(from methanol), $[\alpha]_D^{20} - 23°$ (c 1; DMFA), R_f 0.64 (system 3); composition $C_{24}H_{28}O_{11}N_4$. On hydrolysis, p-nitrophenyl, glycine, and glucosamine were found in a ratio of 1 : 1.96 : 0.92.

p-Nitrophenyl 2-(γ-O-methyl-N-benzyloxycarbonyl-L-glutaminoylamino)-2-deoxy-β-D-glucopyranoside (IVb) was obtained similarly from 0.5 mmole of (IIa) and 1 mmole of the γ-methyl ester of N-benzyloxycarbonyl-L-glutamic acid; yield 55%, mp 198°C (from methanol), $[\alpha]_D^{20} - 20°$ (c 1; DMFA), R_f 0.7 (system 3), composition $C_{23}H_{34}O_{13}N_4$. On hydrolysis, p-nitrophenol, glycine, glutamic acid, and glucosamine were found in a ratio of 1 : 1 : 0.96 : 0.9.

SUMMARY

1. A method has been developed for the synthesis of p-nitrophenyl β-D-glucosaminides acylated with glucine and alanine.

2. p-Nitrophenyl N glycyl-β-D-glucosaminide has been found to be a substrate of a neutral β D-hexosaminodase.

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L-ASPARAGINASE BOUND IN A POLYACRYLAMIDE GEL

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UDC 577.155.32.678.745.842-148

In recent years, interest has risen in the immobilization of various enzymes, including enzymes of medical significance, in order to create new medicinal forms. The immobilization of L-asparaginase (L-asparagine aminohydrolaze, EC 3.5.1.1) has been performed hitherto mainly with the aid of adsorption [1] or by covalent attachment to the matrix [2-4].

On the inclusion of the enzyme in the lattice of a polyacrylamide gel, the molecule of L-asparagine – the substrate of L-asparagine – has small dimensions in comparison with the enzyme molecule. However, there is very little information on the properties of L-asparagine included in a polyacrylamide gel.

In order to fix L-asparaginase in the structure of a polyacrylamide gel we have used a modification of the method proposed by Miller [5]. The aim of the present work was to investigate the stability and some kinetic parameters of L-asparaginase bound in a polyacrylamide gel.

To elucidate the influence of the concentration of acrylamide and of the cross-linking agent N,N'-bisacrylamide on the inclusion of L-asparaginase in the gel, we performed a series of experiments in which the amount of acrylamide was varied from 3 to 15% at a constant concentration of N,N'-methylenebisacrylamide of 1.5%, and the amount of cross-linking agent was varied from 1 to 5% in a 6% polyacrylamide gel. The best results on the binding of L-asparaginase in a polyacrylamide gel were achieved at a 6% concentration of acrylamide with 1.5% of the cross-linking agent N,N'-methylenebisacrylamide.

A distinguishing feature of L-asparaginase is its capacity for retaining its activity over a wide pH range, with a maximum at pH 7.0-8.0. The bell-shaped curve of the pH dependence of the activity of the native enzyme contracts for the enzyme included in the gel, and in place of the plateau of the optimum activity pH a well-defined sharp peak of activity appears on the curve at pH 8.5.

Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, Riga. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 382-384, May-June, 1976. Original article submitted May 5, 1975.

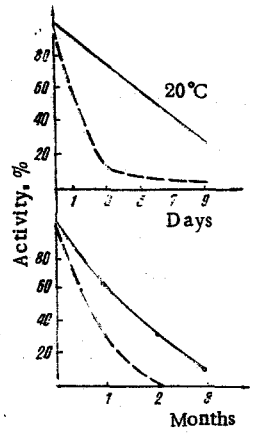

Fig. 1. Stability of soluble (– –) and immobilized (——) L-asparaginase on storage.

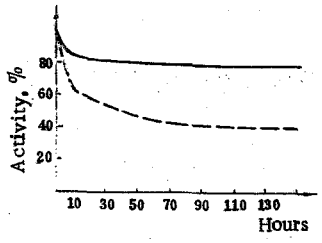

Fig. 2. Stability of L-asparaginase in physiological solution at 37°C (––– soluble; —— immobilized enzyme).

