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Immunological characterization of the mitochondrial 2-oxoglutarate carrier from liver and heart

Organ specificity

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Antibodies have been prepared against the 2-oxoglutarate transport proteins purified from bovine heart and rat liver mitochondria. The anti-heart antiserum cross-reacts with the 2-oxoglutarate carrier (OGC) from beef, pig, rat and rabbit heart, but not with the OGC from liver of the same animals. Conversely, the anti-liver antiserum recognizes the carrier protein from liver of all species tested but not from heart. Immunoinactivation of oxoglutarate transport activity by the antibodies is also tissue specific. Peptide maps of purified OGC show structural differences between the carrier from heart and liver of the same animal species. These results indicate the existence of isoforms of the OGC in heart and liver.

2-Oxoglutarate carrier; Antibody; Western blotting; Mitochondria

1. INTRODUCTION

The OGC, an intrinsic protein of the inner mitochondrial membrane, catalyzes an exchange between 2-oxoglutarate and malate, which is important for several metabolic processes such as gluconeogenesis from lactate and the malate-aspartate shuttle (for reviews see [1,2]). Unlike other mitochondrial anion-transporting systems such as the dicarboxylate and the citrate carrier, the OGC has a high activity both in liver and heart. In our laboratory we have isolated and functionally reconstituted the 2-oxoglutarate transport protein from both heart and liver mitochondria [3–5]. Whereas the functional properties of the OGC have been investigated both in mitochondria and in proteoliposomes, little is known about the structure of this carrier. We have recently shown that essential sulphydryl groups are located at the cytosolic face of the OGC in the vicinity of the substrate-binding site [6]. In order to obtain further insight into the structure and function of the OGC we have prepared specific antibodies against the carrier purified from bovine heart and rat liver mitochondria. The anti-heart antiserum cross-reacts with the OGC from heart of various species but does not recognize the carrier protein from liver even from the same species. A completely opposite cross-reactivity is exhibited by the anti-liver antiserum. It is concluded that the antigenic properties of the OGC are markedly tissue specific.

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Abbreviations: OGC, 2-oxoglutarate carrier; Pipes, 1,4-piperazine-diethanesulfonic acid; SDS, sodium dodecyl sulphate

2. MATERIALS AND METHODS

Nitrocellulose membrane filters (BA 85) were purchased from Schleicher and Schuell, peroxidase-conjugated anti-rabbit Ig and [1-^{14}C]2-oxoglutarate from Amersham International, Tween 20 from Sigma, microtitration polystyrene plates from Nunc, proteinase K and pronase from Boehringer, protein A-Sepharose CL-4B from Pharmacia. All other reagents were of analytical grade.

Mitochondria from different organs and species were prepared by standard procedures. The OGC from heart and liver of several mammals was purified in 3% Triton X-114 essentially as described previously [3,5]. For the preparation of the antisera, the OGC proteins from bovine heart and rat liver were electroeluted from SDS preparative gels containing 17.5% acrylamide and an acrylamide:bisacrylamide ratio of 150. After collection of preimmune sera, 0.2 mg of OGC from bovine heart or rat liver was dissolved in 0.5 ml of 0.9% NaCl, emulsified with an equal volume of complete Freund's adjuvant and injected subcutaneously at multiple sites into the backs of rabbits. Four weeks after the first injection, a booster injection of 0.2 mg of antigen emulsified in incomplete Freund's adjuvant was given intramuscularly into the flank. Blood was collected from the marginal ear vein after 2 weeks and serum was obtained by centrifugation of blood that had been left to clot at room temperature for 2 h. Where specified, IgG was purified from the antisera by affinity chromatography on protein A-Sepharose CL-4B. The reactivity of the antibodies was tested by ELISA and by immunoblotting according to standard procedures. For the immunoinactivation experiments, the purified OGC from bovine heart or rat liver (15 μg of protein) was incubated with purified IgG in a final volume of 0.5 ml containing 0.8% (w/v) Triton X-114, 20 mM Pipes and 20 mM Na_2SO_4, pH 7.0. After 3 h incubation in ice, the samples were assayed for oxoglutarate transport activity in reconstituted liposomes, as described previously [5]. Protease digestion of purified

