Specific Dicyclohexylcarbodiimide Inhibition of the E–P + H_2O ⇌ E + P_i Reaction and ATP ⇌ P_i Exchange in Sarcoplasmic Reticulum Adenosinetriphosphatase†

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ABSTRACT: Treatment of sarcoplasmic reticulum adenosinetriphosphatase (ATPase) with *N,N'*-dicyclohexylcarbodiimide is known to produce total inhibition of calcium binding and enzyme activity. However, we now find that treatment with lower reagent:protein ratios produces selective inhibition of hydrolytic P_i cleavage, enzyme phosphorylation with P_i, and ATP ⇌ P_i exchange, while calcium binding and enzyme phosphorylation with ATP remain largely unaffected. This specific inhibition is attributed to derivatization of residues which are normally involved in acid–base-assisted catalysis of the hydrolytic reaction and its reversal, but are not involved in calcium binding or in the mechanism of phosphoryl transfer from ATP to the enzyme. This specific inhibition is prevented by the presence of micromolar calcium during the incubation with the inhibitor, evidently through an allosteric effect of calcium binding on the catalytic site. We also find that the initial adducts formed between ATPase residues and *N,N'*-dicyclo[^{14}C]carbodiimide undergo further degradation with release of radioactive product into the medium, while the protein residues remain inactivated probably by linkage with neighboring residues. Therefore, the stoichiometry of radioactive labeling underestimates the actual number of inactivated residues.

N,N'-Dicyclohexylcarbodiimide (DCC) has been widely used as a reagent for modifying carboxylate side chains of proteins in order to inhibit H^+ translocation and/or catalytic activity in several enzyme systems (Hoppe & Sebald, 1981; Fillingame, 1980; Pennington & Fischer, 1981; Phelps & Hatefi, 1981).

In sarcoplasmic reticulum (SR) this reagent has been found to inhibit calcium binding to high-affinity sites whose occupancy by calcium is an absolute requirement for enzyme activation; therefore, the concomitant inhibition of adenosinetriphosphatase (ATPase) activity has been attributed to a primary interference of the carboxylate derivatization with calcium binding (Pick & Racker, 1979; Murphy, 1981). However, DCC also inhibits the *Neurospora* H^+-ATPase which operates with a catalytic mechanism analogous to that of the Ca^{2+}-ATPase but does not require Ca^{2+} for activation (Bowman et al., 1978; Sussman & Slayman, 1983), indicating that catalysis of the hydrolytic reaction can be inhibited by a mechanism other than calcium binding. Therefore, we have looked for conditions permitting blockage of specific residues of SR ATPase with DCC, in an attempt to inhibit selectively one of the partial reactions of the catalytic cycle without inhibiting calcium binding. We have incubated SR ATPase with different concentrations of DCC for various time intervals and then studied the effects of these incubations on the partial reactions of the enzyme cycle. In addition we have determined the stoichiometry of ATPase labeling with [^{14}C]DCC and compared it with various patterns of inactivation.

Materials and Methods

SR vesicles were prepared from rabbit leg muscle as previously described (Eletr & Inesi, 1972). *N,N'*-Dicyclohexyl[^{14}C]carbodiimide ([^{14}C]DCC) was obtained from Amersham or R.P.I.

Treatment with DCC. SR vesicles (1–5 mg of protein/mL) were incubated at 2 °C in a medium containing 200 μM DCC, 30 mM MES,[1] pH 6.2, 100 mM KCl, 20 μg of A23187/mL, and either 1 mM EGTA or 50 μM $CaCl_2$. The reaction was stopped by increasing the pH to 8.0 and $CaCl_2$ concentration to 1 mM.

Fractionation of [^{14}C]DCC-Treated Vesicles by HPLC. SR vesicles (4–7 mg) treated with [^{14}C]DCC were dissolved in 1.0 mL of 50 mM phosphate buffer (pH 7.0) containing 1% SDS and 0.1 M Na_2SO_4. A small volume (0.10–0.15 mL) of the dissolved sample was injected in a Waters HPLC system equipped with a size exclusion column type TSK G 3000 SW. The sample was then eluted with the 50 mM phosphate buffer (pH 7.0) containing 1% SDS and 0.1 M Na_2SO_4, at a flow rate of 0.4 mL/min (room temperature). The light absorption of the elution medium was monitored continuously with a Waters 440 UV detector (280 nm), and fractional samples (0.1 mL) were collected for measurement of radioactivity by scintillation counting.

