Dependence of the Conformational State of the Isolated Adenine Nucleotide Carrier Protein on the Detergent Used for Solubilization†

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Received June 28, 1985

ABSTRACT: The mitochondrial adenine nucleotide (AdN) carrier can assume two conformational states that are trapped by the specific inhibitors of AdN transport carboxyatractyloside (CATR) and bongkrekic acid (BA). When the AdN carrier protein was extracted from beef heart mitochondria by the zwitterionic detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and purified in the same detergent, the fluorescence of the tryptophanyl residue(s) of the protein was partially quenched by ATP (or ADP), but not by nontransportable nucleotides; CATR, which alone was ineffective, was able in the presence of ATP (ADP) to further quench the fluorescence, and BA reversed the quenched fluorescence to the original level. With 3′-*O*-naphthoyl-ATP (N-ATP) as an extrinsic fluorescence probe, it was shown that BA could release bound N-ATP but that CATR was ineffective. These results indicate that the AdN carrier in CHAPS is able to react readily with BA, but not with CATR. The opposite situation occurs with the carrier solubilized and purified in (laurylamido)-*N*,*N*-dimethylpropylamine oxide (LAPAO) [Brandolin, G., Dupont, Y., & Vignais, P. V. (1985) *Biochemistry 24*, 1991–1997]. These data taken together were interpreted to mean that the CATR and BA conformations of the isolated AdN carrier depend on the micellar structure in which it is embedded; the carrier in LAPAO is in the CATR conformation, and the carrier in CHAPS is in the BA conformation. For the transition between the CATR and BA conformations to occur in the carrier in CHAPS and in the carrier in LAPAO, ATP or ADP is required; nontransportable nucleotides were ineffective. The energy of activation of the ATP (ADP) induced transition is 40 kJ/mol between 0 and 10 °C.

Through fluorescence studies, it has been shown that the membrane-bound adenine nucleotide (AdN)[1] carrier and the isolated AdN carrier in the detergent LAPAO can adopt two conformations recognized and trapped by the inhibitors carboxyatractyloside (CATR) and bongkrekic acid (BA) (Brandolin et al., 1981, 1982, 1985; Block et al., 1983). The transition between the two conformations is very slow in the absence of transportable nucleotides but is considerably accelerated by addition of ADP or ATP. This paper deals with the effect of ATP (ADP), CATR, and BA on the intrinsic fluorescence of the AdN carrier purified in 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and on the fluorescence of bound N-ATP. The AdN carrier in CHAPS exhibited binding properties for CATR and BA opposite to those of the carrier in LAPAO. The results indicated that without added ligands the BA conformation is preferentially adopted by the carrier in CHAPS and the CATR conformation by the carrier in LAPAO, suggesting that the micellar structure of the detergent can stabilize either of the two possible conformations of the AdN carrier.

Experimental Procedures

Chemicals. ATP was purchased from Pharmacia P-L Biochemicals, 1-naphthoic acid from Fluka, AcA 202 from Industrie Biologique Française, CHAPS (Hjelmeland, 1980) from Sigma, and hydroxylapatite from Bio-Rad. Bongkrekic acid was prepared as described in a previous report (Lauquin & Vignais, 1976). 3′-*O*-Naphthoyl-ATP (N-ATP) was synthesized by the method of Schäfer & Onur (1979), based on the use of the imidazolide derivative of 1-naphthoic acid (Gottikh et al., 1970). A critical micellar concentration (cmc) of 0.39% (w/v) for CHAPS was determined under our experimental conditions by a fluorescence titration (De Vendittis et al., 1985).

The following proteins from Pharmacia P-L Biochemicals were used as molecular weight standards: phosphorylase *b* (94000), bovine serum albumin (67000), ovalbumin (43000), carbonic anhydrase (30000), soybean trypsin inhibitor (20100), and α-lactalbumin (14400).

Purification of the AdN Carrier Protein in CHAPS. Beef heart mitochondria were purchased in 0.25 M sucrose as described by Smith (1967) and stored in liquid nitrogen. After being thawed, an aliquot of the mitochondrial suspension corresponding to 30 mg of protein was centrifuged at 25000*g* for 5 min. The supernatant was withdrawn and the pellet solubilized at 0 °C in 2 mL of 10 mM Mes buffer, pH 6.5, containing 2% (w/v) CHAPS, 0.1 M Na_2SO_4, and 1 mM EDTA. Following centrifugation at 25000*g* for 15 min, the supernatant was loaded on a 10-mL hydroxylapatite column equilibrated with a buffer consisting of 0.6% CHAPS, 0.1 M Na_2SO_4, and 10 mM Mes, pH 6.5. The pass-through fraction was further purified by filtration on a 15-mL column of AcA 202 gel equilibrated with the same buffer as that used for

† This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale (INSERM/U.191), from the Centre National de la Recherche Scientifique (CNRS/U.A. 1130), and from the Université Scientifique et Médicale de Grenoble.

