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Kinetics of Inhibition and Binding of Dicyclohexylcarbodiimide to the 82,000-Dalton Mitochondrial K^+/H^+ Antiporter*

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Inhibition of K^+/H^+ antiport by *N,N'*-dicyclohexylcarbodiimide in Mg^{2+} depleted mitochondria follows first order kinetics, exhibiting a half-time of 13 min when mitochondria are incubated with 50 nmol/mg inhibitor at 0 °C. ^{14}C radiolabeled *N,N'*-dicyclohexylcarbodiimide binds to the 82,000-dalton protein, and the second order rate constant for binding is found to be approximately the same as the second order rate constant for inhibition. These findings provide additional confirmation of the identification of this porter with the 82,000-dalton protein and permit us to estimate that rat liver mitochondria contain about 8 pmol/mg of K^+/H^+ antiporter with a turnover number of 700 s^{-1}.

The K^+/H^+ antiporter of rat liver mitochondria is protected from *N,N'*-dicyclohexylcarbodiimide inhibition and binding by quinine and by endogenous Mg^{2+}. An 82,000-dalton, [^{14}C]*N,N'*-dicyclohexylcarbodiimide-binding protein is also observed in rat liver submitochondrial particles, establishing this as an integral protein of the inner membrane. Submitochondrial particles, presumed to be inverted in membrane orientation, are protected from radiolabeling by external Mg^{2+}, supporting the contention that the Mg^{2+} binding site is localized to the matrix side of the K^+/H^+ antiporter.

During steady-state respiration, electrophoretic potassium influx into mitochondria is balanced by electroneutral potassium efflux via the Mg^{2+}-regulated K^+/H^+ antiporter (1). Gauthier and Diwan (2) were the first to report that DCCD[1] inhibits K^+ uptake into respiring mitochondria, a finding later confirmed by Jung *et al.* (3). It is difficult in energized mitochondria to exclude interference with other steps in the complex sequence leading to K^+ uptake; nevertheless, Jung *et al.* (3) have concluded that DCCD inhibits K^+ uniport but not cation/proton antiport. On the basis of a simpler assay, in which Mg^{2+}-depleted mitochondria undergo rapid KOAc uptake in the absence of respiration (4), we have concluded that DCCD blocks K^+ transport by virtue of an irreversible interaction with the mitochondrial K^+/H^+ antiporter (5). This interpretation is strengthened by the demonstration that K^+ uptake under these conditions is mediated almost entirely by K^+/H^+ antiport and not by K^+ uniport (6).

Armed with this result, we now wish to characterize more thoroughly the interaction between DCCD and the K^+/H^+ antiporter. In particular, it remains to be shown that the kinetics of DCCD binding to the 82,000-dalton protein previously identified as the K^+/H^+ antiporter (5) are consistent with this identification. The experiments reported here show that the rate constant for *inhibition* of antiport activity is the same, within experimental error, as the rate constant for *binding* to the 82,000-dalton protein and that all conditions which protect the antiporter from inhibition by DCCD also prevent labeling of the 82,000-dalton protein by DCCD. We have used these results to obtain an estimate of 700 s^{-1} for the turnover number of this carrier.

EXPERIMENTAL PROCEDURES

Mitochondrial Preparations—Rat liver mitochondria, isolated by differential centrifugation as previously described (7), were stored at 0 °C at 50 mg of protein/ml in 0.25 M sucrose. Submitochondrial particles were isolated by sonication of mitoplasts following the protocol described by Hackenbrock and Hammon (8).

Mg^{2+}-depleted mitochondria (5) were prepared by adding one part of stock suspension to four parts of 25 °C medium containing K^+ salts of TES (27 mM) and EDTA (5.5 mM). The resulting mixture is 110 mosm and pH 7.8. Rotenone (1 μg/mg) and A23187 (1 nmol/mg) were added, the suspension was incubated at 25 °C for 2 min to allow K^+/H^+ antiport to come to equilibrium, and then it was placed on ice. This pretreatment results in Mg^{2+} depletion to 2–4 nmol/mg, as confirmed by atomic absorption spectroscopy. For DCCD-treated preparations, DCCD was added after 3 min on ice.

K^+/H^+ antiport activity was estimated from the rate of swelling of Mg^{2+}-depleted mitochondria suspended at 0.1 mg/ml in 110 mosm KOAc medium at 25 °C containing K^+ salts of acetate (55 mM), TES (5 mM), EDTA (0.1 mM), and EGTA (0.1 mM), pH 7.8. Under these conditions, the K^+ transport rates comprising the control activities for the experiments of this paper were 258 ± 23 nmol/(mg·min) (n = 20, four different mitochondrial preparations). The rate of swelling was monitored by changes in the inverse absorbance of the suspension at 520 nm, as previously described (5–7).

