Biochimica et Biophysica Acta, 733 (1983) 274–282
Elsevier

BBA 71800

INTERACTION OF *ESCHERICHIA COLI* F_1-ATPase WITH DICYCLOHEXYLCARBODIIMIDE-BINDING POLYPEPTIDE

TIP W. LOO, HELGA STAN-LOTTER, DONALD MACKENZIE, ROBERT S. MOLDAY and **PHILIP D. BRAGG**

Department of Biochemistry, University of British Columbia, Vancouver, B.C., V6T 1W5 (Canada)

(Received March 11th, 1983)

Key words: F_1-ATPase; Dicyclohexylcarbodiimide-binding polypeptide; Protein orientation; (E. coli)

Antibody raised against the *N,N'*-dicyclohexylcarbodiimide (DCCD)-binding polypeptide of *Escherichia coli* bound to the cytoplasmic surface of the cell membrane. A weak reaction was seen with everted vesicles of the thermophile PS3. Rat-liver mitochondrial membranes did not react with the antibody. Reaction of the isolated DCCD-binding polypeptide with the antibody was prevented by oxidation of methionine residues or cleavage of the polypeptide with cyanogen bromide. Modification of the arginine residues of the DCCD-binding polypeptide did not affect interaction with the antibody. Purified F_1-ATPase of *E. coli* bound to the isolated DCCD-binding polypeptide as shown by solid-phase radioimmune assay. Binding involved the α and/or β subunits of F_1 and the arginine residues of the polar central region of the DCCD-binding polypeptide. Our results are consistent with a looped arrangement of the DCCD-binding polypeptide in the membrane in which the carboxyl- and amino-terminal regions of the molecule are at the periplasmic surface and the polar central region, interacting with F_1, is at the cytoplasmic surface of the cell membrane.

Introduction

The ATPase complexes of mitochondria, chloroplasts and the bacterial cell membrane are involved in the formation of ATP by oxidative and photophosphorylation [1,2]. The complex is composed of an intrinsic membrane portion, F_0, through which protons move to the active site of the enzyme on the extrinsic membrane portion, F_1. In *E. coli*, F_0 is composed of three kinds of polypeptides [3]. There is good evidence that one of these polypeptides, the dicyclohexylcarbodiimide (DCCD)-binding polypeptide, is involved in the proton pathway through F_0. Thus, proton movement through F_0 is prevented by reaction of this polypeptide with DCCD and also by mutation in which certain of its aminoacyl residues are modified [4–6].

An understanding of the arrangement of these polypeptides in the membrane is obviously an important prerequisite to determining the mechanism of proton translocation through F_0. The amino acid sequence of the DCCD-binding polypeptide is suggestive of structure. Four regions of the molecule can be recognized (Fig. 1) [7,8]. Seven amino-terminal, predominantly polar amino acids are followed by a nonpolar region (residues 8–33). Amino acids 34–52 are predominantly polar. The fourth region (residues 53–79) is nonpolar. It has been suggested [7] that the two nonpolar regions of the molecule are transmembranous, forming a looped structure in which the middle polar region is exposed at the cytoplasmic surface of the membrane. The looped arrangement is supported by the findings that: (i) alteration of aminoacyl re-

Abbreviations: DCCD, N,N^1-dicyclohexylcarbodiimide; F_1, F_1-ATPase protein of the ATPase complex; F_0, F_0 protein of the ATPase complex.

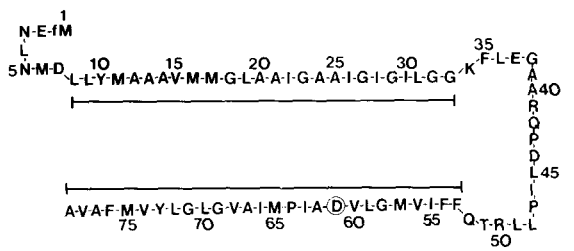

Fig. 1. Amino acid sequence of DCCD-binding polypeptide [8]. The two nonpolar regions of the molecule are shown by the bars. DCCD is bound by the circled aspartic acid residue.

sidue 28 in the first nonpolar segment influenced the reaction of DCCD with aspartyl-61 in the other nonpolar segment [5]; (ii) tyrosine residues 10 and 73 were accessible on the periplasmic (outer) surface of the cell membrane [9]; (iii) antiserum against the DCCD-binding polypeptide blocked the leakage of protons through F_0 in F_1-stripped everted membrane vesicles [10].

The accessibility of the DCCD-binding polypeptide at the cytoplasmic surface suggests that it could react with F_1. However, there is presently no direct evidence for the identity of the F_1-binding polypeptide(s).

In the present paper, we confirm that the DCCD-binding polypeptide is exposed on the cytoplasmic surface of the inner membrane. Modification of methionine residues, but not of arginine residues, interferes with reaction of the antibody with the isolated DCCD-binding polypeptide. We also show that F_1 can bind to the isolated DCCD-binding polypeptide. Binding involves α and β subunits of the F_1 and arginine residues of the DCCD-binding polypeptide. Thus, our results are consistent with the proposed 'loop' model for the arrangement of the DCCD-binding polypeptide in the cell membrane of *E. coli*.

