Des-N-methylhypognavinol. 16.898 mg Probe verbrauchte 0.67 ccm 0.02*N* NaOH (F=1.225). Gef.: CH_3, 0.35. Der Säure-Teil der titrierten Lösung wurde papierchromatographisch geprüft. Rf 0.17. Kontrollversuch mit AcOH: Rf 0.18.

Zusammenfassung

Durch Selen-Dehydrierung des Des-N-methylhypognavinols wurde eine schwach basische aromatische Fraktion in sehr kleiner Menge erhalten, deren UV-Spektrum demjenigen des 3-Azaphenanthrens ähnlich ist. Durch Oxydation des Tribenzoyl-des-N-äthylhypognavinols und darauffolgende Verseifung wurde ein 6-gliedriges Laktam $C_{22}H_{29}O_5N$ erhalten. Anhydro-hydroxy-des-N-methylhypognavinol $C_{21}H_{27}O_4N$, ein Carbinolamináther, wurde durch Oxydation des Des-N-methylhypognavinol mit Kaliumferricyanid erhalten. Für die Konstitution des Hypognavinols wurde die Formel (VIII) oder (IX) und für Hypognavin die Formel (X) oder (XI) aufgestellt.

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13. β-Hydroxyglutamic Acid Decarboxylase in *Escherichia coli*.

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The enzymatic decarboxylation of β-hydroxyglutamic acid by various strains of *Escherichia coli* has already been reported by Umbreit and Heneage.[1] They seemed to conclude that β-hydroxyglutamic acid decarboxylase is different from glutamic acid decarboxylase, which is always found to be present in the bacteria, for the following reasons. First, the two enzymes are found in various ratios distributed in the strains of *E. coli*. Second, the formation ratio of two enzymes seems to be associated with the condition of bacterial growth. The third reason lies in the peculiar effect of pH upon the activity of β-hydroxyglutamic acid decarboxylase.

The work described in this paper was undertaken with the object of obtaining a more precise information on the distinction between the β-hydroxyglutamic and glutamic acid decarboxylases in *E. coli*.

Materials and Methods

Enzyme Preparation and Measurement of Activity—The source of enzyme was an acetone-dried powder of *E. coli*, strain No. 1 (isolated by Dr. D. Mizuno of the National Institute of Health, Tokyo), cultured in a synthetic medium containing 0.01*M* sodium glutamate, 1% glucose, 0.05*M* phosphate, 0.1% NH_4Cl, 0.01% $MgSO_4 \cdot 7H_2O$, and 0.001% $FeSO_4 \cdot 7H_2O$, at 30° for 16~18 hrs. Conventional manometric techniques were used to measure the decarboxylase activity at 30°. The reaction mixture in each vessel was 2.0 cc. and the Q_{CO_2} values represented CO_2 μL./mg. Kjeldahl-N/hr. in all experiments.

Materials—β-Hydroxy-DL-glutamic acid used in these studies was synthesized by the method of Izumi.[2] L-Glutamic acid was the commercial product of Takara Pharmaceutical Co. 4-Amino-3-hydroxybutyric acid was synthesized by Dr. N. Ikeda of the Faculty of Pharmacy, University of Chiba.

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1) W. W. Umbreit, P. Heneage: J. Biol. Chem., **201**, 15(1953).
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Results and Discussion

The pH- and pS-activity curves of the amino acid decarboxylases are shown in Fig. 1. The optimal pH value for β-hydroxyglutamic and glutamic acid decarboxylases was at 5.0 for both. The K_m of these enzymes was $1.2\times10^{-2}M$ for β-hydroxy-DL-glutamic acid and $1.1\times10^{-3}M$ for L-glutamic acid. As shown in Fig. 2, approximately 0.5 mole of CO_2 was formed

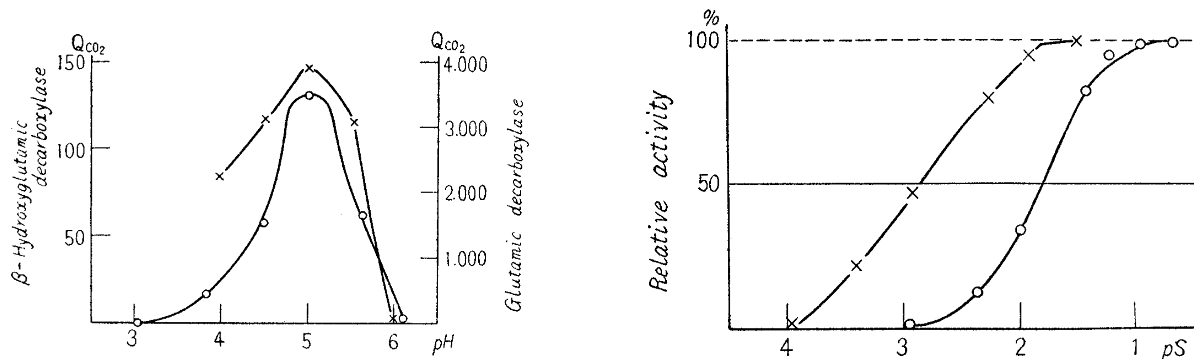

Fig. 1. pH- and pS-Activity Curve

—○——○— β-Hydroxy-DL-glutamic acid decarboxylase
—×——×— Glutamic acid decarboxylase

