Inactive Form of Erythrocyte Carbonic Anhydrase B in Patients with Primary Renal Tubular Acidosis

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ABSTRACT Evidence was found for an inactive form of carbonic anhydrase type B in the erythrocytes of two children with primary renal tubular acidosis. The addition of zinc chloride to hemolysates from these patients resulted in a marked increase in the activity of this enzyme. No such effect was noted with hemolysates of control subjects. No significant differences were observed in the zinc levels of hemolysates of these patients and of normal individuals. However, the level of zinc in the carbonic anhydrase B isolated from one of these patients was low, suggesting a modified form of the enzyme. The restoration of activity upon the addition of zinc was reversed by ethylenediamine tetraacetate, but no such effects were noted for the carbonic anhydrase B of normal individuals. Thus the abnormal carbonic anhydrase B has decreased zinc binding.

The ultraviolet difference spectrum of the carbonic anhydrase B of normal individuals and that of a patient showed a peak at 305 nm which decreased upon the addition of zinc. The abnormal form of carbonic anhydrase B was not distinguishable from that of normal individuals by either immunological or electrophoretic criteria.

INTRODUCTION

Carbonic anhydrases (EC 4.2.1.1.) which catalyze the reversible hydration of CO_2 (1) play an important role in CO_2 gas transport and acid/base equilibrium. These enzymes have zinc in their active centers.

There are two major carbonic anhydrase isozymes designated as the B (CA-B)[1] and C (CA-C) types. They show no immunological cross reactions (2) and their synthesis is controlled at different genetic loci (3, 4). A quantitative immunochemical estimation of these isozymes was established by Funakoshi and Deutsch (2), and it has been reported that although the level of CA-B in human erythrocytes varies considerably under certain pathological or physiological conditions (5–11), no significant changes occur in the levels of CA-C. These immunological measurements reflect the total amount of the active and inactive enzyme protein in human erythrocytes. We have previously compared the results of a kinetic estimation of the CA-B-dependent esterase activity by an immunoadsorbent method with an immunochemical estimation of the levels of CA-B and simultaneously determined the specific activity (units per milligram of CA-B) of this enzyme (6). In the present study we present evidence for the presence of an inactive form of erythrocyte CA-B in patients with renal tubular acidosis (RTA) with the above described enzymic and immunologic techniques.

METHODS

Case reports. The properties of the erythrocyte carbonic anhydrases of two children with metabolic acidosis from unrelated Japanese families were investigated. Some general data on their blood electrolytes and the urea levels of these hyperchloremic acidotic patients are presented in Table I. Some pertinent clinical data on these children follow.

Case 1. The patient was a 3-year-old male with growth

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[1] *Abbreviations used in this paper*: apo-CA-B, apocarbonic anhydrase B type; CA-B, carbonic anhydrase B type; CA-C, carbonic anhydrase C type; RTA, renal tubular acidosis.

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TABLE I
Blood Data of Two Patients with Primary RTA

	Case 1	Case 2
Sodium, *meq/liter*	139.0	145.0
Potassium, *meq/liter*	5.3	3.5
Chloride, *meq/liter*	118.0	120.0
Calcium, *mg/dl*	4.5	6.7
Phosphorus, *mg/dl*	8.0	6.5
pH	7.23	7.22
Bicarbonate, *meq/liter*	11.8	9.5
P_{CO_2}, *mm/Hg*	21.4	24.0
Urea nitrogen, *mg/dl*	22.0	15.5

retardation in which severe hyperchloremic acidosis was noted 17 days after birth. The following studies were performed at 2 yr of age. There was no glucosuria or aminoaciduria present in urine and creatinine clearance was 126 ml/min per 1.73 m^2 body surface. Tubular reabsorption of phosphate was 99% and the maximum concentration ability test was recorded at the specific gravity of 1,012. After ammonium chloride was given orally in a dose of 0.1 g/kg body weight (12), a fixed high urinary pH (above 7.38) was observed when serum bicarbonate concentration was reduced to 9.8 meq/liter, suggesting primary RTA (13). No nephrocalcinosis was noted on roentgen analysis.