Material and Methods

Growth of cells and preparation of membrane vesicles. The following strains of *E. coli* were used in this work: ML308-225, WS1 (*thi, his, pro*), N_{144} (ATPase-negative mutant of WS1), DL-54 (ATPase-negative mutant of ML308-225), and AN382 (*thi, arg, uncB401*) [23]. The cells were grown to the exponential phase on a minimal salts medium with 0.8% (w/v) glucose, 0.1% (w/v) casein amino acids (Difco), 12 μM ferric citrate, and supplemented with 1 mg/l of thiamine and 50 mg/l of arginine, histidine and proline where required. Everted membrane vesicles were prepared by disruption of cells in a French press at 1400 kg/cm^2 as described previously [11] and suspended in 50 mM Hepes-KOH buffer (pH 7.5)/10 mM $MgCl_2$/10% (v/v) glycerol. Everted membrane vesicles were stripped of the F_1-ATPase by treatment with 2 M urea [12]. PS3, a kind gift of Dr. Y. Kagawa, was grown and everted membrane vesicles prepared as described by Yoshida et al. [13]. The vesicles were stripped with 2 M urea as above.

Preparation of E. coli F_1-ATPase and DCCD-binding polypeptide. F_1 was released from everted membrane vesicles of strain ML308-225 and purified as before [14] except that $MgCl_2$ was replaced by 20 mM $CaCl_2$ in the precipitation step with 20% (v/v) methanol, and separation of the enzyme on a sucrose gradient was omitted. The DCCD-binding polypeptide was extracted from cells of strain ML308-225 and purified as described previously [12]. Antisera to F_1 and the DCCD-binding polypeptide were prepared as described before [10].

Preparation of subunits of E. coli F_1. F_1 lacking the δ and ε subunits was made by controlled treatment of the ATPase with TPCK-trypsin [14]. F_1 was dissociated into its subunits as described by Dunn and Futai [15] and the α and β subunits separated as described previously [16].

Solid phase radioimmune assays. The procedures of MacKenzie and Molday [17] were used. In the 'competitive inhibition assay' flex vinyl microtitre plate wells were pretreated with 25-μl portions of 0.1% (w/v) polylysine at 22°C for 4–6 h. The wells were subsequently washed with water to remove unbound polylysine. DCCD-binding polypeptide (25 μl) at a concentration of 0.13 mg/ml in 100 mM sodium borate buffer (pH 8.8)/2% (w/v) Triton X-100 was incubated in the wells for 8–10 h at 22°C. Unbound polypeptide was removed by extensive washing with phosphate-buffered saline (0.137 M NaCl/2.68 mM KCl/1.47 mM KH_2PO_4/8.09 mM Na_2HPO_4 (pH 7.5)) containing 10 mM $MgCl_2$. Nonspecific binding sites were then quenched by incubation overnight at 4°C with radioimmune assay buffer consisting

of 2% (w/v) bovine serum albumin, 2% (v/v) fetal calf serum and 0.1% (w/v) NaN_3 in phosphate-buffered saline/10 mM $MgCl_2$. The assay wells were then incubated with 25 μl portions taken from a mixture of varying concentrations of the free antigen (vesicles, modified DCCD-binding polypeptide, etc.) which had been preincubated with appropriately diluted antiserum (1 : 500 in buffer) for 60 min. After 1.5 h at 22°C, the wells were washed with phosphate-buffered saline/10 mM $MgCl_2$, and then incubated with 25 μl of affinity-purified ^{125}I-labelled goat anti-rabbit immunoglobulin (15–40 μg/ml radioimmune assay buffer; $(1–2)\cdot 10^6$ dpm/μg) for 60 min. The wells were rinsed extensively with phosphate-buffered saline/10 mM $MgCl_2$ and cut out, and the bound radioactivity was determined in a Beckman Gamma 8000 counter. Two basic versions of the 'binding assay' were used which differed in the nature of the free antigen. This was either *E. coli* F_1 or the DCCD-binding polypeptide. Binding of the free antigen to the fixed antigen was then detected by using an antiserum to the free antigen. For example, polylysine-treated microtiter wells were incubated with 25 μl portions of the antigen to be fixed (F_1, 0.75 mg protein/ml; DCCD-binding polypeptide, 0.13 mg/ml). Following quenching of nonspecific binding sites as in the competitive inhibition assay, the wells were incubated with F_1 (0.75 mg/ml in 50 mM Hepes-KOH buffer (pH 7.5)/10 mM $MgCl_2$/10% (v/v) glycerol/3% (w/v) bovine serum albumin) or DCCD-binding polypeptide (0.13 mg/ml in 100 mM sodium borate buffer (pH 8.8)/10 mM $MgCl_2$/3% (w/v) bovine serum albumin/2% (w/v) Triton X-100) depending on the nature of the fixed antigen. The wells were washed with phosphate-buffered saline/10 mM $MgCl_2$ and then reacted with 25 μl of serial dilutions in radioimmune assay buffer of the antiserum to the free antigen. The extent of binding of the rabbit antiserum was measured with ^{125}I-labelled goat anti-rabbit immunoglobulin as described above. Controls for the nonspecific binding of F_1 or DCCD-binding polypeptide to the wells were run by omitting the fixed antigen in the procedure. Controls for potential cross-reactivity between F_1 and the antiserum to the DCCD-binding polypeptide, or between the DCCD-binding polypeptide and the antiserum to F_1, were run by omitting the free antigen in the procedure.

