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SELECTIVE TISSUE DISTRIBUTION OF CARBONIC ANHYDRASE ISOZYMES IN THE OX

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Summary

By affinity chromatography the isozymic distribution of carbonic anhydrase (carbonate hydro-lyase, EC 4.2.1.1) has been studied in extract from various bovine tissues. Carbonic anhydrase II forms isolated from erythrocyte, kidney and brain are indistinguishable by specific activity, amino acid composition, fingerprint, electrophoretic and immunological behaviour. By these criteria they differ from carbonic anhydrase I isolated from rumen epithelium.

Two types of carbonic anhydrase (carbonate hydro-lyase EC 4.2.1.1) differing markedly in their kinetic properties were recognized in the red cells of most mammals. They were designated as carbonic anhydrase B or carbonic anhydrase I for the "low activity" form and carbonic anhydrase C or carbonic anhydrase II for the "high activity" form. Only carbonic anhydrases of type II were identified from non-mammalian species and it was postulated that the "low activity" may have arisen through a duplication of the gene governing the synthesis of the "high activity" isozyme held to be the evolutionarily older molecule [1]. As yet, too little is known about carbonic anhydrases in other tissues [2] from which isolation in the purified state was complicated by contaminating erythrocytes. Many questions have arisen concerning the synthesis and the physiological significance of these two types of carbonic anhydrase. In this respect, ox appears a "good" animal. Indeed only "high activity" carbonic anhydrases were identified in bovine erythrocytes [3,4]. On the other hand two "low activity" forms were isolated from bovine rumen epithelial

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tissue [5]. We have undertaken the separation of carbonic anhydrases of "low" and "high activity" from some bovine tissues using a specific method such as affinity chromatography. The electrophoretic migration, amino acid composition, fingerprint and immunological behaviour of carbonic anhydrase I isolated from rumen and carbonic anhydrase II isolated from erythrocyte, kidney and brain are compared.

Bovine hemolysate (A) and soluble fraction of renal parenchyma (B) were prepared as previously described [6,7]. Methods for preparation of the soluble fraction from brain tissue (C) and rumen epithelium (D) were respectively identical to those for rat brain (Filippi, D., Sciaky, M., Limozin, N. and Laurent, G., unpublished) and ovine rumen epithelium (Venot-Giraud, N., Di Costanzo, J. and Laurent, G., unpublished). After concentration of these preparations ethanol/chloroform extracts (ECE) were carried out according to the procedures previously applied to human hemolysate [6]. Isolation of carbonic anhydrase I and II was performed by affinity chromatography as described by Marriq et al. [8]. Concentration of carbonic anhydrase solutions was measured by absorbance at 280 nm assuming $E^{1\%}_{280}$ values of 17.4 for carbonic anhydrase I and 19.0 for carbonic anhydrase II. Carbonic anhydrase activity was determined by the Wilbur and Anderson method [9]. Amino acid analyses were performed with a Beckman Multichrom analyzer. Cystein was obtained after performic oxydation [10]. Electrophoresis on cellulose acetate strips was carried out at pH 9.2 in 0.02 M veronal buffer. Fingerprints of tryptic peptides were obtained as previously described [11]. Immunological behaviour was investigated by the technique of Ouchterlony [12].

The elution patterns obtained by affinity chromatography of the ethanol/chloroform extracts (Fig. 1) show that the active proteins are concentrated in one peak eluted by KCNO buffer for erythrocyte, kidney and brain and by NaI buffer for rumen epithelium preparations in accordance with their specific activity (Table I). The carbonic anhydrases II from erythrocyte, kidney and brain are indistinguishable by their electrophoretic migration (Fig. 2), amino