SDS-Polyacrylamide Gel Electrophoresis and Fluorography—Mitochondria were incubated at 10 mg/ml with [^{14}C]DCCD. At predetermined times, the reaction was stopped by adding 0.1 ml of the suspension to 0.9 ml of ice-cold acetone (9). The precipitate was washed three times with 90% acetone and resuspended in 1% SDS. SDS-PAGE was performed by the method of Laemmli (10) and fluorographs were prepared as described by Bonner and Laskey (11).

Estimates of bound [^{14}C]DCCD were obtained from slices of dried gels from labeled mitochondria. 3-mm strips were cut from each lane in the region containing the 82,000-dalton protein, as determined from molecular weight standards. 11 lanes were sampled in this way for each time point. These were pooled and oxidized in a Packard

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[1] The abbreviations used are: DCCD, *N,N'*-dicyclohexylcarbodiimide; k_1, pseudo-first order rate constant; k_2, second order rate constant; mosm, milliosmolal; TES, *N*-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SMPs, submitochondrial particles; TEA^+, tetraethylammonium; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid.

model 306 tissue oxidizer, and the evolved $^{14}CO_2$ was trapped with 8 ml of Carbasorb II (Packard Instrument Co.). These samples were counted in a Beckman Instruments model LS 230 liquid scintillation counter and adjusted for counting efficiency, as determined by internal [^{14}C]toluene standards. The manufacturer's stated specific activity, 54 Ci/mol (2 Bq/pmol), was used to determine the amount of DCCD contained in each pooled sample, and this was normalized to the amount of original mitochondrial protein transferred to the gels. The resulting number is an estimate of the number of moles of DCCD incorporated into the 82,000-dalton protein/mg of total mitochondrial protein. [^{14}C]DCCD, obtained from Research Products International, was dissolved in ethanol following evaporation of the original solvent (pentane) with nitrogen gas at 25 °C. Molecular weight standards were purchased from Bio-Rad. All chemicals used were reagent grade and commercially available.

RESULTS

Kinetics of DCCD Inhibition of the K^+/H^+ Antiporter—Incubation of Mg^{2+}-depleted mitochondria with DCCD results in a rapid loss of K^+/H^+ antiport activity (Fig. 1). The pseudo-first order rate constants for DCCD inhibition (k_1), determined from the semilog plots of Fig. 1*A*, are proportional to DCCD concentration (Fig. 1*B*), indicating inhibition by 1 mol of DCCD/mol of antiporter (12). The second order rate constant for DCCD inhibition (k_2), obtained from the slope of the curve in Fig. 1*B*, is 1.2×10^{-3} (nmol/mg)$^{-1}$ min^{-1}. An independent experiment of the same type gave a similar result, 1.1×10^{-3} (nmol/mg)$^{-1}$ min^{-1}. Expressing the concentration of DCCD in units of nanomoles/milligram rather than in molar units is based on the observation that k_2 is constant over a 4-fold range of protein concentrations when the amount of DCCD per milligram of protein is held constant and DCCD concentration is varied. The dependence of the second order rate constant on the concentration of membranes, which has also been observed for DCCD inhibition of the mitochondrial ATPase (13), probably reflects the partitioning of the hydrophobic DCCD molecule into the mitochondrial membranes (13, 14).

Kinetics of [^{14}C]DCCD Binding to the 82-kDa Protein—[^{14}C]DCCD binding to the 82-kDa protein, measured following SDS-PAGE of mitochondrial proteins as described under "Experimental Procedures," also follows first order kinetics (see Fig. 2). Assuming one DCCD-binding site per 82-kDa protein, the data in Fig. 2 yield an estimate of k_2 equal to 0.84 $\times 10^{-3}$ (nmol/mg)$^{-1}$ min^{-1}, in good agreement with the estimate for inhibition of antiport activity (1.1×10^{-3} (nmol/mg)$^{-1}$ min^{-1}). Extrapolation provides an estimate of 7–8 pmol/mg for the total number of DCCD-binding sites in rat liver mitochondria. Assuming one DCCD-binding site per antiport protein and using estimates of the number of mitochondria per milligram (15, 16), this works out to about 800 K^+/H^+ antiporters/mitochondrion. We have observed maximal rates of K^+/H^+ antiport of about 300 nmol/mg·min (17) from which we can calculate a turnover number of 680 s^{-1} for this porter.

