PREPARATION OF ADSORBED LIPASE AND ITS PROPERTIES

K. D. Davranov, M. Rizaeva,
M. Z. Zakirov, and Zh. Diyarov

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Recently, the attention of many workers has been directed to the preparation of immobilized enzymes and to the study of their properties. This is due to the fact that immobilized enzymes are widely used in scientific investigations and for the solution of practical problems.

The present paper describes methods for obtaining lipase adsorbed on ion-exchange materials based on cellulose and the results of a study of some properties of the adsorbed lipase.

Table 1 gives the results of experiments on the adsorption of total protein and lipase on cellulose-based adsorbents. The best adsorbent is DEAE-cellulose. With an increase in the concentration on the enzyme solution, its adsorption capacity rises.

The lipase is not eluted from the adsorbent during the water elution of a certain amount of inactive proteins.

Then the influence on the sorption process of such factors as the pH, the ionic strength, the temperature, and the concentration of the enzyme solution from which sorption was performed was investigated and the optimum time of contact of the protein with DEAE-cellulose and the sorption capacity of the latter were determined.

The results relating to the influence of the ionic strength of the buffer solutions on the activity of the immobilized enzyme obtained showed that with a decrease in the ionic strength of the buffer solution the activity of the immobilized preparation obtained rises, a change in the buffer concentration from 0.05 to 0.001 M leading

TABLE 1. Adsorption of Protein on Cellulose-Based Ion-Exchange Materials

Absorbent, mg	Supernatant liquid after treatment		Eluate		Adsorbed	
	1	2	1	2	1	2
DEAE-cellulose						
	776,0	89,5	50,76	9,4	373,24	30,1
	689,7	57,0	69,30	9,4	441,00	52,6
	588,6	48,0	76,14	9,4	535,26	61,6
CM-cellulose						
	1109,8	72,0	53,76	9,6	36,44	37,4
	997,5	71,2	58,14	10,4	144,46	37,4
	891,0	67,5	64,68	14,2	244,32	37,5
ECTEOLA-cellulose						
	768,2	63,0	47,5	23,6	384,30	32,4
	658,3	61,7	64,1	18,4	477,60	38,9
	554,4	58,5	71,78	14,55	573,72	45,9

Note. Volume of enzyme solution 100 ml; amount of protein in the initial solution 1200 mg; lipase activity 119 units/ml; 1 – amount of protein, mg; 2 – total activity of the lipase, ml of 0.1 N KOH.

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TABLE 2. Influence of the pH of the Solution on the Activity of the Immobilized Lipase Obtained

pH	Activity of the complex obtained, ml of 0.1 N KOH		Protein content	
	units/g	% on initial	mg/g	% on initial
4,4	35,8	88,2	26,25	71,S
4,S	36,6	87,2	40,1	82,08
5,0	37,4	78,3	35,6	81,4
6,5	39,5	87,7	36,5	83,4
7,8	43,2	96,0	38,0	75,0
8,0	43,7	97,0	44,8	78,8
9,5	—	—	45,0	81,2

TABLE 3. Adsorption of Protein and Lipase Activity on DEAE-Cellulose

DEAE-cellulose, mg	Supernatant liquid		Eluate	
	1	2	1	2
1000	689,7	57,0	69,30	9,4
2000	588,6	48,0	76,14	9.4
2500	539,5	25,0	75,80	7,0
3000	462,5	7,0	79,25	—
3500	395,7	—	—	—

See note to Table 1.

to a 1.5- to 2-fold increase in the activity of the immobilized material. This shows that the presence of ions of an electrolyte in the solution has an adverse influence on the sorption of the enzyme (Fig. 1a).

An important factor affecting the ion-exchange sorption process is the pH of the medium. With a change in the pH from 4.4 to 5.0, the activity of the complex falls from 88.2 to 78.3% of the activity of the initial enzyme, but with a further rise in the pH the activity increases: at pH 7.8 to 96% and at pH 8.0 to 97%. With an even further rise in the pH of the enzyme solution the enzyme is inhibited (Table 2).

Results of an investigation of the influence of the time of contact of the support with the enzyme solution on the activity of the resulting immobilized enzyme are given in Fig. 1b. With an increase in the time of contact from 5 to 15 min the activity of the immobilized enzyme rose from 30 to 75%, respectively, on the activity of the initial enzyme. A further increase in the time of contact (to 25 min) did not affect the activity of the immobilized lipase obtained, but after this the activity fell.

Figure 1c shows the dependence of the lipase-sorption process on the temperature. A change in the temperature had different effects on the activity of the complex obtained and on the sorption of protein. With a rise in the temperature from 1 to 15°C the amount of protein sorbed on the support increased, and with a further rise in the temperature it decreased. The total activity of the immobilized enzyme increased from 70 to 80% with a rise in the temperature from 1 to 15°C and then, with a rise in the temperature to 30°C, it fell to 55% of the activity of the initial enzyme.

Experiments with different amounts of adsorbent at a constant concentration of protein and lipase activity (Table 3) show that with a rise in the amount of DEAE-cellulose as a constant volume of enzyme solution (100 ml) the adsorption of the enzyme rises, and at a ratio of adsorbent and enzyme solution of 3500 mg : 100 ml complete adsorption takes place.

On elution with 0.1 M phosphate buffer containing 1.0 M NaCl, the lipolytically active protein passes into the solution quantitatively, the specific activity of the enzyme rising 3- to 4-fold in this process in comparison with the initial extract.

