# ADSORPTION IMMOBILIZATION OF PREVIOUSLY MODIFIED L-ASPARAGINASE

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In the present work we have shown the possibility of applying adsorption immobilization with preliminary modification of the protein [1-3] to an enzyme — the asparaginase from *E. coli* (L-asparagine amidohydrolase, EC 3.5.1.1), which possesses a tetrameric structure and has mol. wt. 133,000.

For the experiments we used highly purified preparations of the L-asparaginase from *E. coli* [4] with an activity of 170-180 IU/mg. In selecting the optimum time of the modification of the asparaginase, we measured its activity at two different pH values (8.0 and 9.0) during its modification with a dye. A pH of 8 is the optimum for the action of the enzyme, and pH 9 was chosen on the assumption of a displacement of the optimum pH on the introduction of the negatively charged dye into the protein in order to follow the modification of the enzyme in a similar manner to that described previously [1]. A mixture of 2 mg of asparaginase in 2 ml of 0.1 M borate buffer, pH 8.3, and 1 mg of dye was incubated at room temperature. During incubation, samples were taken and their activities were determined at two pH values (Fig. 1). The activity at pH 9.0 rose during the first 5 min and then fell; the activity at pH 8.0 changed only slightly during the first 10 min and then decreased. Such results show a rapid modification of the enzyme in the first few minutes, leading to a displacement of the pH optimum and a comparatively small loss of activity during this time. On prolonged incubation with the dye, apparently, a far-reaching modification of the enzyme takes place with a great loss of its activity.

In order to obtain immobilized asparagine, modification was performed under the conditions described above and, after 10 min, portions of anion-exchange resin equilibrated with 0.1 M borate buffer, pH 8.3, were added. No more support was added after the decoloration of the supernatant liquid. The anion-exchange resin with the sorbed enzyme was washed with 0.5 M NaCl in order to eliminate the feebly sorbed and also the unmodified protein. Washing with solutions having a higher ionic strength is undesirable because of the dissociation of the subunits of the enzyme [5], while, as preliminary experiments showed, a 0.5 M solution of NaCl is sufficient for the desorption of the unmodified enzyme.

As carriers we tested DEAE-Sephadex A-50, DEAE-cellulose DE-32, and Amberlite CG-400 1. On washing with 0.5 M NaCl, part of the sorbed protein was eliminated, and this amounted to from 1 to 50% of the whole of the activity, depending on the sorbent used. The specific activities of the preparations of immobilized asparaginase are given below.

| Carrier | Desorption on washing with 0.5 M NaCl, % of the total activity in the experiment | Specific activity of the preparation, units/g |
|---|---|---|
| DEAE-Sephadex A-50 | 1 | 1750 |
| DEAE-cellulose DE-32 | 50 | 130 |
| Amberlite CG-400 1 | 11 | 200 |

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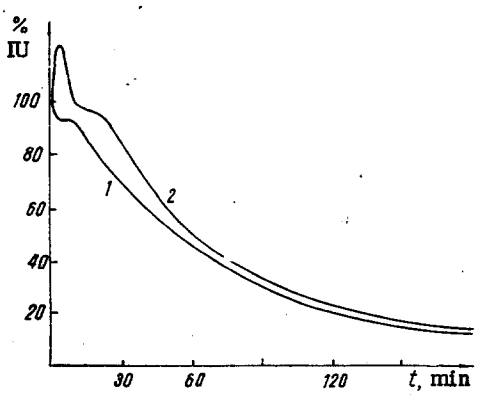


Fig. 1 Change in the activity of asparaginase during its modification with a dye: 1) activity at pH 8.0; 2) activity at pH 9.0 [activity expressed as a percentage of the initial activity (before the addition of the dye) taken as 100%].

The results obtained show that the adsorption method of immobilization with preliminary modification of the protein by a dye can be applied to asparaginase — an enzyme with a subunit structure. As a carrier it is possible to use practically any anion-exchange resin. The choice of anion-exchange resin carrier for immobilization should be dictated in this case not by the method but rather by the characteristic features of the problem of the practical utilization of the preparation.

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