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SERINE–GLYOXYLATE AMINOTRANSFERASE FROM KIDNEY BEAN *(PHASEOLUS VULGARIS)*

II. THE REVERSE REACTIONS

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SUMMARY

1. The serine–glyoxylate aminotransferase (EC 2.6.1.45) isolated from *Phaseolus vulgaris*, which catalyzes aminotransferase reactions between serine–glyoxylate and serine–pyruvate, was shown to catalyze the reverse reactions hydroxypyruvate–glycine and hydroxypyruvate–alanine.

2. The equilibrium constant for the reaction of serine and glyoxylate to form hydroxypyruvate and glycine was between 32 and 119. The K_m values for hydroxypyruvate and glycine were $1.1 \cdot 10^{-3}$ and $1.1 \cdot 10^{-2}$ M, respectively. The equilibrium constant for the reaction of serine and pyruvate to form hydroxypyruvate and alanine was 43. The K_m values for hydroxypyruvate and alanine were $6.3 \cdot 10^{-4}$ and $2 \cdot 10^{-2}$ M, respectively.

3. The reverse reactions were inhibited by low concentrations of hydroxylamine, but were less sensitive to *N*-ethylmaleimide and *p*-hydroxymercuribenzoate. The reverse activities were not specifically affected by NH_4^+.

INTRODUCTION

It has been proposed [1] that glycollate is metabolized in leaves in the following manner:

4 glycollate $\longrightarrow$ 4 glyoxylate $\longrightarrow$ 4 glycine

$2CO_2$, $2NH_3$

glucose $\leftarrow\leftarrow\leftarrow$ 2 glycerate $\longleftarrow$ 2 hydroxypyruvate $\leftarrow$ 2 serine

The proposed pathway is supported by several labelling experiments [2–5] and some of the enzymes involved are restricted in plants to the peroxisomes [6].

Abbreviation: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid.

The above scheme contains two aminotransferase reactions; the formation of glycine from glyoxylate and the formation of hydroxypyruvate from serine. Although several glyoxylate aminotransferases have been demonstrated in plants [7–9], serine–glyoxylate aminotransferases (Reaction 1) have received most attention [8–11]:

serine + glyoxylate $\rightleftharpoons$ hydroxypyruvate + glycine (1).

The serine–glyoxylate aminotransferase isolated from spinach [10] and kidney bean [11] may utilize pyruvate as an alternate amino acceptor (Reaction 2):

serine + pyruvate $\rightleftharpoons$ hydroxypyruvate + alanine (2).

In 1963 Willis and Sallach [12] reported that extracts prepared from a number of higher plants were capable of synthesizing serine from alanine and hydroxypyruvate and glycine was an alternate amino donor. However, previous attempts to demonstrate the reverse of Reactions 1 and 2 using purified serine–glyoxylate aminotransferase preparations have been unsuccessful [8, 10]. The present communication reports an investigation of the reverse reactions catalyzed by a partially purified serine–glyoxylate aminotransferase isolated from kidney bean leaves.

EXPERIMENTAL

Materials

Serine–glyoxylate aminotransferase was isolated from the leaves of 14-day old, greenhouse grown, kidney bean seedlings (*Phaseolus vulgaris* L. cv. "Red Kidney"), using the method of Smith [11]. ^{14}C-Labelled compounds were obtained from Amersham-Searle, Arlington Heights, Ill.; and Aquasol liquid scintillation cocktail from New England Nuclear, Boston, Mass. Analytical grade chemicals were obtained from commercial suppliers.

Assay of reactions catalyzed by serine–glyoxylate aminotransferase

Standard reaction mixtures (1 ml) contained the following components in μmoles: *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES), 60 (pH 8.0) and either [$^{14}C_3$]serine, 1 (spec. act., 0.125 Ci/mole) and glyoxylate, 1 or [$^{14}C_3$]serine, 1 (spec. act., 0.125 Ci/mole) and pyruvate, 1 or [$^{14}C_3$]alanine, 1 (spec. act., 0.06 Ci/mole) and hydroxypyruvate, 1 or [$^{14}C_2$]glycine, 1 (spec. act., 0.06 Ci/mole) and hydroxypyruvate, 1.