For experiments in which the fixed antigen (F_1, chemically modified or normal DCCD-binding polypeptide, subunits of F_1) was titrated with the free antigen (DCCD-binding polypeptide or F_1), the experiments were carried out as described above except that the concentration of free antigen was varied with a constant amount of fixed antigen. (The amounts are given in the legends to Figs. 6 and 7.) The antisera against F_1 and the DCCD-binding polypeptide were diluted 1 : 300 in radioimmune assay buffer. 25 μl were used in each well. Net binding of free to fixed antigen was always corrected for any nonspecific binding of the free antigen or for cross-reactivity with heterologous antiserum.

Preparation of rat-liver mitochondrial membranes. Phosphate-washed purified inner mitochondrial membranes were prepared from rat-liver mitochondria as described by Soper and Pedersen [18]. The purified membranes at 5 mg protein/ml of phosphate-buffered saline/10 mM $MgCl_2$ were sonicated at a power of 50 W in a Branson W185D sonifier for 15 s periods for a total of 2.5 min. The temperature was kept at 0°C. Large fragments were removed by centrifugation at $12000\times g$ for 10 min. The supernatant was either centrifuged at $250000\times g$ for 2 h to sediment 'sonicated washed mitochondrial membranes' or incubated with 4 M urea for 30 min at 22°C prior to centrifugation as above. The 'urea-treated, washed mitochondrial membranes' were washed by recentrifugation of a suspension in phosphate-buffered saline/10 mM $MgCl_2$. All membranes were resuspended in phosphate-buffered saline/10 mM $MgCl_2$.

Chemical modification of DCCD-binding polypeptide. The DCCD-binding polypeptide at a concentration of 0.1–0.2% (w/v) was incubated at 0°C with performic acid for 20 h in the dark [19]. The performic acid was removed by evaporation at 35–40°C under reduced pressure. The oxidized protein was dissolved in 90% formic acid or in 100 mM sodium borate buffer (pH 8.8)/2% (w/v) Triton X-100. The extent of oxidation was determined by amino acid analysis.

Cleavage of the DCCD-binding polypeptide with cyanogen bromide followed the method of Sebald et al. [20]. The cleaved products were taken

up in 80% formic acid or in the borate/Triton X-100 buffer for use.

DCCD-binding polypeptide (0.3 mg) in 1 ml 100 mM sodium borate buffer (pH 8.8)/2% (w/v) Triton X-100 was incubated at 22°C in the dark for 3 h with either 1 ml 150 mM phenylglyoxal or 1 ml 200 mM 2,3-butanedione in borate/Triton buffer. The reaction was stopped by the addition of 1 ml 300 mM L-arginine in borate buffer. After 30 min, the reaction mixture was lyophilized. The lyophilized material was taken up at a concentration of 0.15 mg/ml in borate/Triton buffer.

For all experiments using modified DCCD-binding polypeptide control experiments were carried out using unmodified DCCD-binding polypeptide which had been taken through the procedures in the absence of the modifying agents.

Enzymatic modification of DCCD-binding polypeptide. The DCCD-binding polypeptide (0.2 mg) in 0.2 ml 0.1 M glycine/38 mM Tris buffer (pH 8.8)/2% (w/v) Triton X-100 was incubated for 24 h with TPCK-trypsin. Soybean trypsin inhibitor (8 μg) in 0.2 ml of the same buffer was added and incubation continued for a further 30 min. The mixture was lyophilized and dissolved for use at a concentration of 1 mg polypeptide/ml in the same buffer but without Triton X-100. The digested polypeptide was readily soluble. The DCCD-binding polypeptide (0.2 mg) was also treated with *Staphylococcus aureus* V8 protease (10 μg) under conditions similar to the above except that incubation at 37°C was carried out for 6 h and the reaction was terminated by heating the reaction mixture at 100°C for 3 min. The reaction mixture was lyophilized and the digested polypeptide dissolved for use in buffer as above.

Protein assays. The DCCD-binding polypeptide was assayed as described by Fillingame [21]. The protein content of preparations of F_1 and its α and β subunits was measured by the procedure of Bradford [22].