Effects of Mg^{2+} and H^+ on DCCD Inhibition—Both Mg^{2+} and H^+ have been proposed as physiological regulators of K^+/H^+ antiport (1, 17–19), and both prevent labeling of the antiport protein by radioactive DCCD (5). Partial depletion of matrix Mg^{2+} from 38 to 20 nmol/mg results in partial release of K^+/H^+ antiport activity (data not shown) and a corresponding 79% decrease in the k_1 for DCCD inhibition (see Fig. 3). Incubating mitochondria at pH 6.7 with DCCD produces a similar effect (Fig. 3), decreasing the k_1 by 83%. These data support the conclusion (5) that inhibitors of K^+/H^+ antiport also protect the antiporter from inhibition by DCCD. We postulate that matrix magnesium and protons inhibit the antiporter by binding to an allosteric site on the protein (17). Since this site is most likely located in a hydrophilic region and since DCCD most likely interacts within a hydrophobic region (14, 20–28), we consider it likely that Mg^{2+} and H^+ protect by inducing a conformational change in the antiporter protein (5). Note that the pH dependence is contrary to the expectation that reaction with an uncharged carboxylic group should proceed faster at acid pH (29–31).

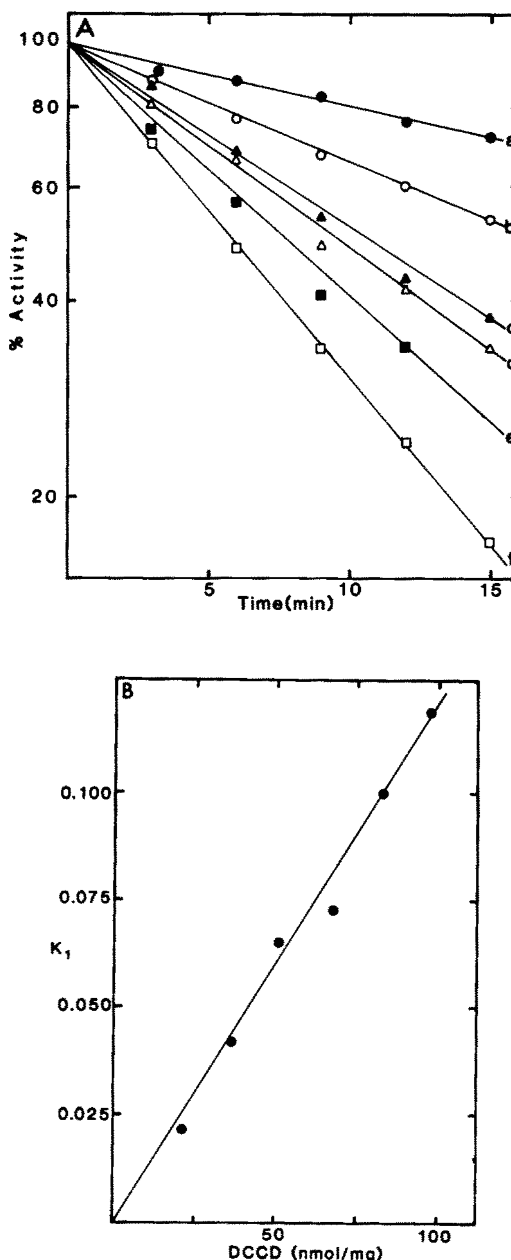

FIG. 1. **The kinetics of DCCD inhibition of mitochondrial K^+/H^+ antiport activity.** *A*, the percent of K^+/H^+ antiport activity is plotted *versus* time of incubation with DCCD, as described under "Experimental Procedures." The *curves* correspond to DCCD concentrations (nmol/mg) of: *a*, 21.5; *b*, 36.3; *c*, 51.2; *d*, 67.7; *e*, 82.6; and *f*, 97.5. *B*, values of the first order rate constant, k_1, for DCCD inhibition, determined from the curves in *A*, are plotted *versus* the amount of DCCD added during the incubation. The slope yields $k_2 = 1.2 \times 10^{-3}$ (nmol/mg)$^{-1}$ min^{-1}.

Effect of Quinine on DCCD Inhibition—Quinine is a reversible inhibitor of the antiporter (4) and also protects the antiporter from DCCD inhibition and binding (5). To facilitate quantitative comparison of inhibition and protection, we have examined the dose response curve for each effect. From Fig. 4 it can be seen that the dose response curve for quinine