[1] Abbreviations: AdN, adenine nucleotide; $NaDodSO_4$, sodium dodecyl sulfate; CHAPS, 3-[(cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; LAPAO, (laurylamido)-*N*,*N*-dimethylpropylamine oxide; CATR, carboxyatractyloside; BA, bongkrekic acid; ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; N-ATP, 3′-*O*-(1-naphthoyl)adenosine triphosphate; Mes, 2-(*N*-morpholino)ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; IgG, immunoglobulin G; Tris, tris(hydroxymethyl)aminomethane.

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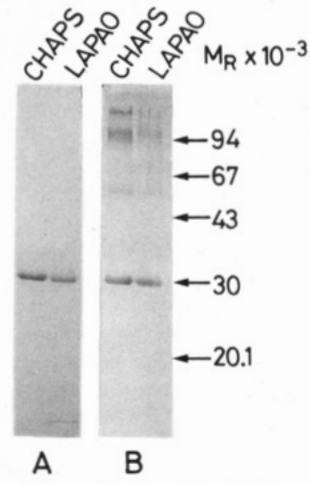

FIGURE 1: Immunocharacterization of the AdN carrier protein purified in CHAPS and LAPAO, followed by $NaDodSO_4$–polyacrylamide gel electrophoresis. Beef heart mitochondria (30 mg of protein) were lysed by incubation in 2 mL of a medium composed of 2% CHAPS (w/v) or 1% LAPAO (w/v), 0.1 M Na_2SO_4, 10 mM Mes, pH 6.5, and 1 mM EDTA. The AdN carrier was purified as described under Experimental Procedures. Each track in the gel corresponds to the migration of 3 μg of solubilized protein. (A) Coomassie Blue staining. (B) Immunocharacterization after electrotransfer onto a nitrocellulose sheet (see Experimental Procedures). The molecular weights on the right-hand side are those of marker enzymes (cf. Experimental Procedures).

hydroxylapatite chromatography.

The purity of the AdN carrier protein was assessed by $NaDodSO_4$–polyacrylamide gel electrophoresis (Laemmli, 1970), followed by immunocharacterization. Prior to gel electrophoresis, the AdN carrier protein was exhaustively dialyzed against distilled water and the precipitate was resolubilized with 10% $NaDodSO_4$. A parallel preparation of the AdN carrier in 0.05% LAPAO was performed as described by Brandolin et al. (1982); in this case the protein and LAPAO were precipitated by trichloroacetic acid, followed by resolubilization of LAPAO in cold ethanol. In both cases, protein was solubilized in 10% $NaDodSO_4$.

When the protein purified in CHAPS was subjected to $NaDodSO_4$ gel electrophoresis, its migration was slightly slower than that of the AdN carrier in LAPAO, and the recovery was 2 times lower than that of the carrier purified in LAPAO ($\simeq$3% vs. $\simeq$6% after AcA 202 chromatography). A further characterization by an immunoenzymatic reaction was therefore performed to ascertain the identity of the carrier protein in CHAPS. The proteins separated by $NaDodSO_4^-$ polyacrylamide gel electrophoresis were transferred onto nitrocellulose paper (Towbin et al., 1979); the transferred proteins were reacted with a rabbit antiserum raised against the purified AdN carrier obtained in LAPAO and solubilized in $NaDodSO_4$. After a second incubation with a peroxidase-conjugated antibody, where the antibody was an anti-rabbit IgG raised in goat, the peroxidase attached to the immunoreactive proteins on the nitrocellulose sheets was revealed with diaminobenzidine supplemented with nickel and cobalt to intensify the coloration (De Blas & Cherwinski, 1983). An intense colored band of M_r 31 000 was revealed, together with bands whose molecular weight values were multiples of 31 000, corresponding most likely to different states of aggregation of the AdN carrier (Figure 1). These results show conclusively that the same molecular species, namely the AdN carrier protein, can be extracted and purified in CHAPS or LAPAO.