Case 2. The patient was a 3-yr-old male with growth retardation in which metabolic acidosis was noted at 3 mo of age. The following data were obtained at 2 yr of age. The patient evidenced hyperchloremic acidosis. His urine showed no sign of glucosuria or aminoaciduria, and his normal glomerular filtration rate was 93.0 ml/min per 1.73 m^2 body surface. Phenolsulfonphthalein was 45% at 15 min, maximum concentration ability test was 1,014, and there was no nephrocalcinosis. An ammonium chloride loading test was not performed because of parental objection. The alkaline urine (pH 7.21–7.37) and the serum bicarbonate levels (9.52–12.0 meq/liter) suggested a diagnosis of primary RTA (13).

To control acidosis these two children were treated with a base supplement of 4 meq/kg per day, consisting of sodium bicarbonate and buffered citrate solution for 2 yr.

Subjects. The erythrocytes of these patients and their families, along with those of 25 healthy children (2–14 yr of age) and 10 healthy adults (20–40 yr of age), were utilized as controls.

Preparation of blood samples. Heparinized, fresh, venous blood was centrifuged at low speed to remove the erythrocytes, and the packed cells were washed two times with 10 vol of 0.9% saline by centrifugation. 2 vol of deionized water were then added to the packed cells and the material was frozen (−20°C) and thawed (37°C) to effect hemolysis. The stroma was removed by centrifugation at 10,000 *g* for 30 min. The hemoglobin content of each hemolysate was measured by the cyanmethemoglobin method (14) and then adjusted to ≅3% for use in the enzymic and immunological assays.

Determination of the levels of CA-B and CA-C. CA-B and CA-C isozymes were assayed according to a single radial immunodiffusion technique (15) with a slight modification of the method described by Funakoshi and Deutsch (2). The CA-B and CA-C standards employed were purified by methods previously reported (16). Specific antisera to CA-B and CA-C were obtained by immunization of individual horses, with antigens being employed as in previous studies (17). The levels of CA-B and CA-C are expressed as milligrams of enzyme per grams of hemoglobin.

Assay of enzyme activity. The esterase activity of the carbonic anhydrase was measured by a slight modification of Armstrong et al. (18) with 1 mM *p*-nitrophenyl acetate as substrate. A unit of enzyme activity releases 1 μmol of *p*-nitrophenol per min at 25°C.

The specific activity of CA-B and CA-C. A modification of the immunoadsorbent method of Axen et al. (19) was employed to determine the specific activity of CA-B and CA-C as described previously (6). Affinity columns of Sepharose 4-B (Pharmacia Fine Chemicals, Piscataway, N. J.) coupled with anti-human CA-B horse IgG and with anti-human CA-C IgG were utilized for the determination of the specific activity of these isozymes. This was expressed as units per milligram of isozyme.

Experiment of Zn^{2+} addition. Different levels of $ZnCl_2$ in 0.45% bovine serum albumin, 4.5 mM Tris-HCl buffer, pH 7.4, were incubated at 37°C for several hours containing 5 μM, purified or crude enzyme. Absorbance measurements and determinations of enzyme activity were made with a Hitachi model 624 spectrophotometer (Hitachi, Ltd., Co., Tokyo, Japan).

Apocarbonic anhydrase B (apo-CA-B). Apo-CA-B was obtained from purified holo-CA-B of normal individuals as described by Lindskog and Nyman (16) with *o*-phenanthroline.

Determination of zinc concentration. The concentrations of zinc in hemolysates were determined with Hitachi model 518 atomic absorption spectrophotometer, with the dithizone method described by Malström (20).