Results

Reaction of antibody with membrane vesicles

The ability of antibody to the DCCD-binding polypeptide to react with this polypeptide in everted membrane vesicles was demonstrated by the competitive inhibition assay [17]. (F_1 was removed from everted vesicles by treatment with 2 M urea.) In this assay, the DCCD-binding polypeptide of the membrane vesicles competes for the antibody with DCCD-binding polypeptide immobilized in microtiter wells. As shown in Fig. 2, under the conditions of our experiments approx. 50% inhibition of binding was obtained with a concentration of 10 μg everted vesicle protein/ml. A similar concentration of protein was required when F_1-stripped everted vesicles from the *E. coli unc* mutants DL-54, N_{144} and AN382 were used. The last two mutants have defects in F_0 not involving the DCCD-binding polypeptide [1,23].

The ATPase complex of the thermophilic bacterium PS 3 has been well characterized [24]. In spite of considerable homology between the DCCD-binding polypeptides of *E. coli* and PS3 [8], the antibody reacted only weakly with F_1-stripped everted membrane vesicles of PS 3 (Fig. 2). Everted inner mitochondrial membrane vesicles (submitochondrial particles) from rat liver, or purified mitochondrial membranes from which F_1 had been stripped, did not react with the antibody to the *E. coli* DCCD-binding polypeptide, at least at the concentration of antigen used (Fig. 2).

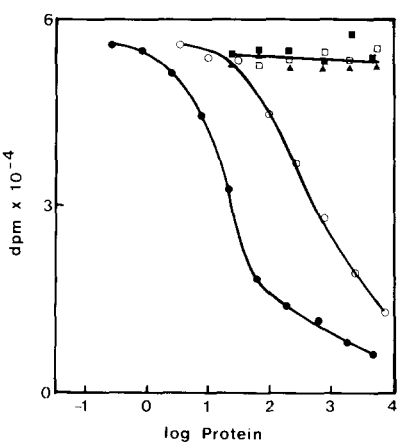

Fig. 2. Inhibition of antibody binding to immobilized DCCD-binding polypeptide (3.3 μg protein) by various amounts of membrane vesicles of *E. coli*, PS3 and rat liver mitochondria. ●, F_1-stripped everted vesicles of *E. coli*; ○, F_1-stripped everted vesicles of PS3; ■, phosphate-washed mitochondrial inner membranes; □, sonicated phosphate-washed mitochondrial inner membranes; ▲, urea-treated phosphate-washed mitochondrial inner membranes. Protein concentration is expressed as μg/ml.

Reaction site for the antibody on the DCCD-binding polypeptide

The DCCD-binding polypeptide was modified by treatment with several group-specific reagents [25]. The modified polypeptide was then used in the competitive inhibition assay against immobilized unmodified DCCD-binding polypeptide. Cleavage of the polypeptide at methionine residues with cyanogen bromide, or oxidation of methionine residues with performic acid to greater than 95%, resulted in a reduction in the affinity of the DCCD-binding polypeptide for the antibody by almost two orders of magnitude (Fig. 3). The affinity for the antibody was reduced 3-fold by cleavage of the polypeptide with TPCK-trypsin or *S. aureus* V8 protease. Modification of arginine residues of the DCCD-binding polypeptide with phenylglyoxal or 2,3-butanedione had no effect on its reaction with the antibody.

Binding of F_1 by isolated DCCD-binding polypeptide

Purified F_1 from *E. coli* was bound by the isolated DCCD-binding polypeptide from this organism. This was shown in two ways. In the first method, DCCD-binding polypeptide immobilized in microtitre plate wells ('fixed antigen') was reacted with F_1 ('free antigen'). Bound F_1 was titrated with various dilutions of anti-F_1 serum. The extent of binding of the antibody was then measured with ^{125}I-labelled goat anti-rabbit immunoglobulin. Nonspecific binding of antiserum to the fixed antigen (and to the wells) and nonspecific binding of F_1 to the wells were corrected for by omitting the F_1 and the DCCD-binding polypeptide, respectively. As shown in Fig. 4 (upper panel), nonspecific binding of anti-F_1 serum was negligible. Significant nonspecific binding of F_1 could be detected but this was much less than the binding of F_1 found in the presence of the DCCD-binding polypeptide. The extent of nonspecific binding could be decreased by lowering the concentration of F_1 used in the experiment. In the second method (Fig. 4, lower panel), the fixed antigen was purified F_1 and the binding of DCCD-binding polypeptide to this was measured using antiserum to the polypeptide. Again, significantly more DCCD-binding polypeptide was bound to F_1 than in the control.

