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# Effect of Dicyclohexylcarbodiimide on Unisite and Multisite Catalytic Activities of the Adenosinetriphosphatase of *Escherichia coli*†

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ABSTRACT: The inhibitory effect of dicyclohexylcarbodiimide (DCCD) on the activity of the adenosinetriphosphatase of *Escherichia coli* ($ECF_1$) has been examined in detail. DCCD reacted with $ECF_1$ predominantly in $\beta$ subunits with a maximum of 2 mol of reagent per mole of $ECF_1$ being incorporated in these subunits. Ninety-five percent inhibition of steady-state or multistate ATPase activity required incorporation of 1 mol of DCCD per mole of enzyme into $\beta$ subunits. Seventy-five percent inhibition of the initial rate of unisite catalysis was only obtained after incorporation of 2 mol of DCCD per mole of $ECF_1$ into $\beta$ subunits. Analyses of the kinetics of unisite catalysis and nucleotide binding experiments both indicate that DCCD binds outside the substrate ATP binding site. Inhibition by this reagent appears to be due in part to an effect on the catalytic sites but mainly to the blocking of cooperativity between these sites.

$F_1$ ATPases from bacteria, plant chloroplasts, and animal and plant mitochondria are similar complexes of $M_r$ 350 000-380 000 made up of five different subunits, $\alpha$, $\beta$, $\gamma$, $\delta$, and $\epsilon$, now generally agreed to be present in the stoichiometry of 3:3:1:1:1, respectively (Futai & Kanazawa, 1983; Senior & Wise, 1983; Fillingame, 1980). The active site for both ATP synthesis and ATP hydrolysis in the enzyme is thought to be located on $\beta$ subunits with a possible contribution from the $\alpha$ subunits (Futai & Kanazawa, 1983; Senior & Wise, 1983). On the basis of considerations of stoichiometry, therefore, there should be three catalytic sites per complex. Direct evidence for at least two and probably three sites of ATP hydrolysis has been obtained by substrate binding experiments and from kinetic studies of the ATPase activity of the enzyme (Cross & Nalin, 1982; Nalin & Cross, 1982; Grubmeyer et al., 1982; Grubmeyer & Penefsky, 1981a,b).

$F_1$ ATPase from all of the sources listed above show dramatic cooperativity between active sites, and this is reflected in the rate of ATP hydrolysis under different conditions of enzyme to substrate concentration ratios. Thus, the rate of ATP hydrolysis is very slow when there is more enzyme than substrate, the rate-limiting step of this single-site or unisite catalysis being the slow off rate of product ADP (Grubmeyer & Penefsky, 1981b; Grubmeyer et al., 1982; Gresser et al., 1982). When there is an excess of substrate to enzyme, i.e., under steady-state or multisite assay conditions, the rate of ATPase activity is much faster, i.e., by a factor of $10^4$-$10^6$ over that in unisite catalysis (Grubmeyer et al., 1982). The much enhanced rate of catalysis when two or more active sites per

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complex contain bound nucleotide has been explained in terms of binding of substrate in one site dramatically increasing the off rate of product at the second site (Grubmeyer et al., 1982; Gresser et al., 1982).

Another clear indication of cooperativity between catalytic sites comes from studies using inhibitors of ATP hydrolysis (Satre et al., 1979; Yoshida et al., 1981; Steinmeier & Wang, 1979; Ferguson et al., 1975). One potent inhibitor of ATPase activity is the hydrophobic carbodiimide dicyclohexylcarbodiimide (DCCD).[1] This reagent has been shown to react with $F_1$ ATPase mainly in the $\beta$ subunit and inhibit ATPase activity when bound in substoichiometric amounts with respect to the number of $\beta$ subunits per complex (Satre et al., 1979; Wang et al., 1984; Yoshida et al., 1981; Esch et al., 1981).

Here we describe studies on the effect of DCCD on single-site and multisite ATPase activity of $F_1$ ATPase from *Escherichia coli* ($ECF_1$) as a function of the stoichiometry of modification. Evidence is presented that DCCD inhibits multisite ATP hydrolysis and unisite catalysis differently and that the reagent binds to $ECF_1$ in a maximum of 2 mol per mole of enzyme or 2 mol/3 mol of $\beta$ subunit.

