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Stereospecific Decarboxylation of Specifically Labeled Carboxyl-^{14}C Aminomalonic Acids by L-Aspartate β-Decarboxylase*

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ABSTRACT: Studies with specifically labeled carboxyl-^{14}C aminomalonic acids obtained by oxidation of L-serine-*1*-^{14}C and L-serine-*3*-^{14}C showed that L-aspartate β-decarboxylase catalyzes the removal of the carboxyl group of aminomalonic acid that is in the position analogous to the CH_2COOH group of L-aspartate rather than the α-carboxyl group. The findings constitute experimental proof of Ogston's hypothesis concerning aminomalonic acid.

We have previously reported that L-aspartate β-decarboxylase decarboxylates animomalonic acid to glycine and that this reaction is stereospecific (Palekar *et al.*, 1970). Thus, we found that enzymatic decarboxylation of aminomalonic acid in the presence of H_2O-*t* gave (*S*)-Gly-*t*; in the corresponding nonenzymatic reaction (*SR*)-Gly-*t* was formed. (*S*)-Gly-*t* is also formed in the reaction catalyzed by serine hydroxymethylase (Wellner, 1970). Thus, in the enzyme-catalyzed decarboxylation of aminomalonic acid, the carboxyl group that is lost may be considered to be in a position on the enzyme that is analogous to the CH_2OH group of L-serine and to the CH_2COOH group of L-aspartate. However, one cannot exclude the possibility that the carboxyl group of aminomalonic acid that is lost is in the position analogous to to that of the α-carboxyl group of aspartate and therefore that addition of a proton is accompanied by inversion of configuration. In the present studies, which were carried out in order to answer this question, we have used specifically labeled carboxyl-^{14}C aminomalonic acids prepared by oxidation of L-serine-*1*-^{14}C and L-serine-*3*-^{14}C. In this paper the carboxyl-labeled aminomalonic acid obtained by oxidation of L-serine-*1*-^{14}C will be referred to as aminomalonate-*1*-^{14}C; the product obtained by oxidation of L-serine-*3*-^{14}C will be designated as aminomalonate-*3*-^{14}C.

Materials

L-Aspartate β-decarboxylase was isolated from *Alcaligenes faecalis*, strain N (ATCC 25094), as described by Tate and Meister (1968). Hog kidney D-amino acid oxidase was kindly supplied by Dr. Daniel Wellner. L-Serine-*3*-^{14}C was obtained from Amersham Searle, and DL-serine-*1*-^{14}C was obtained from the New England Nuclear Corp. L-Serine-*1*-^{14}C was obtained from the racemate by selective destruction of the D isomer by treatment with D-amino acid oxidase followed by adsorption of the L-serine on Dowex 50 (H^+) followed by elution with 2 M ammonium hydroxide.

Methods

The reactions were carried out in stoppered tubes in which a polyethylene well containing 0.1 ml of 1 N potassium hydroxide was suspended. The reactions were stopped by injecting 1 ml of ethanol into the tubes, which were then placed on a shaker for 90 min. The potassium hydroxide was mixed with 10 ml of liquid scintillation medium (Bray, 1960) for the determination of $^{14}CO_2$. The denatured protein was removed by centrifugation and the supernatant solution was evaporated to dryness *in vacuo*. The residue was dissolved in 0.2 ml of water and the pH was adjusted to 6 by addition of sodium hydroxide. This solution was applied to the top of a column of Dowex 1 (acetate form); the glycine was eluted with 1 ml of water and the eluate was evaporated to dryness. The residue was dissolved in 0.4 ml of water. Aliquots of this solution were taken for the determination of glycine-^{14}C by scintilla-

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TABLE I: Decarboxylation of Specifically Labeled Carboxyl-^{14}C Aminomalonic Acids by L-Aspartate β-Decarboxylase.