For comparison with the native (soluble) lipase, we determined the influence of the pH and the temperature on the DEAE-cellulose–lipase. Fig. 2a shows the dependence of the activity of the immobilized lipase on the pH of the medium. It was found that immobilization did not change the nature of the dependence of the acti-

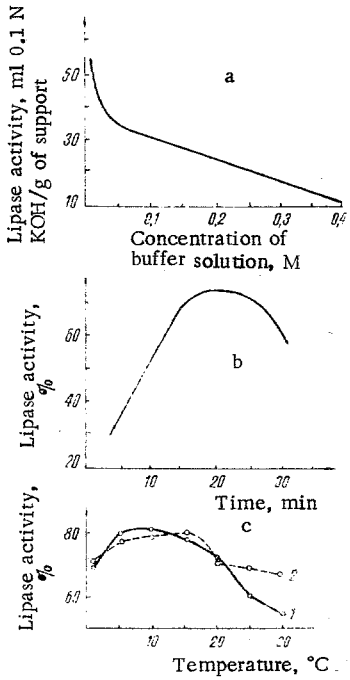

Fig. 1. Influence of the concentration of buffer solution (a), on the time of contact of the support with the enzyme solution (b), and of the sorption temperature (c) on the activity of the resulting immobilized enzyme (1 – activity; 2 – protein).

vity of the lipase on the pH but it appreciably broadened the pH ranges of its activity: while for the native lipase they were 4.6–4.8 and 7.8–8.0 [2], for the immobilized enzyme they were 4.4–5.6 and 7.0–8.6, the pH optima for both the native and the immobilized enzymes being 4.8 and 7.8, respectively.

Figure 2b shows the dependence of the activity of the immobilized lipase on the temperature at the optimum pH. The general nature of the dependence of the lipase activity on the temperature had not changed as the result of immobilization.

In contrast to the native lipase, having a temperature optimum of 40°C under the given conditions, the immobilized lipase had its optimum activity at 45–47°C. Consequently, immobilization led to some increase in the heat stability of the lipase, which is characteristic for a number of other immobilized enzymes [3].

EXPERIMENTAL

The enzyme was obtained by a method described previously [1]. The protein was determined from the absorption at 280 nm on an SF-16 spectrophotometer. The lipase activity was determined as described previously [2].

The materials used were diethylaminoethylcellulose (DEAE-cellulose), (TU [Technical Specification] 6-09-64471), carboxymethylcellulose (CMC) (TU-10P-108-67), and ECTEOLA-cellulose (MRTU [Interrepublican Technical Specification] 6-09-6490-69) – materials of Soviet manufacture.

Preparation of the Adsorbed Lipase. A weighed amount of moist DEAE-cellulose (W=80-85%) was placed in a glass beaker and then the required amount of enzyme solution with the given concentration of enzyme and the necessary pH value was added. The beaker was placed on a magnetic stirrer in a thermostat and was left for a predetermined time. After incubation, the contents of the beaker were transferred to a porous glass filter and the support with the sorbed enzyme was separated from the liquid. The filtrate was collected in a test-tube and the concentration of protein in it was determined, while the immobilized enzyme was washed with distilled water until there were no longer traces of protein in the wash waters. From the preparation of immobilized enzyme dried in vacuum on the glass filter a sample was taken for an activity determination. The amount of sorbed protein was calculated from the difference in the initial concentration and the concentration in the filtrate after sorption.

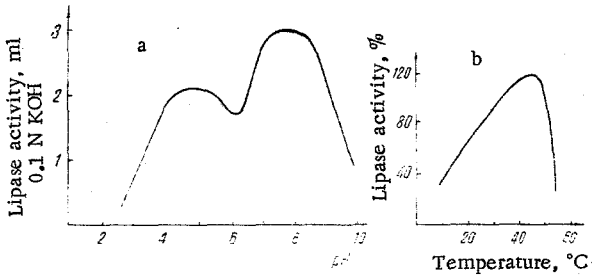

Fig. 2. Dependence of the activity of the immobilized lipase on the pH and on the temperature (0.1 M phosphate buffer, pH 7.8).

The influence of the ionic strength of the buffer solutions on the ion-exchange sorption process was investigated in phosphate buffer (pH 7.8) at room temperature (18-20°C) with a time of contact of the support with the enzyme solution of 15 min. The influence of the pH of the enzyme solution on the production of an active insoluble enzyme was determined at 5°C with a contact time (of the enzyme solution with the support) of 15 min. The pH of the reaction mixture was changed by adding dilute solutions of ammonium hydroxide or acetic acid.

The influence of the temperature on the preparation of the active immobilized enzyme was studied at pH 7.8 with a contact time of 15 min. The dependence of the activity of the immobilized preparation on the time of contact was investigated at pH 7.8 at room temperature.

The sorption capacity of DEAE-cellulose for the lipase was determined at pH 7.8, temperature 20°C, and a time of contact (of the support with the enzyme solution) of 15 min.

SUMMARY

1. Under certain conditions, lipase immobilized on cellulose-based ion-exchange materials can be obtained. The best adsorbent is DEAE-cellulose.

2. The optimum conditions for the immobilization of lipase on DEAE-cellulose are pH 8.0, time of contact with the support 15-20 min, temperature 10°C.

3. The immobilized lipase (DEAE-cellulose–lipase) retains the main functional properties of the native enzyme.

LITERATURE CITED

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