Incubation was for 30 min at 30 °C, and the reaction was terminated by the addition of 0.2 ml of 1.5 M trichloroacetic acid. The reaction mixture was applied to a Dowex 50 H^+-form column (7.0 cm $\times$ 0.9 cm) and the radioactive product was washed from the column with 20 ml of distilled water. A 5 ml aliquot of the effluent was added to 10 ml of Aquasol and radioactivity determined using a Packard liquid scintillation counter with external standardization.

The reaction mixtures for the determination of equilibrium constants were prepared in a volume of 2 ml containing double the constituents listed above and excess protein (approximately ten times the amount used to obtain linear rates under standard conditions). Reaction mixtures were incubated at 25 °C and 0.5-ml aliquots

were removed at 2, 4 and 8 h and added to 0.2 ml of 1.5 M trichloroacetic acid. This solution was applied to Dowex 50 H^+-form columns. The product was washed from the columns with 20 ml of water and unreacted radioactive substrate was eluted with 15 ml of 3 M NH_4OH.

RESULTS AND DISCUSSION

General properties

The isolation method used in this study was similar to the one used by Smith [11], who reported a 100-fold increase in specific activity, and a serine–glyoxylate aminotransferase fraction free of alanine–glyoxylate and glutamate–glyoxylate activities. Alanine–hydroxypyruvate and glycine–hydroxypyruvate aminotransferase activities were linear with time and enzyme concentration when assayed under conditions at which less than 3% of the least concentrated substrate was reacted. The pH optima were approx. 8.5 for alanine–hydroxypyruvate and 9.0 for glycine–hydroxypyruvate in either HEPES or *N*,*N*-bis(2-hydroxyethyl)glycine buffer.

The relative rates of the four activities of serine–glyoxylate aminotransferase were determined (Table I) and indicated that of the two reverse reactions, alanine–hydroxypyruvate was more active than glycine–hydroxypyruvate, while both proceeded at a higher rate than the serine–pyruvate reaction; which needed ten times as much pyruvate as serine to drive the reaction to detectable levels. Of the four activities, serine–glyoxylate has the highest specific activity.

TABLE I

RELATIVE RATES OF THE FOUR ACTIVITIES CATALYZED BY SERINE–GLYOXYLATE AMINOTRANSFERASE

All of the assays were run with 1 μmole of each substrate except for the serine–pyruvate assay, where 10 μmoles of pyruvate was required to assay the reaction accurately. Volume of reaction mixture, 1 ml.

Substrates		Spec. act. (μmoles/mg per h)
Amino donor	Amino acceptor	
Serine	Glyoxylate	156.5
Serine	Pyruvate	9.5
Alanine	Hydroxypyruvate	10.5
Glycine	Hydroxypyruvate	6.5

Inhibitors

Alanine–hydroxypyruvate and glycine–hydroxypyruvate aminotransferase activities were inhibited by hydroxylamine at low concentrations, but were only inhibited by sulfhydryl reagents (*p*-hydroxymercuribenzoate and *N*-ethylmaleimide) at relatively high concentrations (Table II). These observations are in agreement with inhibition studies of the serine–glyoxylate aminotransferase activity of the enzyme [9–11]. Smith [11] had demonstrated an inhibition of serine–glyoxylate aminotransferase activity by NH_4^+, with 100% inhibition at 10^{-2} M and 92% inhibition at 10^{-3} M. When the effect of NH_4^+ on the alanine–hydroxypyruvate and glycine–

TABLE II

EFFECT OF VARIOUS INHIBITORS ON ALANINE–HYDROXYPYRUVATE AND GLYCINE–HYDROXYPYRUVATE ACTIVITIES

Inhibitor	Concn (M)	Inhibition (%)	
		Ala–hydroxypyruvate	Gly–hydroxypyruvate
Hydroxylamine	10^{-5}	98	85
	10^{-6}	30	11
p-Hydroxymercuribenzoate	10^{-3}	53	32
	10^{-4}	28	8
N-Ethylmaleimide	10^{-3}	14	16
	10^{-4}	5	1
NH_4NO_3	10^{-2}	51	49
	10^{-3}	12	13
	10^{-4}	6	12
NH_4Cl	10^{-2}	46	42
	10^{-3}	6	9
	10^{-4}	0	0
Ammonium acetate	10^{-2}	30	37
	10^{-3}	4	2
	10^{-4}	4	1
KNO_3	10^{-2}	47	22
KCl	10^{-2}	43	24
Sodium acetate	10^{-2}	30	20

hydroxypyruvate activities was investigated, it was found that no true NH_4^+ inhibition was present. The inhibition was of a much lower magnitude, and was subsequently shown to be caused by non-specific ion effects, as demonstrated by the inhibition levels produced by the corresponding non-ammonium salts (Table II).