Expt	^{14}C of Aminomalonic Acid	$^{14}CO_2$ (cpm)	Glycine-^{14}C (cpm)	Glycine Total[d] (mμmoles)	Glycine Rel Sp Act.[e]
1. Enzymatic decarboxylation[a]	1-^{14}C	154 (40)[f]	1380 (1240)[f]	69.5	99[f]
	3-^{14}C	3950 (3720)[f]	620 (308)[f]	62.0	7.5[f]
2. Decarboxylation with heat-inactivated enzyme[b]	1-^{14}C	114	137	11.5	55
	3-^{14}C	230	312	7.5	55
3. Decarboxylation in acid[c]	1-^{14}C	1130	1030	98.0	49
	3-^{14}C	4420	4340	119.0	48

[a] The reaction mixture (0.5 ml) contained 0.2 M sodium acetate buffer (pH 5.0), L-aspartate β-decarboxylase (1.6 mg), and either aminomalonate-*1*-^{14}C (920 mμmoles; 20,000 cpm) or aminomalonate-*3*-^{14}C (861 mμmoles; 65,000 cpm). The reaction mixtures were incubated for 30 min at 25°; the formation of carbon dioxide and glycine was determined as described in the text. [b] The enzyme was inactivated by heating at 100° for 1 min prior to the experiment, which was carried out as described for expt 1. [c] The reaction mixture contained 0.5 ml of 0.5 N acetic acid and either aminomalonate-*1*-^{14}C (184 mμmoles; 4000 cpm) or aminomalonate-*3*-^{14}C (172 mμmoles; 13,000 cpm). Incubation was carried out for 4 hr at 37°. [d] Determined by the ninhydrin method (Rosen, 1957). [e] (Specific activity of glycine/specific activity of aminomalonate) × 100. [f] Corrected for nonenzymatic decarboxylation (expt 2).

tion counting and for total glycine by the ninhydrin procedure (Rosen, 1957). Glycine was further identified by thin layer chromatography in three solvent systems (Palekar *et al.*, 1970).

Preparation of Specifically Labeled Aminomalonic Acids from L-*Serine-1-*^{14}C *and* L-*Serine-3-*^{14}C. Carbobenzoxy chloride (1.1 mmoles; 187 mg) was added over a period of 30 min with constant stirring to a solution of the labeled L-serine (0.96 mmole; 101 mg) in 1.25 ml of water containing 210 mg of $NaHCO_3$ at 0°. The mixture was stirred for 2 hr, after which the excess carbobenzoxy chloride was removed by extraction with ether and the aqueous layer was acidified by addition of 5 N HCl. This solution was extracted repeatedly with ethyl acetate and the combined ethyl acetate extracts were evaporated *in vacuo* to yield an oil which was suspended in 2 ml of water. The pH of this solution was adjusted to 6.8 by addition of solid $NaHCO_3$, and 208 mg of potassium permanganate was added gradually. The mixture was stirred at 4° for 16 hr and then filtered on a sintered glass funnel. The residue was washed with 5 ml of 50% methanol. The combined filtrate and washing containing the carbobenzoxyamino acid were subjected to hydrogenolysis at room temperature and pressure using palladium black as catalyst. After removal of the catalyst the filtrate was evaporated *in vacuo* to dryness and the residue was chromatographed on a sheet of Whatman No. 3MM paper (descending; solvent chloroform–methanol–17% ammonium hydroxide, 2:2:1, v/v). Standards consisting of aminomalonic acid, serine, and glycine were also chromatographed. The chromatogram was scanned for radioactivity with a Nuclear-Chicago Actigraph III Model 1002 strip scanner. The area corresponding to aminomalonic acid was cut out and the amino acid was eluted with the solvent. The identity of the aminomalonic acid product was confirmed by thin-layer chromatography on silica gel G in the following solvent systesm: (a) chloroform–methanol–17% ammonium hydroxide (2:2:1, v/v); (b) 1-propanol–34% ammonium hydroxide (7:3, v/v); and (c) 1-propanol–water (7:3, v/v). Aminomalonic acid synthesized as described previously (Palekar *et al.*, 1970) was used as reference. The R_F values of aminomalonic acid in these solvent systems a, b, and c were 0.44, 0.06, and 0.22, respectively, and those of glycine were 0.72, 0.3, and 0.35, respectively. Under the conditions employed, the conversion of serine to aminomalonic acid was far from complete, but sufficient amounts of product were obtained to carry out the necessary experiments. Substantial quantities of serine and some glycine were also recovered.

