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The carbonic anhydrases of some guinea-pig tissues

Highly purified isoenzymes of carbonic anhydrase (EC 4.2.1.1) have been obtained from the erythrocytes of several mammalian species[1], and two forms of carbonic anhydrase have recently been isolated from rat prostate in a nearly homogeneous form[2]. We report here observations on the distribution and kinetic properties of some carbonic anhydrases that we have purified from the stomach, colonic mucosa and erythrocytes of the guinea-pig. Each isoenzyme obtained from these tissues was homogeneous during ion-exchange chromatography, electrophoresis and sedimentation equilibrium runs. In addition we have identified some components of partially purified carbonic anhydrase obtained from the small-intestinal mucosa.

From haemolysates of guinea-pig erythrocytes, the enzymes were prepared using a two-step procedure: precipitation of the bulk of the haemoglobin with $(NH_4)_2SO_4$, followed by chromatography on DEAE-cellulose. From particle-free supernatants of gastric and intestinal homogenates, carbonic anhydrases were obtained using a three-step procedure: $(NH_4)_2SO_4$ precipitation, gel filtration using Sephadex G-75, and chromatography using DEAE-cellulose. Details of these methods will be described elsewhere.

In the tissues of the guinea-pig, we find two main types of carbonic anhydrase that are distinguished most clearly by their kinetic properties. Table I shows estimates of the V values of these enzymes measured by an electrometric method[3]. The V values were normalised by dividing by the molar concentrations of enzyme in the reaction mixture, $[E_0]$, which were, in turn, estimated from the $A_{280\ nm}$ of stock enzyme solutions assuming that $E^{1\%}_{1cm} = 16.0$ and that the molecular weights of the enzymes are 30 000. One type of enzyme possesses a very high $V/[E_0]$ when compared

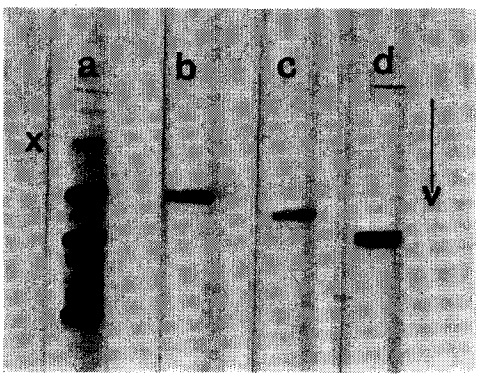
TABLE I

PROVISIONAL KINETIC DATA FOR ISOENZYMES OF GUINEA-PIG CARBONIC ANHYDRASE

Measurements are of hydration of CO_2 at 0° and pH 7.2[3].

Source of enzyme	*Isoenzyme*	$V/[E_0]$ ($sec^{-1} \times 10^{-3}$)	K_m (mM)	*Yields from 12 250-g animals (mg)*
Erythrocytes*	High activity carbonic anhydrase	152	10.3	16
	High activity carbonic anhydrase-2	104	10.2	2
	Low activity carbonic anhydrase	8	21.9	35
Stomach**	High activity carbonic anhydrase	146	9.7	4
	High activity carbonic anhydrase-2	—	7.7	0.2
	Low activity carbonic anhydrase	—	—	—
Colonic mucosa***	High activity carbonic anhydrase	150	10.1	1
	High activity carbonic anhydrase-2	—	—	—
	Low activity carbonic anhydrase	9	25.1	2

* Guinea pigs were decapitated and bled.
** The whole of the stomach from bled animals was used.
*** Obtained from bled animals.



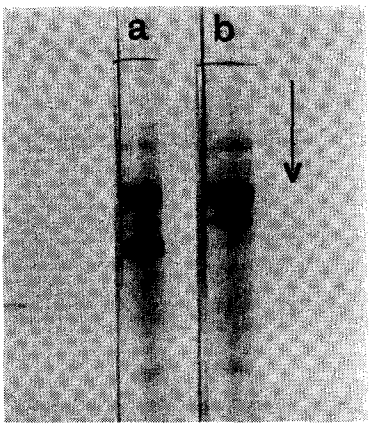


Fig. 1. Acrylamide gel electrophoresis. (a) 100 μg crude erythrocyte carbonic anhydrase; (b) 20 μg erythrocyte high activity enzyme; (c) 10 μg erythrocyte high activity carbonic anhydrase-2; (d) 40 μg erythrocyte low activity enzyme. Cathode at top of photograph; anode at bottom. Voltage gradient: 40 V/cm. Gels were run for 2 h. Current through each gel approximately 2 mA. Gels were stained in 1% naphthalene black in 5% acetic acid, for 2 h.