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OGC proteins (5–10 μg) was performed, after precipitation with 10% TCA, in 100 μl of buffers (0.1 M sodium borate-HCl, pH 7.5, 5 mM $CaCl_2$, 0.1% SDS and 0.1 μg of pronase, or 50 mM Tris-HCl, pH 8.0, 0.1% SDS and 0.5 ng of proteinase K) for 30 min at 40°C (pronase) or at room temperature (proteinase K). The reaction was stopped by addition of 2% SDS plus 100 mM dithioerythritol and boiling for 2 min. The proteolytic products were separated by electrophoresis on a linear gradient acrylamide gel (10–20%) and stained with the silver nitrate method [7]. Protein was determined by a modified Lowry method [8].

3. RESULTS

In preliminary experiments the two polyclonal antisera raised against the purified OGC from bovine heart and rat liver were characterized by ELISA. The anti-heart antiserum produced a positive response against 0.1 μg of purified OGC from bovine heart up to a dilution of 1:6000. With the anti-liver antiserum the immunoreaction was still detectable at 1:5000 dilution in the presence of the same amount of purified OGC from rat liver. In both cases no reaction was found with the preimmune sera even at a dilution of 1:100.

In order to investigate whether the antisera were able to affect the activity of the OGC, purified IgG was incubated with the solubilized OGC isolated from bovine heart or rat liver. After this incubation the mixtures were tested for oxoglutarate transport activity in reconstituted liposomes. The anti-heart IgG inhibited the reconstituted OGC activity when incubated with the bovine heart protein, but not with the rat liver protein (Fig. 1). Conversely, the anti-liver IgG strongly decreased the reconstituted transport activity of the OGC from rat liver, without affecting the activity of the OGC from bovine heart (Fig. 1). It should be noted that, under the experimental conditions used in these experiments (presence of Triton X-114 and non-optimal salt concentrations), the OGC proteins were not immunoprecipitated by the specific antibodies. This is supported by the following observations: (a) centrifugation of the incubation mixtures was not required to observe the reported inhibitions nor increased the degree of inhibition, and (b) the immunoblots of the supernatants and pellets obtained from the incubation mixtures by centrifugation showed the presence of the immunoreactive material only in the supernatants. Clearly the antigen/antibody complex is unable to reconstitute the transport activity in liposomes. As shown in Fig. 1A and B, incubation of the purified IgG with the liposomes reconstituted with the bovine heart or the rat liver OGC did not cause any inhibition of the oxoglutarate transport activity, probably because the antigenic determinants are not sufficiently exposed on the external surface of the liposomes.

The ability of the immune sera to react with the mitochondrial OGC of different species and tissues was investigated by Western blot analysis. In these experiments the total mitochondrial proteins or the

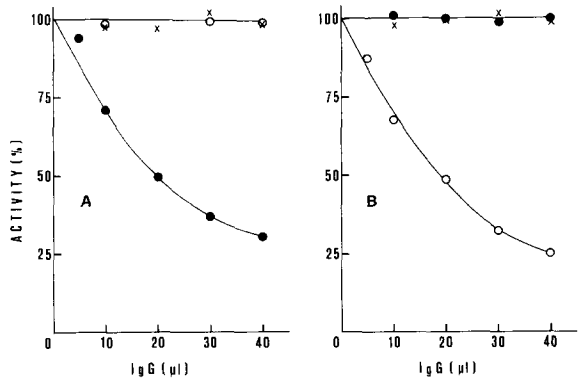

Fig. 1. Effects of antibodies on the reconstituted oxoglutarate transport activity of bovine heart OGC and of rat liver OGC. The OGC from bovine heart in A and from rat liver in B were incubated in the solubilized state with anti-heart OGC IgG (•) or with anti-liver OGC IgG (○). In (×) the OGC from bovine heart (A) and from rat liver (B) were incubated after reconstitution into liposomes with anti-heart OGC IgG or anti-liver OGC IgG, respectively. The control value of uninhibited oxoglutarate transport was 17 mmol/6 min per g protein in A (at 1 mM external substrate) and 6 mmol/10 min per g protein in B (at 0.1 mM external substrate).