## Experimental Procedures

[$^{14}$C]DCCD was obtained from Research Products International. [$^{14}$C]ADP and [$^3$H]AMP-PNP were purchased from ICN. [$\gamma$-$^{32}$P]ATP was obtained from Amersham. EDC was purchased from Sigma.

*Source of Cells and Preparation of $ECF_1$.* *E. coli* strain KY7485 containing a $\lambda$-transducing phage which carries all of the genes of the unc operon was kindly provided by Dr. R. A. Fillingame, University of Wisconsin. The $F_1$ complex was purified from this strain after induction of the $\lambda$ phage (Foster et al., 1980) according to the procedure described by Foster & Fillingame (1979). Protein concentrations were determined according to Lowry et al. (1951) with bovine serum albumin as a standard. A molecular weight of 380 000 for $ECF_1$ [see Senior & Wise (1983)] was used in calculations of molarity.

*Assay of Unisite Catalysis ATPase Activity.* $ECF_1$ (0.5 $\mu$M) in 50 mM Tris-sulfate, 1 mM $KH_2PO_4$, and 0.5 mM EDTA, pH 8.0, was incubated for 1 min at 25 °C with vigorous magnetic stirring. The reaction was started by adding [$\gamma$-$^{32}$P]ATP (0.15 $\mu$M). At required times, the reaction was stopped with 60% perchloric acid. [$\gamma$-$^{32}$P]ATP was separated from labeled inorganic phosphate, [$^{32}$P]$P_i$, by the precipitation method of Sugino & Miyoshi (1964) as modified by Grubmeyer & Penefsky (1981b). The rate of ATP hydrolysis was plotted as a function of time (seconds) and the initial rate obtained from this plot by drawing a tangent to the curve at the initial time points.

*Multisite ATPase Activity.* Steady-state ATPase activity was assayed in a 1-mL solution containing 25 mM Tris–acetate (pH 7.5), 25 mM KCl, 5 mM $MgCl_2$, 5 mM KCN, 2 mM phosphoenolpyruvate, 2 mM ATP, 0.5 mM NADH, 20 units of L-lactic acid dehydrogenase, and 30 units of pyruvate kinase at 37 °C. The absorbance change at 340 nm was followed in a Beckman DU7 spectrophotometer.

*$NaDodSO_4$–Polyacrylamide Gel Electrophoresis.* Samples for gel electrophoresis were dissolved in a buffer containing 6% $NaDodSO_4$, 10% $\beta$-mercaptoethanol, 20% glycerol, and 0.125 M Tris, pH 6.2. Slab gels prepared with a 5% polyacrylamide stacking gel and a 12–18% polyacrylamide gradient gel were run according to Laemmli (1970) with 0.2% $NaDodSO_4$ in the electrophoresis buffer. Staining with Coomassie brilliant blue R and destaining were carried out according to Downer et al. (1976). Gels were scanned in a Zeidel Model SL-50h-XL soft laser scanning densitometer at 595 nm and the areas of each peak integrated with an Apple II computer. Gels were sliced into 1-mm-thick slices by using a Mickel gel slicer. Slices, dissolved in 0.5 mL of 15% $H_2O_2$ at 60 °C for 8 h, were mixed with 4 mL of Beckman Ready-sol EP LSC cocktail and counted in a Beckman LS 7000 scintillation counter.

*Reaction of $ECF_1$ with DCCD.* $ECF_1$ (1 mg/mL) was reacted with DCCD (50–150 $\mu$M) at room temperature in 50 mM Tris–sulfate and 0.5 mM EDTA, pH 7.0. At selected times, the reaction was terminated by eluting aliquots of the reaction mixture through a 1-mL Sephadex G50 column using the column centrifugation technique as described by Penefsky (1977). Complete removal of unreacted DCCD was ensured by a second cycle of elution through such a column, or DCCD was separated by gel filtration on a Sephadex LH 20 column (0.5 × 10 mm) using the same buffer system employed in the column centrifugation method.

[$^{14}$C]DCCD-labeled $ECF_1$ was reacted with EDC (6.0 mM) for 60 min at room temperature in 50 mM Mops, 50 mM KCl, 1 mM $MgCl_2$, and 10% glycerol, pH 7.2.