Determination of [^{14}C]DCC Bound to SR Protein. SR vesicles were incubated with [^{14}C]DCC and at fractional times 0.5–0.7 mg of SR protein was placed on a 0.45-nm HAWP Millipore filter and washed once with 1.0 mL of 50% ethanol and several times with 2:1 chloroform–methanol in order to remove the membrane lipids. The filter was then dissolved in dimethylformamide for determination of radioactivity. Approximately 10% of the protein was lost during the washing, and the results were corrected accordingly.

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[1] Abbreviations: MES, 2-(*N*-morpholino)ethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; HPLC, high-performance liquid chromatography; SDS, sodium dodecyl sulfate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; MOPS, 3-(*N*-morpholino)propanesulfonic acid.

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Chromatographic Analysis of [^{14}C]*DCC and Its Breakdown Products.* Carbodiimides were analyzed by a slight modification of the method of Partis et al. (1983) using a Waters HPLC system equipped with a C_{18} radial pack (Waters; 4.6 mm × 10 cm) column; 50–70-μL samples of a 200 μM [^{14}C]DCC solution were injected and eluted with methanol at 0.5 mL/min flow rate (room temperature). Ultraviolet absorption of the eluate was monitored with a Waters 440 UV detector at 214 nm, and radioactivity was monitored in fractional elution samples (0.1 mL). Most of our [^{14}C]DCC solutions contained 5–15% hydration products.

Assays. (*1*) *The ATPase activity* was assayed by measuring P_i cleavage from ATP in a media containing 30 mM Tris-HCl (pH 7.5) or 20 mM MOPS (pH 6.8), 0.1 mM $CaCl_2$, 80 mM KCl, 3 mM $MgCl_2$, and 10–15 μg of protein/mL. The reaction was started by addition of 2 mM ATP and quenched with molybdovanadate reagent (Lin & Morales, 1977) after a 2-min incubation at 35 °C.

(*2*) *Phosphorylation with ATP* was obtained in media containing 30 mM Tris, pH 7.5, 10 mM $CaCl_2$, 80 mM KCl, 5 mM $MgCl_2$, and 0.1 mg of protein/mL. The reaction was initiated by addition of 100 μM [γ-^{32}P]ATP and quenched after 10 s at room temperature with 2 volumes of 10% (w/v) trichloroacetic acid containing 4 mM P_i, and samples of 1 mL were filtered through Millipore filters (pore size 0.45 μm). The filters were washed 5 times with the same acid medium and counted in a scintillation counter (Knowles & Racker, 1975). Controls were obtained by quenching the enzyme with trichloroacetic acid before the addition of ATP.

(*3*) *Phosphorylation with* P_i was obtained in media containing 50 mM Tris–maleate, pH 6.0, 0.5 mM EGTA, 0.5 mg of SR protein/mL, 15 mM $MgCl_2$, and 2 mM P_i. Following a 30-s incubation at 25 °C, the reaction was quenched with 0.25 M PCA and 4 mM P_i. The denatured protein was centrifuged and washed repeatedly, and the sediment was dissolved for determination of radioactivity and protein.

(*4*) *ATP* $\rightleftharpoons$ P_i *exchange* was obtained in reaction mixtures containing 50 mM Tris–maleate, pH 6.8, 10 mM $MgCl_2$, 6 mM ATP, 0.2 mM ADP, 4 mM [^{32}P]P_i, 2 mM $CaCl_2$, and 50 μg of protein/mL. After acid quenching, [^{32}P]P_i was extracted from the reaction mixture as described by Carvalho et al. (1976), and samples of the aqueous phase were withdrawn for determination of [γ-^{32}P]ATP by scintillation counting.

(*5*) *Calcium binding* was determined by equilibration of $^{45}Ca^{2+}$ with SR vesicles in chromatography columns (Hummel & Dryer, 1962; Inesi et al., 1980).