Prior reaction of immobilized DCCD-binding polypeptide with F_1 did not affect its subsequent reaction with antibody (Fig. 5). (A similar result was obtained for the reaction of immobilized F_1

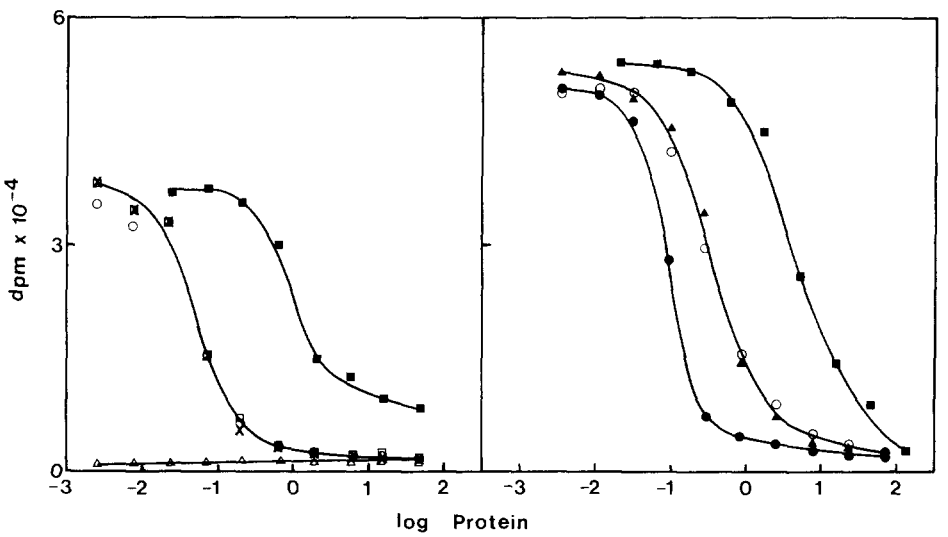

Fig. 3. Inhibition of antibody binding to immobilized DCCD-binding polypeptide by various amounts of protease-treated or chemically modified DCCD-binding polypeptide. Left panel: ×, untreated DCCD-binding polypeptide; □, 2,3-butanedione-treated polypeptide; ○, phenylglyoxal-treated polypeptide; ■, performic acid-treated polypeptide; △, antibody replaced in control experiments by preimmune serum. Right panel: ●, untreated DCCD-binding polypeptide; ○, TPCK-trypsin treated polypeptide; ▲, protease V8-treated polypeptide; ■, cyanogen bromide-treated polypeptide. The amount of immobilized DCCD-binding polypeptide was 3.3 μg. Protein concentration is expressed as μg/ml.

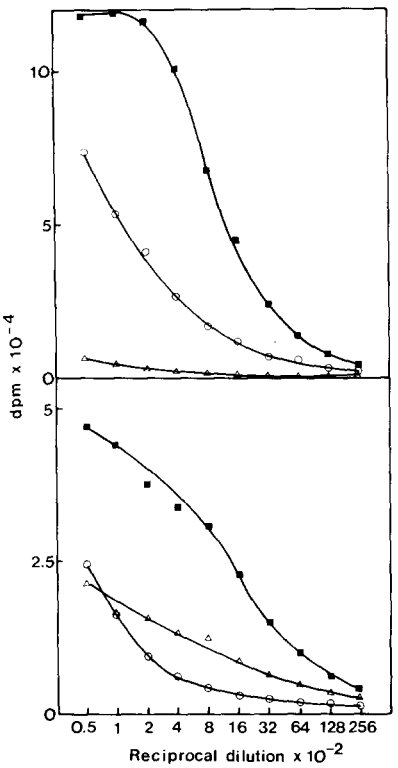

Fig. 4. Binding of F_1 to DCCD-binding polypeptide. Upper panel: Immobilized DCCD-binding polypeptide (3.3 μg) in the presence (■) or absence (△) of F_1 (19 μg protein) was titrated with various dilutions of anti-F_1 serum. ○, F_1 added but the immobilized DCCD-binding polypeptide omitted. Lower panel: Immobilized F_1 (19 μg protein) in the presence (■) or absence (○) of DCCD-binding polypeptide (3.3 μg polypeptide) was titrated with various levels of anti-DCCD-binding polypeptide serum. △, DCCD-binding polypeptide present but immobilized F_1 omitted. The binding of antibody to F_1 (upper panel) or to the DCCD-binding polypeptide (lower panel) was measured indirectly with ^{125}I-labelled goat anti-rabbit immunoglobulin as described in Materials and Methods.

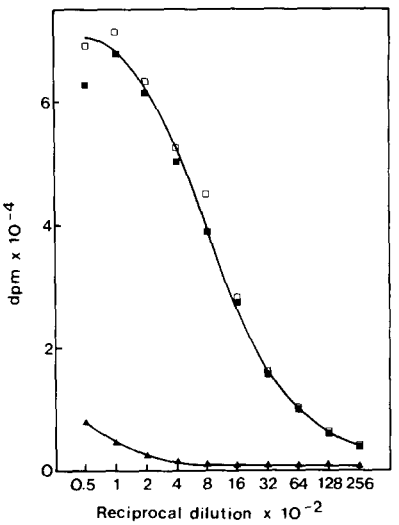

Fig. 5. Effect of F_1 on binding of anti-DCCD-binding polypeptide serum to the DCCD-binding polypeptide. Immobilized DCCD-binding polypeptide (3.3 μg protein) was titrated with various dilutions of its antiserum in the absence (■) and presence (□) of F_1 (19 μg protein). ▲, immobilized DCCD-binding polypeptide and F_1 omitted. The binding of antibody to the DCCD binding polypeptide was measured indirectly with ^{125}I-labelled goat anti-rabbit immunoglobulin as described in Material and Methods.

with its antibody.) There are two possible explanations for this. Either the reaction of the DCCD-binding polypeptide with its antibody is sufficiently strong to displace prebound F_1 or the F_1-binding site on the polypeptide is separate from the binding site for the antibody. As described below, our results favour the latter explanation.

