DECARBOXYLATION OF 3,4-DIHYDROXYPHENYLALANINE BY OXYHEMOGLOBIN

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SUMMARY

A lysate of red blood cells catalyzes the decarboxylation of 3,4-dihydroxyphenylalanine. This activity appears to be due to a reaction with oxyhemoglobin and not the presence of aromatic L amino acid decarboxylase in erythrocytes.

The physiologic decarboxylation of 3,4-dihydroxyphenylalanine (DOPA) is catalyzed by aromatic L amino acid decarboxylase. This enzyme activity is widely distributed in mammalian tissues, although until recently it was not detected in blood. A recent report by Tate et al. (1) indicates that human erythrocytes contain DOPA decarboxylase activity. However, in a preliminary examination of this DOPA decarboxylase activity in this laboratory, it became apparent that compounds which are potent inhibitors of aromatic L amino acid decarboxylase were ineffective against this activity. It now appears that the apparent DOPA decarboxylase activity in erythrocytes may be the result of a nonspecific reaction of oxyhemoglobin with DOPA.

MATERIALS AND METHODS

Heparinized or nonheparinized whole blood was obtained from normal human volunteers. DL-DOPA-carboxyl-^{14}C and omnifluor were purchased from New England Nuclear Corp. The DOPA was diluted to a specific activity of 1.6×10^9 dpm (as L-DOPA)/mmole L-DOPA. L-α-Methyl-3,4-dihydroxyphenylalanine hydrazine (MK 486), N-methyl-N-(2 hydroxybenzyl) hydrazinium (1^+) hydrogen oxalate (NSD 1039) and pargyline hydrochloride were gifts of Merck and Company, Smith and Nephew, Ltd., and Abbott Laboratories, respectively.

Hemoglobin was prepared by disruption of washed whole red blood cells in 2 volumes of ice-cold distilled water followed by centrifugation. Ammonium sulfate fractionation of this lysate was done by the addition of saturated ammonium sulfate solution prepared in 0.01 M phosphate buffer, pH 6.8. Further purification of hemoglobin was performed by chromatography of the 50-71% saturated ammonium sulfate fraction on Sephadex G-100 or on CM Sephadex C-50 (2). Hemoglobin derivatives were prepared using a solution of hemoglobin purified by CM Sephadex C-50 chromatography. Methemoglobin was prepared by addition of a 3 molar excess of potassium ferricyanide to a solution of hemoglobin followed by overnight "flow" dialysis (3) against 1000 volumes of 0.05 M phosphate buffer, pH 6.0. A solution of hemoglobin was also converted to carboxy-hemoglobin in a Thunberg tube by alternate evacuation and flushing with carbon monoxide and allowing the solution to stand overnight in an atmosphere of carbon monoxide. Deoxygenated hemoglobin was prepared by addition of 0.3 mg sodium dithionite solution to the incubation medium under a nitrogen atmosphere just before starting incubation. All steps were done at 0 to 4°C.

The release of $^{14}CO_2$ from L-^{14}C-DOPA was measured (4) by trapping the radioactive CO_2 on a small piece (1.7 x 7 cm) of Whatman 3 MM filter paper treated with 2.5 ml of 20% phenethylamine methanol solution. This adsorbent was placed in a counting vial which was inverted and connected by a rubber tube to a test tube containing the reaction mixture (4). Reaction mixtures contained 60 mμmoles L-DOPA, 40 mμmoles pyridoxal phosphate, 60 mμmoles pargyline, 70 mμmoles ascorbic acid, 1.25 μmoles Na-EDTA, 50 μmoles sodium phosphate, pH 7.0, and enzyme in a total volume of 0.6 ml. Six mμmoles MK 486 or 90 mμmoles NSD 1039 were added in certain experiments. Preincubation and incubation were done at 37°C for 10 minutes each. The reaction was stopped by injecting 0.6 ml of 6% trichloroacetic acid through the connecting rubber tube with a syringe. Enzyme prepara-