Electrophoresis. SR vesicles (1 mg/mL) were incubated with [^{14}C]DCC as described above, collected by centrifugation at 140000*g* for 30 min, and resuspended in a medium containing 0.6 M sucrose, 100 mM KCl, 50 μM $CaCl_2$, and 30 mM Tris-HCl, pH 7.0. The samples were then centrifuged at 140000*g* for 1 h, and the pellets were dissolved in 2% SDS (w/v), 0.2 M sucrose, 50 mM Tris, pH 6.8, and 1% β-mercaptoethanol. Electrophoresis was carried out by the method of Weber & Osborn (1969), or the Laemmli method (1970) on a 7–15% acrylamide gradient containing SDS. The gels were stained by the Coomassie blue reagent by using the Fairbanks method (1971). Usually 30–40 μg of protein in 60–80 μL was used for each well in the slab. Fluorography for detection of radioactivity was performed according to Bonner & Laskey (1974).

RESULTS

Various Patterns of ATPase Inhibition by DCC. In agreement with the findings of Pick & Racker (1979) and Murphy (1981) we found the preincubation of SR vesicles with DCC at high DCC:protein ratios in the presence of EGTA inhibits both calcium binding and ATPase activity. We then proceeded to vary the DCC:protein ratios, hoping to obtain selective inhibition of specific partial reactions of the ATPase cycle. We found that incubation of SR vesicles at a concentration of 1 mg of protein/mL of 200 μM DCC produced total ATPase (P_i production) inhibition but only 50% reduction of calcium uptake (Figure 1).

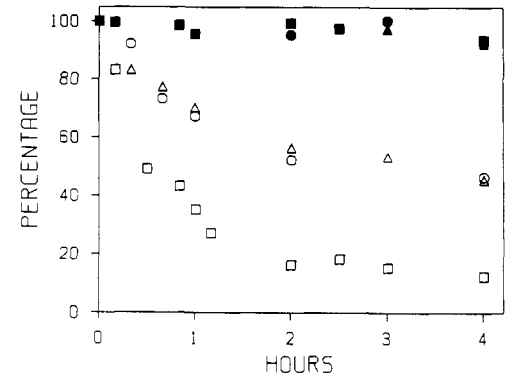

FIGURE 1: Calcium binding, enzyme phosphorylation with ATP, and ATPase activity, following derivatization of SR ATPase with DCC at high DCC:protein ratios. SR vesicles (1 mg of protein/mL) were incubated with 200 μM DCC in the presence (filled symbols) or in the absence of 10 μM Ca^{2+} (empty symbols). Calcium binding (○, ●) was measured in the presence of 10 μM Ca^{2+}, yielding a maximal level of 8.8 nmol of calcium bound/mg of protein. Phosphoenzyme (△, ▲) was obtained by incubating the enzyme with [γ-^{32}P]ATP, yielding a maximal level of 3.9 nmol/mg of protein. The ATPase activity (□, ■) of control samples was 7 μmol of P_i released/min by 1 mg of protein. See Materials and Methods for technical details.

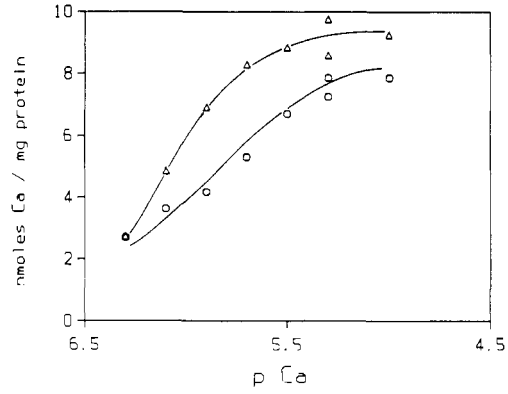

FIGURE 2: Calcium binding to SR vesicles incubated with DCC at low DCC:protein ratios. SR vesicles (5 mg of protein/mL) were incubated with 200 μM DCC in the presence of Ca^{2+} (△) or EGTA (○) for 5 h. Calcium binding (in the absence of ATP) was measured at equilibrium as previously described (Inesi et al., 1980).

