Synthesis of d-β-Glutamine from β-Glutamic Acid by Glutamine Synthetase*

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Previous studies in this laboratory have shown that β-glutamic acid (β-aminoglutaric acid) is a substrate for glutamine synthetase, which catalyzes the synthesis of β-glutamine and the corresponding hydroxamic acid according to the following equation (1).

\[
\text{COOH} \quad \xrightarrow{\text{CH}_2} \quad \text{CONH}_2 (\text{NH}_2 \text{OH})
\]

\[
\text{CH}_2 \quad \xrightarrow{\text{CH}_2} \quad \text{CH}_2
\]

\[
\text{CHNH}_2 + \text{ATP} + \text{NH}_3 (\text{NH}_2 \text{OH}) \rightarrow \text{CHNH}_2 + \text{ADP} + \text{Pi}
\]

\[
\text{CH}_2 \quad \xrightarrow{\text{CH}_2} \quad \text{COOH}
\]

In contrast to β-glutamine and β-glutamylhydroxamic acid, β-glutamic acid does not possess an asymmetric carbon atom, but has, according to the nomenclature of Schwartz and Carter (2), a meso carbon atom.

The present experiments were designed to determine whether glutamine synthetase distinguishes between the carboxyl groups of β-glutamic acid. It is noteworthy in this connection that the enzyme exhibits significant activity toward d-glutamic acid as well as L-glutamic acid (3, 4), and it might therefore be expected to catalyze synthesis of both isomers of β-glutamine from β-glutamic acid. In the present studies, the enzymatically synthesized β-glutamine was found to exhibit stereospecific optical activity. Efforts to obtain the pure optically active isomers of L- and d-β-glutamine for comparative purposes by resolution of di-β-glutamine with alkaloids were not successful. Therefore, another approach to the problem was employed which was based on earlier work indicating that chymotrypsin acts stereospecifically on dimethyl-β-hydroxy glutarate and diethyl-N-acetyl-β-glutamate (5, 6). Chymotrypsin cleaves a specific ester bond of the latter compound to yield a monoethyl ester of N-acetyl-β-glutamic acid (5, 6). Chymotrypsin cleaves a specific ester bond of the latter compound to yield a monoethyl ester of N-acetyl-β-glutamic acid. This isomer is identical with the dextrorotatory isomer obtained by the action of chymotrypsin on diethyl-N-acetyl-β-glutamate (6). We have also acetylated the β-glutamine synthesized from β-glutamic acid by sheep brain glutamine synthetase (8); this N-acetyl-β-glutamine exhibits optical rotation which is equal and opposite to that obtained via the chymotrypsin route. These relationships are shown in Fig. 1. Analogous results have been obtained with the corresponding hydroxamic acids.

**EXPERIMENTAL PROCEDURE**

**N-Acetyl-L-β-glutamine—N-Acetyl-L-α-aminoglutaric acid monoethyl ester obtained by the action of chymotrypsin on the corresponding diethyl ester (5) (100 mg; 0.46 mmole) was dissolved in 10 ml of concentrated ammonium hydroxide and allowed to stand at 26°C for 15 hours. The solution was concentrated in a flash evaporator, and the amorphous residue was dissolved in a minimum quantity of water. An excess of Dowex 50 (H+) was added and the suspension was stirred until all the ammonia (as determined with Nessler’s reagent) was removed from solution. The resin was removed by filtration and the filtrate was concentrated in a flash evaporator to a crystalline residue. The crystals were triturated with acetone; after filtration, 78 mg of product (90% yield) were obtained; m.p. 192–193°C. Analysis showed**

C<sub>8</sub>H<sub>14</sub>O<sub>4</sub>N<sub>2</sub>

Calculated: C 44.7, H 6.38, N 14.9

Found: C 44.9, H 6.42, N 14.9

**N-Acetyl-D-β-glutamine—This compound was prepared from racemic N-acetyl-β-aminoglutaric acid monoethyl ester (5) as described above for the corresponding L isomer. The yield was 90%; m.p. 171–172°C. Analysis showed**

C<sub>8</sub>H<sub>14</sub>O<sub>4</sub>N<sub>2</sub>

Calculated: C 44.7, H 6.38, N 14.9

Found: C 44.9, H 6.51, N 14.8

**N-Acetyl-L-β-glutamylhydroxamic acid—N-Acetyl-L-β-aminoglutaric acid monoethyl ester (5) (100 mg) was dissolved in 20 ml of water containing 200 mg of hydroxylamine hydrochloride, and the solution was brought to pH 11 by gradual addition of 2 N NaOH. After standing at 26°C for 30 min, the pH was adjusted to 8 by addition of 2 N HCl, and the solution was concentrated in a flash evaporator to an amorphous residue. The residue was extracted with water, filtered, and the filtrate was adjusted to pH 3 by addition of 2 N HCl and then concentrated in a flash evaporator**

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was removed by filtration, and the filtrate was concentrated in a flash evaporator to a crystalline product. The crystals were triturated with acetone and then recovered by filtration. The yield of product was 261 mg (90%); m.p. 193-194°. Analysis showed

\[ \text{C}_4\text{H}_7\text{O}_2\text{N}_2 \]
Calculated: C 44.7, H 6.88, N 14.9
Found: C 44.9, H 6.80, N 14.7

The infrared absorption curve of this product, carried out in Nujol mull, was identical with those of the corresponding \(L\) and \(D\) forms.

