AFFINITY CHROMATOGRAPHY OF E. coli L-ASPARAGINASE ON SILICATE SUPPORTS

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One of the methods of isolating enzymes that has recently come into wide use is affinity chromatography, which is characterized by high biospecificity, rapidity, and a high yield of enzyme preparation.

Kristiansen et al. [1, 2] were the first to use affinity chromatography to obtain the enzyme L-asparaginase from E. coli. A cell extract of the enzyme was purified on Sepharose 6B with D-asparagine chemically bound through putrescine or hexamethylenediamine. Unfortunately, the authors did not give either the yield or the degree of purification of the enzyme. In a method for purifying L-asparaginase proposed by Weetall [3], the affinity sorbent was porous glass modified with γ-aminopropyltriethoxysilane with anti-L-asparaginase antibody attached by the thiocyanate method. However, the initial bacterial extract purified on an immunosorbent with a degree of purity of 2-7 had a low yield of enzyme activity (6-11%).

We have proposed a method for the affinity chromatography of E. coli L-asparaginase using as support for the affinity sorbent Silochromes C-80 and C-120, and also carbonized calcium metasilicate. As ligand we selected one of the strongest and at the same time simplest inhibitors of L-asparaginase – D-asparagine.

The silicate support was treated with γ-aminopropyltriethoxysilane to introduce an amino group, and then D-asparagine was attached to it by the simple and convenient glutaraldehyde method.

Figure 1 shows the results of the affinity chromatography of L-asparaginase on a column of Silochrome-C-80-D-asparagine. The first fractions, eluted with 0.01 M K phosphate buffer, contained the enzyme to be purified together with ballast proteins not sorbed on the affinity sorbent. Then elution was carried out with a 0.5 M buffer containing 0.1 M D-asparagine. The fraction then collected contained L-asparaginase with a specific activity of 180 IU/mg. Elution with 2 M NaCl gave fraction III, containing a small amount of L-asparaginase and proteins that had obviously been sorbed nonspecifically on the affinity sorbent.

We then made a more detailed study of the possibility of using affinity sorbents for purifying L-asparaginase in bulk. For specific desorption we used both D-asparagine and L-aspartic acid. The results of the experiments are given in Table 1.

As can be seen from Table 1, a higher yield of enzyme was obtained by its desorption with 0.5 M K phosphate buffer containing 0.1 M D-asparagine, although desorption with 0.05 M buffer raised the specific activity of the enzyme.

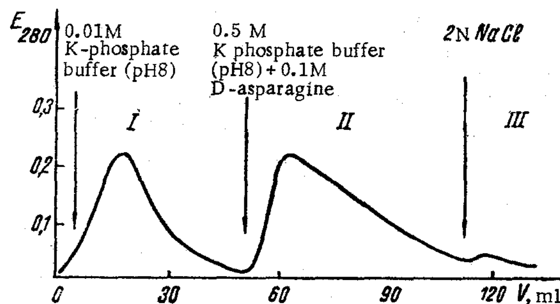

Fig. 1. Affinity chromatography of L-asparaginase in a column containing Silochrome-C-80-D-asparagine (the arrows show the composition of the eluting solution).

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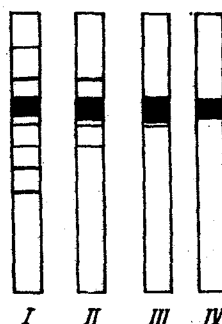

Fig. 2. Electrophoretograms of L-asparaginase: I) initial L-asparaginase product; II) after purification as described previously [4]; III) after affinity chromatography and desorption with 0.5 M K phosphate buffer containing D-asparagine; IV) after affinity chromatography and desorption with 0.05 M K phosphate buffer containing D-asparagine.