Kinetics

Double reciprocal plots of initial velocity against alanine (or glycine) concentration at a series of fixed concentrations of hydroxypyruvate yielded a set of parallel lines, one for each concentration of keto acid. This is in agreement with the "ping-pong" reaction mechanism postulated for aminotransferase reactions [13] and supports previous observations of the two forward reactions [9, 11]. The K_m and V values for the four reactions catalyzed by serine–glyoxylate aminotransferase were calculated by the method of Velick and Vavra [14] and are presented in Table III.

Equilibria

To determine the K_{eq} for Reaction 1, two separate assays were performed, one with serine and glyoxylate as the substrate pair, and a second with glycine and hydroxypyruvate as the substrates. Reaction mixtures were incubated at 25 °C until equilibrium was reached. With the reaction mixture containing serine and glyoxylate a K_{eq} of 32 was calculated. The K_{eq} derived from reaction mixtures with glycine and hydroxypyruvate as substrates was 119.

These figures are not greatly dissimilar when one considers that the first represents conversion of 85% of the serine and glyoxylate to glycine and hydroxypyruvate,

TABLE III

SUMMARY OF KINETIC CONSTANTS FOR THE FOUR REACTIONS CATALYZED BY SERINE–GLYOXYLATE AMINOTRANSFERASE

Aminotransferase assayed	Kinetic constants
Serine–glyoxylate	K_m serine, $7.1 \cdot 10^{-4}$ M (ref. 11)
	K_m glyoxylate, $6.0 \cdot 10^{-4}$ M (ref. 11)
	V, 362 μmoles/mg per h*
Glycine–hydroxypyruvate	K_m glycine, $1.1 \cdot 10^{-2}$ M
	K_m, hydroxypyruvate $1.1 \cdot 10^{-3}$ M
	V, 111 μmoles/mg per h
Serine–pyruvate	K_m serine, $3.9 \cdot 10^{-4}$ M (ref. 11)
	K_m pyruvate, $3.8 \cdot 10^{-2}$ M (ref. 11)
	V, 49 μmoles/mg per h*
Alanine–hydroxypyruvate	K_m alanine, $2 \cdot 10^{-2}$ M
	K_m hydroxypyruvate, $6.3 \cdot 10^{-4}$ M
	V, 125 μmoles/mg per h

* Calculated from $v = \frac{V \cdot AB}{K_a \cdot B + K_b \cdot A + AB}$, using v from Table I.

while the second represents the conversion of 9% of the glycine and hydroxypyruvate to serine and glyoxylate.

It was impractical to determine the K_{eq} for the reaction in which serine and pyruvate are converted to alanine and hydroxypyruvate using serine and pyruvate as substrates. This was due to the fact that the reaction did not reach equilibrium even after a 20 h incubation period. At which time, only 20% of the substrates had been converted to products, and there was a loss of total counts. Using alanine and hydroxypyruvate as substrates, the K_{eq} was determined to be 43. There have been previous reports concerning the difficulty of determining equilibrium constants for glyoxylate aminotransferase reactions [8, 15].

CONCLUSION

The serine–glyoxylate aminotransferase isolated from *Phaseolus vulgaris* catalyzes four reactions. The major function of the enzyme is the conversion of glyoxylate and serine to glycine and hydroxypyruvate. This conclusion is supported by the position of the equilibrium, the relatively low K_m values for serine and glyoxylate and the relatively high maximum velocity for this reaction (Table III). The reverse reactions may be physiologically important in the dark, when the glyoxylate concentration is lower.

The transfer of an amino group from serine to glyoxylate is the only reaction of the four which is specifically inhibited by NH_4^+, which supports the earlier conclusion [11] that NH_4^+ affects the binding of glyoxylate to the enzyme. The physiological significance of this inhibition is not known.

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