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Separation of the diastereoisomers of 3-hydroxyglutamic acid by ion-exchange chromatography

As a preliminary to a study of the metabolism of 3-hydroxyproline, we needed a chromatographic method for the separation of the diastereoisomers of 3-hydroxyglutamic acid. Ion-exchange chromatography has been used for the separation of the diastereoisomers of several amino acids with two asymmetric carbon atoms (for a review, see ref. 1). No separation of the diastereoisomers of 3-hydroxyglutamic acid occurred under those conditions in which there was complete separation between the diastereoisomers of 4-hydroxyglutamic acid and of 3-hydroxyaspartic acid[2-4], and another procedure was therefore worked out.

Materials and methods

DL-DL-*allo*-3-Hydroxyglutamic acid was obtained from Fluka AG, Buchs, Switzerland. Dowex AG 50W-X8 (200–400 mesh, hydrogen form) was obtained from BioRad Laboratories, Inc., Richmond, Calif., U.S.A. Acetone-dried cells of *E. coli* and *Cl. welchii* were obtained from Sigma Chemical Company, St. Louis, Mo., U.S.A. and Worthington Chemical Corp., Freehold, N.J., U.S.A.

The quantitative determination of the amino acids was performed according to MOORE AND STEIN[5]. The manometric determination of decarboxylase activity in 0.2 *M* sodium acetate buffer, pH 5.0, was carried out by the Warburg technique. Ion-exchange chromatography was carried out in glass columns at room temperature; 0.07 *M* HCl was used as eluting agent, and the rate of elution was 0.15 ml per min per cm^2. Fractions were collected by means of an automatic fraction collector with a drop counter. Paper chromatography (descending) was carried out with pyridine–water (4:1) as described by UMBREIT AND HENEAGE[6].

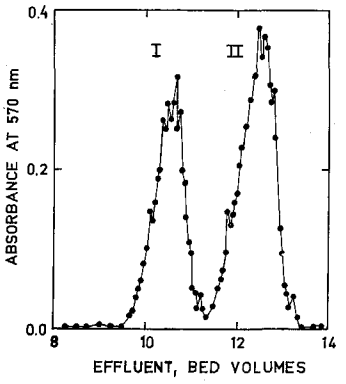

Fig. 1. Chromatography on a column of Dowex AG 50W-X8 (200–400 mesh, hydrogen form, 3.0 × 56 cm) of 600 mg of DL-DL-*allo*-3-hydroxyglutamic acid with 0.07 *M* HCl. The concentration of amino acid was determined by means of the ninhydrin reaction according to MOORE AND STEIN[5].

TABLE I

RATES OF DECARBOXYLATION OF THE MATERIAL IN PEAK I AND PEAK II WITH DIFFERENT SOURCES OF ENZYME

The incubations were carried out at 37° in Warburg vessels with 0.2 *M* sodium acetate buffer (pH 5.0), 10–30 mg of acetone-dried cells and an initial concentration of 0.010 *M* substrate in a total volume of 2 to 3 ml.

*Enzyme (batch)**	*Initial rate of CO_2 evolution, μl CO_2 per min per mg of enzyme*		*Ratio I/II*
	Peak I	*Peak II*	
E. coli (Sigma, type I, batch No. 73B-8110)	0.54	0.056	9.6
E. coli (Sigma, type II, batch No. 75B-9160)	2.58	0.32	8.1
E. coli (Worthington, GLD 6442)	2.3	0.32	7.2
Cl. welchii (Sigma, type II, batch No. 43B-647)	0.23	0.032	7.2

* Acetone-dried cells.

Results

Fig. 1 shows the separation of the diastereoisomers of 3-hydroxyglutamic acid on a cation exchanger with dilute hydrochloric acid as eluting agent. The commercial preparations of 3-hydroxyglutamic acid have been found to contain various amounts of 4-amino-3-hydroxybutyric acid, which, however, was eluted far more slowly than the dicarboxylic acid under these conditions. The material in each peak (peaks I and II, Fig. 1) was rechromatographed, taken to dryness and recrystallized from hot ethanol. The melting points for the hydrochlorides were: I, 194–195°; II, 190–192°. These materials were treated with different batches of commercial preparations of acetone-dried cells of *E. coli* and *Cl. welchii*. As shown in Table I, the material in peak I was decarboxylated at about an 8-fold higher rate than that in peak II, when such concentrations were used that the initial rate of decarboxylation was independent of the substrate concentration. In separate experiments it was ascertained that there was no gas uptake or release when 4-amino-3-hydroxybutyrate was incubated with the acetone-dried cells, either with or without potassium hydroxide in the center well. About 46% of the material in peak I was decarboxylated, as was about 41% of that in peak II. 4-Amino-3-hydroxybutyric acid could be demonstrated as the reaction product, using chromatography on Dowex 50 and on paper.

Discussion

The diastereoisomers of 3-hydroxyglutamic acid have previously been separated by fractional crystallization of the hydrochlorides of the free amino acid or its diethyl ester of the N-acetyl diethyl ester derivatives[7,8]. The *allo*-form[7] (*threo*-form[9]) is decarboxylated at a severalfold higher rate[6,8,9] than the *normal* form (*erythro*-form), and KANEKO AND YOSHIDA[10] have demonstrated the formation of (−)-4-amino-3-hydroxybutyric acid as the reaction product of the decarboxylation of the *threo*-form. This compound has the same configuration as the natural form of carnitine[10]. The difference in the susceptibility to enzymic decarboxylation between the diastereoisomers of 3-hydroxyglutamic acid is similar to that found with the diastereoisomers

of 5-hydroxylysine[11]. The ratio between the initial rates of decarboxylation of the two diastereoisomers of 3-hydroxyglutamic acid was of the same order of magnitude as that reported by other authors[6,8,9], and the material in peak I (Fig. 1) may therefore be designated as *threo*-3-hydroxy-DL-glutamic acid, and that in peak II as *erythro*-3-hydroxy-DL-glutamic acid.

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Reproduzierbare Gradientenchromatographie an Kolonnen mit schrumpfenden Austauschern

Die Methoden der chromatographischen Fraktionierung von Proteingemischen oder solchen anderer hochmolekularer Naturstoffe haben seit Einführung passend substituierter Materialien mit Cellulose-[1] oder Dextrangerüst als Ionenaustauscher eine weltweite Anwendung gefunden. Die technische Weiterentwicklung derartiger Austauscher führte zu Produkten, welche auch ohne Anwendung von Druck befriedigende Durchflussgeschwindigkeiten während der Chromatographie liefern.

Bei steigender Ionenstärke des Elutionsmittels zeigen diese Austauscher indessen eine mehr oder minder ausgeprägte Schrumpfung ihres Volumens, die bei starkem Anstieg der Ionenstärke während eines Chromatographieprozesses 40–60% des ursprünglichen Bettvolumens ausmachen kann. Während Fraktionierungen mit stufenweiser Elution durch ein derartiges Verhalten des Gel-Bettes nicht wesentlich beeinträchtigt werden, muss eine Gradientenchromatographie durch eine solche Volumenverminderung des Austauschers empfindlich gestört werden: Oberhalb des