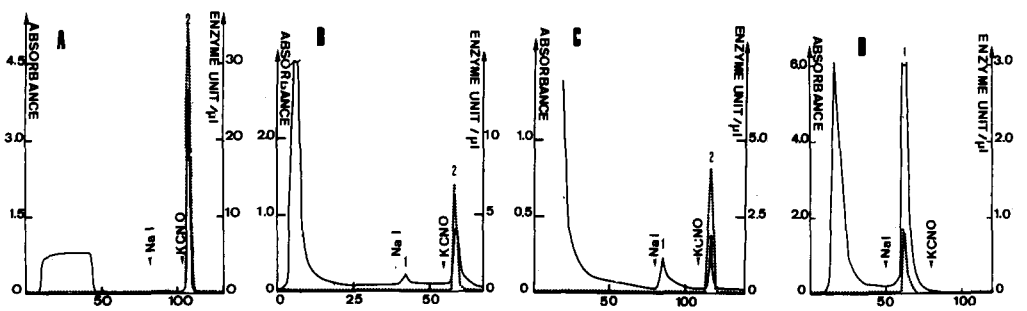

Fig. 1. Affinity chromatography on sulfanilamide "CH-Sepharose 4B". (A) 50 ml of ethanol/chloroform extract (ECE) from 25 ml of erythrocytes; (B) 32 ml of ECE from 80 g of renal parenchyma; (C) 45 ml of ECE from 200 g of brain tissue; (D) 36 ml of ECE from 60 g of rumen epithelium. The columns (0.9 X 15 cm for A, B, C and 1.6 X 20 cm for D) were equilibrated and elution started with 0.1 M Tris/sulphate, pH 7.5. Carbonic anhydrase I forms were eluted with 0.1 M Tris/sulphate/0.1 M NaI, pH 7.5 (first arrow) and carbonic anhydrase II forms with 0.1 M Tris/sulphate 0.02 M KCNO, pH 6.6 (second arrow). Abscissa: fraction number; ordinate: (———) absorbance at 280 nm, (. . . . . .) enzyme activity.

TABLE I

COMPARATIVE SPECIFIC ACTIVITY OF CARBONIC ANHYDRASES FROM SOME BOVINE TISSUES

Carbonic anhydrase	Enzyme (units/mg)
Erythrocyte	14 800
Kidney	15 000
Brain	13 600
Rumen	220

TABLE II

AMINO ACID COMPOSITION OF "HIGH ACTIVITY" AND "LOW ACTIVITY" CARBONIC ANHYDRASES (CA) FROM SOME BOVINE TISSUES

All values are numbers of residues per molecule

Amino acids	High activity carbonic anhydrase			Low activity carbonic anhydrase (Rumen)
	Red cells	Kidney	Brain	
Asp	32.1	31.2	33.0	31.6
Thr *	14.4	14.2	14.3	10.2
Ser *	16.6	17.2	17.4	26.7
Glu	24.2	22.2	25.2	23.8
Pro	18.8	18.4	18.9	19.9
Gly	20.5	20.4	20.8	19.9
Ala	17.4	16.9	17.9	23.1
Val **	20.5	19.9	20.2	15.0
Met	3.0	2.6	2.6	0
Cys	0	0	0	3.1
Ile **	5.0	4.9	5.4	9.7
Leu	26.6	26.4	26.8	26.6
Tyr	8.2	8.7	7.4	6.6
Phe	11.3	11.7	11.6	11.2
Lys	18.5	17.9	18.9	19.6
His	10.9	11.5	11.0	10.5
Arg	9.0	9.0	9.0	7.0
Trp ***	7.0	6.9	6.8	7.8

* Values extrapolated to zero time.

** Values at 144 h.

*** Evaluated by the method of Bencze and Schmid [13].

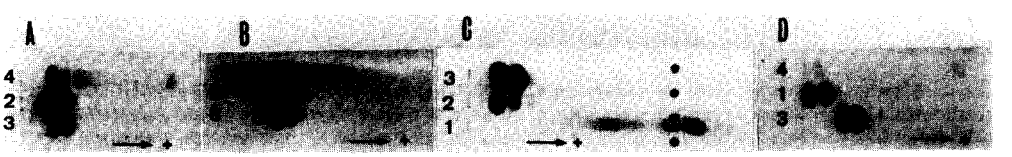

Fig. 2. Cellulose acetate electrophoresis of fractions isolated by affinity chromatography. (1) NaI fraction, (2) KCNO fraction, (3) reference mixture of erythrocyte bovine carbonic anhydrases II, (4) ethanol chloroform extract.