*Binding of [$^3$H]AMP-PNP and [$^{14}$C]ADP to $ECF_1$.* Binding of [$^3$H]AMP-PNP to $ECF_1$ or DCCD-modified $ECF_1$ (1 mg/mL) was determined as described by Cross & Nalin (1982) using the column centrifugation technique. Binding of [$^{14}$C]ADP to $ECF_1$ or DCCD-modified $ECF_1$ was measured as described by Satre et al. (1979). Enzyme (1 mg/mL) was incubated for 60 min in 50 mM Tris–sulfate, 2 mM $MgSO_4$, and 0.5 mM EDTA, pH 7.5, with different concentrations of [$^{14}$C]ADP ($1.1 \times 10^4$ cpm/nmol). Free ADP was removed by the centrifuge column method, the $ECF_1$ was collected, and aliquots were withdrawn for protein determination and for counting.

## Results

*Reaction of $ECF_1$ with DCCD and Removal of Unreacted Reagent.* $ECF_1$ was reacted with DCCD at a range of different concentrations and then protein separated from unreacted reagent prior to assaying for activity and before quantitation of the stoichiometry of modification. The standard procedure for removing unbound DCCD has been to use the Sephadex G50 centrifuge column technique of Penefsky (1977). In our hands, this method did not completely remove unreacted reagent when present in high concentration if only a single pass of the reaction mixture through the column was used. For example, when $ECF_1$ that had been reacted with 150 $\mu$M DCCD for 5 h was applied, the protein eluting from the centrifuge column contained 5 mol of reagent per mole of enzyme. A second cycle of elution reduced the amount of DCCD associated with the protein to 1.9 mol per mole of $ECF_1$ without changing the extent of inhibition induced by the reagent. When samples eluting after the first and second cycles were analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis, they each retained close to 1.9 mol of DCCD in the resolved subunits with more than 85% of the reagent in $\beta$ subunits.

An alternative approach to separating labeled $ECF_1$ from unreacted DCCD was by gel filtration on Sephadex LH 20. When a sample of $ECF_1$ that had been reacted with 150 $\mu$M DCCD for 5 h was eluted on a column of Sephadex LH 20

[1] Abbreviations: DCCD, dicyclohexylcarbodiimide; Mops, 3-(*N*-morpholino)propanesulfonic acid; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; $ECF_1$, water-soluble extrinsic ATPase sector of the $F_1F_0$ complex of *Escherichia coli*; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; AMP-PNP, 5′-adenylyl imidodiphosphate; EDTA, ethylenediaminetetraacetic acid.

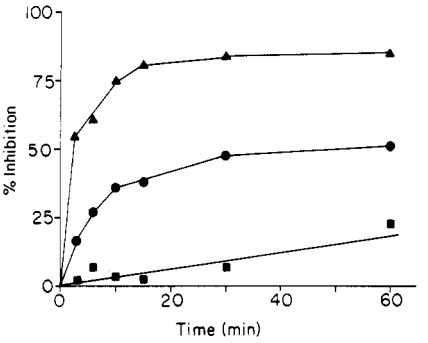


FIGURE 1: Inhibition of $ECF_1$ by DCCD in the absence and presence of ATP or $Mg^{2+}$. $ECF_1$ (1 mg/mL) in 50 mM Tris–sulfate, pH 7.0, and 0.5 mM EDTA was incubated with 150 $\mu$M DCCD at room temperature without additions (●) or in the presence of 2 mM ATP (▲) or 5 mM $MgSO_4$ (■). At the indicated times, an aliquot was withdrawn for assay of ATPase activity.

(0.5 × 10 cm), the enzyme so isolated contained 1.8 mol of DCCD/mol, and there was no further removal of reagent during $NaDodSO_4$–polyacrylamide gel electrophoresis of the protein.

*ATP Stimulation of DCCD Inhibition of Multisite Catalysis of $ECF_1$.* DCCD was found to inhibit the multisite ATPase activity of $ECF_1$ in a time- and concentration-dependent manner as reported previously (Satre et al., 1979; Lotscher & Capaldi, 1984). There are two phases to this inhibition during time course experiments, a rapid phase completed within 10–15 min at room temperature and pH 7.0 and a slower phase. Thus, the inhibition of multisite activity in the presence of 150 $\mu$M DCCD reached 30% after 10 min (Figure 1) and then increased slowly until by 5 h of incubation the enzyme was 90% inhibited. Figure 1 compares a time course of reaction of DCCD with the enzyme in the presence and absence of $Mg^{2+}$ and ATP. The metal ion was found to shield the enzyme from inhibition. In contrast, ATP significantly stimulated inhibition of $ECF_1$ by the reagent. The effect of ATP was related to an increased incorporation of DCCD into the $\beta$ subunit in the presence of ATP as shown in Figure 2.