Electrophoresis. Vertical starch gel electrophoresis was carried out in Tris-EDTA-borate pH 8.6 buffer according to the method employed by Deutsch et al. (17). A potential gradient of 6–8 V/cm was applied over a period of 16 h.

Purification of CA-B. CA-B was isolated from a patient with RTA and from a normal control subject according to the method described previously (6). 1 ml of packed blood cells was employed as starting materials. The purity of the protein isolated was checked by sodium dodecyl sulfate gel electrophoresis according to the method of Weber and Osborn (21).

Chemicals. *p*-Nitrophenyl acetate was obtained from the Sigma Chemical Co., St. Louis, Mo., and DEAE-cellulose was obtained from the Green Cross Corp., Osaka, Japan. Sepharose 4-B was obtained from Pharmacia Fine Chemicals, Inc., as previously mentioned. All reagents used were of analytical grade.

RESULTS

The level and specific activity of CA-B and CA-C in patients with primary RTA. The level of CA-B and CA-C, the total esterase activity, CA-B and CA-C-dependent esterase activity, and the specific activity of the isozymes in the erythrocytes of patients and of control subjects are summarized in Table II. In case 1 the level of CA-B was in the upper range of normal (13.4 mg/g Hb) but its specific activity was low (0.29 U/mg) compared to normal subjects (0.51 U/mg). In case 2 the level of CA-B was within normal limits (10.1 mg/g Hb) but its specific activity was significantly lower (0.15 U/mg). Neither the level nor the specific activity of CA-C in these patients was different from those of normal subjects.

TABLE II

The Level and Specific Activity of CA-B and CA-C in Erythrocytes

Subject	No. of cases	Immunological level		Esterase activity			Specific activity	
				Total activity	Dependent activity			
		CA-B	CA-C		CA-B	CA-C	CA-B	CA-C
		mg/g Hb		*U/g Hb*			*U/mg isozyme*	
Case 1	1	13.4	1.40	9.9	3.9	6.0	0.29	4.29
Case 2	1	10.1	1.33	5.7	1.5	4.2	0.15	3.16
Case 1								
Father	1	16.4	2.25	16.4	9.3	7.1	0.57	3.16
Mother	1	16.0	1.79	16.5	8.5	8.0	0.53	4.47
Case 2								
Father	1	9.0	1.43	11.2	6.0	5.2	0.67	3.64
Mother	1	9.8	1.67	10.2	3.9	6.3	0.40	3.77
Normal								
Children	25	10.0* ±0.39	1.72* ±0.09	10.5* ±0.36	5.1* ±0.36	5.4* ±0.17	0.51* ±0.04	3.14* ±0.22
Adult	10	13.5* ±0.78	1.76* ±0.06	14.8* ±0.54	8.9* ±0.67	5.9* ±0.35	0.66* ±0.05	3.35* ±0.19

* Values represented are expressed as mean±SE.

The results of family studies of these patients shown in Table II show that the level and the specific activity of CA-B for the patients in case 1 are normal. Slightly low levels of CA-B was noted for both parents in case 2, and a low specific activity was noted for the mother of case 2.

Two possible reasons could account for the low specific activity of CA-B. One might relate to a structural modification which would reduce its catalytic activity without affecting cross-reactivity with antibody; the other could be explained by the presence of an inhibitor. To investigate these possibilities the following experiments were performed.

The concentration of zinc in erythrocytes of patients. The zinc content of the erythrocytes in case 1 was 34.2 μg/g Hb, and 40 μg/g Hb in case 2. The levels in normal children ranging from 26.6 to 41.8 μg/g Hb with a mean±SE of 32.1±0.93 μg/g Hb were relatively low compared to the 34.5–50.3 μg/g Hb with a mean±SE of 41.5±0.50 μg/g Hb values noted for adults. No differences were observed in the zinc content of the erythrocytes of the two patients and those of normal children, and the zinc content for the parents of these patients were within a normal level.