In experiments in which we lowered the DCC:protein ratio even further (5 mg of protein/mL of 200 μM DCC) we found that the maximal levels and the apparent affinity of calcium binding (Figure 2), as well as the phosphoenzyme levels formed by reacting the enzyme with ATP (Figure 3), were only minimally reduced. On the other hand, the phosphoenzyme levels formed by reacting the enzyme with P_i, the ATPase activity (i.e., P_i production) and the ATP $\rightleftharpoons$ P_i exchange were strongly inhibited (Figure 4). These experiments indicate that by limiting the amount of DCC available per enzyme unit, it is possible to produce specific inhibition of the E–P + H_2O $\rightleftharpoons$ E + P_i reaction. It is of interest that the ATP $\rightleftharpoons$ P_i exchange undergoes the strongest reduction (Figure 4), since it reflects a simultaneous inhibition of both directions of the hydrolytic reaction, while the ATPase (P_i production) activity

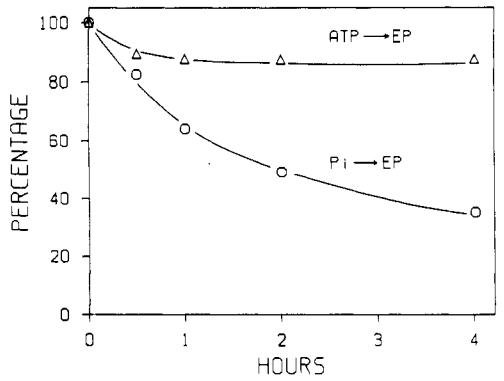

FIGURE 3: Phosphoenzyme formation in SR vesicles incubated with DCC at low DCC:protein ratios. SR vesicles (5 mg/mL) were incubated with 200 μM DCC for various time intervals in the presence of EGTA. Enzyme phosphorylation with ATP in the presence of Ca^{2+} (△) or with P_i in the absence of Ca^{2+} (○) was obtained as described under Materials and Methods. The control phosphoenzyme levels were 3.5 (steady-state level in the presence of ATP) and 2.5 (equilibrium level in the presence of P_i) nmol/mg of protein.

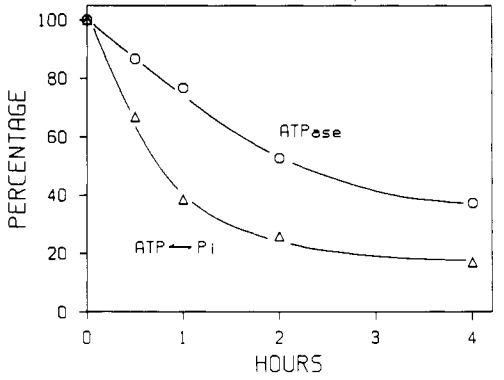

FIGURE 4: Ca^{2+}-dependent ATPase activity (P_i production) and ATP $\rightleftharpoons$ P_i exchange catalyzed by SR vesicles following incubation with DCC at low DCC:protein ratios. The steady-state ATP hydrolysis was obtained in the presence of 20 mM MOPS, pH 6.8, 80 mM KCl, 2 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.1 mM EGTA, 1 mM PEP, 50 μg of pyruvate kinase/mL, 10 μM A23187, 50 μg of SR protein/mL, and 1 mM ATP. ATP $\rightleftharpoons$ P_i exchange was obtained and measured as explained under Materials and Methods.

reflects inhibition of only the forward direction. It is also interesting that in conditions producing selective inhibition of the hydrolytic reaction, protection was obtained if the preincubation with DCC was carried out in the presence of 10 μM Ca. This indicates that calcium binding to the high-affinity sites produces a structural change limiting the DCC reactivity of enzyme residues which are not involved in calcium binding but have a critical role in the catalytic mechanism.

DCC Binding to the SR Protein. In order to determine the relationship between various patterns of enzyme ihibition and DCC incorporation, we measured radioactive labeling of the SR protein following incubation with [^{14}C]DCC. In preliminary experiments by HPLC chromatography we found that DCC partitions rapidly into the lipid membrane phase and then slowly reacts with protein residues. Since at all times a significant amount of label is associated with the lipids, it is necessary to remove the lipids by repeated washings with chloroform/ethanol before measuring the label actually bound to the protein. We then found that in conditions producing selective inhibition of the E–P + H_2O $\rightleftharpoons$ E + P_i reaction (i.e., following incubation at low DCC:protein ratios) only negligible incorporation of radioactive label was obtained. On the other hand, significant labeling occurred during incubation with high DCC:protein ratios in the presence of calcium (Figure 5). When the incubation with high DCC:protein ratios was carried