*Effect of DCCD on Unisite Catalysis by $ECF_1$.* The initial rate of unisite catalysis of unmodified $ECF_1$ was in the range of 0.004–0.008 mol of ATP hydrolyzed per mole of $F_1$ per second under our experimental conditions. This is considerably slower than the unisite catalysis of beef heart $F_1$ which can only be followed by fast kinetics. The rate of unisite catalysis slows significantly after 20 s (Figure 3). The slower second phase of unisite catalysis has been attributed to near-equilibration of tightly bound substrate (ATP) and tightly bound products (ADP + $P_i$) on the enzyme (Grubmeyer et al., 1982). DCCD altered the kinetics of both phases of unisite catalysis of $ECF_4$ as shown in Figure 3 for a sample of enzyme containing 1.6 mol of DCCD/mol of $ECF_1$. This preparation modified by DCCD retained 65% of the initial rate of unisite catalysis of the control (unmodified) enzyme. It showed a faster second phase, and within 120 s, the amount of ATP hydrolysis by control and DCCD-reacted $ECF_1$ was almost the same.

*Stoichiometry of Inhibition of Unisite and Cooperative ATPase Activity by DCCD.* The inhibition of unisite and multisite or cooperative ATPase activity as a function of bound DCCD is shown in Figure 4A. These data are replotted in Figure 4B to compare the relative inhibition of unisite catalysis and multisite catalysis. The same plot has been used by Matsuno-Yagi & Hatefi (1984) to compare the relative inhibition of oxidative phosphorylation and multisite ATPase

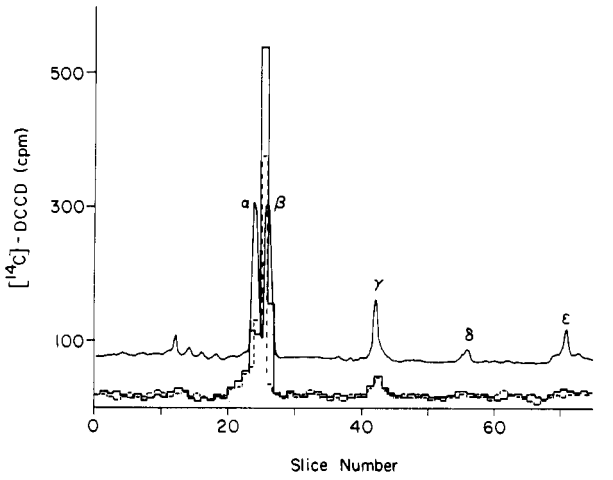


FIGURE 2: Radioactive profile of the subunits of $ECF_1$ after inhibition by [$^{14}$C]DCCD in the absence and presence of ATP. $ECF_1$ (1 mg/mL) was incubated with [$^{14}$C]DCCD (12 000 cpm/nmol) in the same condition described in Figure 1 at room temperature for 1 h in the absence or presence of 2 mM ATP. Unreacted reagent was removed by the column centrifugation method. The samples were subjected to $NaDodSO_4$ gel electrophoresis. The distribution of radioactivity was determined after the gel was stained and destained with Coomassie blue. The densitometric trace at 595 nm is also shown. The histogram shows [$^{14}$C]DCCD incorporation in the presence (—) and absence (- - -) of 2 mM ATP.

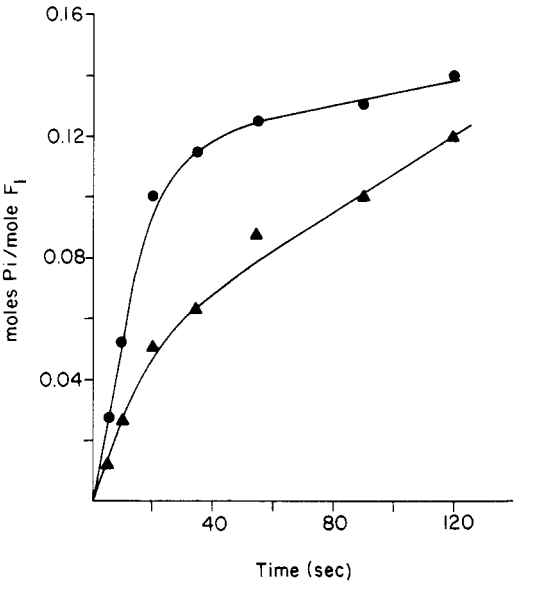


FIGURE 3: Kinetics of unisite catalysis by DCCD-modified $ECF_1$. (●) Unmodified $ECF_1$; (▲) enzyme containing 1.6 mol of DCCD per mole.