The kinetic behavior of L-asparaginase included in a polyacrylamide gel is largely identical with that of the native soluble L-asparaginase. The curves of the dependence of the initial rate of the reaction of the concentration of the substrate practically coincide with the two forms of the enzyme. The catalytic reactions of both the soluble and the bound enzyme follow the Michaelis–Menten equation.

The Michaelis constants (K_m) practically coincide. Below we give the values of K_m and information on the inhibition of soluble L-asparaginase and the enzyme included in the polyacrylamide gel:

L-Asparaginase	K_m(app.)	Inhibition by $3 \cdot 10^{-3}$ M S-benzyl-N-benzyloxy-carbonyl-L-cysteine, %
Soluble	$1.16 \cdot 10^{-5}$	95
Immobilized	$(1.60\text{-}2.00) \cdot 10^{-5}$	23

The reaction for the slight increase in the value of K_m for the bound L-asparaginase is obviously the diffusion resistance to the approach of the substrate molecule to the enzyme.

We also confirmed the inhibition of the activity of the enzyme included in polyacrylamide gel by one of the strongest competing inhibitors of soluble L-asparaginase–S-benzyl-N-benzyoxycarbonyl-L-cysteine. In a concentration of $3 \cdot 10^{-3}$ M, this compound inhibits the activity of the soluble enzyme by 95%, but of the immobilized enzyme by only 25%.

It may be assumed that the reduction in the sensitivity of the included enzyme to the action of a competing inhibitor is connected with the retarded diffusion of the S-benzyl-N-benzyloxycarbonyl-L-cisteine, the molecules of which are larger than those of L-asparagine.

The dependences of the rates of the reactions catalyzed by the two forms of the enzyme on the temperature between 0 and 5°C coincide. However, with a rise in the temperature the difference in the rates of inactivation increases, and at 60°C an appreciable stabilizing action of the binding of the soluble L-asparaginase appears. At 65°C, the two forms of the enzyme are inactivated to the same extent.

Aqueous suspension of the enzyme included in the gel show an increased stability on storage both at 0-4°C and at room temperature (Fig. 1).

An investigation of the stability of L-asparaginase included in polyacrylamide gel at 37°C in physiological solution, which stabilizes the native enzyme to a certain extent, showed a considerable advantage of the immobilized enzyme in comparison with the soluble L-asparaginase (Fig. 2). Thus, the L-asparaginase included in the polyacrylamide gel differs little in its kinetic behavior from the native enzyme but possesses an increased stability, thanks to which it may successfully replace the native enzyme in many analytical and enzyme investigations.

EXPERIMENTAL

In the investigations we used L-asparaginase from E. coli with a specific activity of 180 MU/mg of protein. The enzymatic activity of the L-asparaginase was determined by direct Nesslerization [6].

For the inclusion of the L-asparaginase in the lattice of the polyacrylamide gel, to 1 ml of aqueous solution of the enzyme containing 1300 MU with a specific activity of 180 MU/mg of protein we added 4 ml of 0.1 M tris-HCl buffer with pH 8.6, 320 mg of acrylamide, and 73 mg of N,N'-methylenebisacrylamide. The mixture was stirred for 5 min, and then 0.01 ml of tetramethylethylenediamine and 5 mg of ammonium persulfate were added. Polymerization of the mixture took place with a rise in the temperature. After 5 min, the reaction mixture was cooled and the test tube was immersed in an ice bath for 10 min, after which the mixture was allowed to stand at room temperature for 2 h. The gel so obtained was comminuted by its repeated passage through a medical syringe with a needle having a diameter of 1 mm and the particles of the gel were then washed with 10 volumes of 0.1 M tris-HCl buffer with pH 8.6 until enzymatic activity had disappeared completely from the wash waters. In this way, 5 ml of moist 6% polyacrylamide gel containing 195 MU/ml of L-asparaginase was obtained.

SUMMARY

The enzyme L-asparaginase from E.coli has been included in polyacrylamide gel, and some of its properties have been investigated: stability, pH dependence, heat stability, K_m. It has been shown that the enzyme-gel obtained has a better stability then the native enzyme.

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