TABLE 1

ISOLATION AND SOME CHARACTERISTICS OF DECARBOXYLASE ACTIVITY FROM HUMAN ERYTHROCYTES

	DOPA Decarboxylase Activity		5-HTP Decarboxylase Activity
	units/mg protein	units/mmole Hb	units/mg protein
1. Fraction of hemolyzed blood cells			
a. 0-50% ammonium sulfate fraction	7.9	425	< 0.2
" + MK 486	7.6	406	
" + NSD 1039	7.2	383	
b. 50-71% ammonium sulfate fraction	10.0	485	< 0.2
" + MK 486	8.9	431	
" + NSD 1039	9.8	476	
c. Purified hemoglobin*	14.4	358	
" + MK 486	10.7	272	
" + NSD 1039	15.8	391	
" - Pyridoxal phosphate	18.6	461	
2. Guinea pig kidney extract**	8,830		
" + MK 486	190		
" + NSD 1039	10		

*Purified hemoglobin was prepared by the chromatography of the 50 to 71% ammonium sulfate fraction on CM Sephadex C-50 (see Fig. 2).

**The tissue was homogenized in 3 volumes of ice cold water, centrifuged at 30,000 x g for 30 minutes at 4°C and the supernatant fraction served as the enzyme.

tions boiled for 1 minute served as controls. Radioactivity was determined in a Packard liquid scintillation spectrometer using Omnifluor solution. 5-Hydroxytryptophan (5-HTP) decarboxylase activity was measured by a previously described method (5). One unit of DOPA or 5-HTP decarboxylase activity is defined here as μμmoles L-DOPA decarboxylated or serotonin formed/min. Protein and hemoglobin concentrations were measured by the optical density at 280 nm and 577 nm (6), respectively.

RESULTS

As reported previously (1) hemolyzed blood cells had considerably more apparent DOPA decarboxylase activity than intact whole blood cells. This activity precipitated not only in the 0-50% saturated ammonium sulfate fraction, in which a large part of aromatic L amino acid decarboxylase in tissues precipitates (5), but also in the higher ammonium sulfate fraction (Table 1). Chromatography of the 50 to 71% saturated ammonium sulfate precipitate, after dissolution, on either Sephadex G-100 (Fig. 1) or on

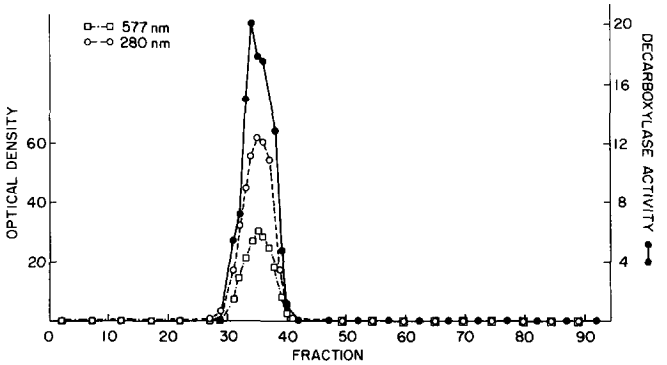

Fig. 1. Chromatography of the 50 to 71% ammonium sulfate fraction of a red cell lysate on Sephadex G-100. The precipitate of 50 to 71% ammonium sulfate saturation of a lysate derived from 50 ml whole blood was dissolved in small volumes (about 1/5 volumes of precipitate) of 0.005 M Tris-HCl buffer, pH 7.0. The Sephadex G-100 column (2.5 x 180 cm) was equilibrated and eluted with 0.05 M Tris-HCl buffer, pH 7.0. Fractions of 6.7 ml were collected. DOPA decarboxylase activity is expressed as units/100 ml eluate.