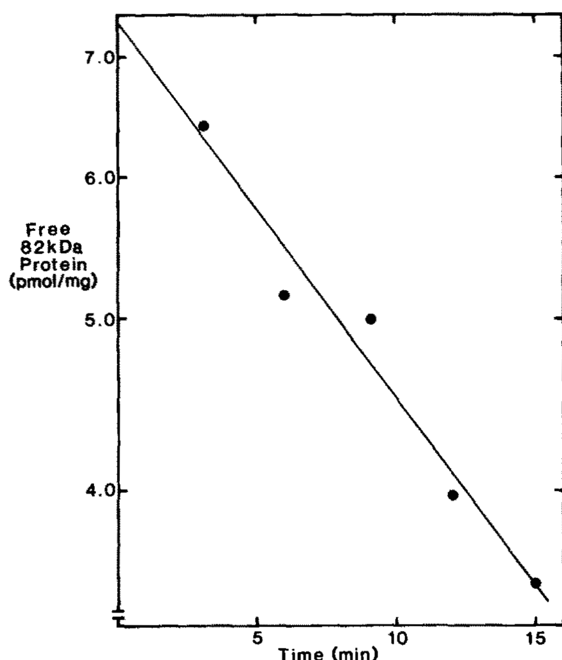

FIG. 2. **The kinetics of DCCD binding to the 82,000-dalton polypeptide.** Mg^{2+}-depleted mitochondria were reacted with [^{14}C] DCCD, and the amount of DCCD incorporated into the 82,000-dalton protein was estimated as described under "Experimental Procedures." Assuming a 1:1 stoichiometry (see text), "free 82-kDa protein" is given by $A(\infty) - A(t)$, where $A(t)$ is the amount of DCCD incorporated after t minutes of incubation. In two preparations, $A(\infty)$, determined by iteration, was found to be 7.3 and 7.7 pmol/mg, and these values were found to be within 5% of $A(60)$, the observed amount of DCCD incorporated at 60 min. The slope of the curve corresponds to $k_2 = 0.84 \times 10^{-3}$ (nmol/mg)$^{-1}$ min^{-1}.

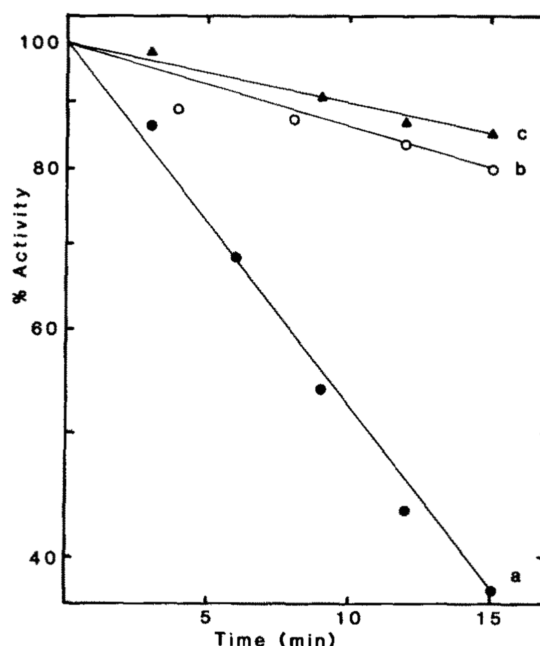

FIG. 3. **Mg^{2+} and H^+ protection of the K^+/H^+ antiporter.** The percent of K^+/H^+ antiport activity is plotted *versus* time of incubation in the presence of 50 nmol/mg of DCCD. *Curve a,* data were obtained following incubation with DCCD at pH 7.8 in 110 mosm medium containing 5.5 mM EDTA, as described under "Experimental Procedures." *Curve b,* data were obtained as in *curve a,* except that 5.5 mM EDTA was replaced by 0.1 mM EGTA and 15 mM sucrose. *Curve c,* data were obtained as in *curve a,* except that pH was 6.7 during incubation with DCCD.

protection against DCCD inhibition is parallel to that for quinine *inhibition* of K^+/H^+ antiport. The I_{50} is 65 μM (range, 40–70 μM) for protection and 28 μM (range, 18–30 μM) for inhibition. Since the protection experiments were carried out at mitochondrial concentrations of 5–10 mg/ml, while inhibition experiments were at 0.1 mg/ml, it occurred to us that the difference in I_{50} values could be explained by partitioning of the hydrophobic drug into the membranes. This was verified directly by assaying K^+/H^+ antiport at mitochondrial concentrations up to 2 mg/ml, using an optical probe with a reduced light path. By extrapolation, an I_{50} for quinine inhibition at 10 mg/ml of 40–60 μM was found, indicating agreement between the two values. The kinetics of quinine protection are consistent with a number of different mechanisms. The effective membrane concentration of DCCD could be reduced through formation of an inactive complex with quinine or through salting-out from the membrane phase by quinine. We consider it more likely that quinine protects by virtue of a direct interaction with the antiporter protein. Although this mechanism remains hypothetical, it is supported by the agreement, after correction for mitochondrial concentration, between the I_{50} for protection and the I_{50} for inhibition. The effect of DCCD concentration on quinine protection is shown in Fig. 5, in which $1/k_1$ is plotted *versus* [quinine]. If we assume that quinine protects by virtue of its own interaction with the antiporter, then the negative x intercept equals the K_I for quinine (32). These values are found to be 39 and 48 μM for the experiments reported in Fig. 5.

