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 thiocyante 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 Silochrome 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).](image-url)
**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**

<table>
<thead>
<tr>
<th>Silicat support</th>
<th>Amount of sorbent, g</th>
<th>Volume of K phosphate buffer, ml</th>
<th>Time of separation, min</th>
<th>Specific activity before purification, IU/mg</th>
<th>Yield of L-asparaginase, %</th>
<th>Specific activity after purification, IU/mg</th>
<th>Degree of purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silochrome C-80</td>
<td>0.25</td>
<td>230</td>
<td>5</td>
<td>0.5 M buffer + 0.1 M D-asparagine</td>
<td>90</td>
<td>190</td>
<td>4.8</td>
</tr>
<tr>
<td>Silochrome C-80</td>
<td>0.5</td>
<td>860</td>
<td>10</td>
<td>0.05 M buffer + 0.1 M D-asparagine</td>
<td>60</td>
<td>235</td>
<td>5.8</td>
</tr>
<tr>
<td>Silochrome C-80</td>
<td>0.85</td>
<td>230</td>
<td>5</td>
<td>0.5 M buffer + 0.1 M L-aspartic acid</td>
<td>40</td>
<td>190</td>
<td>4.7</td>
</tr>
<tr>
<td>Silochrome C-120</td>
<td>0.5</td>
<td>460</td>
<td>5</td>
<td>0.5 M buffer + 0.1 M D-asparagine</td>
<td>50</td>
<td>193</td>
<td>4.8</td>
</tr>
<tr>
<td>Carbonized calcium</td>
<td>0.5</td>
<td>860</td>
<td>5</td>
<td>0.05 M buffer + 0.1 M D-asparagine</td>
<td>20</td>
<td>244</td>
<td>6.0</td>
</tr>
</tbody>
</table>

*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.