**N-Acetyl-D-β-glutamylhydroxamic acid**—A sample of enzymatically synthesized glutamylhydroxamic acid (150 mg) isolated by the procedure used for the corresponding amide (1) was acetylated as described above. The yield was 132 mg (70%), m.p. 137-138°. Analysis showed

\[ \text{C}_4\text{H}_7\text{O}_2\text{N}_2 \]
Calculated: C 41.1, H 5.88, N 13.7
Found: C 41.4, H 5.93, N 13.7

**Determination of Optical Rotation**—Determinations of optical rotation were carried out in water in a Cary model 60 spectropolarimeter at 25° with an optical path of 1 cm. The optical rotations of the several compounds isolated here are given in Table I. The values for the \(L\) and \(D\) isomers of N-acetyl-β-glutamine and the corresponding hydroxamic acids are, within experimental error, equal and opposite.

**Discussion**

The findings show clearly that the enzyme distinguishes between the carboxyl groups of β-glutamic acid and thus catalyzes the synthesis of only one optical isomer of β-glutamine and of its hydroxamic acid. It is of interest that \(D\)-β-glutamine rather than its enantiomorph is formed; although the enzyme acts on both optical isomers of glutamic acid, it amidates \(L\)-glutamic acid more rapidly than \(D\)-glutamic acid, and it is highly active on \(L\)-glutamic acid.

\[ ^1 \text{We thank Mr. Herbert M. Kagan for assistance in these determinations.} \]
stereospecific toward α-methylglutamic acid, acting only on the L isomer (9). The ability of both isomers of glutamic acid to act as substrates suggests that the functional groups of these amino acids can attach to the same enzyme sites. Although higher homologues of glutamic acid (α-aminoadipic acid, α-aminopimelic acid) exhibit some activity, aspartic acid is completely inactive, and it therefore appears that the enzymatically active conformation of the glutamic acid carbon chain is that in which the molecule is extended, or at least one in which the distance between the carboxyl groups is significantly greater than in aspartic acid. Assuming an extended chain conformation, it is possible to construct models of the isomers of glutamic acid in which the spatial positions of the nitrogen and carboxyl carbon atoms are similar (9). Thus, in Fig. 2, the model of α-glutamine is obtained by turning a mirror image model of L-glutamine until the amino group is in the same position as the amino group of the model of L-glutamine. Then the α-carboxyl group, γ carbon atom, and amide group of the model of D-glutamine are rotated to the positions shown.2

2 It may be noted that while the α hydrogen atom of L-glutamine is visible in the model shown in Fig. 2, the α hydrogen atom of α-glutamine is hidden since it is on the under surface of the model. Thus, the α hydrogen atoms are on opposite sides of these molecules; as discussed elsewhere (9), this probably accounts for the strict stereospecificity of glutamine synthetase toward α-methylglutamic acid. If the models as shown are assumed to be on the-
tamine is constructed (Fig. 2), the β-amino group is found to be oriented in the same direction as the α-amino group of L-glutamine. Furthermore, the relative positions in space of the nitrogen atom and the carboxyl carbon atoms of D-β-glutamine closely approximate those of L-glutamine. A model of L-β-glutamine was also constructed, and it was found that the carboxyl and amino groups of this model cannot be brought into the same relationship with each other as those of L-glutamine. Thus, only one of the optical isomers of β-glutamine has a conformation closely equivalent to that shown in the model of L-glutamine. Consideration of these models therefore leads to an explanation of the enzymatic findings, i.e. that both L- and D-glutamic acid are substrates, (the former being more active) and that the preferred conformation of β-glutamic acid on the enzyme is that which leads to D-β-glutamine synthesis.

SUMMARY

Glutamine synthetase from sheep brain catalyzes the synthesis of D-β-glutamine and D-β-glutamylhydroxamic acid from β-glutamic acid (β-aminoglutaric acid). The optical rotations of the N-acetyl derivatives of the enzymatically synthesized products are equal and opposite to those of the corresponding L-enzyme surface, replacement of the α hydrogen atom of D-glutamic acid by a methyl group would be expected to interfere with its attachment to the enzyme, while similar substitution of the α hydrogen atom of L-glutamic acid would not.

isomers, which were obtained from 3-L-acetylamino-4-carboxethoxybutanoic acid. The ability of glutamine synthetase to distinguish between the carboxyl groups of β-glutamic acid is considered in relation to other data on the specificity of this enzyme. A tentative explanation is proposed for the findings based on spatial similarity between extended conformations of L-glutamine and D-β-glutamine.

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