TABLE 1. Results of the Purification of L-Asparaginase by Affinity Chromatography in Bulk

Silicate support	Amount of affinity sorbent, g	Amount of L-asparaginase deposited, IU	Volume in sorption and desorption, ml	Time of sorption and desorption, min	Specific desorption*	Yield of L-asparaginase, %	Specific activity of the L-asparaginase, IU/mg	Degree of purification
Silochrome C-80	0,25	230	5	30	0.5 M buffer + 0.1 M D-asparagine	90	190	4,8
Silochrome C-80	0,5	860	10	45	0.05 M buffer + 0.1 M D-asparagine	60	235	5,8
Silochrome C-80	0,85	230	5	30	0.5 M buffer + 0.1 M L-aspartic acid	40	190	4,7
Silochrome C-120	0,5	460	5	30	0.5 M buffer + 0.1 M D-asparagine	50	193	4,8
Carbonized calcium metasilicate	0,5	860	5	45	0.05 M buffer + 0.1 M D-asparagine	20	244	6,0

*K phosphate buffer was used.

Very good results were obtained in the purification of L-asparaginase on an affinity sorbent consisting of carbonized calcium metasilicate as support, but the yield of purified enzyme was fairly low.

The L-asparaginase isolated by affinity chromatography was tested for purity by electrophoresis in polyacrylamide gel (Fig. 2) and was compared with the pure enzyme obtained by a method developed by us previously [4].

The proposed method provides the possibility of replacing a multistage purification of L-asparaginase by a single-stage method – affinity chromatography. At the same time, the high stability of the sorbent, its resistance to microflora, and the simplicity of regeneration with 2 M NaCl has enabled it to be used repeatedly for several years.

EXPERIMENTAL

For the purification of L-asparaginase we used the crude enzyme produced industrially with a specific activity of 40 IU/mg. As the support we used Silochrome C-80, Silochrome C-120, and carbonized calcium metasilicate.

The L-asparaginase activity was determined as described elsewhere [5], and the protein content by the Lowry method [6]. Disk electrophoresis in polyacrylamide gel was performed by Davis's method [7], and the enzymatic activity in the gel was determined by staining [8].

Synthesis of the Affinity Sorbent. The treatment of the silicates with γ-aminopropyltriethoxysilane and subsequent coupling with glutaraldehyde was performed in accordance with a method we have described previously [9]. Then, to 9.1 g of activated silicate in 70 ml of 0.01 M K phosphate buffer (pH 8.0) we added 350 mg of D-asparagine and stirred the mixture with the aid of a magnetic stirrer at room temperature for 24 h. After this, the product was filtered off and washed with water.

The affinity sorbent was stored in the refrigerator at +5°C.

The Sorption and Desorption of the L-Asparaginase on the Affinity Sorbent. 1. In a column. For this work we used a column with dimensions of 1.0 × 20 cm. For separation, 10-20 mg of the crude enzyme in 1 ml of 0.01 M K phosphate buffer (pH 8.0) was deposited on the column. The conditions of desorption are shown in Fig. 1.

2. In bulk. To 9.0 g of the affinity sorbent was added 90 ml of 0.01 M K phosphate buffer (pH 8.0) containing 450 mg of the initial crude L-asparaginase. The mixture was stirred with a magnetic stirrer for 30-45 min at room temperature. Then it was filtered and the residue was washed with the same buffer until the solution was free from protein.

The L-asparaginase was desorbed by treatment with 90 ml of 0.5 M or 0.05 M K phosphate buffer (pH 8.0) containing 0.1 M D-asparagine or L-aspartic acid. The mixture was stirred for 30-40 min, and the solid was filtered off and washed on the filter with two more portions of the above mentioned solution.

Regeneration of the Sorbent. For the complete desorption of the proteins, the affinity sorbent was washed with 2 M NaCl until proteins had disappeared completely from the solution. Then it was washed several times with water to eliminate the excess of NaCl.

The D-asparagine and L-aspartic acid were freed from impurities by dialysis against water.

SUMMARY

1. The synthesis is proposed of an affinity sorbent for the purification of L-asparaginase using silicate materials as supports and the inhibitor D-asparagine as ligand.

2. By the method of affinity chromatography on a column and in bulk, from a crude preparation of L-asparaginase with a specific activity of 40 IU/mg a highly purified enzyme has been obtained with a specific activity of 200-230 IU/mg with a yield of about 90% in one stage.

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