Fig. 2. Acrylamide gel electrophoresis. (a) 60 μg crude carbonic anhydrase from colonic mucosa; (b) 40 μg crude carbonic anhydrase from stomach. See legend to Fig. 1.

with values reported for other sorts of enzymes, while a second type of carbonic anhydrase possesses a $V/[E_0]$ which is approximately 1/20 th that of the former. These carbonic anhydrases will be referred to as "high activity" and "low activity" carbonic anhydrases, respectively. We have also obtained an isoenzyme (high activity carbonic anhydrase-2) for which values of $V/[E_0]$ are approximately 70% of "high activity" carbonic anhydrase. A fourth carbonic anhydrase has been observed during ion-exchange chromatography of erythrocyte, stomach and colonic carbonic anhydrases (labelled X in Fig. 1) but not yet obtained in sufficient quantity for detailed study.

Crude samples of carbonic anhydrase from erythrocytes, colonic and small-intestinal mucosae and stomach were analysed by acrylamide gel electrophoresis[4]. The crude erythrocyte enzyme yielded three major protein bands (Fig. 1). The band which had the lowest mobility was identified, after chromatography of the crude enzyme on DEAE-cellulose, as a "high activity" carbonic anhydrase. The band with an intermediate mobility was identified as a "low activity" carbonic anhydrase. The fastest moving band was haemoglobin.

The crude colonic enzyme showed two main bands (Fig. 2): the faster band was a "low activity" carbonic anhydrase, while the slower band was a "high activity" carbonic anhydrase. Erythrocytes and colonic mucosae yielded twice as much of the "low activity" as of the "high activity" isoenzyme. In contrast, the crude gastric enzyme showed only one major protein band (Fig. 2). After using ion-exchange chromatography of the crude gastric enzyme, this was identified as a "high activity" enzyme. Unlike colonic mucosa and erythrocytes, gastric tissue yielded a negligibly small amount of "low activity" carbonic anhydrase.

In accordance with their chromatographic behaviour, the "high activity" carbonic anhydrases from erythrocytes, colonic mucosa and stomach were electro-

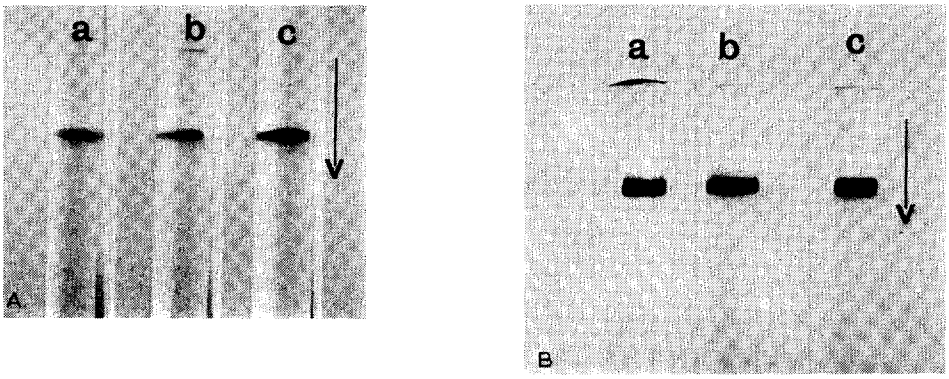


Fig. 3. Acrylamide gel electrophoresis. A. (a) 10 μg stomach high activity carbonic anhydrase; (b) 10 μg colonic high activity enzyme; (c) 10 μg stomach high activity enzyme *plus* 10 μg colonic high activity enzyme. B. (a) 30 μg colonic low activity enzyme; (b) 30 μg erythrocyte low activity enzyme; (c) 15 μg colonic low activity enzyme *plus* 15 μg erythrocyte low activity enzyme. See legend to Fig. 1.

phoretically indistinguishable, as were the "low activity" carbonic anhydrases of the colonic mucosa and erythrocytes (Fig. 3).

Crude carbonic anhydrase obtained from mucosal tissue taken from the entire length of the small intestine below the ligament of Treitz, exhibited a prominent "high activity" band and a much less prominent "low activity" band. The isoenzyme (labelled X in Fig. 1) which has been found in very small quantities in the stomach, colon and erythrocytes, was also present in the small-intestinal mucosa in an amount comparable with that of "high activity" carbonic anhydrase.

From data obtained from sedimentation equilibrium runs, it appears that each isoenzyme, the low and high activity enzymes and high activity carbonic anhydrase-2 (from whatever tissue source) has a molecular weight of about 30 000. Using isoelectric focussing[5] (LKB Instruments Ltd.), we found that the isoelectric pH's of the "high activity" and "low activity" colonic enzymes differed by more than 2 pH units, being pH 5.2 and pH 7.4, respectively. Amino acid analyses indicate that all the guinea-pig carbonic anhydrases possess a high proportion of proline residues, that all "low activity" carbonic anhydrases contain approximately 1.5 times as many serine residues as the "high activity" enzymes (*cf.* ref. 6), and that both "high activity" and "low activity" carbonic anhydrase molecules contain a single half-cystine residue.

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