^{14}C]DCCD. For dissociation of the sample (II), 2-mercaptoethanol was omitted. For further details see Methods.

purities were present. For comparison, the subunit profile of isolated F_1 is given in parallel (Fig. 4B, I).

The ATPase complex was then immunoprecipitated from [^{14}C]DCCD-labelled bovine heart and rat liver mitochondria (2.0 nmol [^{14}C]DCCD/mg protein). Profile was obtained by means of fluorography of the SDS-polyacrylamide gel electrophoresis analysed complexes. The radioactivity of both complexes was associated with two polypeptides of M_r 9000 and 16 000 (Fig. 4B, IV, V). This pattern corresponded closely to that obtained in the chloroform-methanol extract (see Fig. 4A). The same result was also obtained with other types of mammalian mitochondria (not shown). Hence, of the three polypeptides that react in mitochondria with DCCD, only the 9000 and 16 000 polypeptides belong clearly to the ATPase complex and can represent the DCCD-binding moiety of F_0.

Discussion

The present study demonstrates the non-identical nature of the three polypeptides of M_r 9000, 16 000 and 33 000 reacting with DCCD, while inhibiting

ATPase activity. The hydrophobic 9000 and 16 000 polypeptides are a component of the ATPase complex. The 9000 polypeptide reacts with DCCD proportionally to the inhibition of the enzyme but the interaction of the 16 000 polypeptide does not correspond to the inhibitory effect.

The 33 000 polypeptide is different from the other two polypeptides. It is hydrophilic and cannot be recovered in the isolated ATPase complex. An analogous DCCD-reacting polypeptide was explained by Sebald et al. [20] as an oligomer of the DCCD-binding subunit of ATPase. Also Tzagoloff and coworkers showed [10,11] that the 45 000 translation product of mitochondrial protein synthesis can be converted by chloroform-methanol to the 7800 product (subunit 9), the former being probably an oligomer of the latter. In our experiments, however, the 33 000 polypeptide did not disaggregate on chloroform-methanol extraction. Furthermore, its non-hydrophobic nature explains why such a polypeptide has never been recovered by organic solvent-based procedures for purification of the DCCD-binding protein [5,20].

Nevertheless the 33 000 polypeptide does not belong to the ATPase complex, it is already labelled at low, inhibitory concentrations of DCCD. This is in contrast to the labelling of other membrane polypeptides, e.g. the beta subunit of F_1 [34] and subunit III of cytochrome-*c* oxidase [35], which occurs at high concentrations of DCCD. Furthermore, there apparently exist a relationship with the phosphorylating assembly considering that the 33 000 polypeptide was reduced in brown adipose tissue mitochondria in parallel with the reduction of F_1 and of the 9000 and 16 000 polypeptides [21].

It has been repeatedly demonstrated that mitochondrial ATPase is inhibited even if only one of the six monomers of the DCCD-binding protein is occupied by the inhibitor [15,20]. When the binding of DCCD to bovine heart ATPase complex was further analysed, a single covalently labelled polypeptide was observed in some laboratories [6,8,15], while in others two reactive polypeptides were detected [16—19].

The results, referred to here, support the latter finding and show that, in agreement with Norling et al. [16], the inhibitory effect directly corresponds only to the reaction of DCCD with a polypeptide of M_r 9000, which apparently represents the monomers of the DCCD-binding protein. The involvement of the less reactive sites (the 16 000 polypeptide) in the inhibition of ATPase appears to be more complicated. According to M_r and the convertibility of the 16 000 polypeptide to the lower molecular weight component [16,17] these sites could be a dimer of the 9000 polypeptide or a complex of the 9000 polypeptide with another subunit of F_0. The labelling of the 9000 and 16 000 polypeptides is, however, clearly different. The distribution of radioactivity found between the two polypeptides is not a random process, which would be expected if all of the monomers present in the ATPase complex exhibited the same reactivity towards DCCD and the same ability to aggregate.

It is therefore suggested that the two distinct classes of binding sites reflect different topology of individual monomers of the DCCD-binding protein within F_0, which either occurs in situ or results from the binding of DCCD. It seems interesting that the DCCD-induced change in the reactivity of individual monomers, as suggested by Altendorf [23] and demonstrated by Sigrist-Nelson and Azzi [37] for the chloroplast DCCD-binding protein, is also compatible with a non-symmetrical arrangement of the monomers within F_0.