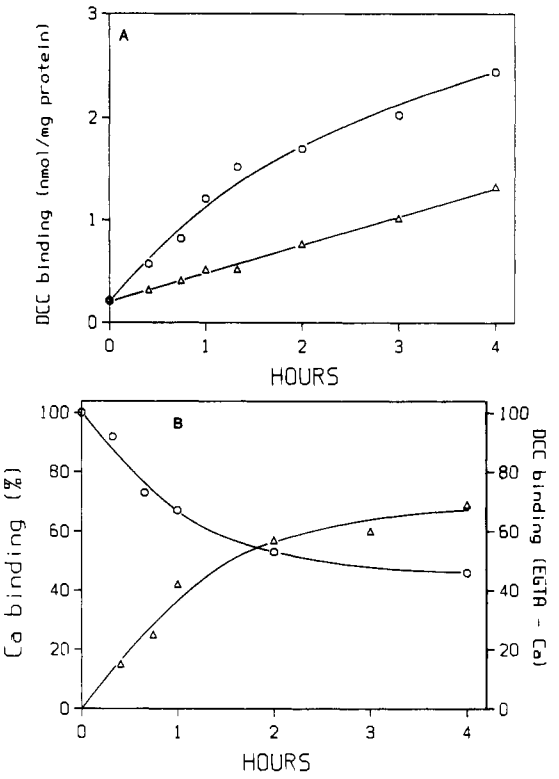

FIGURE 5: Time course of [^{14}C]DCC labeling and inhibition of calcium binding in SR vesicles incubated in the presence of high DCC:protein ratios. (A) SR vesicles (1 mg/mL) were incubated with 200 μM [^{14}C]DCC in the presence of calcium (△) or EGTA (○), and serial samples were taken for determination of [^{14}C]DCC labeling in the protein. (B) The excess labeling (△) obtained in the presence of EGTA (over the labeling in the presence of calcium) is compared to the inhibition of calcium binding (○). Maximal values for excess labeling in the presence of EGTA were obtained following a 15-h incubation.

out in the absence of calcium (thereby producing 50% inhibition of calcium binding), an additional 1.2 nmol of label was incorporated per mg of protein (Figure 5). The time course of this additional labeling and that of calcium binding inhibition proceeded in parallel (Figure 5B), indicating a casual relationship between these two events. We also demonstrated by electrophoresis and autoradiography that the radioactive label was in fact bound to the ATPase protein.

It should be pointed out that the stoichiometry of specific labeling is lower than expected [see also Pick & Racker, (1979)], considering that the SR vesicles used in these studies contain 9–10 nmol of calcium binding sites/mg of protein, and 50% inhibition of calcium binding should be accompanied by incorporation of 4–5 nmol of radioactive label/mg of protein.

The lack of radioactive labeling following incubations producing inhibition of the E–P + H_2O $\rightleftharpoons$ E + P_i reaction and the low stoichiometry of labeling following incubations producing inhibition of both calcium binding and ATPase activity led us to suspect that the initial adducts formed between protein residues (possibly carboxyl) and [^{14}C]DCC undergo nucleophilic atrack by neighboring residues with elimination of radioactive urea (Carraway & Koshland, 1972). In this manner specific protein residues may be blocked without radioactive labeling. We checked this possibility by incubating SR vesicles with [^{14}C]DCC, sedimenting the reacted vesicles by centrifugation and analyzing the supernatant by HPLC. It should be pointed out that the DCC has a tendency to be degraded as a consequence of hydration, and even in our best samples of DCC we found that approximately 10% of that total radioactivity was eluted in a separate peak attributed to dicyclohexylurea (Partis et al., 1983). Following incubation with

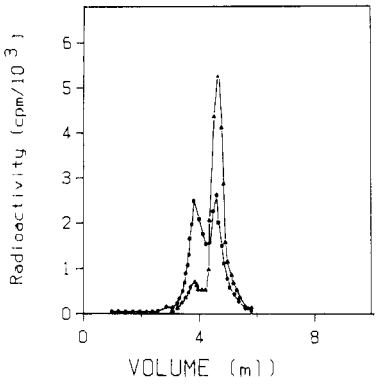

FIGURE 6: HPLC analysis of degradation products following incubation of [^{14}C]DCC with SR vesicles. Previous to HPLC chromatography, 200 μL of a solution containing 200 μM [^{14}C]DCC, 1 mM EGTA, 30 mM MES, pH 6.2, 100 mM KCl, and 4 μg of A23817 was incubated for 2 h (0 °C) in the absence (▲) and in the presence (●) of 200 μg of SR protein. At the end of the incubation the samples were centrifuged, and 60 μL of each supernatant was subjected to HPLC on a C_{18} column as explained under Materials and Methods. Fractional samples were collected for determination of radioactivity.

