ESTERASE AND HYDRASE ACTIVITY OF CARBONIC ANHYDRASE-I FROM PRIMATE ERYTHROCYTES*

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Carboxylic esterase activity toward the acetate and butyrate esters of α- and β-naphthol is associated with two forms of erythrocyte carbonic anhydrase (CA-I, CA-II) in man (Tashian et al., 1963) and other primate species. Of 22 primate species tested, erythrocyte CA-I from the rhesus macaque (Macaca mulatta) and doguera baboon (Papio doguera) were among those forms showing high specific esterase activity; in comparison, human and chimpanzee (Pan troglodytes) CA-I exhibit moderate esterase activity. This report compares the hydrolase and hydrase activities of partially-purified CA-I from human, chimpanzee, baboon, and rhesus hemolysates.

METHODS

Isolation and purification of CA-I. Hemolysates were prepared by adding one volume of distilled water to one volume of washed, packed cells; and purification of CA-I was initiated by chloroform-ethanol extraction (Waygood, 1955). The hemoglobin-free fractions containing CA-I were purified further by passage through columns of diethylaminoethyl (DEAE) cellulose and carboxymethyl (CM) cellulose equilibrated with 10^{-3} M phosphate buffer, pH 7.0.

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Purification data for human CA-I are presented in Table I. Esterase activity other than that associated with the CA forms in the chloroform-ethanol, DEAE, and CM fractions was corrected for by subtracting the esterase activity (22%, 6%, and 0% respectively) not inhibited by 10^{-4} M acetazolamide. By starch-gel electrophoresis and staining procedures (see legend, Fig. 1), CA-II was found in the DEAE-cellulose fractions, but not in those treated with CM-cellulose. Therefore, the actual specific esterase and hydrase activity of CA-I in the first two fractions would be lower than the values in Table I. The degree of reduction would depend on the findings that human CA-II has higher specific hydrase activity than CA-I (see discussion), and that human hemolysates contain about 5 times as much CA-I as CA-II (Gibbons and Edsall, 1963).

TABLE I

Partial Purification of Carbonic Anhydrase-I from Human Erythrocytes

Fraction	Protein[1]	Specific esterase activity[2]	Specific hydrase activity
	mg	unit/mg protein	unit/mg protein
Chloroform-ethanol extract	2.8	730	750
DEAE-cellulose	2.3	950	1010
CM-cellulose	1.9	1110	1060

[1]From 1 ml of packed cells

[2]See Figs. 2 and 3 for definition of activity units

Protein concentration was estimated by the method of Lowry et al. (1951) standardized with bovine serum albumin.

Enzyme assays. Hydrase activity was estimated by a modification of the CO_2-veronal-bromthymol blue method of Mattenheimer and deBruin (1962).

Carboxylic ester hydrolase activity was estimated by incubating the enzyme in a mixture (final volume: 0.4 ml) containing 10^{-3} M β-naphthyl acetate and 75 μg Blue RR (double zinc salt of 4'-amino-2',5'-dimethoxybenzanilide) in 0.2 M tris-HCl buffer, pH 7.0. The mixtures were incubated for 45 min. at 37°; the pink pigment was then extracted into 1.6 ml of chloroform and read photometrically at 535 mμ. Standards of β-naphthol were run under the above conditions, and hydrolysis of β-naphthyl acetate followed by an estimation of liberated β-naphthol.

RESULTS AND DISCUSSION

Starch-gel electrophoresis of the lyophilized CA-I samples showed only the presence of esterase-staining components which could be inhibited by acetazolamide. At pH 8.5, the major component of human and chimpanzee CA-I migrates slowly anodally and that of rhesus and baboon CA-I, slowly cathodally (Fig. 1). The electrophoretically distinct minor components (-1 and +1 to +3) which appear in concentrated samples may represent preparation artifacts of the major component.

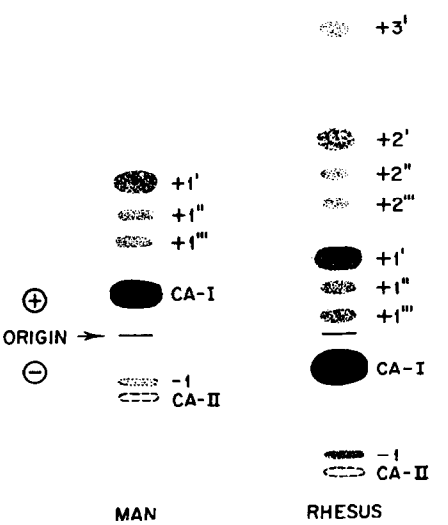

Figure 1. Diagram of esterase stain patterns of major and minor components of partially-purified erythrocyte CA-I after starch-gel electrophoresis in borate/NaOH buffer (gel: pH 8.5, 0.02 M; bridge: pH 8.5, 0.3 M), voltage 7 v/cm for 17 hr at 4°. Protein concentration: 6 mg/ml. Staining procedure as described in Tashian and Shaw (1962). Degree of staining approximates stippling density. Several other minor cathodal components and CA-II are removed during CM-cellulose treatment; the positions of CA-II are included for reference only. Distance from origin to +3 = 10 cm.