purified OGCs were solubilized in 1% SDS, electrophoresed, transferred onto nitrocellulose and incubated with the anti-heart or the anti-liver OGC antiserum. Fig. 2A shows that the anti-heart antiserum cross-reacted with a single band in heart mitochondria of all the species tested. The immunodecorated band had an M_r of 31500 in beef and pig and 31 kDa in rat and rabbit. These molecular weights are identical to those of the OGC isolated from porcine and bovine hearts [3,4] and of the OGC isolated from rat heart (see Fig. 3). In contrast, there was no cross-reactivity of the bovine heart antiserum with the pure OGC isolated from the liver of the same species (Fig. 2A) nor with any protein of solubilized liver mitochondria from beef (Fig. 2A), pig, rat and rabbit (not shown). As shown in Fig. 2B, the liver antiserum reacted with the OGC present in liver mitochondria of all the species tested, although with a lower affinity for beef and pig. The OGC from rat liver had an M_r of 32500 as previously demonstrated [5], whereas the OGC from bovine and porcine liver had the same mobility as the OGC previously isolated from the heart of pig and beef ([3,4] and Fig. 3 of the present paper). In striking contrast with the above results, the rat liver antiserum did not react at all with the heart OGC of the same species (Fig. 2B) as well as with the OGC of bovine, porcine and rabbit hearts (not shown). In other experiments (not shown) it was found that both the heart and the liver antiserum reacted only weakly with the renal OGC, i.e. with a single band of 31.5 kDa in kidney mitochondria from beef, pig, rat and rabbit. It should be noted that the absence of cross-reactivity between heart and liver, and the weak reactivity with kidney, were confirmed by immunoblot experiments in which