Effect of Zn^{2+} on the specific activity of carbonic anhydrase in hemolysate from patients. Increases in the esterase activities after the addition of Zn^{2+} were observed in hemolysate from patients with RTA. On the other hand, the addition of Zn^{2+} had no effect on the hemolysates of control subjects or those of parents of these patients. Table III shows the effect of the addition of Zn^{2+} on the specific activity of the isozymes. The increase in the total esterase activity was directly attributed to increases in the activity of CA-B. The specific activity of CA-B obtained after the addition of Zn^{2+} in these cases were fairly close to those in normal individuals. These facts suggest that about 41 and 62% of CA-B were present in an inactive form in the hemolysates of cases 1 and 2, respectively, and that the rest of CA-B was normal active form. The inactive CA-B was considered to be converted to active form by the addition of Zn^{2+}.

Effect of EDTA on inactive CA-B. The activity restored by the addition of Zn^{2+} was readily lost by freezing and thawing. This suggests the possibility that the binding of Zn^{2+} to the inactive enzyme was weak. The effect of EDTA was studied on the inactive form of CA-B by adding to the incubation mixture various concentrations of EDTA and then incubating for 30 min at 37°C before assay. As shown in Table IV the increase in activity resulting from the addition of Zn^{2+} was reversed by the addition of 50 mM EDTA. Levels of EDTA of 0.25 M or higher were necessary to lower carbonic anhydrase activities in the hemolysates of patients and of normal subjects in which no Zn^{2+} had been added. To measure the relationship between the active and inactive form of CA-B, the reversibility of the inactive form to active form was also

TABLE III

Effect of Zn^{2+} Addition on the Specific Activity of CA-B and CA-C

Subject	No. of cases	Added Zn^{2+}	Total activity	Specific activity			Inactive CA-B
				CA-B	CA-C	Inactive CA-B*	
			U/g Hb		*U/mg isozyme*		*% of total*
Case 1		—	9.9	0.29	4.29	0.20	41
		+	12.6	0.49	4.29		
Case 2		—	5.7	0.15	3.16	0.24	62
		+	8.1	0.39	3.16		
Case 1							
Father		—	16.4	0.57	3.16	0	0
		+	16.4	0.57	3.16		
Mother		—	16.5	0.53	4.47	0	0
		+	16.5	0.53	4.47		
Case 2							
Father		—	11.2	0.67	3.64	0	0
		+	11.2	0.67	3.64		
Mother		—	10.2	0.40	3.77	0	0
		+	10.2	0.40	3.77		
Normal children	25	—	10.5±0.36‡	0.51±0.04‡	3.14±0.22‡	0	0
		+	10.5±0.36	0.51±0.04	3.14±0.22		
Normal adult	10	—	14.8±0.54‡	0.66±0.05‡	3.35±0.19‡	0	0
		+	14.8±0.54	0.66±0.05	3.35±0.19		

The incubation mixture was composed of hemolysate containing 50 μM CA-B, 0.45% bovine serum albumine, pH 7.4, 4.5 mM Tris-HCl buffer, 100 μM $ZnCl_2$ in a final volume of 1 ml. The incubation was carried out for 6 h at 37°C before assay.

* The specific activity of inactive CA-B was expressed as the difference in the specific activity before and after the Zn^{2+} addition.

‡ Values are expressed as mean±SE.

investigated. The assay mixture, preincubated with Zn^{2+}, in these cases was treated with 50 mM EDTA and dialysed against 1,000 vol of 10 mM Tris-HCl, pH 7.4, to remove EDTA, and Zn^{2+} was added again. The activity was completely restored thus indicating that the inactive form of CA-B has weak Zn^{2+} binding.