Fluorescence Assays. Fluorescence assays were carried out with a high-sensitivity spectrofluorometer (Biologic, Grenoble, France). A 1 × 1 cm fluorescence quartz cuvette was used, inserted in a temperature-controlled cell holder; the content of the cuvette was mechanically stirred. Routinely, the AdN carrier preparation in 0.4 mL was diluted with 1.2 mL of 135 mM glycerol and 10 mM Tris, pH 7.4, to which was added 0.4 mL of the buffer consisting of 0.6% CHAPS, 0.1 M Na_2SO_4, and 10 mM Mes, pH 6.5. Reagents were injected with Hamilton automatic syringes in small volumes (2–10 μL). The fluorescence of the tryptophanyl residues was excited at 300 nm (1-nm band-pass) with a 75-W XE lamp. The emitted light was measured at right angles through a 3345 Corning filter, the band-pass being centered at 345 nm. For measurements with N-ATP, fluorescence was excited at 312 nm (1-nm band-pass), and the emitted light was recovered through a K-40 Balzer filter, with a band-pass of 50 nm, centered at 410 nm. The temperature was usually set at 10 °C.

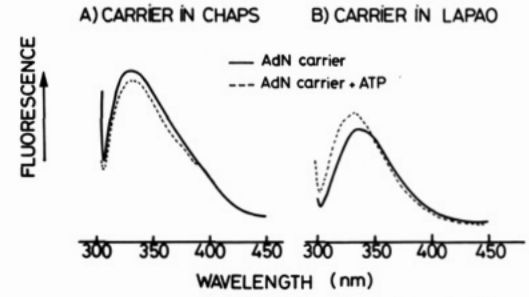

FIGURE 2: Fluorescence emission spectrum of the AdN carrier in CHAPS and LAPAO. The AdN carrier protein was prepared as described under Experimental Procedures and used at a concentration of 0.2 mg/mL at pH 6.5. Excitation was at 300 nm, with a slit width set at 2 nm. The emission slit width at 345 nm was 5 nm. ATP and CATR were added at the final concentration of 2 μM. (A) Emission spectrum in CHAPS. (B) Emission spectrum in LAPAO.

RESULTS

Effect of ATP, CATR, and BA on the Intrinsic Fluorescence of the AdN Carrier Solubilized in CHAPS. The fluorescence emission spectrum of the AdN carrier protein in CHAPS, when excited at 300 nm, was typical of tryptophanyl residues with a λ_{max} at 330 nm. Upon addition of ADP or ATP, the emission fluorescence was quenched (Figure 2A). This effect was caused by an ADP- or ATP-specific change in the tryptophanyl environment since other nucleotides like IDP, UDP, or their triphosphate homologues were ineffective. It must be recalled that the emission spectrum of the AdN carrier in LAPAO is increased and displaced to the red upon addition of ADP or ATP with a peak at 340 nm (Figure 2B) (Brandolin et al., 1981).

In the following experiments (Figure 3), the time course of the changes in the fluorescence emission was followed, using a filter selecting the emitted light at 345 nm (cf. Experimental Procedures). Routinely, the concentration of the carrier was between 0.2 and 0.4 μM. Addition of 2 μM ATP (or ADP) resulted in an abrupt decrease of the fluorescence level; a further abrupt decrease to the end point was elicited by addition of 2 μM CATR (Figure 3A). CATR did not modify the shape of the emission spectrum (not shown). Reversing the order of addition gave a different response (Figure 3B); CATR alone had no effect; a subsequent addition of ATP resulted, however, in a large fluorescence quenching, virtually equal to the sum of the two plateaus of quenching caused by the successive additions of ATP and CATR in the first experiment.

As shown in Figure 4A, the fluorescence decrease elicited by ATP was reversed by BA. A concentration of 0.25 μM BA, i.e., of the same order as that of the carrier protein, was

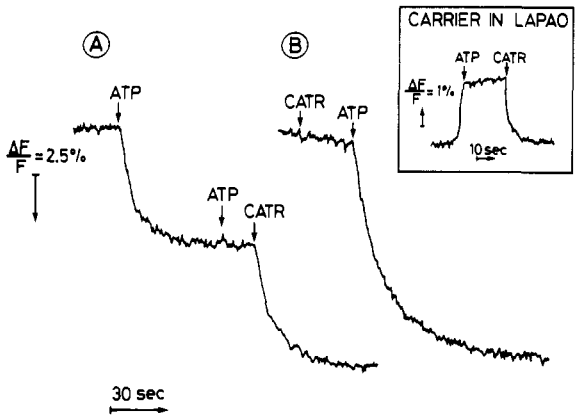

FIGURE 3: Effect of ATP (ADP) and CATR on the intrinsic fluorescence of the AdN carrier protein in CHAPS. Conditions were as described under Experimental Procedures. Assays were performed at 10 °C. When indicated, ATP and CATR were added at a final concentration of 2 μM. Insert: Effect of ATP and CATR on the intrinsic fluorescence (at 355 nm) of the AdN carrier in LAPAO.