Effect of chemical modification of the DCCD-binding polypeptide on its reaction with F_1

By contrast with its inhibitory effect on the binding of antibody, performic oxidation of methionine residues of the DCCD-binding polypeptide had little effect on the binding of F_1 (Fig. 6A). However, modification of its arginine residues with phenylglyoxal and 2,3-butanedione reduced the affinity of the DCCD-binding polypeptide for F_1 with a relatively small effect on the total F_1-binding capacity of the polypeptide (Fig. 6C). Double-reciprocal plots of binding versus concentration gave half-saturation values (under the conditions of this experiment) of 0.13, 0.36 and 0.66 μg for untreated, phenylglyoxal-, and 2,3-butanedione-treated DCCD-binding polypeptide, respectively. This suggests that F_1 binds to the polypeptide in the region of its arginine residues. These are located in the central polar region (residues 41–50) (Fig. 1) of the DCCD-binding polypeptide molecule. This region should remain intact when the polypeptide is cleaved by cyanogen bromide at methionine residues 17 and 57. As shown in Fig. 6B, the DCCD-binding polypeptide had increased affinity for F_1 following cleavage by cyanogen bromide. Half-saturation of

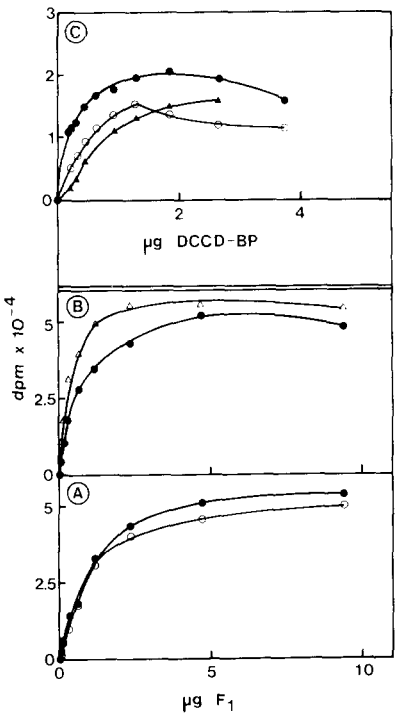

Fig. 6. Effect of chemical modification of the DCCD-binding polypeptide on the binding of F_1. (A) Titration of immobilized untreated (●) or performic-acid treated (○) DCCD-binding polypeptide (3.3 μg protein) with F_1. (B) Titration of immobilized untreated (●) or cyanogen bromide-treated (△) DCCD-binding polypeptide (3.3 μg protein) with F_1. (C) Titration of immobilized F_1 (19 μg protein) with untreated (●), phenylglyoxal-treated (○), and 2,3-butanedione-treated (△) DCCD-binding polypeptide (3.3 μg protein). The binding of antibody to F_1 (A, B) or to the DCCD-binding polypeptide (C) was measured indirectly with ^{125}I-labelled goat anti-rabbit immunoglobulin as described in Material and Methods.

the binding sites on the untreated and cleaved polypeptide under the conditions of our experiment were given by 0.55 and 0.2 μg F_1, respectively. The F_1-binding capacities of the polypeptides were similar.

Reaction sites on F_1 for DCCD-binding polypeptide

The subunits of F_1 responsible for binding to the DCCD-binding polypeptide were explored. Treatment of F_1 with TPCK-trypsin followed by reisolation of the enzyme on a sucrose gradient resulted in complete removal of the δ and ε subunits and cleavage of the amino-terminal 15 residues of the α subunits [26,27]. The γ subunit was not significantly cleaved in our preparations. The trypsin-treated F_1 was bound by the DCCD-binding polypeptide almost as effectively as the native enzyme (Fig. 7; left panel). Pronase treatment of