Effect of the patient hemolysate on the specific activity of normal control hemolysate. To determine if there is any factor in the hemolysates of patients that inactivates CA-B, the hemolysate from a normal control was mixed with an equal volume of hemolysate of a patient. This mixture was incubated for 6 h at 37°C and the activity was assayed. Failure to modify the specific activity of CA-B indicated that hemolysates of these patients do not inactivate CA-B. The addition of Zn^{2+} to the mixed hemolysate results in an increase of 17% in the specific activity of CA-B. This enhanced activity would appear to relate to the activation of the CA-B in the patient's hemolysate.

Starch gel electrophoresis of hemolysate from patients. Fig. 1 shows the starch gel electrophoretic pattern of the carbonic anhydrase in hemolysates of the two patients and of a normal control. The electrophoretic properties of the samples appear to be identical.

Purification of CA-B from erythrocyte of a patient.

TABLE IV

Effect of EDTA on the Esterase Activity of Hemolysates

Subject	Zn^{2+} Addition	EDTA concentration*		
		0	50	250
Case 1	—	9.9	9.9	6.9
	+	12.6	9.9	6.9
Case 2	—	5.7	5.7	4.0
	+	8.1	5.7	4.0
Normal children	—	10.2	10.2	7.1
	+	10.2	10.2	7.1

Values are expressed as the esterase activity in units per gram of hemoglobin. Each value represents the average of duplicate analyses.

* The EDTA concentrations are expressed in millimoles.

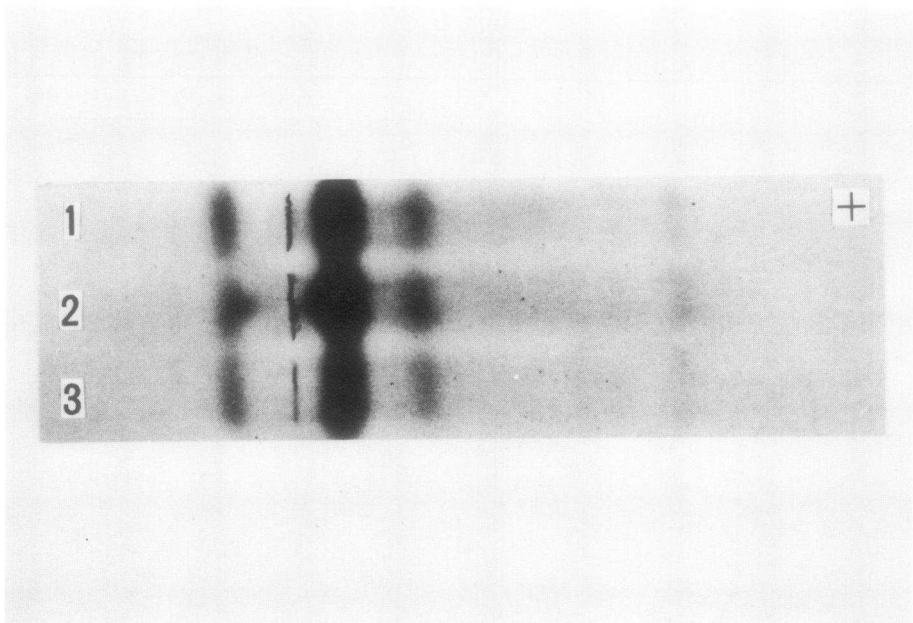

FIGURE 1 Starch gel electrophoresis of ethanol-chloroform extracts in patients with RTA. 1, case 1; 2, a normal control; 3, case 2.

CA-B was isolated from 1 ml of erythrocytes of patient 1, in a yield of 30% (980 μg) as described in Methods. An isoelectric focusing experiment showed a component with the identical isoelectric point as normal CA-B, in a pH 5.85. Sodium dodecyl sulfate gel electrophoresis showed a single component with the mobility of CA-B from a normal control (Fig. 2).

