Localization of Immunoreactive Regions in the Beef Heart Adenine Nucleotide Carrier Using Rabbit Antisera against the Carboxyatractyloside-Liganded and the Sodium Dodecyl Sulfate Denaturated Carrier Forms†

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ABSTRACT: The existence of different antigenic determinants in the beef heart adenine nucleotide (AdN) carrier was demonstrated by exploring the reactivity of fragments of the carrier protein with rabbit antisera directed against either (a) the beef heart AdN carrier denatured with sodium dodecyl sulfate ($NaDodSO_4$ carrier) or (b) the beef AdN carrier liganded by the specific inhibitor carboxyatractyloside (CATR–carrier). The antigenic determinants reacting with antiserum to the CATR–carrier appeared to be close to the N- and C-terminal ends of the carrier protein, whereas those reacting with antiserum to the $NaDodSO_4$ carrier were located preferentially in the central region of the protein. The same antisera were used to study the immunogenic specificity of the beef liver AdN carrier, the rat heart AdN carrier, and the rat liver AdN carrier. The beef liver and rat heart AdN carriers were found to react with both antisera whereas the rat liver AdN carrier reacted essentially with the CATR–carrier antiserum.

Carboxyatractyloside (CATR),[1] a specific inhibitor of the mitochondrial adenine nucleotide carrier (AdN carrier), binds to the carrier to give a stable CATR–carrier complex. This complex is highly antigenic (Bojanovski et al., 1976; Buchanan et al., 1976; Lauquin et al., 1978; Eierman et al., 1977; Kolarov et al., 1978; Schultheiss & Klingenberg, 1984, 1985). The beef heart AdN carrier has been purified from a detergent extract of the mitochondrial membrane both in its free form and in a form liganded by CATR [see Vignais et al. (1985) for review]. The rabbit antiserum directed against the CATR–carrier complex was found to react strongly with this complex but reacted poorly with the free form of the carrier (Buchanan et al., 1976; Lauquin et al., 1978). It is known that the AdN carrier assumes a different conformation in the presence of CATR than in the absence of CATR (Block et al., 1983; Brandolin et al., 1985). The difference in immunogenicity between the free and CATR-complexed forms of the AdN carrier suggests that the antigenic determinants presented by the carrier depend upon the conformation assumed by the carrier.

The present paper describes the mapping of antigenic determinants in the beef heart AdN carrier protein, on the basis of the use of antisera directed against the CATR–carrier complex (CATR–carrier antiserum) and the carrier protein denatured by sodium dodecyl sulfate ($NaDodSO_4$ carrier antiserum) and the reaction of these antisera with cleavage products obtained by limited fragmentation of the AdN carrier. The beef heart carrier antisera were also used to determine whether acceptor sites in the AdN carrier proteins from beef liver, rat heart, and rat liver are capable of reacting with antibodies directed against the beef heart AdN carrier.

Materials and Methods

Carboxyatractyloside was obtained from Boehringer. Bongkrekic acid (BA) was prepared as previously described (Lauquin & Vignais, 1976). Bovine serum albumin, dithionitrobenzoic acid, Triton X-100, and Nonidet P40 were purchased from Sigma Chemical Co. Guanidinium chloride and citraconic anhydride were from Pierce. Acrylamide and bisacrylamide were obtained from Eastman Kodak and BA 85 nitrocellulose sheets, 0.45 μm, from Schleicher and Schüll. Hydroxylapatite was from Bio-Rad and Ultrogel ACA 202 from IBF. ^{125}I-Labeled protein A was from Amersham and [^{14}C]NaCN from the Commissariat à l'Energie Atomique, Saclay.

Beef heart mitochondria and rat heart mitochondria were prepared as described by Smith (1967) and Chance and Hagihara (1963), respectively. Rat liver mitochondria were prepared by the method of Hogeboom et al. (1984); the same method was used for the preparation of beef liver mitochondria.

The AdN carrier protein was isolated from beef heart mitochondria as reported by Riccio et al. (1975) with modifications (Boulay et al., 1983). Cleavages at methionyl residues by cyanogen bromide (Gross, 1967) and at cysteinyl residues by cyanylation at alkaline pH (Vanaman et al., 1970) were performed on the citraconylated AdN carrier as described by Boulay et al. (1983). Prior to $NaDodSO_4$–polyacrylamide gel electrophoresis, the AdN carrier protein was decitraconylated in 50% formic acid for 5 h at room temperature. The acidolytic cleavage at the Asp-203–Pro-204 bond was carried out on the carrier protein, after thionitrobenzoylation and citraconylation, in 70% formic acid at 35 °C for 48 h (Boulay, 1983).