CM Sephadex C-50 (Fig. 2) indicated that the elution profile of the apparent DOPA decarboxylase activity was identical with that of hemoglobin which

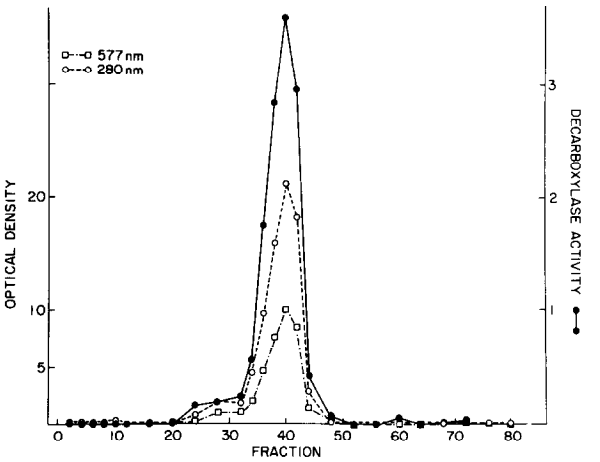

Fig. 2. Chromatography of the 50 to 71% ammonium sulfate fraction of the lysate on a CM Sephadex C-50 column. The ammonium sulfate precipitate was dissolved in water and dialyzed overnight against 0.01 M phosphate buffer, pH 6.8, and applied to the column (25 x 1.7 cm, equilibrated with 0.01 M phosphate buffer, pH 6.8). Four ml fractions were collected. The first 4 fractions were eluted with 0.01 M phosphate buffer, pH 6.8, and the next 76 fractions were eluted with an increasing gradient between 0.01 M phosphate buffer, pH 6.8, and 0.04 M Na_2HPO_4, pH 8.9, using 150 ml of each buffer in a Vari-grad mixer. DOPA decarboxylase activity is expressed as units/100 ml eluate.

obviously represented the major protein. Throughout the purification the DOPA decarboxylase activity per unit of hemoglobin was approximately constant. Two potent inhibitors of aromatic L amino acid decarboxylase, MK 486 and NSD 1039, did not inhibit this reaction but strongly inhibited guinea pig kidney decarboxylase (Table 1). The omission of pyridoxal phosphate from the reaction mixture did not diminish the decarboxylation reaction. No 5-HTP decarboxylase activity was detected in ammonium sulfate fractions (Table 1). Hemoglobin derivatives such as carboxy-hemoglobin, methemoglobin or deoxygenated hemoglobin, prepared from purified fresh hemoglobin, did not cause any significant decarboxylation of DOPA (Table 2). From these results, the inescapable conclusion is that oxyhemoglobin reacts with DOPA resulting in the release of the carboxyl group.

TABLE 2

DOPA DECARBOXYLATING ACTIVITY OF SOME HEMOGLOBIN DERIVATIVES

	DOPA DECARBOXYLASE ACTIVITY units/mg protein
Purified hemoglobin*	8.4
Methemoglobin	< 0.1
Carboxyhemoglobin	< 0.4
Deoxyhemoglobin	< 0.3

*Purified hemoglobin was prepared by chromatography of 50 to 71% ammonium sulfate saturation fraction on a CM Sephadex C-50 column with a pH gradient elution. The main part of this freshly prepared purified hemoglobin is considered to be oxyhemoglobin.

DISCUSSION

The present study indicates that there is probably no detectable aromatic L amino acid decarboxylase in red blood cells and that reaction with oxyhemoglobin is responsible for the decarboxylation of DOPA which occurs in hemolysates of erythrocytes. It should be noted that the molar concentration of hemoglobin is far in excess of the amount of DOPA decarboxylated possibly indicating a stoichiometric rather than a catalytic reaction.

A recent investigation by Dairman and Christenson (7) also support the contention that the DOPA decarboxylase activity of human erythrocytes is not catalyzed by aromatic L amino acid decarboxylase. These workers find that either D or L-DOPA is equally well decarboxylated and that the activity is not inhibited by antibodies to aromatic L amino acid decarboxylase. This being true, the absolute values for the decarboxylase activity of hemoglobin reported herein are approximately 2-fold higher than they should be since the specific activity of the substrate was calculated on the basis of only the contribution of the L-^{14}C-DOPA.

The role of oxyhemoglobin in the destruction of exogenously administered DOPA cannot be evaluated at present. The reason for the apparent lowering of this activity during DOPA therapy in Parkinsonism (1) is not clear, although in retrospect the decarboxylase values reported would have been more meaningful had they been expressed per mole of hemoglobin.

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