The activity and the amount of zinc in the purified CA-B are shown in Table V. The specific activity of the CA-B from case 1 was 0.27 U/mg as compared with 0.62 U/mg for a control. The zinc level was 1.12 μg/mg CA-B in case 1 and 2.34 μg/mg in the control. These results show the presence of 0.51-g atoms Zn per molecule enzyme in case 1 and 1.07 in the control. The specific activity:zinc ratio was almost the same in each case which suggests that the low specific activity of CA-B in case 1 is a result of the absence of zinc in this isozyme. This is in agreement with an immunologically active but enzymatically inactive form of CA-B in the patients.

Effect of metals on the activity of purified CA-B from erythrocyte of a patient. The purified CA-B (5 μM) from case 1 was incubated with various metal ions (10 μM) for 6 h at 37°C, and the changes in activity were determined. The addition of Zn^{2+} resulted in a marked increase in the specific activity from 0.27 to 0.44 U/mg CA-B. Activation by Co^{2+} and Mn^{2+} but not by Cu^{2+} was noted as shown in Table VI. The same effect of these metals was observed with apo-CA-B prepared from a normal control.

The ultraviolet difference spectrum of CA-B from a patient. The ultraviolet difference spectrum of the purified CA-B of a patient and a control had a peak at 305 nm as shown in Fig. 3. After incubation of the purified CA-B from case 1 with Zn^{2+} for 6 h at 37°C, the absorbance at 305 nm decreased by 50%. This returned to its original level after treatment with 50 mM EDTA.

Kinetic study of the Zn^{2+} binding to inactive CA-B. The activation of the enzyme by Zn^{2+} was reached within 6 h as shown in Fig. 4. The effect of various levels of Zn^{2+} on the restoration of the esterase activities of the hemolysate and the purified CA-B from case 1 is shown in Fig. 5. More Zn^{2+} was required for maximum activation of the hemolysate than was required for the purified CA-B from the same patient. The crude hemolysate required more than an equimolar concentration of Zn^{2+} to obtain the maximum levels of the activation, whereas the isolated CA-B from case 1 (5 μM) required only 2 μM Zn^{2+}. As mentioned earlier, in case 1, 41% of CA-B was present as an inactive form, therefore the Zn^{2+} concentration (2 μM) required to obtain the maximum level of activation was considered to be that required for the inactive form. It is possible that some of the added Zn^{2+} was bound to other proteins in hemolysates. This raises the question why the addition of Zn^{2+} activated CA-B, because the hemolysates of the patients have the same level of zinc as normal children. To determine the level of protein-bound zinc, 0.3 ml of hemolysate of patients and of normal controls was extensively

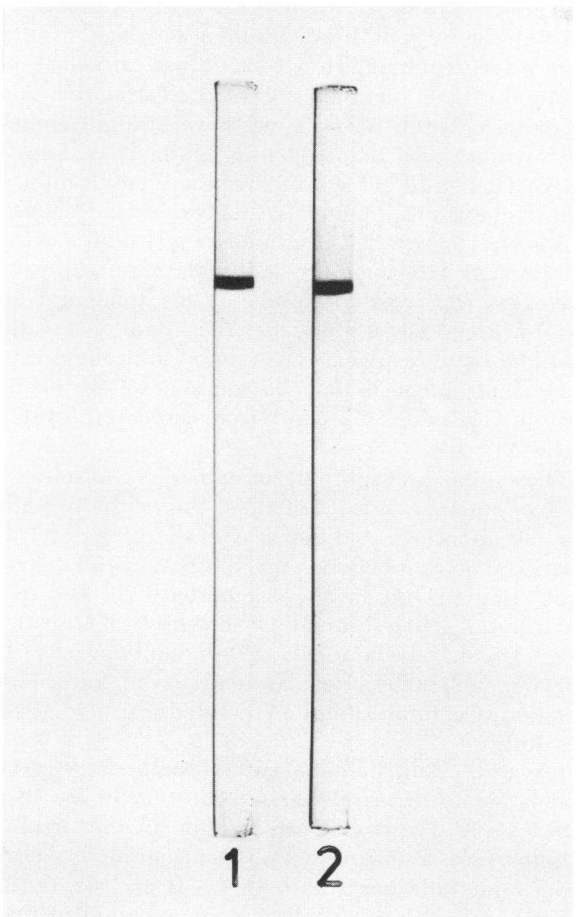

FIGURE 2 Sodium dodecyl sulfate gel electrophoresis of purified CA-B. 1, case 1; 2, a normal control.

dialyzed against 30 vol of distilled water for 72 h. Almost 97% of the zinc in both source hemolysates was nondializable.