Rabbits were given multiple injections of AdN carrier protein, which was either denatured with $NaDodSO_4$ or combined with CATR. The CATR-liganded carrier was prepared from beef heart mitochondria preincubated with CATR, following the same procedure as for the native AdN carrier. Before immunization, a sample of preimmune serum was

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[1] Abbreviations: AdN, adenine nucleotide; $NaDodSO_4$, sodium dodecyl sulfate; CATR, carboxyatractyloside; BA, bongkrekic acid; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; BSA, bovine serum albumin; P_i, inorganic phosphate.

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collected; this preimmune serum was not reactive against our carrier preparations. For each injection, 0.2 mg of the lyophilized beef heart AdN carrier protein was dissolved in 100 μL of 5% $NaDodSO_4$ and then diluted with 900 μL of saline buffer. The carrier protein solution was mixed with an equal volume of complete Freund's adjuvant by repeated passage through a needle. The emulsion was injected by multiple intradermic injections into the backs of the rabbits. The injections (0.2 mg) were repeated 3 times every third week. Three weeks after the last intradermic injection, three booster injections were administered intramuscularly every other day. Bleeding was performed 10 days after the last injection. The collected blood was allowed to clot at 37 °C and was left overnight at 4 °C. After centrifugation, the supernatant was filtered through a sterile Millex filter, 0.45 μm, and stored at 4 °C with 1 mM EDTA and 0.01 NaN_3.

Electrophoretic separation of the peptides obtained by chemical cleavage of the AdN carrier protein was performed in 10–20% acrylamide slab gels (Cabral & Schatz, 1979). Samples were dissolved in a buffer consisting of 50% glycerol, 2% $NaDodSO_4$, 0.1 M sodium phosphate, pH 7.5, 7% β-mercaptoethanol, and traces of bromphenol blue. After separation on $NaDodSO_4$–polyacrylamide gel, the peptides were electrophoretically transferred to a nitrocellulose sheet (Towbin et al., 1979). The electrophoretic buffer solution consisted of 192 mM glycine, 20 mM Tris–base, 0.05% $NaDodSO_4$, and 20% methanol, pH 8.3. The transfer proceeded for 3 h at 4 °C with an electrical field of 10 V/cm giving 1.5 A between two planar carbon electrodes (13 × 20 cm). After protein transfer, the nitrocellulose sheet was soaked for 1 h at room temperature with a saline buffer (150 mM NaCl, 10 mM sodium phosphate, pH 7.5) supplemented with 3% (w/v) bovine serum albumin to saturate nonspecific protein binding sites. The nitrocellulose sheet was then incubated overnight at 4 °C with the rabbit antiserum diluted 50-fold in saline–BSA buffer. After five washes in the same saline buffer, supplemented with 1% (w/v) Nonidet P40, it was incubated with 2 μCi of ^{125}I protein A diluted in the saline–BSA buffer for 2 h at room temperature. After five further washes in the saline–Nonidet, it was dried and processed for autoradiography with Fuji RX film.

A dot immunobinding assay (Jahn et al., 1984) was performed essentially as described by Kuhn-Nentwig and Kadenbach (1985). A grid of squares (1.5 × 1.5 cm) was drawn on a nitrocellulose sheet with a soft pencil. The nitrocellulose sheet was rinsed for 2 min in boiling distilled water and dried for 15 min at 60 °C before use. Five microliters of solutions of varying concentrations of AdN carrier in $NaDodSO_4$ was spotted on the centers of the squares. After heating for 15 min at 60 °C, the nitrocellulose sheet was fixed for 15 min in 10% (v/v) acetic acid and 25% (v/v) isopropyl alcohol and rinsed twice with distilled water and phosphate–saline buffer (150 mM NaCl, 20 mM NaP_i, pH 7.4). The sheet was incubated for 1 h in a "blocking" solution consisting of phosphate–saline buffer supplemented with 3% (w/v) bovine serum albumin. Then, they were reacted with the antiserum and ^{125}I protein A as described above for the Western blot. The sheet was finally dried; the squares were cut out and counted for radioactivity.