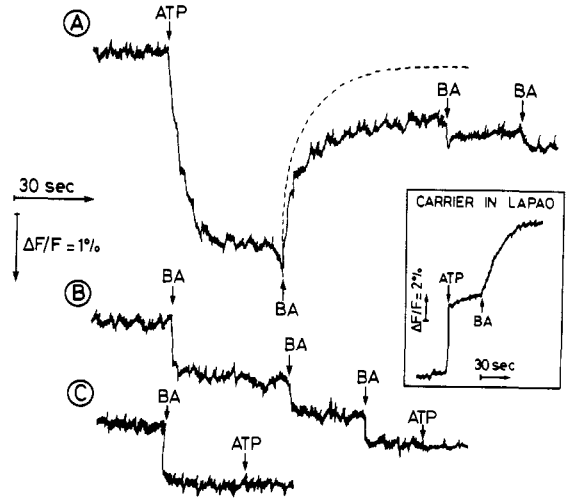

FIGURE 4: Effect of BA on the intrinsic fluorescence of the AdN carrier protein in CHAPS. Conditions were as described under Experimental Procedures. Assays were performed at 10 °C. When indicated, ATP and BA were added at final concentrations of 2 and 0.25 μM, respectively. Insert: Effect of ATP and BA on the intrinsic fluorescence of the AdN carrier in LAPAO.

able to induce a substantial, but not complete, reversal of the fluorescence. A parallel experiment with BA in the absence of ATP showed that a first addition of 0.25 μM BA induced a small but measurable quenching, which was 2 times higher than the quenching elicited by subsequent additions of BA (Figure 4B). The quenching caused by sequential additions of BA except the first one is probably unspecific. The larger quenching observed upon the first addition of BA may comprise a fraction of unspecific quenching and a fraction of specific quenching due to BA binding. When the trace in Figure 4A corresponding to the BA-induced reversal of the quenching caused by ATP was corrected for the quenching due to the addition of BA (dotted line), the reversal of fluorescence was found to be virtually complete. Finally, when BA was added first, followed by ATP, the addition of ATP did not affect the fluorescence level (Figure 4C).

From the results of the above experiments, it is clear that, in the presence of ATP or ADP, CATR and BA have opposing effects on the intrinsic fluorescence of the AdN carrier in CHAPS: the quenching elicited by ATP is enhanced by CATR and reversed by BA. Further, the effects depend on the detergent used. For example, in a previous work carried

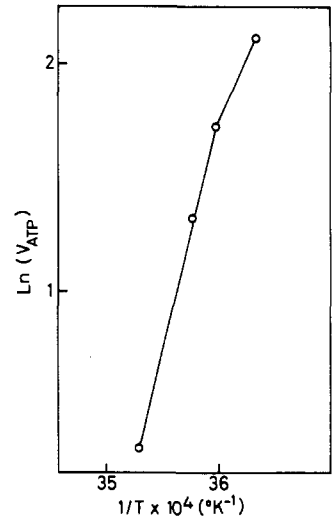

FIGURE 5: Arrhenius plot of the rate of the ATP-dependent quenching of the intrinsic fluorescence of the AdN carrier in CHAPS. Conditions were as described under Experimental Procedures. The temperature was set at four given values between 2 and 10 °C. ATP was added at a final concentration of 0.5 μM.

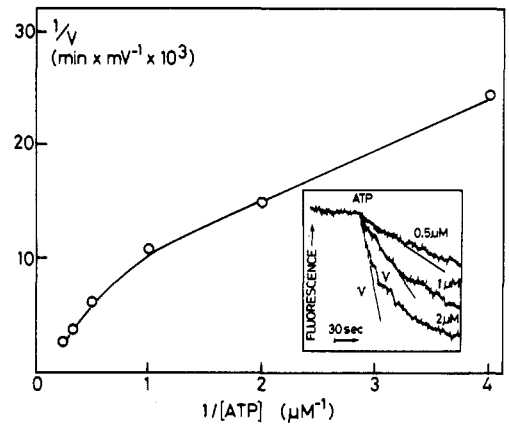

FIGURE 6: Effect of ATP concentration on the initial rate of quenching of the intrinsic fluorescence of the AdN carrier in CHAPS. Assays were performed as described under Experimental Procedures at 2 °C.

out with LAPAO (Brandolin et al., 1985), the fluorescence level of the carrier alone was similar to that of the carrier in the presence of CATR with or without ATP; a higher fluorescence level was obained upon addition of BA plus ATP than in the presence of ATP alone. In summary, the fluorescence of the carrier in CHAPS was markedly modified by CATR when ATP was present but was only slightly modified by BA whether or not ATP was present. On the other hand, the fluorescence of the carrier in LAPAO was modified by BA plus ATP but not by CATR. Furthermore, two extreme fluorescence levels of the carrier in CHAPS can be distinguished, a high fluorescence level corresponding to the carrier alone or in the presence of BA with or without ATP and a lower fluorescence level corresponding to the carrier in the presence of CATR plus ATP.