Effect of Osmolality on DCCD Inhibition and Binding—Bernardi and Azzone (33) have shown that K^+/H^+ antiport activity increases as the medium osmolality decreases, and we

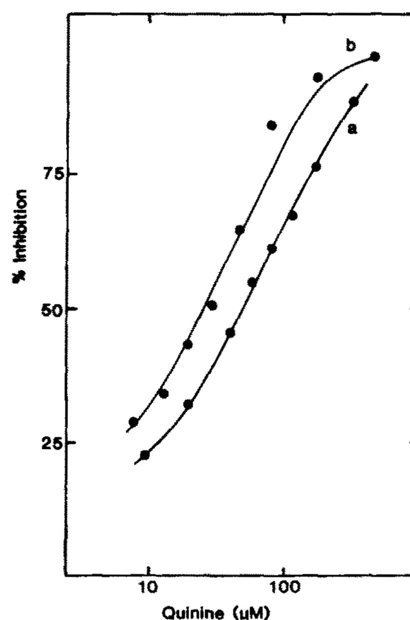

FIG. 4. **Dose response curves for quinine inhibition and protection of the K^+/H^+ antiporter.** *Curve a,* the first order rate constant, k_1, for DCCD inhibition of K^+/H^+ antiport was determined at various concentrations of quinine, which was present *during incubation with DCCD* (50 nmol/mg). The data are plotted as the percent reduction of k_1 *versus* the log of quinine concentration (μM). *Curve b,* mitochondria were depleted of Mg^{2+}, and K^+/H^+ antiport activity was assayed at various concentrations of quinine, which was present *during the assay.* The percent inhibition of K^+/H^+ antiport activity is plotted *versus* the log of quinine concentration.

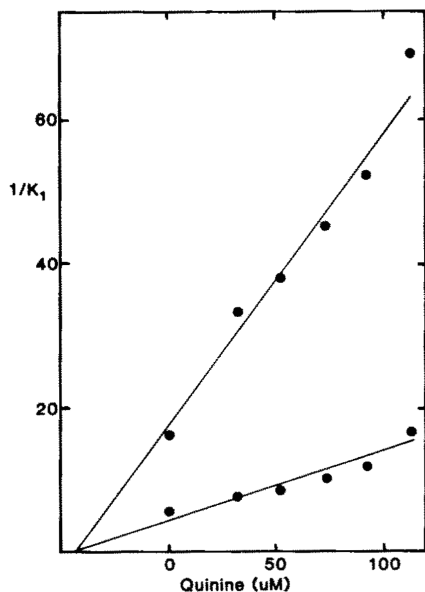

FIG. 5. **Quinine protection against DCCD inhibition of the K^+/H^+ antiporter.** The first order rate constant, k_1, for DCCD inhibition of the K^+/H^+ antiporter was determined at two concentrations of DCCD and at various levels of quinine during the incubation. Linear regressions on the data indicate negative intercepts of 39 μM (*upper curve*, 27 nmol/mg DCCD) and 48 μM (*lower curve*, 80 nmol/mg DCCD). The lines are drawn with a negative intercept of 39 μM for both sets of data.

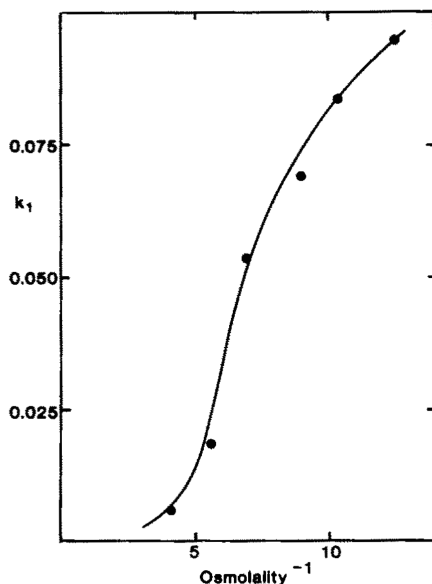

FIG. 6. **Increasing osmolality protects the K^+/H^+ antiporter against DCCD inhibition.** Mitochondria were incubated with DCCD (50 nmol/mg) as described under "Experimental Procedures" except that the osmolality was adjusted by addition of K^+ TES after Mg^{2+} depletion and before addition of DCCD. The first order rate constant for inhibition, k_1, is plotted *versus* inverse osmolality.

have verified this finding in our own laboratory. The rate constant for DCCD inhibition also increases as medium osmolality decreases (see Fig. 6), supporting with the conclusion of Brierley *et al.* (34) that the K^+/H^+ antiporter is protected from DCCD inhibition by increased osmotic strength. Our studies, including those of Fig. 6, show nearly complete protection in 250 mosm medium.