Results

Mapping of Antigenic Determinants in the Beef Heart AdN Carrier with Rabbit Antisera Directed against the Beef Heart CATR–Carrier and the Beef Heart $NaDodSO_4$ Carrier. The beef heart AdN carrier protein can be readily cleaved at the level of the Asp-203–Pro-204 bond by acidolysis (Aquila et al., 1982), at the methionyl residues (Met-200, -214, -237, -238, -239, -249, and -281) by treatment with CNBr and at the cysteinyl residues (Cys-56, -128, -159, and -256) by treatment with sodium cyanide at alkaline pH (Boulay et al., 1983; Boulay & Vignais, 1984). It is noticeable that four cysteinyl residues are scattered all over the sequence of the carrier protein, whereas the seven methionyl residues are all located at the C-terminal end. Cleavage at the methionyl residues yields a large peptide fragment called CB_1 and a number of very small peptides. On the basis of the amino acid sequence of the whole carrier protein (Aquila et al., 1982) and of the sequences of small peptides yielded by chemical cleavage of the CB_1 fragment (Boulay et al., 1983), a peptide map of the beef heart AdN carrier has been constructed (Figure 1). As the peptide fragments arising from the above cleavages are well identified and their mobility in $NaDodSO_4$–polyacrylamide gel electrophoresis is well assessed, it is possible to readily draw conclusions concerning the number and the identity of the immunoreactive peptides after immunoblotting.

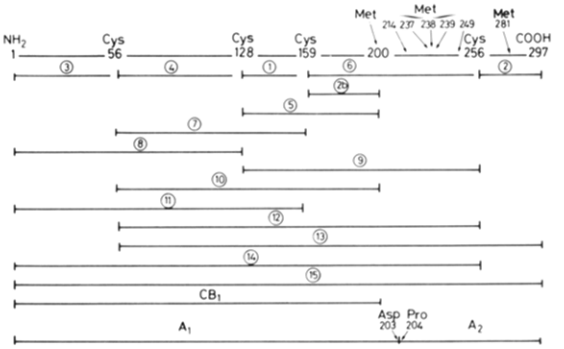

FIGURE 1: Alignment of peptides obtained by cleavage of the beef heart AdN carrier protein at cysteinyl and methionyl residues and at the acidolabile Asp-203–Pro-204 bond.

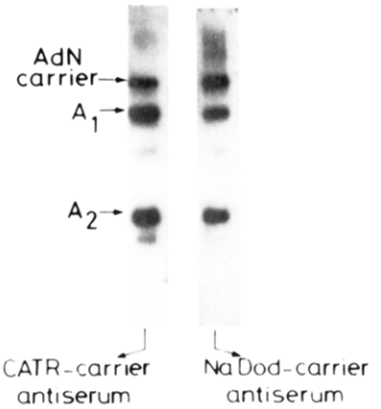

FIGURE 2: Autoradiography of an immunoblot showing the immunoreactive fragments obtained by acidolytic cleavage of the beef heart AdN carrier at the Asp-203–Pro-204 bond. The immune complexes formed with the CATR–carrier antiserum and the $NaDodSO_4$ carrier antiserum (1/50 dilution) were revealed by ^{125}I protein A as described under Materials and Methods.

As mentioned above, acidolytic cleavage at the Asp-203–Pro-204 bond results in two fragments A_1 and A_2 spanning Ser-1–Asp-203 and Pro-204–Val-297, respectively. After separation by $NaDodSO_4$–polyacrylamide gel electrophoresis, the two peptides were transferred to a nitrocellulose sheet for assay of antigenicity with specific antisera. As shown in Figure 2, they reacted with both the CATR–carrier antiserum and the $NaDodSO_4$ carrier antiserum.