The rate of the ATP-induced fluorescence decrease of the AdN carrier in CHAPS was temperature-dependent. From the Arrhenius plot in Figure 5, an energy of activation of about 40 kJ/mol between 0 and 10 °C was calculated similar to that found for the transition between the CATR and BA conformations in the membrane-bound carrier (Block et al., 1983). The technique used for mixing was insufficiently rapid to resolve kinetics at temperatures above 10 °C.

Increasing ATP concentrations led to an increase in the rate of fluorescence quenching of the AdN carrier in CHAPS

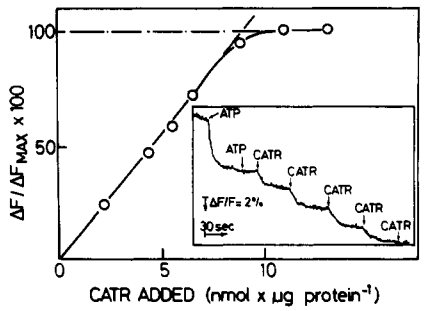

FIGURE 7: Titration of CATR binding sites in the AdN carrier in CHAPS. In the insert is represented a typical experiment of titration, starting by addition of a saturating concentration of ATP and followed by addition of small increments of CATR.

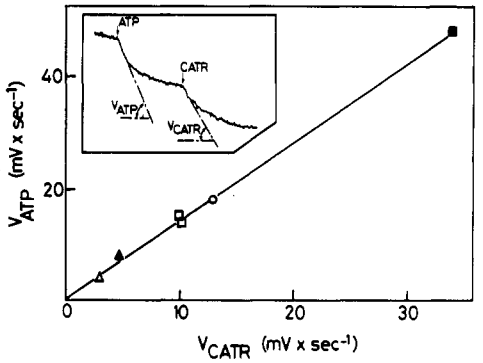

FIGURE 8: Relationship between the initial rate of quenching of the intrinsic fluorescence of the AdN carrier in CHAPS, caused by ATP and by CATR. Assays were performed as described under Experimental Procedures. The temperature was set at 10 °C. The CATR concentration was 2 μM. The ATP concentrations were as follows: △, 0.1 μM; ▲, 0.2 μM; □, 0.3 μM; ○, 0.5 μM; ■, 1 μM.

(insert, Figure 6). The reciprocal plot of the rate of fluorescence quenching vs. the ATP concentration was not linear, suggesting positive cooperativity for ATP binding. To accelerate the ATP-induced quenching of fluorescence at very low concentrations of ATP, and to make measurements of the extent of quenching possible over short periods of time (not exceeding 2 min), the temperature was raised to 20 °C. Under these conditions, substoichiometric concentrations of ATP with respect to that of the ATP carrier were found to elicit a slow, but extensive, quenching of fluorescence virtually of the same size as that observed with saturating concentrations of ATP (not shown).

Following the addition of a saturating concentration of ATP to the AdN carrier in CHAPS, sequential additions of small amounts of CATR led to a stepwise decrease of fluorescence down to a limiting value (Figure 7, insert); it should be recalled that CATR added first did not affect fluorescence (Figure 3). The end point of the titration of the CATR binding sites illustrated in Figure 7 was 9 nmol of CATR/mg of carrier protein. At smaller concentrations of ATP, lower rates of fluorescence quenching were observed upon addition of CATR. A linear relationship was found between the rate of quenching elicited by different concentrations of ATP and the rate of quenching elicited by the subsequent addition of a saturating concentration of CATR, the latter rate being about 40% lower than the former one (Figure 8). This corrobates the conclusion (Figure 2) that the recognition of ATP (or ADP) by the AdN carrier in CHAPS is compulsory for CATR binding and that the CATR-induced fluorescence change subsequent to the addition of ATP could reflect additional molecular events in the AdN carrier.