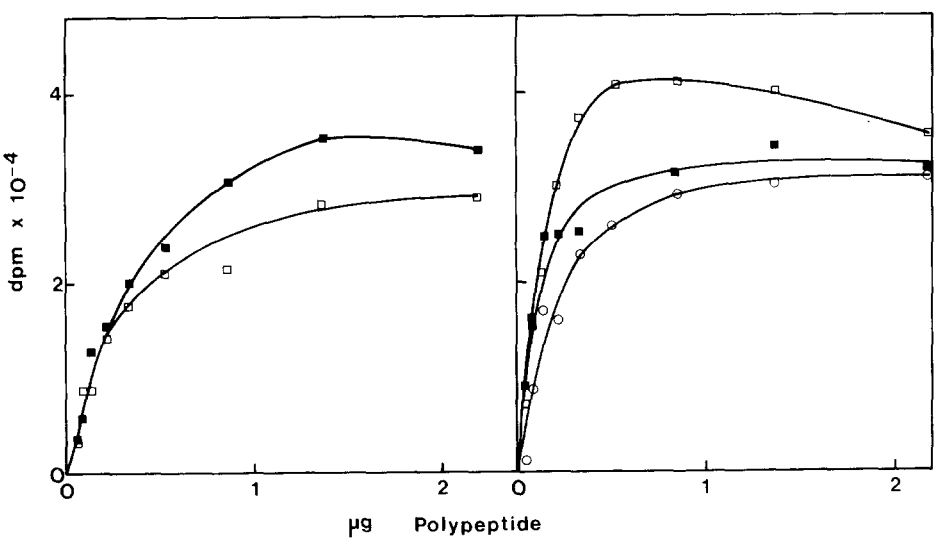

Fig. 7. Binding of F_1 subunits to DCCD-binding polypeptide. Left panel: Titration of immobilized F_1 (■) (19 μg protein) and TPCK-trypsin-treated F_1 (□) (14 μg protein) by DCCD-binding polypeptide. Right panel: Titration of immobilized F_1 (■) (19 μg protein), α subunit (□) (6 μg protein), and β subunit (○) (9 μg protein) by DCCD-binding polypeptide. The binding of antibody to the DCCD-binding polypeptide was measured indirectly with ^{125}I-labelled goat anti-rabbit immunoglobulin as described in Material and Methods.

F_1 reduced the extent of binding dramatically due to extensive cleavage of all subunits of the enzyme. The role of the major subunits of F_1 in binding was investigated following purification of the α and β subunits from the salt-dissociated enzyme by the procedure of Dunn and Futai [15]. These subunits were immobilized in polylysine-treated microtitre plate wells and the extent of binding of the DCCD-binding polypeptide measured using its antibody. Both subunits bound the polypeptide effectively (Fig. 7, right panel).

Discussion

Although the antibody was raised against the isolated DCCD-binding polypeptide, it could still recognize and bind to this polypeptide when it was assembled as an oligomer in the membrane [10]. Competitive inhibition assays have verified that the antibodies can react with the cytoplasmic surface of the cell membrane of *E. coli*. Oxidation of methionine residues in the isolated DCCD-binding polypeptide almost completely abolished its ability to react with the antibody. Cleavage of the polypeptide at methionine residues with cyanogen bromide had a similar effect. These results are consistent with antibody-binding site(s) being close to one or more methionine residues. If the binding site on the isolated polypeptide is the same as that of the membrane-bound polypeptide, then the most likely methionine residue is that at position 57 (Fig. 1). This assignment depends on the validity of the evidence [9] that tyrosine residues 10 and 73 are on the periplasmic surface of the membrane. Then the DCCD-binding polypeptide molecule must have a looped arrangement in the membrane and the other methionine residues at positions 6, 11, 16, 17, 65 and 75 could not be close to the cytoplasmic surface of the membrane (Fig. 1). Modification of arginine residues with phenylglyoxal and 2,3-butanedione had little effect on the binding of the antibody. Thus, it is unlikely that the antibody reacts with residues 41–50 of the polar segment, of the polypeptide molecule.

F_1 binds to the isolated DCCD-binding polypeptide. Modification of the arginine residues affected binding, but oxidation of methionine residues did not alter the reaction of F_1 with the polypeptide. This places the F_1-binding site near residues 41–50 in the polar segment of the polypeptide. This site is consistent with the 'looped model' in which this region is proposed to be at the cytoplasmic surface of the cell membrane [7]. The independence of the F_1-binding and antibody-binding sites was confirmed directly. Binding of F_1 to the DCCD-binding polypeptide did not prevent binding of the antibody. The reverse was also true.

The significance of the binding of F_1 by the DCCD-binding polypeptide is unclear. Arginine residues are involved in this interaction, and they also appear to be involved in the interaction of F_1 with F_0 in everted vesicles. We have found (unpublished results) that treatment of F_1-stripped everted vesicles with phenylglyoxal, an arginine-modifying reagent, completely abolished rebinding of F_1. This suggests that the binding of F_1 to the isolated DCCD-binding polypeptide may be physiologically significant. Binding to the polypeptide involves the α and/or β subunits of F_1, since both of these subunits can bind independently. This is not surprising in view of their extensive sequence homology [27]. However, δ and ε subunits of F_1 have been implicated in the binding of the F_1 to the membrane in *E. coli* [28]. These subunits are not required for interaction with the isolated DCCD-binding polypeptide. Mutants (*unc D*) have been isolated in which β subunits are retained by the membrane in the absence of the other subunits [29]. The polypeptide with which the β subunits interact has not been identified, but clearly F_1 is able to form linkages with F_0 not involving the δ and ε subunits. Recently, Andreo et al. [30] have concluded also that the δ subunit of chloroplast F_1 is not absolutely required for binding to the membrane but is required to block leakage of protons through F_0. Drozdovskaya et al. [31] have found that a significant portion of the α, β and γ subunits of beef-heart mitochondrial F_1 interact with membrane proteins.