The immunoblots in Figure 3 illustrate the reactivity of the CATR–carrier antiserum, and that of the $NaDodSO_4$ carrier antiserum, against peptides yielded either by direct cleavage

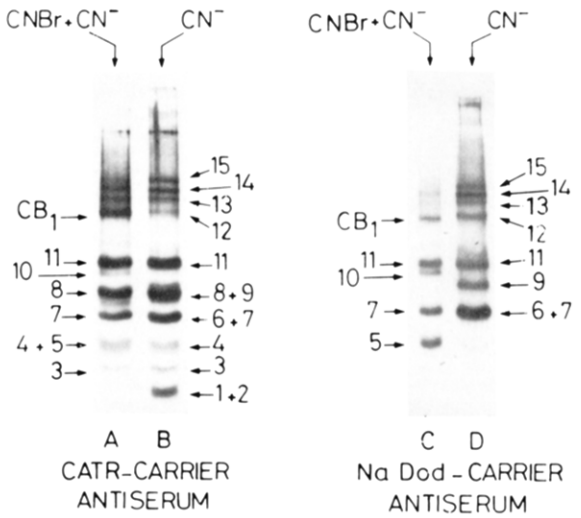

FIGURE 3: Autoradiography of immunoblot showing the immunoreactive fragments obtained by simple cleavage of the beef heart AdN carrier at cysteinyl residues by cyanide (tracks B and D) and by double cleavage at cysteinyl residues and methionyl residues by cyanide and cyanogen bromide (tracks A and C). After electrotransfer, the peptide fragments were reacted with the CATR–carrier antiserum (1/50 dilution, tracks A and B) or with the $NaDodSO_4$ carrier antiserum (1/50 dilution, tracks C and D), and the immune complexes were radioactively labeled with ^{125}I protein A (cf. Materials and Methods).

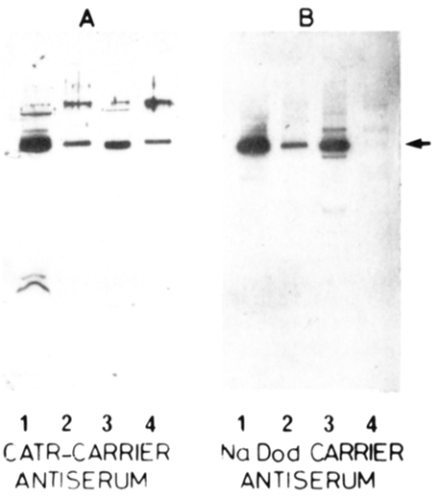

FIGURE 4: Autoradiography of an immunoblot showing the reaction of the AdN carrier in $NaDodSO_4$ lysates of mitochondria (150 μg of protein) obtained from beef heart (track 1), beef liver (track 2), rat heart (track 3), and rat liver (track 4) with the CATR–carrier antiserum and the $NaDodSO_4$ carrier antiserum (1/50 dilution). The immune complexes were revealed by ^{125}I protein A. The AdN carrier–antibody complexes are indicated by the arrow.

at cysteinyl residues (tracks B and D) or by cleavage at both methionyl residues and cysteinyl residues (tracks A and C). Some bands in Figure 3 (track B) corresponded to pairs of peptides (peptides 1 and 2, 6 and 7, 8 and 9) that could not be resolved on the gels. Identification of the immunoreactive peptides was made possible by comparing the gel pattern with and without cleavage at methionyl residues (tracks A and B, C and D in Figure 4). For example, in track B (Figure 3), the first immunoreactive band corresponded to a mixture of peptide 1 (Cys-128–Asn-158) and peptide 2 (Cys-256–Val-297), the latter of which contains Met-281. The disappearance of this band after cleavage of the carrier protein at methionyl residues (track A, Figure 3) leads us to conclude that only peptide 2 reacts with the CATR–carrier antiserum. Peptide 2b (Cys-159–Met-200), which arises from cleavage at both cysteinyl and methionyl residues, has virtually the same size as peptides 1 and 2 and should migrate to the same level in track A of Figure 3; it should have given a positive reaction if it were immunoreactive, which is not the case. Peptide 3 (Cys-56–Leu-127), yielded by cleavage at cysteinyl residues (track A, Figure 3), was immunoreactive. Peptide 5 (Cys-128–Met-200), obtained by combined cleavage at cysteinyl and methionyl residues, was expected to migrate to the same level as peptide 4 in track B. From the positive response on the gel at this level, it was difficult to draw a definite conclusion as to the antigenicity of peptide 5, since peptide 4 itself gave a positive response. However, since there was no increase in the intensity of the band corresponding presumably to both peptides 4 and 5, one can conclude that peptide 5 is not immunoreactive. The fact that peptide 5 consists of peptides 1 and 2b, which are not immunoreactive, may be indicative but does not provide definite evidence that peptide 5 is not immunoreactive; in fact, Cys-159 could be required for the formation of an antigenic site. The band corresponding to peptides 6 (Cys-159–Asp-255) and 7 (Cys-56–Asn-158) (track A, Figure 3) became less intense after cleavage at methionyl residues (track A, Figure 3). This is explained by the disappearance of peptide 6, which contains a number of methionyl residues (Met-200, -214, -237, -238, -239, and -249). Of the two peptides 6 and 7, the reactive one is therefore peptide 7, which is consistent with the fact that peptide 7 contains the reactive peptide 4. In the band corresponding to peptides 8 (Ser-1–Leu-127) and 9 (Cys-128-Asp-255), peptide 8 must be immunoreactive, as it contains the immunoreactive peptides 3 and 4. Along this line, it is noteworthy that fragment A_2 (Pro-204–Val-297) obtained by acidolytic cleavage of the AdN carrier (Figure 2) loses its reactivity against the CATR–carrier antiserum after succinylation with succinic anhydride (data not shown). As most of the lysine residues are located at the C-terminal end of the carrier molecule, their modification by succinylation is expected to affect essentially peptide 2 (Cys-256–Val-297); furthermore, peptides 1 (Cys-128–Asp-158) and 2b (Cys-159–Met-200) are not immunoreactive. It remains that the only possible reactive sequence in peptide 9 is Met-200–Cys-256; however, this could not be experimentally determined. Finally, peptide 3 (Ser-1–Asp-55) exhibited a weak reactivity.