Effect of CATR and BA on the Release of Bound N-ATP from the AdN Carrier Solubilized in CHAPS. In a previous

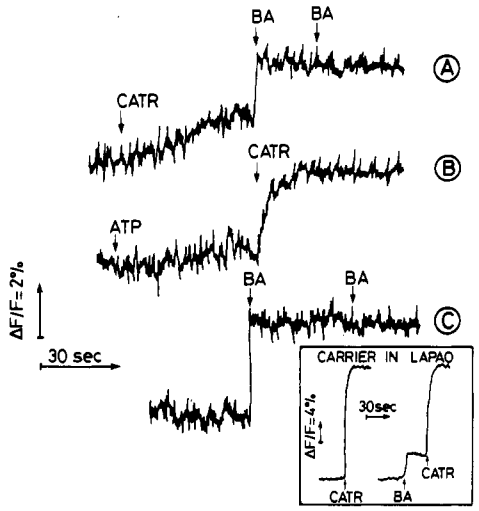

FIGURE 9: Fluorescence changes linked to the release of bound N-ATP upon addition of ATP, CATR, and BA to the AdN carrier in CHAPS. Assays were performed as described under Experimental Procedures, at a temperature of 10 °C. N-ATP was used at a final concentration of 1 μM. The final concentrations of CATR, ATP, and BA were 1, 2, and 1 μM, respectively. Insert: For comparison, the effect of CATR, ATP, and BA on the N-ATP bound to the AdN carrier in LAPAO is shown.

paper (Block et al., 1983), the binding properties of N-ADP (N-ATP) to the membrane-bound carrier in beef heart mitochondria were explored. Binding of N-ADP was accompanied by fluorescence quenching; conversely, the specific release of N-ADP (or N-ATP) from the membrane-bound AdN carrier by means of CATR or BA resulted in fluorescence enhancement. Binding of N-ATP by the AdN carrier purified in LAPAO (Dupont et al., 1982) also resulted in fluorescence quenching; upon addition of CATR, fluorescence was abruptly increased; in contrast, BA was not very effective (Figure 9, insert). As shown in Figure 9, a different response to CATR and BA was obtained for bound N-ATP in the case of the AdN carrier purified in CHAPS. In fact, 1 μM CATR alone elicited a very slow rise of fluorescence, and a subsequent addition of 1 μM BA resulted in a jump of fluorescence to the final level, reflecting the abrupt release of bound N-ATP. The effect of 1 μM BA was maximal since it was not increased by a further addition of BA (Figure 9, trace A). When added prior to CATR, BA was able to induce at once a rapid and larrge fluorescence response (Figure 9, trace C). When ATP was added first at a final concentration of 2 μM, not sufficient to release bound N-ATP, 1 μM CATR, which alone was poorly effective, elicited a fluorescence signal of the same size as that obtained by addition of BA, although slower (Figure 9, trace B).

In summary, the effective release of bound N-ATP upon addition of CATR and BA depended on the detergent used to solubilize and purify the AdN carrier, namely, CHAPS or LAPAO. When the carrier was solubilized in CHAPS, BA, but not CATR, was effective in the release of N-ATP; when the carrier was solubilized in LAPAO, CATR but not BA was effective. These results indicate that BA, but not CATR, has free access to its binding site in the AdN carrier in CHAPS and that the converse holds for the carrier in LAPAO.

In the following experiment (Figure 10), the binding affinity of N-ATP for the AdN carrier was assessed as follows. Aliquot fractions of the AdN carrier solubilized in CHAPS were incubated with increasing concentrations of N-ATP, and the bound N-ATP was released upon addition of a saturating concentration of BA. A typical saturation curve of the fluorescence increase vs. N-ATP concentration was obtained,

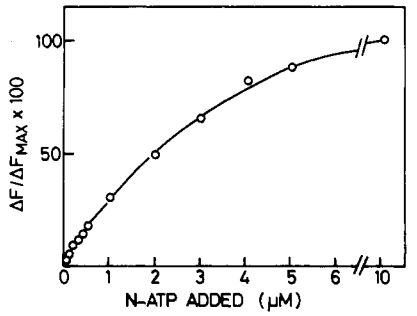

FIGURE 10: Titration of the carrier-bound N-ATP by BA. Experimental conditions were as in Figure 9. Specifically bound N-ATP was released by addition of 0.5 μM BA; it was proportional to the fluorescence increase depending on addition of BA.