DISCUSSION

The presence of a mutant form of CA-B which is immunologically active but enzymatically inactive was demonstrated in two patients with primary RTA.

TABLE V
The Specific Activity and Zinc Content of Purified CA-B

Subject	Specific activity	Zinc	Specific activity/ zinc	Zinc/CA-B
	U/mg CA-B	*μg/mg CA-B*	*U/mg*	*g atoms/30,000 g CA-B*
Case 1	0.27	1.12	0.24	0.51
Control	0.62	2.34	0.26	1.07

The mol wt of CA-B used was 30,000 (1).

TABLE VI
Specificity of Metal Ions

Metal ion	Relative specific activity	
	o-Phenanthroline-treated CA-B (normal source)	Purified CA-B (case 1)
Zn^{2+}	100.0	100.0
Co^{2+}	74.5	74.8
Mn^{2+}	25.0	24.8
Cu^{2+}	0	0

Values are expressed as the percent of the activity observed in the presence of Zn^{2+}.

Schapira et al. reported previously the presence of a mutant form of CA-B in a family with RTA (22). The purified CA-B from this patient was unstable in 8 M urea or high temperature (56°C). In our cases the stability of the enzyme against urea or temperature was normal (data not shown).

A study of a variant form of human CA-B has also been reported by Tashian and Carter (23). They found that its esterase activity increased with the addition of Zn^{2+}. This variant CA-B, however, was found to contain one zinc atom per molecule of enzyme. The CA-B in our cases thus appears to be a novel type of a mutant CA-B.

Funakoshi and Deutsch have reported that about 83% of erythrocyte zinc is bound to CA-B (2). Our studies of CA-B isolated from normal individuals showed it to retain 1 g atom of zinc per molecule and accounts for ≅83% of the zinc of the hemolysate. However, 40–60% of the zinc in the hemolysates of RTA patients was bound to CA-B even though the level of zinc was within normal range. Our dialysis experiment indicated that considerable zinc must be bound to proteins other than CA-B.

It has been reported that substitution of metals for zinc modifies the activity of CA-B (16). In our study, metal ions such as Co^{2+} or Mn^{2+} also activated the

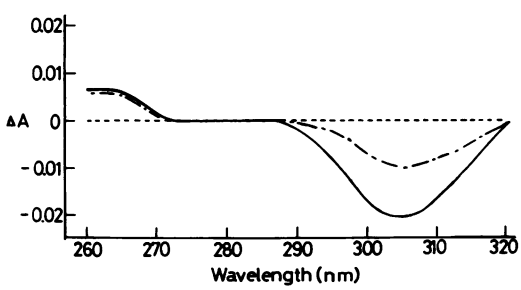

FIGURE 3 Ultraviolet difference spectra of CA-B solutions (2 μM in 0.005 M Tris-HCl buffer, pH 7.4) were measured in 1-cm cells at 25°C. The solid curve represents purified normal CA-B vs. purified CA-B from case 1. The dashed curve represents normal CA-B vs. CA-B from case 1 to which Zn^{2+} had been added.