To investigate the possibility that changes in matrix pH, rather than changes in matrix volume, could be responsible for the osmotic effects (Fig. 6), we substituted TEA^+ for matrix K^+ and Mg^{2+}. TEA^+ is not transported by the antiporter (35) and TEA^+ uptake is exceedingly slow in the absence of respiration; therefore, matrix pH should remain unaffected by changes in osmolality in these mitochondria. Nevertheless, these preparations are also found to be protected against DCCD inhibition at high osmolalities (data not shown). Experiments were also carried out at constant osmolality using mitochondria loaded with varying amounts of TEA^+ salts to change matrix volume. Fig. 7 shows that the k_1 for DCCD inhibition depends on the amount of TEA^+ loading, suggesting that the protective effect of osmolality is not due to changes in medium osmolality *per se*. We tentatively conclude that *matrix volume* affects both the activity of the antiporter (33) and its inhibition by DCCD (see Figs. 6 and 7 and Ref. 34). In line with previous arguments (36), we could postulate that an unknown matrix solute interacts with the antiporter such that increasing its concentration results in increasing binding to the antiporter with consequent inhibition and protection. It should be noted, however, that the putative allosteric regulatory sites are nearly empty of Mg^{2+} and protons during these experiments, and nonspecific interactions may occur under these conditions.

Despite the fact that its mechanism remains obscure, the volume effect has been exploited as an independent test to verify the identification of the K^+/H^+ antiporter with the 82-kDa [^{14}C]DCCD-binding protein (5). The fluorographs presented in Fig. 8 show that Mg^{2+}-depleted mitochondria incubated with [^{14}C]DCCD in 110 mosm medium exhibit a labeled band at 82 kDa (*lane 1*). In contrast, the 82-kDa protein is *not* labeled when Mg^{2+}-depleted mitochondria are incubated in 250 mosm medium (*lane 2*) or when Mg^{2+}-containing mitochondria are incubated in 110 mosm medium (*lane 3*). Therefore, K^+/H^+ antiport and the 82-kDa protein are both protected from DCCD by isotonic conditions (272 mosm) in the absence of Mg^{2+} and by Mg^{2+} in hypotonic medium (110 mosm), providing additional evidence that the 82-kDa protein represents the K^+/H^+ antiporter.

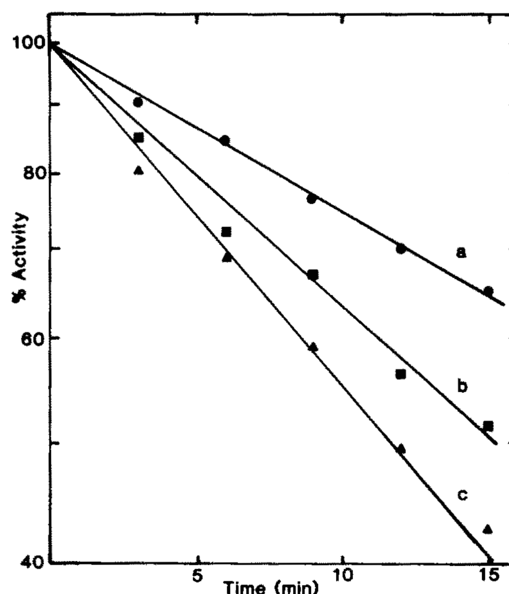

FIG. 7. **The effect of tetraethylammonium loading on DCCD inhibition.** Mitochondria were loaded with tetraethylammonium, anions, and water for various times in 270 mosm medium, as described under "Experimental Procedures," and the time course of DCCD inhibition was measured. The mitochondria were allowed to respire for 3 min (*curve a*), 5 min (*curve b*), and 7 min (*curve c*) before the addition of A23187 and initiation of DCCD exposure.

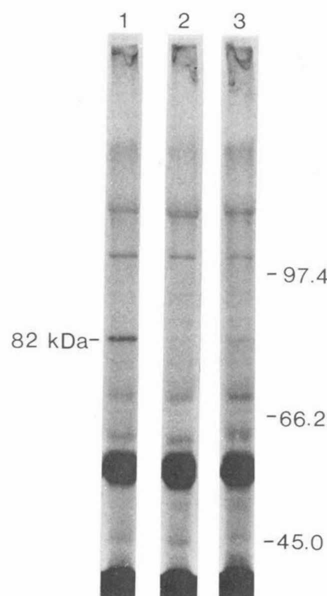

FIG. 8. **Increased osmolality prevents [^{14}C]DCCD labeling of the 82-kDa protein.** Fluorographs were obtained following SDS-PAGE with 7.5% gels. *Lane 1*, Mg^{2+}-depleted mitochondria were incubated with [^{14}C]DCCD in 110 mosm medium. *Lane 2*, Mg^{2+}-depleted mitochondria were incubated with [^{14}C]DCCD in medium adjusted to 250 mosm with K^+ TES after Mg^{2+} depletion. *Lane 3*, Mg^{2+}-containing mitochondria were incubated with [^{14}C]DCCD in 110 mosm medium in which EDTA was replaced by EGTA.