The immunoblots corresponding to tracks C and D of Figure 3 resulted from the reaction of the $NaDodSO_4$ carrier antiserum with the same peptide fragments as those present in tracks A and B of Figure 3, which were reacted with the CATR–carrier antiserum as previously discussed. A number of bands that gave a positive reaction with the CATR–carrier antiserum did not react with the $NaDodSO_4$ carrier antiserum and vice versa. This was the case for the band corresponding to peptides 1 (Cys-128–Asn-158) and 2 (Cys-256-Val-297). The band corresponding to peptides 8 and 9 gave a positive reaction (track D, Figure 3); after cleavage at methionyl residues, the reactivity of this band disappeared (track C, Figure 3). Peptide 9, which contains a number of methionyl residues, was therefore the reactive peptide. The band corresponding to peptides 6 and 7 (track D, Figure 3) was fainter after CNBr treatment (track C, Figure 3) but did not disappear, which is consistent with the presence of antigenic determinants in both peptides. The positive reactivity of peptide 7 (Cys-56–Asn-158), compared to the absence of reactivity of peptide 8 (Ser-1–Leu-127), implied that an antigenic determinant is located in peptide 1. However, as shown above, there is no reaction at the level of the band containing peptides 1 and 2. This apparent contradiction might be explained by the requirement for Cys-128 or the adjacent peptide 4 (Cys-56–Leu-127) for conferring antigenicity to peptide 1. The

Table I: Reactivity of Peptides Obtained by Cleavage of Beef Heart Adenine Nucleotide Carrier, with CATR–Carrier Antiserum and $NaDodSO_4$ Carrier Antiserum[a]

peptide no.	first and last amino acid residues	reactivity with CATR–carrier antiserum	reactivity with $NaDodSO_4$ carrier antiserum
1	Cys-128–Asn-158	–	–
2	Cys-256–Val-297	+	–
2b	Cys-159–Met-200	–	–
3	Ser-1–Asp-55	±	–
4	Cys-56–Leu-127	+	–
5	Cys-128–Met-200	(–)	+
6	Cys-159–Asp-255	(–)	+
7	Cys-56–Asn-158	+	+
8	Ser-1–Leu-127	+	–
9	Cys-128–Asp-255	(–)	+
10	Cys-56–Met-200	+	+
11	Ser-1–Asn-158	+	+
12	Cys-56–Asp-255	+	+
13	Cys-56–Val-297	+	+
14	Ser-1–Asp-255	+	+
15	Ser-1–Val-297	+	+
CB_1	Ser-1–Met-200	+	+

[a] Peptides with numbers grouped with a brace have similar molecular weights and migrate in gels as a single band. Immunochemical analysis was performed as described under Materials and Methods. Parentheses are used when the immunoreactivity of the peptide is uncertain.