Results

Table I summarizes the results of experiments in which the specifically labeled aminomalonic acids were decarboxylated enzymatically and nonenzymatically. In these studies decarboxylation of aminomalonic acid gave only one product, which was identified as glycine by thin-layer chromatography in three solvent systems as well as by ion-exchange chromatography (Spackman *et al.*, 1958). In exp 1, in the study with aminomalonate-*1*-^{14}C, very little radioactivity appeared in the carbon dioxide, while the specific radioactivity of the glycine isolated was about 99% of that of the aminomalonate-*1*-^{14}C used. In contrast, when aminomalonate-*3*-^{14}C was the substrate very little radioactivity appeared in the glycine and very substantial amounts of labeled carbon dioxide were formed. That some radioactivity appeared in the glycine in the experiment with aminomalonate-*3*-^{14}C may be ascribed in part to experimental error and perhaps also to the presence of some D-serine in the preparation of L-serine-*3*-^{14}C employed.[1] It is also possible that some racemization of aminomalonate takes place under the alkaline conditions used in purification of this compound by paper chromatography. In the nonenzymatic decarboxylation reactions (expt 2 and 3) the specific activity of the glycine formed was, as expected, about half of that of the labeled aminomalonate preparations used.

[1] Test of the serine recovered in this preparation of aminomalonate by D-amino acid oxidase showed that about 3% of the serine was of the D configuration.

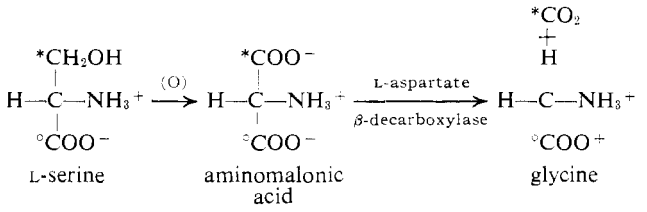

FIGURE 1

Discussion

The data show that L-aspartate β-decarboxylase catalyzes the removal of the carboxyl group of aminomalonic acid that is in a position analogous to the "R" group of an L-amino acid rather than the α-carboxyl group (Figure 1). Thus, decarboxylation of aminomalonate by this enzyme is not analogous to the typical α decarboxylation of amino acids, but seems to reflect the reaction specificity of L-aspartate β-decarboxylase. The present studies conclusively establish that in the formation of (*S*)-Gly-*t* from aminomalonic acid observed earlier (Palekar *et al.*, 1970), addition of the tritium atom occurs with retention of configuration.

The results reported here add another reaction to the now long list of enzyme catalyzed reactions in which it can be demonstrated that an enzyme distinguishes between identical groups of a symmetrical substrate. In his classical note on this subject, Ogston (1948) used two examples to illustrate his hypothesis. One of these concerned the incorporation of carbon dioxide into the α-carboxyl group of α-ketoglutarate in the citric acid cycle and the fallacy of excluding the symmetrical molecule citrate as an intermediate. The other example concerned the reasoning by which aminomalonic acid had been excluded as a possible intermediate in the conversion of serine to glycine. Although the reasoning was faulty, the conclusion was apparently correct as there is now substantial evidence for the participation of a C_1 folate unit in the serine–glycine interconversion. However, our previously reported experiments (Palekar *et al.*, 1970) and the present work provide experimental proof for the particular case argued and used as an illustration by Ogston (1948). It is interesting to note that the present experiments on the decarboxylation of aminomalonate by L-aspartate β-decarboxylase show that it is the carboxyl group of aminomalonate which is derived from C-3 of serine which is released as carbon dioxide. Enzyme activity capable of converting aminomalonate into glycine has been found in the posterior silk glands of silk worms (Shimura *et al.*, 1956) and in rat liver (Thanassi and Fruton, 1962); it is conceivable that these reactions are of physiological significance and therefore that an alternative pathway exists for the conversion of serine to glycine. It would be interesting to learn whether the decarboxylation reactions of aminomalonic acid catalyzed by liver and silk glands exhibit the same or a different specificity as that catalyzed by L-aspartate β-decarboxylase.

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