[^{14}C]DCCD Labeling of Submitochondrial Particles—SMPs lack soluble matrix enzymes and outer membrane proteins, and bands visualized on fluorographs of SMPs represent proteins associated with the inner membrane. SMPs treated with [^{14}C]DCCD in the presence of EDTA are labeled at 82 kDa (Fig. 9, *lane 1*). External Mg^{2+} (1 mM) or quinine (0.5 mM) prevents labeling of this protein (*lanes 2* and *3*, respectively). Assuming the SMPs are inside out, the data in *lane 2* of Fig. 9 show that the Mg^{2+} *protection* site is located on the matrix side of the inner membrane. Since the Mg^{2+} *inhibitory* site has also been localized to the internal surface of the inner membrane (1, 18, 19), these results suggest that the site responsible for Mg^{2+} inhibition of K^+/H^+ antiport is identical to the site for Mg^{2+} protection against DCCD binding to the antiporter.

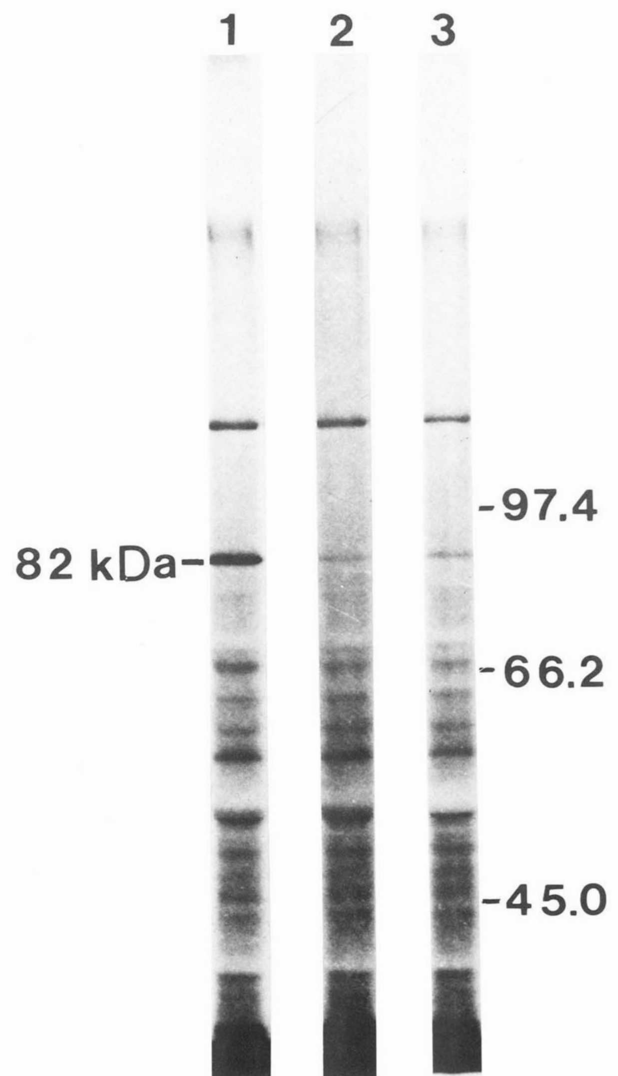

FIG. 9. **[^{14}C]DCCD labeling of an 82-kDa protein in submitochondrial particles.** Fluorographs were obtained following SDS-PAGE with 7.5% gels. *Lane 1*, SMPs were incubated with [^{14}C]DCCD in the presence of EDTA. *Lane 2*, 0.5 mM quinine was included in DCCD incubation. *Lane 3*, 0.1 mM EGTA, 15 mM sucrose, and 1 mM $MgCl_2$ were substituted for EDTA during DCCD incubation.