positive reactivity of peptide 5 (Cys-128–Met-200) contrasts with the absence of reactivity of peptide 1 (Cys-159–Met-200). This suggests that the antigenic site in peptide 5 contains Cys-159. The absence of reactivity of the C-terminal portion of the carrier protein (Cys-256–Val-297), when compared with the positive reactivity of the acidolytic fragment A_2 (Pro-204–Val-297) (see above), suggests the presence of an antigenic determinant against the $NaDodSO_4$ carrier antiserum in the peptide Pro-204–Asp-255. This is consistent with the fact that the acidolytic fragment A_2, which contains only two lysyl residues in the Pro-204–Asp-255 sequences, does not lose its reactivity against the $NaDodSO_4$ carrier antiserum after succinylation (data not shown). Of the two peptides 6 and 7, which migrated to the same level in the gel, only peptide 6 contains methionyl residues; the corresponding band in tracks C and D of Figure 3 showed a marked decrease but did not disappear after cleavage at methionyl residues. We may therefore conclude that peptide 7 is reactive against the $NaDodSO_4$ carrier antiserum and that peptide 6 is probably also reactive. The results concerning the immunoreactivity of all peptides obtained by chemical cleavage of the adenine nucleotide carrier are assembled in Table I.

Cross-Reactivities of the Beef Heart, Beef Liver, Rat Heart, and Rat Liver AdN Carrier. The immunological relationships between the AdN carrier proteins from beef heart, beef liver, rat heart, and rat liver mitochondria were investigated by immunoblotting, by using the same antisera as those described previously, namely, the rabbit antisera obtained after injection of the beef heart AdN carrier liganded by CATR or denatured by $NaDodSO_4$. As illustrated in the immunoblots of Figure 4, the CATR–carrier antiserum reacted against the homologous AdN carrier from beef heart (track 1-A) and also against the AdN carrier present in lysates of beef liver, rat heart, and rat liver mitochondria (tracks 2-A, 3-A, 4-A). On the other hand, the $NaDodSO_4$ carrier antiserum reacted with the homologous AdN carrier protein (track 1-B) and with the AdN carrier from beef liver and rat heart (track 2B, 3-B) but not with the rat liver AdN carrier (track 4-B). Complementary experiments were performed to check the reactivity of fragments of the rat heart AdN carrier with respect to antisera

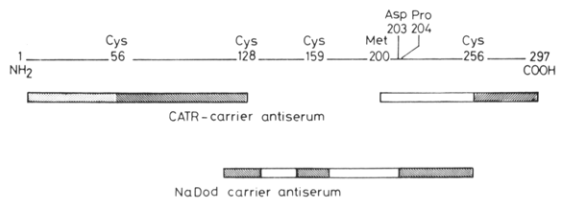

FIGURE 5: Scheme illustrating the localization of antigenic determinants of the beef heart adenine nucleotide carrier against the anti-CATR–carrier antiserum and the anti $NaDodSO_4$ carrier antiserum. The hatched rectangles correspond to unambiguously reactive regions of the protein. The open rectangles correspond to regions whose reactivity is uncertain and the dotted rectangle to a region of low antigenicity. Cys-56 (carrier reacting with the CATR–carrier antiserum) and Cys-128 and -159 (carrier reacting with the $NaDodSO_4$ carrier antiserum) are considered to be part of antigenic sites and to play in these sites a strategic function.

raised against the beef heart CATR–carrier complex and the beef heart $NaDodSO_4$ carrier. The pattern of the reactive peptides was not greatly different from that of the beef heart AdN carrier (not shown).

To verify that the lack of reactivity of the rat liver AdN carrier with the $NaDodSO_4$ carrier antiserum was not due to a low antibody titer in the $NaDodSO_4$ carrier antiserum, compared to that in the CATR–carrier antiserum, a dot immunobinding assay was performed (Jahn et al., 1984). The two antisera, when tested with increasing concentrations of the purified rat heart AdN carrier protein, showed virtually the same titers (data not shown).