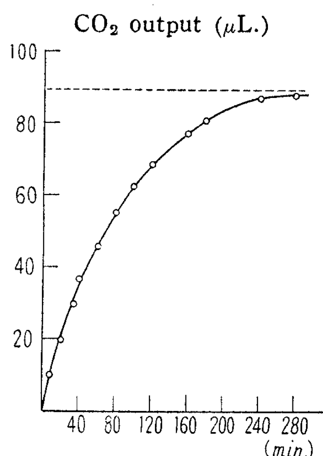

Fig. 2. Enzymic Decarboxylation of β-Hydroxy-DL-glutamic Acid

8 μmoles of sample added; for complete decarboxylation of one isomer, 4 μmoles of CO_2 should be released

from 1.0 mole of β-hydroxy-DL-glutamic acid supplied. The product from enzymatic decarboxylation of β-hydroxyglutamic acid corresponded to 4-amino-3-hydroxybutyric acid in paper chromatogram (solvent, 80% phenol; detection by ninhydrin reagent).

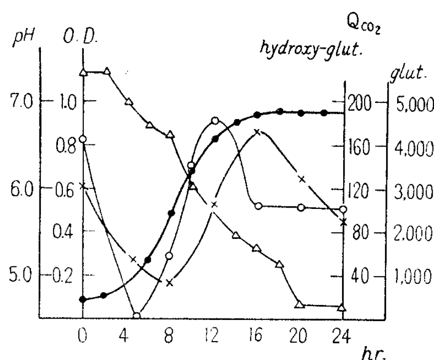

Fig. 3. Changes in the Activity of β-Hydroxy-DL-glutamic and L-Glutamic Acid Decarboxylases during the Growth of *Escherichia coli*

Final concentration of substrates was $4\times10^{-3}M$ in both cases

—●——●— Growth curve
—△——△— pH-curve
—○——○— Activity of β-hydroxyglutamic acid decarboxylase
—×——×— Activity of glutamic acid decarboxylase

Change in the activity of the two enzymes during the course of growth of *E. coli* is shown in Fig. 3. The initial activity depends upon the inoculated bacteria itself which grew in the same synthetic medium at 30°, during 16~18 hrs. During the lag phase of growth curve, each activity per cell decreased. When the culture developed into a logarithmic phase, its

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activity increased markedly and approached the maximum during early stage of stationary phase. The activity dropped again thereafter, but the peak of activity of the two enzymes is different. This fact indicates that β-hydroxyglutamic and glutamic acid decarboxylases are not the same. When aspartate was added to the culture medium in place of glutamic acid, the same data were obtained.

TABLE I. Effect of Carbonyl Agents on the Two Enzymes

Inhibitor added	Final concn. (M)	Inhibition (%)	
		Hydroxyglut.	Glut.
Hydrazine	10^{-2}	70	20
	10^{-3}	40	5
Semicarbazide	10^{-2}	90	10
	10^{-3}	60	0
Hydroxylamine	10^{-2}	100	100
	10^{-3}	100	75

Table I shows the effect of carbonyl agents on the activity of the two enzymes. In general, β-hydroxyglutamic acid decarboxylase was strongly inhibited by these agents.

The present data on optimal pH values of these enzymes did not agree with the values reported by Umbreit and Heneage.[1] This was probably due to the difference of strains and cultural conditions of *E. coli*.

It has been reported that the amino acid decarboxylases[3,4] are formed in an induced system. Glutamic acid decarboxylase may be formed by this mechanism. β-Hydroxyglutamic acid, which must be the inducer of β-hydroxyglutamic acid decarboxylase, was not detected during the course of cultural development of *E. coli*. The mechanism of induced formation of β-hydroxyglutamic acid decarboxylase is not clear, but the following three mechanisms may be presumed. The first mechanism depends upon the non-specific and simultaneous formation of glutamic and β-hydroxyglutamic acid decarboxylases by a single inducer, i.e. glutamic acid. For the second mechanism, the possibility of decarboxylation of β-hydroxyglutamic acid by the glutamic acid decarboxylase may be supposed. The last is the metabolic formation of the inducer, i.e., β-hydroxyglutamic acid in this case, from substrate added, probably glutamic acid, during the cultural development of *E. coli*. However, the present data show that only the first one of these mechanisms is acceptable. Thus, it may be assumed that the β-hydroxyglutamic acid decarboxylase was induced by glutamic acid, simultaneously with the formation of glutamic acid decarboxylase.

Summary

The β-hydroxy-DL-glutamic and L-glutamic acid decarboxylases in *Escherichia coli* were investigated. It was concluded that the two enzymes are different and presumed that these enzymes are induced by glutamic acid simultaneously.

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