activity for DCCD-modified $F_1$ from beef heart mitochondria

DCCD inhibited the cooperative or multisite ATPase activity much more dramatically than single-site catalysis. For example, incorporation of 1 mol of DCCD per mole of $ECF_1$ inhibited multisite ATPase activity by 93% but unisite catalysis by less than half of this amount (42% in the experiment in Figure 4).

The maximum inhibition of single-site ATPase activity obtained in any set of experiments was 76%, and this was a consequence of incorporation of 2 mol of DCCD/mol of enzyme. In no experiments were significantly more than 2 mol of the carbodiimide incorporated into the $\beta$ subunits of $ECF_1$ (moles per mole of enzyme). In one experiment, $ECF_4$ was reacted with 150 $\mu$M DCCD for 24 h, and enzyme was separated from most of the unreacted reagent by gel filtration on Sephadex G50 and then subjected to a second cycle of

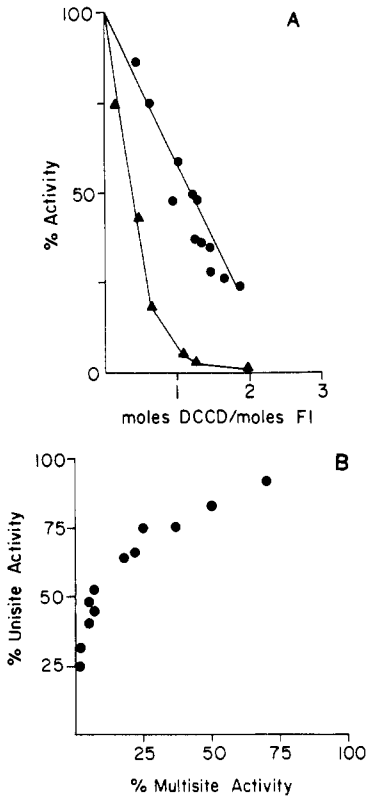


FIGURE 4: Inhibition of unisite and multisite ATPase activity of $ECF_1$ as a function of DCCD incorporated. $ECF_1$ was reacted with [$^{14}$C]DCCD for periods of time up to 15 h under conditions described in the legend to Figure 1 and with 2 mM ATP present. Unreacted DCCD was removed, and samples were assayed for both activities, counted for radioactivity, and subjected to $NaDodSO_4$–polyacrylamide gel electrophoresis. The stoichiometry of modification of $ECF_1$ by [$^{14}$C]DCCD was determined from the number of counts incorporated into $\beta$ subunits on the gel, using a molecular weight of 380 000 for the complex. In panel A, closed circles show data for unisite catalysis, and closed triangles give data for multisite catalysis. Panel B shows the percentage of activity of unisite catalysis relative to the percentage of activity of multisite catalysis at different levels of incorporation of DCCD.

reaction with the carbodiimide. This sample of $ECF_1$ had 2.05 mol of DCCD bound per mole of enzyme in $\beta$ subunits; i.e., only two of the three $\beta$ subunits were able to incorporate DCCD even after prolonged incubation with high concentrations of the reagent.

We have previously shown that the water-soluble carbodiimide EDC cross-links a $\beta$ subunit to the $\epsilon$ subunit and that this can be used to segregate $\beta$ subunits for examination of their relative reactivity to inhibitors. Figure 5 shows a time course of the distribution of [$^{14}$C]DCCD in the $\beta$–$\epsilon$ cross-linked product and into free $\beta$ subunits under conditions where up to a total of 2 mol of [$^{14}$C]DCCD per mole of enzyme had been incorporated into the protein, and where the yield of the cross-link was 45% or more based on the disappearance of $\epsilon$ from the gel profile [also see Lotscher & Capaldi (1984)]. It can be seen that the $\beta$ subunit associated with the $\epsilon$ subunit ($\beta^1$) is unreactive or at best only very poorly reactive with DCCD.

