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THE USE OF ISOCYANIDES FOR THE IMMOBILIZATION OF BIOLOGICAL MOLECULES ON CELLULOSE CARRIERS

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Тийна ЛИННАС, О. КИРРЕТ. ПРИМЕНЕНИЕ ИЗОЦИАНИДОВ ДЛЯ ИММОБИЛИЗАЦИИ БИОЛОГИЧЕСКИХ МОЛЕКУЛ НА ЦЕЛЛЮЛОЗНЫХ НОСИТЕЛЯХ

Support materials, such as polyacrylamide and dextran derivatives used for enzyme immobilization and affinity chromatography are not ideal for solving some problems at large-scale operation in columns. We have investigated the covalent fixation of biologically active substances to carboxymethylcellulose (CM-cellulose), aminoethylcellulose (AE-cellulose) and 4-β-oxyethylsulfonyl-2-aminoanizole ether of cellulose (CE).

In this paper we report experiments performed in order to find conditions which permit high coupling efficiency. A model compound, trypsin, has been immobilized on various cellulose carriers. The dependence of the activity of the carrier on the pH of binding, the binding time and the medium were investigated.

Material and methods

Enzymes. Bovine trypsin (EC 3.4.4.4) «B» was obtained from the Olaine Works (USSR). Immobilized trypsin was assayed with BAEE (N-benzoyl-L-arginine ethyl ester) at 25 °C with the pH-stat method.
Chemicals. BAEE·HCl was obtained from Reanal Co. (Hungary). CM-cellulose and AE-cellulose were produced by the Yerevan Chemical Works, and CE was synthesized in our laboratory by the method described in [1]. Cyclohexylisocyanide was synthesized in our laboratory by the method described in [2].
Binding conditions. Trypsin was dissolved in 7 ml of 0.1 N NaCl solution, at pH 6.0, 1 g of carrier, 50 ml of acetaldehyde and 125 ml of cyclohexylisonitrile being added. The solution was shaken for 4 h at room temperature and washed with 0.1 N NaCl solution (pH 6.0).

Results and discussion

We have investigated the covalent fixation of biologically active substances to polymers using the four-component condensation of an isocyanide, a primary amine, a carbonyl compound and a carboxylic group.

Dependence of the activity of carriers on the pH of binding, binding time and medium (bound enzyme — trypsin, buffer by which the enzyme is bound to the carriers — 0.1 N NaCl)

Carrier	Amount of carrier, g	Amount of bound enzyme, mg	pH of binding	Dependence of activity of carriers on pH after binding, U/g	Storage conditions of carriers	Days							
						0	2	4	10	17	19	27	31
CM-cellulose	1	100	6	35	acetate buffer pH 4.0	55	activity disappears						
CM-cellulose	1	100	6	64	solution of NaCl pH 6.0	64		58	activity disappears				
AE-cellulose	1	100	6	46	acetate buffer pH 4.0	46	activity disappears						
AE-cellulose	5	500	6	222	solution of NaCl pH 6.0	222			161	145	140	125	125
CE	5	500	6	146	0.2 N KH_2PO_4+NaOH buffer pH 4.0	146			117	72.5	activity disappears		
CE	1	100	6	56	acetate buffer pH 4.0	56	activity disappears						
CE	1	100	3	186.5	solution of NaCl pH 6.0								
CE	1	100	4	182.5	„								
CE	1	100	6	191	„								
CE	1	100	8	156	„								

Several biological substances contain amino, carbonyl or anionic groups or combinations of such groups and can thus be attached to a variety of polymeric carriers containing one or more reaction components.

The described procedure obviously implies a great flexibility in the choice of a system for the immobilization of each compound. The reaction may proceed not only in organic solvent systems but also in aqueous solution at physiological pH values, thus decreasing the risk of denaturation or decomposition of the pH-sensitive biological structures.

In this work three different carriers were compared, viz. AE-cellulose and CM-cellulose, in the case of which both amino and carboxylic groups were introduced into the cellulose macromolecule, and ether of cellulose, where the aromatic amino group was introduced into the cellulose macromolecule by alkylation with water-soluble 4-β-oxyethylsulfonyl-2-aminoanizole sulphate ether.

As seen from Table, trypsin was bound to CE at different pH values. The binding at pH 6.0 appeared to be best, the effect being highest, 191 U/g.

The bound carriers were stored in 0.1 N NaOH solution (pH 4.0), 0.2 M KH_2PO_4/NaOH buffer (pH 6.0) and acetate buffer (pH 4.0). The results obtained are presented in Table.

As follows from experimental data, all the three carriers, AE-cellulose, CM-cellulose and CE, stored in acetate buffer, lost their activity on the 2nd day already. CM-cellulose, which was kept in NaCl solution, lost its activity on the 10th day. A comparison of the above carriers showed that AE-cellulose kept in 0.1 N NaCl solution at pH 6.0 appeared to be best, its activity being 222 U/g which is much higher than that of carriers kept under other conditions, and the activity, 125 U/g, was retained even on the 31st day.

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