DISCUSSION

The use of DCCD to probe the structure and function of transport proteins and enzymes of bioenergetic relevance has recently been reviewed by Azzi *et al.* (14). Since DCCD forms covalent bonds with hydrophobically located carboxyl groups, it is somewhat nonselective as a reagent for transport proteins, and numerous labeled bands are seen following SDS-PAGE and fluorography of [^{14}C]DCCD-labeled mitochondria (4). We would have had little chance of identifying the K^+/H^+ antiporter by means of [^{14}C]DCCD *binding* were it not for the observation that DCCD *inhibition* of K^+/H^+ antiport is exquisitely sensitive to the state of the antiporter during exposure to DCCD (4). Thus, conditions leading to DCCD inhibition are found to be the same as those required for [^{14}C]DCCD binding; and, conversely, conditions *preventing* DCCD inhibition are the same as those in which [^{14}C]DCCD binding is prevented. It remains to demonstrate a quantitative, as well as qualitative, correlation between DCCD inhibition and binding. Since the rate constants for DCCD inhibition of mitochondria processes vary widely, as between the F_OF_1-ATPase (13) and the transhydrogenase (37) for example, a kinetic comparison provides a stringent test of the identification of the 82-kDa, [^{14}C]DCCD-labeled protein with the K^+/H^+ antiporter.

DCCD inhibition of K^+/H^+ antiport activity as well as [^{14}C]DCCD binding to the 82-kDa protein both obey first order kinetics with respect to DCCD concentration, and the second order rate constants for binding and inhibition are approximately the same. This agreement strengthens the identification of the K^+/H^+ antiporter (5) and suggests that one DCCD per antiporter is the maximum amount bound. This is in contrast to other systems in which full inhibition of activity has been observed at substoichiometric levels of DCCD bound (20–25, 37). Although alternative explanations for these complex interactions have been offered (24, 25), they are consistent with the existence of an oligomeric complex in which each monomer contains a DCCD-binding site and binding to one site is sufficient for inhibition. Recently, Casey *et al.* (26) have shown that cytochrome *c* oxidase contains one specific DCCD-binding site per molecule which is essential for enzymatic activity. We have observed a similar correlation for the K^+/H^+ antiporter, and conclude that modification by DCCD

of a single specific site on the protein is sufficient to provide complete inhibition of antiport activity.

The second order rate constant for [^{14}C]DCCD binding to the 82-kDa band was found in two separate experiments to be 0.7×10^{-3} and 0.8×10^{-3} $(nmol/mg)^{-1}$ min^{-1} (see Fig. 2). These values are somewhat lower than the corresponding second order rate constant for inhibition. The deviation may be within experimental error, but it is also consistent with a small loss of label via side reactions during preparation of the samples for SDS-PAGE (13, 27, 28). The reaction of DCCD with a carboxyl group initially forms an unstable *O*-acylisourea which is highly susceptible to attack by nucleophiles. If nucleophilic attack occurs before rearrangement to the more stable *N*-acylisourea, the carboxyl group may be regenerated or modified without incorporation of the carbodiimide.

The conclusion that the 82-kDa peptide contains only one DCCD-binding site enables us to estimate that rat liver mitochondria contain 7–8 pmol/mg of K^+/H^+ antiporter protein. From the estimated V_{max} of the antiporter, 300–350 nmol/mg·min (17), we may calculate a turnover number of 40,000–50,000 min^{-1} or 700–800 s^{-1}. This is remarkably close to the value recently obtained by Ligeti *et al.* (38) for the P_i/OH antiporter which is present in 10-fold excess over the K^+/H^+ antiporter. Thus, the difference between the V_{max} for P_i/OH antiport and the V_{max} for K^+/H^+ antiport in intact mitochondria can be accounted for almost completely by the difference in *amounts* of the two antiporters within the membrane.

DCCD inhibits the K^+/H^+ antiporter and binds to the 82-kDa protein only when reacted under conditions favorable for K^+/H^+ antiport activity. When reacted under conditions in which K^+/H^+ antiport itself is reversibly inhibited, DCCD neither inhibits nor binds, and the antiporter is protected from irreversible DCCD attack (5). Protection is provided by matrix Mg^{2+}, by acid matrix pH, by quinine, and by increased osmotic strength. Subthreshhold protection by any of these factors results in reduced values of the pseudo-first order rate constants for DCCD inhibition (see Figs. 3 and 5–7).

The observation that SMPs from rat liver mitochondria are labeled appropriately with [^{14}C]DCCD (Fig. 9) establishes that the 82,000-dalton protein is an integral protein of the inner membrane. Since the K^+/H^+ antiporter is a fundamental homeostatic device of chemiosmotic systems (1, 39), we would expect it to be conserved in nature. The present results confirm that DCCD binding to the K^+/H^+ antiporter is exquisitely sensitive to the state of the antiporter during exposure to DCCD, suggesting that the [^{14}C]DCCD assay may be useful as a screen for the presence of the K^+/H^+ antiporter in membranes from other tissues and organisms. Such studies are underway in our laboratory.

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