The esterase and hydrase activities of CA-I from man, chimpanzee, baboon and rhesus are compared in Figs. 2 and 3. As shown, the esterase activity is higher in rhesus and baboon than in man and chimpanzee, whereas the reverse is true for the hydrase activities. Both hydrase and esterase activities are completely inhibited by the addition of 10^{-4} M acetazolamide; no other hemolysate esterase was observed to be inhibited by acetazolamide.

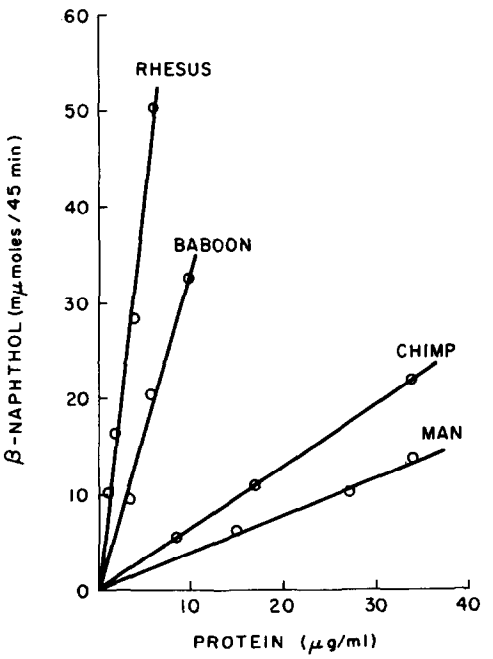

Fig. 2

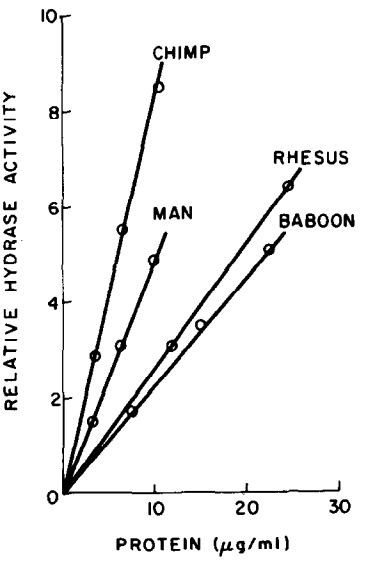

Fig. 3

Figure 2. Formation of β-naphthol from β-naphthyl acetate as a function of the concentration of erythrocyte CA-I.

Figure 3. Rate of enzymatic hydration of CO_2 as a function of concentration of erythrocyte CA-I. Reaction medium in ice bath contains: 1.0 ml 0.02 M veronal buffer, pH 8.0; 0.7 ml 0.01% peptone in H_2O; 0.3 ml 0.2% bromthymol blue in 50% ethanol; and 25 μl enzyme solution. At zero time, 1.0 ml CO_2-saturated H_2O was added. Enzyme activity unit = t_o-t/t-1, where t = catalyzed reaction time and t_o = uncatalyzed reaction time; average t_o = 86 secs.

After starch-gel electrophoresis, CA-I from man and rhesus showed the following order of staining intensity when visualized with Blue RR

salt and naphthol ester substrates: β-naphthyl acetate > α-naphthyl acetate > β-naphthyl butyrate > α-naphthyl butyrate. Apparently the β position and shorter carbon side chain are more readily hydrolyzed by the enzyme.

The CA-I and CA-II forms of human red cell carbonic anhydrase appear to correspond to Fractions I and II reported by Rickli and Edsall (1962); CA-III and CA-V reported by Nyman (1961); and the X_1 and Y proteins of Derrien et al. (1961) and Laurent et al. (1962). These investigators also observed that the component comparable to CA-II has greater specific hydrase activity than the component comparable to CA-I.

The results presented here indicate that the red cell carbonic anhydrase active site in some mammalian species may also hydrolyze carboxylic ester linkages. The recent findings of genetically-determined variants of CA-I in man (Shaw et al., 1962; Tashian et al., 1963) with increased specific esterase activity, suggest that some alteration at or near the active center of the enzyme may have occurred. Whether carbonic anhydrase activity of these variant forms is also altered remains to be determined. Another zinc metalloenzyme, carboxypeptidase A, has been demonstrated to act as both a peptidase and esterase with distinctly different mechanisms indicated (see Vallee et al., 1963).

Studies on the esterase activities of CA-I among various primate species, demonstrated to be homologous by immunochemical analysis, have shown considerable intra- and interspecies variation both in electrophoretic behavior and esterase activity. Comparative studies of the protein structure and enzyme activity of CA-I from other primate species are now in progress.

The carbonic anhydrase of other tissues may not be the same as that found in red cells. Although hydrase activity, and its inhibition by acetazolamide, could be readily demonstrated in human kidney extracts; no component separable by starch-gel electrophoresis and having esterase activity was observed to be inhibited by acetazolamide.

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