*Binding of Nucleotides to DCCD-Modified $ECF_1$.* It has been shown that $ECF_1$ binds up to 3 mol of [$^3$H]AMP-PNP per mole of enzyme in exchangeable sites in the presence of a large excess of this substrate analogue. $ECF_1$ modified with 1 mol of DCCD per mole of enzyme was found to bind the same amount of [$^3$H]AMP-PNP as unmodified enzyme with this amount approaching 3 mol/mol (result not shown). The binding of ADP to DCCD-modified enzyme is shown in Figure

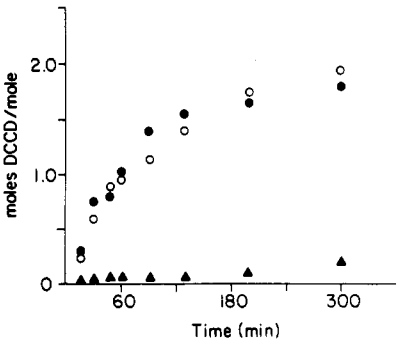


FIGURE 5: Incorporation of [$^{14}$C]DCCD into $\beta$ subunits as a function of time. $ECF_1$ modified by [$^{14}$C]DCCD was reacted with EDC as described under Experimental Procedures and then subjected to $NaDodSO_4$–polyacrylamide gel electrophoresis to resolve the $\beta$–$\epsilon$ cross-linked product. Gels were scanned, and the amount of cross-linked product was calculated by loss of free $\epsilon$ subunit from the profile. The amount of [$^{14}$C]DCCD in the $\beta$–$\epsilon$ cross-linked product (▲) and in free $\beta$ subunits with (○) and without (●) EDC treatment was determined after the gels were sliced.

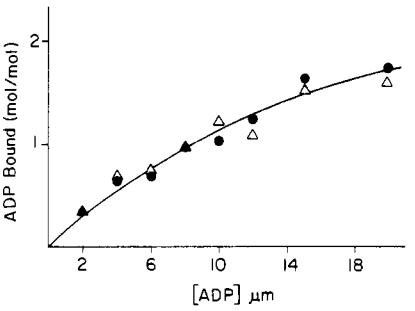


FIGURE 6: Binding of ADP to $ECF_1$ and to DCCD-modified $ECF_1$. Samples of $ECF_1$ reacted with DCCD to 96% inhibition and freed of unbound DCCD were incubated in 50 mM Tris–sulfate, 2.5 mM $MgSO_4$, and 0.5 mM EDTA, pH 7.5, with different concentrations of [$^{14}$C]ADP (11 000 cpm/nmol) at room temperature for 1 h. Free [$^{14}$C]ADP was removed by the centrifuge column technique: (●) control; (△) DCCD-modified $ECF_1$.

6. There was no observable difference between the amount of [$^{14}$C]ADP bound to a sample of $ECF_1$ incorporating 1.5 mol of DCCD per mole of complex and the control untreated enzyme.

## Discussion

DCCD has been shown to inhibit the hydrolysis of ATP by the $F_1$ ATPases from beef heart, from *E. coli*, and from the thermophilic bacterium $TF_1$ by modification of a single glutamic acid residue in the $\beta$ subunit (Satre et al., 1979; Yoshida et al., 1981, 1982; Esch et al., 1981). In the *E. coli* enzyme, the residue modified is Glu-192 (Yoshida et al., 1982). This same residue is altered in the beef heart enzyme (Esch et al., 1981) while Glu-181 is the site of DCCD incorporation in $TF_1$ (Yoshida et al., 1981) (numbering of amino acids is from the sequence of the *E. coli* $\beta$ subunit).