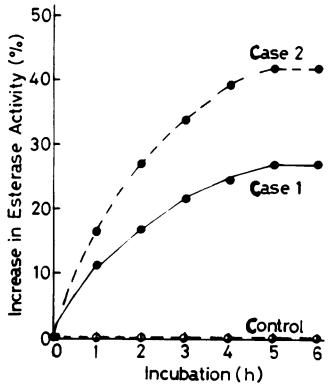

FIGURE 4 Effect of incubation with Zn^{2+} on the esterase activity of hemolysates from patients with RTA. An amount of hemolysate containing 5 μM CA-B was incubated with 0.45% bovine serum albumin and 10 μM $ZnCl_2$ in pH 7.4, 4.5 mM Tris-HCl buffer, in a final volume of 1 ml. Maximum activity was expressed as a percent of initial activity. The initial activity was 9.9 U/g Hb in case 1, 5.7 U/g Hb in case 2, and 10.5 U/g Hb in a control, respectively. Incubation was performed at 37°C.

variant form of CA-B as well as apo-CA-B prepared from normal blood (Table IV). It is unlikely that Zn^{2+} is substituted by other metals in vivo, because 50 mM EDTA did not influence the initial activity and other data indicate that the aberrant CA-B is a zinc-free form.

Our observations suggest that erythrocyte CA-B exist in two distinct forms in these patients with RTA: (*a*) normal native holo-form, which has one atom zinc per one molecule enzyme, and (b) inactive form which is

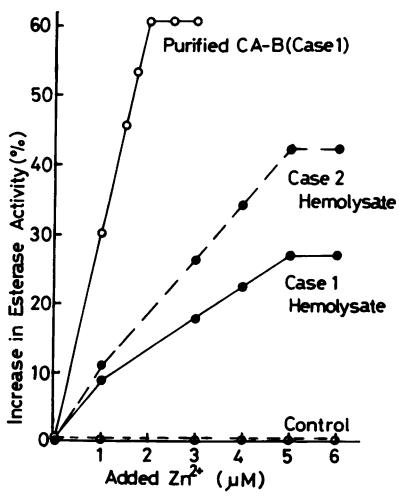

FIGURE 5 Effect of various concentrations of Zn^{2+} on the esterase activities of the hemolysates of RTA patients, of normals, and of purified CA-B from case 1. The mixtures incubated contained 5 μM CA-B and 0.45% bovine serum albumin in pH 7.4, 4.5 mM Tris-HCl buffer, and $ZnCl_2$ to 6 μM, final volume 1 ml. Samples were incubated for 6 h at 37°C. Activity was expressed as a percent of the initial activity as shown in Fig. 4.

activated upon the addition of Zn^{2+}. A modification of CA-B in the two children with RTA may have resulted from prior treatment. However, only bicarbonate and citrate had been given for 2 yr before taking the blood specimens. Quite recently we have studied another RTA patient also demonstrating an inactive form of CA-B. This child has not received any medication, so the possibility of a drug-related effect could be excluded.

It is well known that chloride strongly inhibits CA-B activity (24). However, the hyperchloremic acidosis in our cases was well controlled by the treatment employed. Blood taken 8 mo after the initial observation gave the same results as those reported in the present study. This suggests that the presence of an inactive form of CA-B does not result from increased levels of blood chloride.

The synthesis of carbonic anhydrase is controlled by two codominant, autosomal alles. One of the two alles may be abnormal. A difference in the ultraviolet difference spectrum between the apoprotein and the zinc containing enzyme has been reported (25). The spectral difference noted for the CA-B from an RTA patient (Fig. 3) could be a result of a mutant form of this enzyme, which has a decreased affinity for Zn^{2+}. More detailed structural studies are needed to elucidate this possibility.

It is well established that carbonic anhydrase plays a major role in renal tubular acidification in the distal tubules and hydrogen ion secretion in the proximal tubules (26). Funakoshi and Deutsch reported that kidney medulla contains both CA-B and CA-C (27). In our cases the presence of a mutant form of CA-B in erythrocytes suggests the possibility of a similar form in the kidney medulla, which may relate to the difficulty in acidification of the urine in these patients. Continuing studies in our laboratory are directed to clarification of the above findings in definitive structural modification of the unusual CA-B.

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