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References

1 Beechey, R.B., Robertson, A.M., Holloway, C.T. and Knight, I.G. (1967) Biochemistry 6, 3867—3879
2 Hinkle, P.C. and Horstman, L.L. (1971) J. Biol. Chem. 246, 6024—6028
3 Pansini, A., Guerieri, F. and Papa, S. (1978) Eur. J. Biochem. 92, 545—551
4 Holloway, C.T., Robertson, A.M., Knight, I.G. and Beechey, R.B. (1966) Biochem. J. 100, 79P
5 Cattell, K.J., Lindop, C.R., Knight, I.G. and Beechey, R.B. (1971) Biochem. J. 125, 169—177
6 Steckhoven, F.S., Waitkus, R.F. and VanMoerker, H.T.B. (1972) Biochemistry 11, 1144—1150
7 Knight, I.G., Holloway, C.T., Robertson, A.M. and Beechey, R.B. (1968) Biochem. J. 109, 27P
8 Dianoux, A.C., Bof, M. and Vinais, P.V. (1978) Eur. J. Biochem. 88, 69—77
9 Svoboda, P., Kopecký, J., Houštěk, J. and Drahota, Z. (1979) Biochem. Biophys. Res. Commun. 89, 981—987
10 Tzagoloff, A. and Meagher, P. (1972) J. Biol. Chem. 247, 594—603
11 Sierra, M.F. and Tzagoloff, A. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 3155—3159
12 Sebald, W., Hoppe, J., Wachter, E. (1979) in Function and Molecular Aspects of Biomembrane Transport (Quagliariello, E., Palmieri, F., Papa, S. and Klingenberg, M., eds.), pp. 63—74, Elsevier/North Holland Biomedical Press, Amsterdam
13 Nelson, N., Eytan, E., Notsani, B., Sigrist, H., Sigrist-Nelson, K. and Gitler, G. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 2375—2378
14 Criddle, R.S., Packer, L. and Shieh, P. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 4306—4310
15 Kiehl, R. and Hatefi, Y. (1980) Biochemistry 19, 541—548
16 Norling, B., Glaser, E. and Ernster, L. (1978) in Frontiers of Biological Energetics: From Electrons to Tissue (Dutton, L.P., Leigh, J.S. and Scarpa, A., eds.), pp. 504—515, Academic Press, New York
17 Berden, J.A. and Voorn-Brouwer, M.M. (1978) Biochim. Biophys. Acta 501, 424—439
18 De Young, L., Holtrop, M. and Kroon, A.M. (1979) Biochim. Biophys. Acta 548, 48—62
19 Kužela, Š., Luciaková, K. and Lakota, J. (1980) FEBS Lett. 114, 197—201
20 Sebald, W., Graf, T. and Lukins, H.B. (1979) Eur. J. Biochem. 93, 587—599
21 Svoboda, P., Houštěk, J., Kopecký, J. and Drahota, Z. (1981) Biochim. Biophys. Acta 634, 321—330
22 Fillingame, R.H. (1976) J. Biol. Chem. 251, 6630—6637
23 Altendorf, K. (1977) FEBS Lett. 73, 271—275
24 Smith, A.L. (1967) Methods Enzymol. 10, 81—86
25 Schneider, W.C. and Hageboom, G.H. (1950) J. Biol. Chem. 183, 1123—1128
26 Folch, J., Lees, M., Stanley, S. and Stanley, G.H. (1957) J. Biol. Chem. 226, 497—553
27 Ludwig, B. and Capaldi, R.A. (1979) Biochem. Biophys. Res. Commun. 87, 1159—1167
28 Kopecký, J., Houštěk, J. and Drahota, Z. (1977) Mol. Cell. Biochem. 18, 77—80
29 O'Farrell, P.H. (1975) J. Biol. Chem. 250, 4007—4021
30 Bonner, W.M. and Laskey, R.A. (1974) Eur. J. Biochem. 46, 83—88
31 Lindberg, O. and Ernster, L. (1956) in Methods of Biochemical Analysis (Glick, D., ed.), pp. 1—22, Interscience, New York
32 Pullman, M.E., Penefsky, H.S., Datta, A. and Racker, E. (1960) J. Biol. Chem. 235, 3322—3329
33 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
34 Pougeois, R., Satre, M. and Vignais, P.V. (1980) FEBS Lett. 117, 344—348
35 Casey, P.R., Thelen, M. and Azzi, A. (1980) J. Biol. Chem. 255, 3994—4000
36 Sigrist-Nelson, K. and Azzi, A. (1978) Eur. J. Biochem. 92, 9—14
37 Sigrist-Nelson, K. and Azzi, A. (1979) J. Biol. Chem. 254, 4470—4474