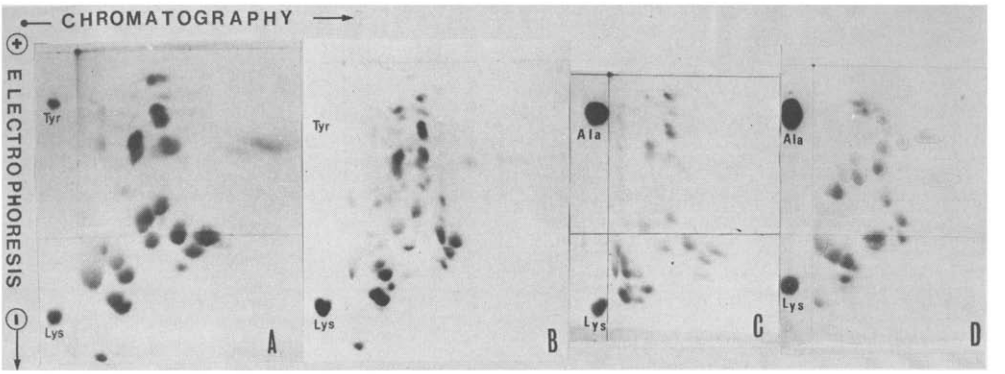

Fig. 3. Peptide maps of the tryptic digest of carbonic anhydrases isolated by affinity chromatography from erythrocyte (A), kidney (B), brain (C) and rumen (D).

acid composition (Table II) and fingerprints of their tryptic digest (Fig. 3). By all these criteria the "low activity" enzyme of rumen differs strongly from the three others.

Affinity chromatography is not an absolutely specific method for separating carbonic anhydrases in a highly purified form; other proteins can be eluted, as it appeared from peak 1 (Fig. 1, B and C). The electrophoretic pattern (Fig. 2) of Fractions 1 and 2 indicates the presence of at least two constituents in each. These represent the multiple molecular forms (genetic variants or secondarily altered forms) of the specifically eluted carbonic anhydrase I or II. On the other hand, fractions separated by methods based on electric charge difference of proteins (ion-exchange chromatography or electrophoresis) can eventually

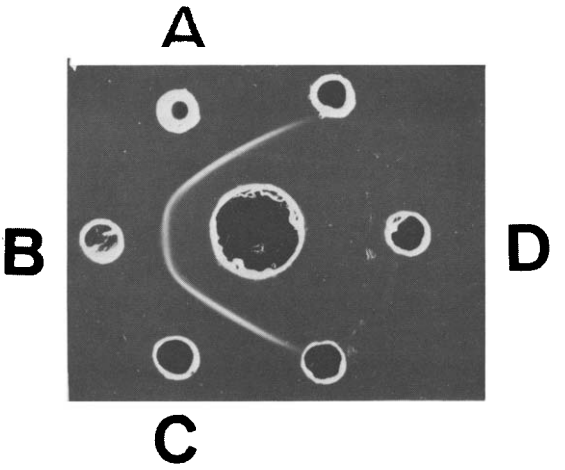

Fig. 4. Ouchterlony double diffusion analysis of bovine erythrocyte carbonic anhydrase II antibody against the carbonic anhydrases isolated by affinity chromatography. Central well: 60 μl of bovine carbonic anhydrase II rabbit antiserum; peripheral wells: 4 μg of carbonic anhydrase from erythrocyte (A), kidney (B), brain (C) and rumen (D).

contain a mixture of carbonic anhydrase forms I and II. The two types of method complement one another. They have been utilized successively to purify bovine erythrocyte carbonic anhydrase II antigen, used for rabbit antiserum preparation [14]. Ouchterlony double diffusion analysis represented in Figure 4 shows a reaction of immunochemical identity for carbonic anhydrases originating from erythrocyte, kidney and brain. No cross-reactivity was noted with carbonic anhydrase from rumen. Elsewhere, rabbit antiserum specific for carbonic anhydrase I of rumen epithelium only reacts with its antigen. It failed also to react against the non-active fraction from kidney and brain (Fig. 1, B and C) eluted by NaI buffer. From the hemoglobin content in the tissue preparations it appears that carbonic anhydrase II from blood is only a very low percentage of the one isolated by affinity chromatography.

These results obtained by specific and sensitive methods demonstrate the absence of "low activity" carbonic anhydrase in erythrocyte, kidney and brain and the absence of "high activity" enzymatic form in the rumen epithelium of the ox. Identical results have been obtained by affinity chromatography directly carried out on hemolysate or tissue supernatants.

It appears that the synthesis of carbonic anhydrase II in various tissues (erythrocyte, kidney and brain) is dependent on the same gene. In keeping with the results of others [5,15] the lack of "low activity" carbonic anhydrases in the red cells is not due to the absence of the corresponding gene in the genome but to a specific repression of this synthesis. This regulation is probably related to different physiological functions of carbonic anhydrases I and II.

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