Discussion

Antibodies Raised in Rabbit by the CATR–AdN Carrier from Beef Heart and Those Raised by the $NaDodSO_4$ AdN Carrier from Beef Heart Are Not Recognized by the Same Determinants of the Beef Heart AdN Carrier. The existence of conformational changes of the AdN carrier protein upon addition of specific ligands is a well-established phenomenon (Block et al., 1983; Brandolin et al., 1985). One of these ligands, CATR, is able to trap the AdN carrier protein in a conformation characterized by typical fluorescence properties (Brandolin et al., 1985). Antisera have been raised against both the beef AdN carrier denatured by $NaDodSO_4$ and the beef heart AdN carrier liganded by CATR. The mapping of antigenic determinants of the beef heart carrier reported in this paper relied on the reactivity of denatured fragments of the carrier protein with the CATR–carrier antiserum and the $NaDodSO_4$ carrier antiserum. It is noteworthy that the reaction of the CATR–carrier antiserum was directed not against conformational determinants but against unfolded amino acid sequences, since it was conducted after gel electrophoresis, in the presence of $NaDodSO_4$. The results showed different patterns of responsiveness to the two antisera. The scheme of Figure 5 summarizes the tentative localization of the antigenic determinants recognized by each antiserum. The fragments responding to the CATR–carrier antiserum appear to be close to the C and N ends of the carrier molecule whereas those responding to the $NaDodSO_4$ carrier antiserum are located preferentially in the middle of the molecule.

Although we are aware of the uncertainties inherent in the detection of segmental sites in a protein by polyclonal antibodies (Berzovsky, 1985), it is clear that marked differences exist in the reactivity of the CATR–carrier antiserum and the $NaDodSO_4$ carrier antiserum against fragments of the homologous AdN carrier. Our finding that the sequence spanning Cys-128–Met-200 does not contain antigenic determinants against the CATR–carrier antiserum might be related

to the occupancy of this region of the carrier by CATR (Boulay et al., 1983). It must be stressed that antigenic determinants may correspond to portions of the immunoreactive peptides. On the other hand, antigenicity may be lost after cleavage at a strategic residue; this could explain why two contiguous peptides may not be immunoreactive when taken separately but become reactive when they are joined to form a larger peptide structure.

It is noteworthy that one of the major antigenic regions reacting with the CATR–carrier antiserum is located around Cys-56 in a very hydrophilic segment of the protein (Saraste & Walker, 1982). As shown in a previous report (Boulay & Vignais, 1984), the region of the AdN carrier encompassing Cys-56 is markedly altered during the transition from the CATR conformation to the BA conformation. We can reasonably speculate that the absence of reaction of the CATR–carrier antiserum against the unliganded and the BA-liganded carriers as shown by the immunodiffusion technique of Ouchterlony (Buchanan et al., 1976) reflects changes in the conformation of this segment or in its accessibility to antiserum.

The absence of reactivity of the Ser-1–Leu-127 fragment against the $NaDodSO_4$ carrier antiserum is more difficult to understand. This antiserum appeared to react essentially with the core of the carrier protein, corresponding to the three α-helices that presumably span the membrane (Saraste & Walker, 1982). One cannot exclude the possibility that the rabbit injected with the carrier protein denatured by $NaDodSO_4$ developed a tolerance against the Ser-1–Leu-127 fragment, which is a relatively well conserved sequence in different species (Baker & Leaver, 1985).

Is the AdN Carrier Protein Tissue Specific? The finding that the AdN carrier from rat liver does not react with the anti-beef heart $NaDodSO_4$ carrier antiserum, whereas the AdN carrier from rat heart is highly reactive, suggests the existence of tissue specificity for the AdN carrier. This tissue specificity is not revealed by the anti-beef heart CATR–carrier antiserum. A dot immunoassay carried out with both the $NaDodSO_4$ carrier antiserum and the CATR–carrier antiserum, against the purified rat heart AdN carrier, showed similar titers for the two antisera and eliminated the possibility that the absence of reactivity of the rat liver AdN carrier to the $NaDodSO_4$ carrier antiserum was due to a low titer of this antiserum. The existence of a tissue specificity for the AdN carrier has already been discussed by different groups (Kolarov et al., 1978; Schultheiss et al., 1985) and has led to the notion of isocarriers. However, the possibility of posttranslational modifications that differ in heart and liver AdN carrier proteins, and the existence of strongly bound cardiolipin (Beyer & Klingenberg, 1985) that hinders antibody acceptor sites in the rat liver AdN carrier, cannot be excluded.

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