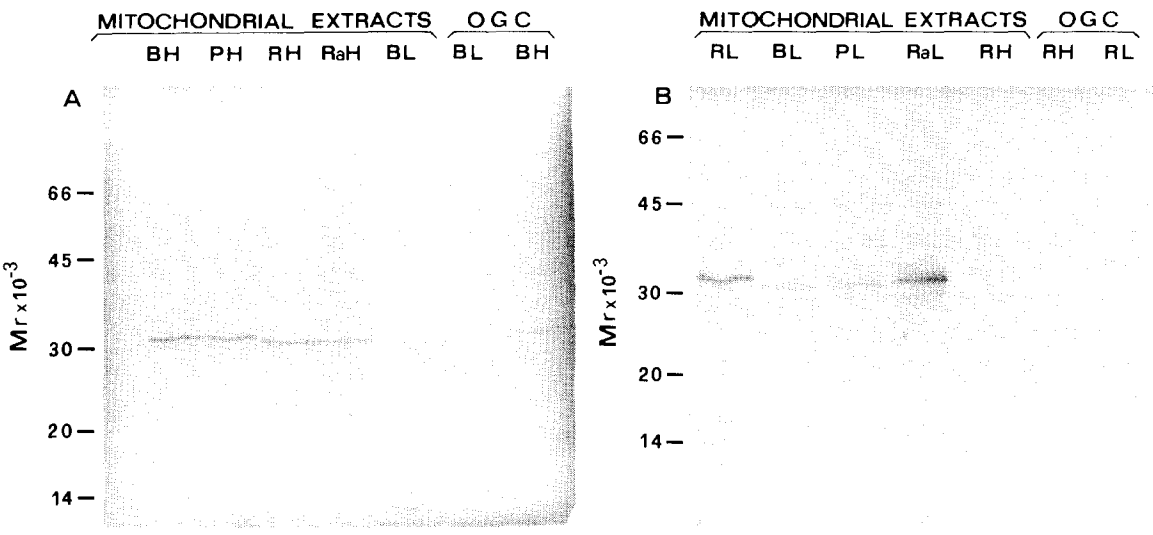

Fig. 2. Immunological cross-reactivity of OGC proteins. Samples were separated on a 17.5% polyacrylamide gel and transferred to nitrocellulose. (A) Immunodecoration with anti-heart OGC antiserum (diluted 1:1000). (B) Immunodecoration with anti-liver OGC antiserum (diluted 1:800). 100 μg of SDS solubilized heart mitochondria from beef (BH), pig (PH), rat (RH) and rabbit (RaH) or 100 μg of SDS solubilized liver mitochondria from beef (BL), pig (PL), rat (RL) and rabbit (RaL) or 1 μg of purified OGC from liver and heart of beef (BL and BH) and rat (RL and RH) were applied to the gel.

we used concentrations of carrier protein and of antibodies 5 times higher than those applied in Fig. 2A and B.

In order to search for structural differences between the liver and the heart OGC more directly, we compared the products obtained by proteolytic fragmentation of the purified proteins. In Fig. 3 the peptide maps produced by pronase digestion of the heart and liver OGC from beef and rat are presented. The peptide patterns of the heart OGC were very similar taking into account that the uncleaved protein from rat, and the fragments derived from it, were all smaller than the corresponding polypeptides from beef. The peptide maps of the OGC from rat and bovine liver were only partly similar. A clear difference, however, could be observed between the peptide patterns of the OGC isolated from the hearts and the livers (Fig. 3). Essentially the same conclusions were reached by comparing the peptide maps of the OGC produced by proteinase K instead of pronase (results not shown).

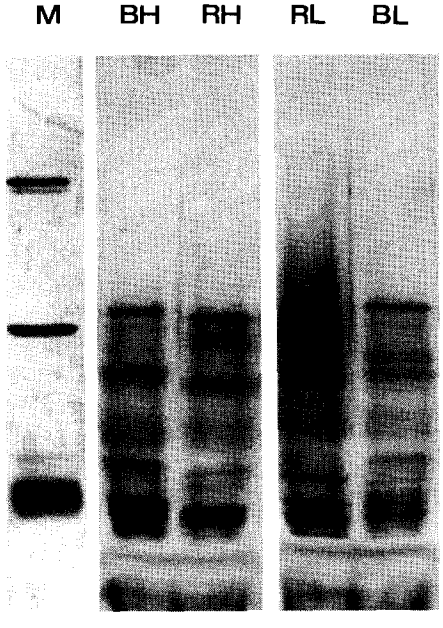

Fig. 3. Peptide maps of OGC obtained by incubating purified carrier proteins with pronase. M, markers (bovine serum albumin, carbonic anhydrase and cytochrome *c*). Other abbreviations as in Fig. 2.

4. DISCUSSION

In this study we have prepared two antisera for the immunochemical characterization of the OGC present in heart and liver of different mammals. The results indicate the existence of an organ specificity of the antigenic properties of the OGC. The anti-heart antibodies react with the OGC in heart of all animals tested but not with the OGC in liver of the same animals. This is documented by both the immunoinactivation and the immunoblot experiments (Figs. 1 and 2). The organ specificity of the OGC is further supported by the parallel experiments where the antiserum raised against the OGC purified from rat liver has been used. In this case, the antibodies react with the carrier protein in all liver samples tested, although with differing affinities, but not in heart samples even from the same species. It appears therefore that the OGC differs immunologically more in the two tissues liver and heart from the same animal than in the same tissue from different animal species. These results demonstrate that the antigenic determinants of the OGC from heart and

liver are tissue specific. The peptide patterns of the OGC from heart and liver of the same organism show indeed some differences in their primary sequence (Fig. 3).

Tissue-specific differences of mitochondrial anion-transporting systems have so far been demonstrated only for the ADP/ATP carrier by immunological studies [9] as well as cDNA sequence analysis [10–15]. It is interesting that in addition to tissue-specific also conformation-specific antibodies could be raised in rabbits against the ADP/ATP carrier [16]. Tissue-specific as well as developmental-specific isoforms were also shown for nucleus-encoded subunits of cytochrome *c* oxidase by immunological studies [17], which were verified by cDNA sequences [18].

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