SR vesicles, the DCC peak decreased and the peak of degradation product increased (Figure 6), demonstrating that the initial protein derivatization undergoes further reactions leading to release of radioactivity into the medium, even though the involved residues remain blocked by formation of mixed anhydrides.

Discussion

Our experiments demonstrate that it is possible to limit the reaction of DCC with SR ATPase to a degree permitting selective inhibition of the phosphoenzyme hydrolytic cleavage and its reversal (i.e., phosphoenzyme formation from P_i in the absence of Ca^{2+}). In these conditions of limited derivatization, calcium binding to high-affinity sites and enzyme phosphorylation with ATP in the presence of Ca^{2+} remain largely unaffected.

The specific inhibition observed in our experiments is likely due to perturbations at the catalytic site and may be interpreted with mechanistic features of enzyme catalysis in SR ATPase. To this effect, we first consider that the phosphorylated enzyme intermediate can be obtained by reacting the enzyme either with ATP in the presence of Ca^{2+} (Yamamoto & Tonomura, 1967; Makinose, 1969) or with P_i in the absence of Ca^{2+} (Masuda & deMeis, 1973). The former is a phosphoryl-transfer reaction, while the latter is the reversal of the phosphoenzyme hydrolytic cleavage which involves water. Another important difference between the two phosphorylation reactions is their pH dependence, inasmuch as the phosphoenzyme levels formed with ATP (in the presence of saturating Ca^{2+}) are independent of pH variations between 6.0 and 8.5 (Inesi & Hill, 1983), while the P_i reaction is markedly pH dependent (Masuda & deMeis, 1974). In this regard, it was previously proposed (Inesi et al., 1984) that protonation of enzyme residues with pK near neutrality favors phosphoenzyme formation from P_i. In their protonated form these residues assist the phosphorylation reaction serving as H^+ donors to form waters with the hydroxyl derived from P_i reaction. On the other hand, in their dissociated form these residues assist phosphoenzyme cleavage by withdrawing H^+ from hydrolytic water, thereby favoring nucleophilic attack of water oxygen on the phosphorus atom. This explains why medium and intermediate $P_i \rightleftharpoons$ HOH oxygen exchange occurs most rapidly at neutral pH (McIntosh & Boyer, 1983), since at this pH, protonation and dissociation of these residues assists equally P_i incorportion onto the enzyme and its hydrolytic cleavage. The selective inhibition produced in our present experiments is consistant with this proposal and suggests that (at low DCC:protein ratios) DCC blocks residues which are involved in catalysis of the $E\text{–}P_i + H_2O \rightleftharpoons E + P_i$ reaction by the mechanism outlined above. This mechanism is of course unrelated to the calcium binding inhibition produced by Pick & Racker (1979), and Murphy (1981), with high DCC:protein ratios on SR ATPase. Rather, it is possible that a similar mechanism is responsible for the inhibition produced by DCC on *Neurospora crassa* H^+-ATPase (Bowman et al., 1978; Sussman & Slayman, 1983) which is not dependent on Ca^{2+} for activation.

It is noteworthy the calcium occupancy of the high-affinity sites on the SR ATPase protects the enzyme not only from inactivation of the same calcium sites but also from inactivation of residues involved in catalysis of the hydrolytic reaction. This demonstrates that calcium binding produces an enzyme change which effectively limits DCC access to the catalytic site.

Finally, an important point must be made with respect to protein labeling with radioactive DCC, which in all cases is lower than expected. It was previously suggested that inactivation of a higher number of binding sites, relative to the number expected from labeling stoichiometry with DCC, may reflect the cooperative inhibition produced by one labeled unit on the other units of an ATPase tetramer (Pick & Racker, 1979). We have demonstrated here that the initial adducts formed between ATPase residues and radioactive DCC undergo further degradation leading to elimination of radioactive label into the medium, even though the protein residues remain inactivated. Therefore, the stoichiometry of labeling cannot be used to estimate the number of inactivated residues.