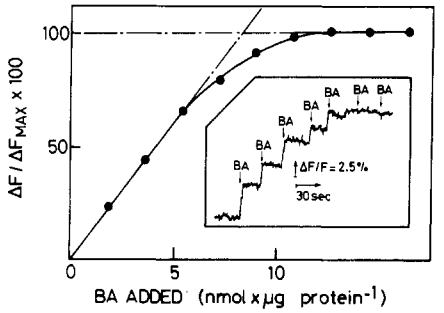

FIGURE 11: Titration of BA binding sites on the AdN carrier in CHAPS. Binding of BA was monitored by the BA-dependent release of N-ATP under the same conditions as those of Figure 9.

with a K_d value of about 2 μM. It should be recalled that the AdN carrier solubilized in LAPAO exhibited two types of N-ATP binding sites with K_d values in the nanomolar and micromolar ranges, respectively (Dupont et al., 1982). The N-ATP binding sites of the carrier in CHAPS ressemble therefore the low affinity binding sites of the carrier in LAPAO.

Stoichiometry of the CATR and BA Binding Sites of the AdN Carrier in CHAPS. For titration of the BA binding sites, advantage was taken of the fast removal of bound N-ATP from the carrier in CHAPS upon increasing concentrations of BA (Figure 11). The end point of the titration of the BA binding sites was about 9 nmol of bound ligand/mg of protein. The same amounts of CATR binding sites (cf. Figure 9) and BA binding sites are therefore present in the AdN carrier in CHAPS. Further, the two different methods used (intrinsic fluorescence change for the CATR binding sites and release of N-ATP used as a fluorescence probe for the BA binding sites) gave the same result.

On the basis of protein concentration and a purity of the AdN carrier in CHAPS of about 80% with either one CATR or one BA bound per carrier dimer (Hackenberg & Klingenberg, 1980; Block et al., 1982), 70% of the AdN carrier in CHAPS was found to be able to bind CATR or BA, a value close to that derived from similar considerations in the case of the AdN carrier in LAPAO (Brandolin et al., 1985).

Discussion

The main conclusion derived from the results reported here is that the nature of the detergent determines the conformational state of the solubilized AdN carrier. From the experiments carried out with the isolated carrier in CHAPS or in LAPAO, it is clear that the two inhibitory ligands, CATR and BA, have opposite effects on the intrinsic fluorescence of the carrier protein. In CHAPS, the fluorescence quenching elicited by a first addition of ATP was accentuated by CATR

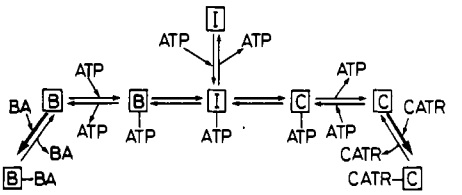

FIGURE 12: Scheme summarizing the different hypothetical conformations assumed by the AdN carrier during the transition from the BA conformation to the CATR conformation. The AdN carrier prepared in CHAPS is in the BA conformation, B; when prepared in LAPAO, the AdN carrier is in the CATR conformation, C. Upon addition of ATP, B or C binds ATP. Upon binding of ATP, B or C gives rise to the common transitory intermediate I–ATP, which is in equilibrium with the free form I. C can be trapped by CATR to give the stable C–CATR conformation; likewise, B reacts with BA to give the B–BA conformation.

but reversed by BA. In LAPAO, the initial fluorescence of the carrier was low; it was increased by ATP to an intermediary level that was quenched by CATR and enhanced by BA (Brandolin et al., 1985). These fluorescence changes are indicative of conformational changes elicited in the carrier protein; more precisely, the two extreme fluorescence levels observed with CATR and BA corroborate the existence of two conformations of the AdN carrier, trapped by CATR and BA, respectively, and detected in the mitochondrial membrane (Block et al., 1983).

Examination of the fluorescence responses as a function of the nature of the detergent led to the surprising finding that the response of the AdN carrier in LAPAO to CATR was opposite to that of the carrier in CHAPS. The same finding held for the response of the carrier to BA; this was particularly well illustrated by the fluorescence changes observed with N-ATP; for example, N-ATP bound to the carrier in CHAPS was released by BA but not by CATR, and conversely N-ATP bound to the carrier in LAPAO was released by CATR but not by BA. This strongly suggests that CATR has access to the carrier in LAPAO and BA has access to the carrier in CHAPS. It is therefore inferred that the carrier in CHAPS is in the BA conformation and the carrier in LAPAO is in the CATR conformation, with binding sites available for BA and CATR, respectively. The fact that LAPAO stabilizes the CATR conformation and CHAPS the BA conformation may come from the different nature of each detergent, CHAPS being a zwitterion and LAPAO a nonionic molecule. A modulation by detergents of the equilibrium between two conformers of another hydrophobic protein, the coat protein of bacteriophage M13, was recently detected by magnetic resonance techniques (Wilson & Dahlquist, 1985).