Two ambiguities in previous studies of the effect of DCCD on $F_1$ ATPase are the stoichiometry and mode of inhibition by the reagent. It has variously been reported that 95% inhibition of multisite ATPase activity of beef heart $F_1$ requires incorporation of 1 mol (Matsumo-Yagi & Hatefi, 1984) and 2 mol (Esch et al., 1981; Pougeous et al., 1979) of DCCD per mole of enzyme. For $ECF_1$, a stoichiometry of inhibition of multisite activity of 1 mol of DCCD per mole of enzyme has been reported by Satre et al. (1979). However, this result has been questioned by Yoshida et al. (1982) on the basis of the possibility that some of the DCCD is bound as an *O*-acylisourea, stable in the assay medium but lost when the stoi-

chiometry of reagent is measured by $NaDodSO_4$–polyacrylamide gel electrophoresis. Our results confirm the results of Satre et al. (1979) that 95% inhibition of the multisite ATPase activity of $ECF_1$ requires binding of only 1 mol of DCCD per mole of enzyme. $ECF_1$ was separated from unbound DCCD by using one or two gel filtration steps until the amount of reagent bound was found to be the same whether measured directly off the column or by $NaDodSO_4$–polyacrylamide gel electrophoresis. Only then was the activity of the modified enzyme measured. It is unlikely that any unstable *O*-acylisourea derivative can survive the conditions of gel electrophoresis.

Our studies also show that the multisite or cooperative ATPase activity of $ECF_1$ is inhibited to a greater extent than unisite catalysis by the incorporation of DCCD into $\beta$ subunits. While multisite ATPase activity was inhibited to 95% by incorporation of 1 mol of DCCD mole of $ECF_1$, unisite catalysis was only inhibited by 75% even after 2 mol of reagent had been bound per mole of enzyme.

Several observations strongly suggest that DCCD reacts outside the nucleotide binding site. The presence of ATP in the incubation medium was found to stimulate rather than protect $ECF_1$ from modification by DCCD. Also, we found that $ECF_1$ which has been modified by DCCD still binds [$^3$H]AMP-PNP and ADP is exchangeable sites with similar affinity to the unmodified enzyme. In this respect, our results are in agreement with the observations of Satre et al. (1979) and DiPietro et al. (1979) and at variance with the findings of Cross & Nalin (1982).

ATP associated with $ECF_1$ under unisite conditions is very tightly bound, and the kinetics of unisite catalysis therefore measure the conversion of bound substrate to bound products on the active sites. The slower initial rates of unisite catalysis after modification by DCCD are consistent with a much slower rate of cleavage of ATP in catalytic sites on those $\beta$ subunits which contain bound DCCD. The extent of this inhibition parallels the number of $\beta$ subunits modified until only about 25% of maximal unisite activity remains. These are conditions under which two of the three $\beta$ subunits have been modified. Even though the initial rate of unisite catalysis was affected significantly by incorporation of DCCD, the amounts of ATP hydrolyzed in DCCD-modified enzyme and control enzyme were almost the same within 120 s. This result can only be explained by ATP being hydrolyzed (although more slowly) at DCD-modified $\beta$ subunits as well as at unmodified subunits. This follows from the fact that DCCD-modified $\beta$ subunits must contain a significant portion of the tightly bound ATP, given that such altered subunits have similar affinities for nucleotides as unmodified subunits.

An important feature of the stoichiometry data is that maximally 2 mol of DCCD is incorporated per mole of $ECF_1$. This result is consistent with our previous studies indicating that one of the three $\beta$ subunits, i.e., that which can be cross-linked to the $\epsilon$ subunit by EDC ($\beta^1$), is unreactive or at best poorly reactive to DCCD (Lotscher & Capaldi, 1984). In experiments described here, enzyme modifed with 2 mol of DCCD per mole of $ECF_1$ contained only 0.05 mol of reagent per mole in the $\beta^1$ subunit. The significance of this structural asymmetry of $ECF_1$ remains to be explored. Finally, it is interesting to compare the relative inhibition of oxidative phosphorylation and multisite ATP hydrolysis after modification of $MF_1$ with DCCD as reported by Matsuno-Yagi & Hatefi (1984) (their Figure 8) with the relative inhibition of single-site catalysis and multisite ATP hydrolysis in our study of $ECF_1$ (our Figure 4). Both the single-site catalysis and ATP synthesis are affected much less by DCCD than is cooperative ATP hydrolysis. Enzyme having less than 5% of multisite ATPase activity retained around 30–40% of single-site catalysis and 30–40% ATP synthesis activity. This raises the interesting possibility that ATP synthesis may not require the same cooperativity now known to be important in multisite ATP hydrolysis.

**Registry No.** ATP, 56-65-5; ATPase, 9000-83-3; DCCD, 538-75-0.

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