Registry No. ATPase, 9000-83-3; DCC, 538-75-0; ATP, 56-65-5; P_i, 14265-44-2.

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One-Step Immunoaffinity Purification of Active Progesterone Receptor. Further Evidence in Favor of the Existence of a Single Steroid Binding Subunit†

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ABSTRACT: A very high capacity immunoaffinity matrix for the purification of progesterone receptor was prepared by cross-linking a monoclonal antireceptor antibody to protein A–Sepharose through the Fc fragment. The monoclonal antibody was selected for its property of losing affinity for the receptor at pH 10.5, i.e., in conditions where the receptor remains stable for extensive periods of time. This made it possible to elute active receptor from the immunosorbent. From crude rabbit uterine cytosol the steroid–receptor complexes were purified in a single step. A 1-mL column (containing 7 mg of monoclonal antibody) bound 1600 pmol of steroid–receptor complexes of which 79.5% were eluted. The overall yield of purification was 49%. The specific activity of the purified steroid–receptor complexes was 6.71 ± 0.79 nmol of bound steroid/mg of protein (mean ± SE of four experiments). The purified receptor consisted of a mixture of 110 000- and 79 000-dalton forms. The latter appeared to be produced by proteolysis of the larger form during purification since immunoblot experiments showed that, at the start of purification, the 110 000-dalton form was present in overwhelming majority (80–95%) in the uterine cytosol and that the 79 000-dalton form only appeared during purification. This conclusion was also supported by the peptide analysis of both forms of receptor: the purified receptor was denatured and labeled with ^{125}I; the 110 000- and 79 000-dalton forms were isolated by gel electrophoresis in denaturing conditions and electroelution and were then submitted to mild or extensive digestions by trypsin, chymotrypsin, and protease V8 from *Staphylococcus aureus*. In all cases, the comparison of the patterns of peptides obtained suggested that the 79 000-dalton protein is a proteolytic product of the 110 000-dalton receptor. The rapidity and the mild elution conditions of this immunoaffinity method enabled us to purify active aporeceptor (ligand-devoid receptor). The purified receptor retained its original properties (affinity for the hormone and DNA-binding and density gradient sedimentation characteristics).

The structure and the biological function of steroid hormone receptors still remain poorly understood [recent review in Eriksson & Gustafsson (1983)]. This is mainly due to their fragility and their very low concentration, which makes it difficult to purify them in sufficient amounts and with a good yield. Moreover, all the purification procedures devised to date yield steroid–receptor complexes (Schrader et al., 1980; Puri et al., 1982; Renoir et al., 1982; Logeat et al., 1981; Greene et al., 1979; Wrange et al., 1979). Only very partial purifications of aporeceptors (ligand-devoid receptors) have been described (Maggi et al., 1981). For this reason, it has been impossible to study the effect of steroids on purified receptors.

We recently prepared monoclonal antibodies to the rabbit progesterone receptor (Logeat et al., 1983) and report here the use of these antibodies to purify rapidly and with a high yield the receptor. The efficiency of the method used relied mainly on two points: (1) The attachment of the antibody through the Fc fragment to protein A–Sepharose, leaving free all the antigen binding sites (Schneider et al., 1982). This ensured a very high capacity of the immunomatrix and also decreased the nonspecific binding of proteins. (2) The use of antibody that is very sensitive to pH conditions. Exposure of the immunosorbent to pH 10.5 allowed elution of the receptor. At this pH, the progesterone receptor was stable for long periods of time. The method was also used to purify steroid-free receptor.

Materials and Methods

Animals. New Zealand rabbits weighing 1 kg were injected during 8 days with diethylstilbestrol (100 μg in 0.5 mL of sesame oil/day) (Rao & Katz, 1977). On day 9, the rabbits were killed; the uteri were excised and rinsed in cold saline solution.

Antibodies. Monoclonal antibodies (IgG_{2a}) were purified from mouse ascite on protein A–Sepharose (Pharmacia). Elution of immunoglobulins was performed with 0.1 M sodium citrate pH 4 buffer. The characteristics of the Mi60-10 antireceptor monoclonal antibody used in this work have previously been described (Logeat et al., 1983). The mouse monoclonal antibodies used as controls and nonrelated to the receptor were a gift of Dr. P. Legrain (Institut Pasteur). They were antiidiotypic antibodies, raised against mouse myeloma

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