For the transition between the BA and CATR conformations to occur, ATP (or ADP) is required; nontransportable nucleotides are ineffective. Most likely, upon binding, ATP (or ADP) brings the carrier protein into an intermediary conformation that can be shifted either to the CATR conformation or to the BA conformation. The energy of activation of this transition is 40 kJ/mol between 0 and 10 °C. These results are interpreted in the scheme of Figure 12, where B represents the carrier in the BA conformation, C the carrier in the CATR conformation, and I the carrier in a transitory conformation. The scheme indicates that the transition between B and C cannot occur unless ATP (or ADP) is bound to B, I, or C. Accumulation of I at substoichiometric concentrations of ATP (or ADP) with respect to the carrier protein is explained by the reversible equilibrium between I and I–ATP: the extent of accumulation of I depends on the equilibrium constants between the different conformations. The fact that the different conformations assumed by the AdN

carrier in the course of the transition between B and C are always in an ATP (or ADP) bound form is consistent with the concept that, in the exchange of ADP or ATP across the mitochondrial membrane, the AdN carrier is always bound with either ADP or ATP.

ACKNOWLEDGMENTS

We thank Dr. G. Brandolin for helpful discussions and criticisms. We gratefully acknowledge the excellent secretarial assistance of J. Bournet.

Registry No. CHAPS, 75621-03-3; LAPAO, 61792-31-2; CATR, 33286-30-5; BA, 11076-19-0; ADP, 58-64-0; ATP, 56-65-5.

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Acetylation of Decarboxylated *S*-Adenosylmethionine by Mammalian Cells†

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Received August 14, 1985

ABSTRACT: Decarboxylated *S*-adenosylmethionine was found to be a substrate for the nuclear acetyltransferases that act on polyamines and on histones. The rate of acetylation of decarboxylated *S*-adenosylmethionine was more than twice that of spermidine at saturating substrate concentrations, and decarboxylated *S*-adenosylmethionine was an active inhibitor of the acetylation of histones by nuclear extracts from rat liver. The acetylation of decarboxylated *S*-adenosylmethionine occurred in vivo in SV-3T3 cells exposed to the ornithine decarboxylase inhibitor 2-(difluoromethyl)ornithine. The decline in putrescine and spermidine brought about by exposure to 2-(difluoromethyl)ornithine was found to be accompanied by a large rise in the content of both decarboxylated *S*-adenosylmethionine and acetylated decarboxylated *S*-adenosylmethionine. These results indicate that decarboxylated *S*-adenosylmethionine is metabolized not only in the well-known reactions in which it serves as an aminopropyl donor for polyamine biosynthesis but also by acetylation in reaction with acetyl coenzyme A. Furthermore, the inhibition of histone acetylation by decarboxylated *S*-adenosylmethionine could contribute to the biological effects brought about by inhibitors of ornithine decarboxylase.

An important role of *S*-adenosylmethionine is to serve as a precursor for polyamine biosynthesis [see reviews by Pegg & McCann (1982) and Tabor & Tabor (1984a) and references cited therein]. In order to serve as a donor of the aminopropyl groups in spermidine and spermine, *S*-adenosylmethionine must first be decarboxylated. Decarboxylated *S*-adenosylmethionine appears to be irreversibly committed to polyamine biosynthesis since it is very poorly if at all active as a substrate for methyltransferases (Zappia et al., 1969; Borchardt et al., 1976; Pegg, 1984a). *S*-Adenosylmethionine decarboxylase is a highly regulated enzyme and is a key step in polyamine biosynthesis (Pegg & Hibasami, 1979; Pegg, 1984a; Tabor & Tabor, 1984b). The cellular content of decarboxylated *S*-adenosylmethionine is normally very low, amounting to only 1 or 2% of the level of *S*-adenosylmethionine itself (Hibasami et al., 1980; Wagner et al., 1982). However, when the synthesis of putrescine and spermidine (which act as aminopropyl acceptors in the polyamine biosynthetic reactions) is prevented by the use of inhibitors of ornithine decarboxylase, there is a several hundred fold rise in the content of decarboxylated *S*-adenosylmethionine (Pegg et al., 1982; Mamont et al., 1982; Wagner et al., 1982; Pegg, 1984b). This rise strongly suggests that polyamine biosynthesis is indeed a major pathway of further metabolism of decarboxylated

† This work was supported by Research Grant GM 26290 from the National Institutes of Health.

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0006-2960/86/0425-0379$01.50/0