In summary, our results are consistent with the looped arrangement of the DCCD-binding polypeptide in the membrane proposed by Altendorf et al. [7] in which the polar central region of this molecule is at the cytoplasmic surface of the cell membrane. This region may interact with the α and/or β subunits of F_1.

Acknowledgment

This work was supported by grants from the Medical Research Council of Canada.

References

1 Downie, J.A., Gibson, F. and Cox, G.B. (1979) Annu. Rev. Biochem. 48, 103–131
2 Fillingame, R.H. (1980) Annu. Rev. Biochem. 49, 1079–1113
3 Foster, D.L. and Fillingame, R.H. (1979) J. Biol. Chem. 254, 8230–8236
4 Friedl, P., Friedl, C. and Schairer, H.U. (1980) FEBS Lett. 119, 254–256
5 Hoppe, J., Schairer, H.U. and Sebald, W. (1980) Eur. J. Biochem. 112, 17–24
6 Wachter, E., Schmid, R., Deckers, G. and Altendorf, K. (1980) FEBS Lett. 113, 265–270
7 Altendorf, K., Hammel, U., Deckers, G., Kiltz, H.H. and Schmid, R. (1979) in Function and Molecular Aspects of Biomembrane Transport (Quagliariello, E., Palmieri, F., Papa, S. and Klingenberg, M., eds.), pp. 53–61, Elsevier/North-Holland, Amsterdam
8 Sebald, W. and Hoppe, J. (1981) Curr. Topics Bioenerg. 12, 1–64
9 Schneider, E., Schmid, R., Deckers, G., Steffens, K., Kiltz, H.H. and Altendorf, K. (1981) in Vectorial Reactions in Electron and Ion Transport in Mitochondria and Bacteria (Palmieri, F., Quagliariello, E., Siliprandi, N. and Slater, E.C., eds.), pp. 231–234, Elsevier/North-Holland, Amsterdam
10 Loo, T.W. and Bragg, P.D. (1982) Biochem. Biophys. Res. Commun. 106, 400–406
11 Bragg, P.D., Davies, P.L. and Hou, C. (1972) Biochem. Biophys. Res. Commun. 47, 1248–1255
12 Loo, T.W. and Bragg, P.D. (1981) Biochem. Biophys. Res. Commun. 103, 52–59
13 Yoshida, M., Sone, N., Hirata, H. and Kagawa, Y. (1975) J. Biol. Chem. 250, 7910–7916
14 Bragg, P.D. and Hou, C. (1980) Eur. J. Biochem. 106, 495–503
15 Dunn, S.D. and Futai, M. (1980) J. Biol. Chem. 255, 113–118
16 Bragg, P.D., Stan-Lotter, H. and Hou, C. (1982) Arch. Biochem. Biophys. 213, 669–679
17 MacKenzie, D. and Molday, R.S. (1982) J. Biol. Chem., 257, 7100–7105
18 Soper, J.W. and Pedersen, P.L. (1979) Methods Enzymol. 55, 328–333
19 Hirs, C.H.W. (1967) Methods Enzymol. 11, 59–62
20 Sebald, W., Machleidt, W. and Wachter, E. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 785–789
21 Fillingame, R.H. (1975) J. Bacteriol. 124, 870–883
22 Bradford, M.M. (1976) Anal. Biochem. 72, 248–254
23 Gutnick, D.L. (1975) in Molecular Aspects of Membrane Phenomena (Kaback, H.R., Neurath, H., Radda, G.K., Schwyzer, T. and Wiley, W.R., eds.), pp. 76–91, Springer-Verlag, Berlin
24 Kagawa, Y., Sone, N., Hirata, H. and Yoshida, M. (1979) J. Bioenerg. Biomembranes 11, 39–78
25 Means, G.E. and Feeney, R.E. (1971) Chemical Modification of Proteins, Holden-Day, San Francisco
26 Bragg, P.D. and Hou, C. (1978) Can. J. Biochem. 56, 559–564
27 Dunn, S.D., Heppel, L.A. and Fullmer, C.S. (1980) J. Biol. Chem. 255, 6891–6896
28 Sternweis, P.C. (1978) J. Biol. Chem. 253, 3123–3128
29 Senior, A.E., Fayle, D.R.H., Downie, J.A., Gibson, F. and Cox, G.B. (1979) Biochem. J. 180, 111–118
30 Andreo, C.S., Patrie, W.J. and McCarty, R.E. (1982) J. Biol. Chem. 257, 9968–9975
31 Drozdovskaya, N.R., Kozlov, I.A., Milgrom, Y.M. and Tsybovski, I.S. (1982) FEBS Lett. 150, 385–389