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PREPARATION OF IMMOBILIZED ALBUMIN AND ITS PROPERTIES

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An immobilized form of human albumin on a polyamide support has been obtained. The complex-forming properties of the immobilized albumin with egg yolk phospholipids and essential phospholipids have been studied.

The production of immobilized albumin is necessary for solving a whole series of problems of both scientific and practical importance. In the first place, albumin bound to a solid matrix may serve as a reagent for the purification of antibodies to this protein. In the second place, it can be used for performing immunoenzyme analysis with the aim of revealing antibodies to albumin and also for monitoring the immune state of the organism. Finally, and most importantly, immobilized albumin can be used as a unique agent for studying the complex forming properties of human serum albumin (HSA) in the norm and in pathology. Here, complex-formation can be studied by the direct method without using the indirect approaches that, as a rule, give distorted results [1].

The production of immobilized albumin on agarose or polystyrene matrices is known [2]. Our task was to obtain albumin preparations immobilized on a polyamide support. Such polymeric matrices have previously been used successfully for the immobilization of a number of such proteins as trypsin, phospholipase, proteinase inhibitor, alphatoxin, protein components of snake venoms, and antibodies [3]. The method of "grafting" the protein onto amino groups with the aid of dialdehydes and the formation of covalent bonds was used for immobilization [4].

As has been shown previously [3], type P-6 polyamide (Kapron) fully satisfies the demands set for a support [2]. Limited hydrolysis of the polyamide in hydrochloric acid permits the creation of the necessary number of reactive amino groups on the surface and in the pores of the support, and by a specially developed technique of precipitating the polyamide from solution it is possible to obtain various forms of sorbent for chromatographic purposes [5].

The support and the human albumen that we selected possess reactive amino groups forming covalent bonds when such dialdehydes as gossypol and glutaraldehyde are used as inserts. Immobilization was achieved by a standard procedure [5]. The amount of albumin bound was determined by the difference (before and after immobilization) in the amounts of albumin by a spectrophotometric method. The proportion of bound HSA amounted to 81% of the initial amount.

It is known that albumin makes up more than half the weight of the proteins of human blood plasma, and one of its main functions is binding of both high- and low-molecular-mass compounds (proteins, toxins, biologically active substances, metabolites, and drugs) and their transport into the liver [1].

We checked whether the binding capacity of the albumin was retained after immobilization. As ligands for binding we used egg yolk phospholipids (EYPs) [7] and also the drug preparation Essentsiale, which likewise has a phospholipid nature (EP). The complex-forming capacity of the immobilized HSA was judged from the amount of phosphorus bound by it (Table 1).

The results obtained permitted the plotting of curves showing the degree of binding of inorganic phosphorus (P_i) [sic — and below] as a function of the concentration of added phospholipids (Fig. 1). As a maximum, 4 mg of immobilized HSA bound 127.5 μl of EYPs (27.84 μg of P_i) and 212 μl of EP (48.8 μg of P_i). Binding was studied with the use of 0.05 M tris-HCl buffer, pH 7.2.

Increasing concentrations of EYPs and EPs were added to test tubes each of which contained 4 mg of immobilized HSA in buffer solution (see Table 1). The tubes were kept at 4°C with constant stirring for 24 h. After the supernatant liquid had been poured off, the deposit was washed with the buffer solution 3-4 times. After each wash, the solid was separated by centrifugation (5000 rpm).

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TABLE 1. Amount of Bound Inorganic Phosphorus Present in Egg Yolk Phospholipids and Essentsiale Phospholipids

Test tube No.	Amount of P_i in the EYPs, μg		Test tube No.	Amount of P_i in the EP μg	
	initial	bound		initial	bound
1	Control		1	Control	
2	11.84	3.92	2	21.28	1.48
3	15.68	9.44	3	25.28	2.36
4	17.6	15.04	4	36.64	5.16
5	27.84	20	5	36.8	6.64
6	30.72	16.64	6	39.04	8.8
			7	48.8	10.32
			8	58.56	9.2

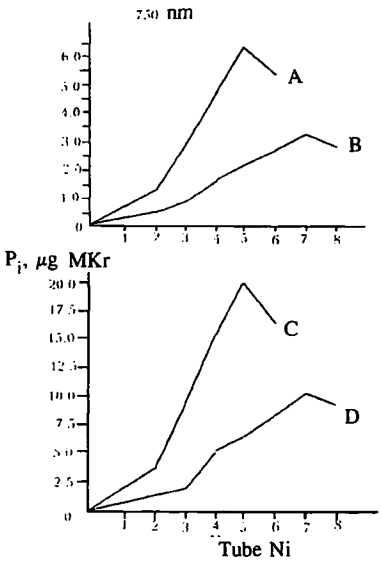

Fig. 1. Curves of the binding of inorganic phosphorus (P_i) in relation to the concentration of added phospholipids: A) curve plotted from the intensities of absorption of the solution for egg yolk phospholipids; B) curve plotted from the intensities of absorption of the solution for Essentsiale phospholipids; C) curve of the binding of inorganic phosphorus in relation to concentrations of egg yolk phospholipids; D) curve of the binding of inorganic phosphorus in relation to concentrations of Essentsiale phospholipids.

The deposits were heated to 100°C and dried. Then, to each tube was added 1.2 ml of perchloric acid ($HClO_4$) and the mixture was kept for an hour at 200°C, after which 1.3 ml of the Vas'kovskii reagent II [7] was added and the tube was placed in a water bath at 100°C for 30 min.

After cooling, the intensity of absorption of each solution was measured on a SF-410 spectrophotometer at a wavelength of 750 nm (see Fig. 1, A, B). The intensities of absorption of the solutions were plotted on a calibration curve [7] and the amounts of bound phosphorus (P_i) were determined (see Fig. 1, C, D).

Thus, an immobilized form of human albumin on a polyamide support has been obtained and its complex-forming properties have been studied with egg yolk phospholipids and Essentsiale phospholipids.

EXPERIMENTAL

Immobilization. A suspension of a weighed sample of polyamide powder (2 g) in 10 ml of 3 N HCl was stirred at 45°C for 2 h. The polyamide was washed with distilled water and with 0.1 N borate buffer, pH 8.5.

After each washing the polyamide was separated off by centrifugation (5000 rpm). To a suspension of the activated polyamide equilibrated in 0.1 M borate buffer was added 10 ml of a 2.5% solution of glutaraldehyde, and the mixture was incubated at room temperature with constant stirring for 15 h. The glutaraldehyde that had not reacted was washed out with the same buffer solution. Then a solution of 100 mg of HSA in 10 ml of buffer solution was added to a suspension of the modified polyamide in the same buffer solution. The mixture was kept at 4°C with constant stirring for 24 h. The nonbound part of the albumin was eliminated by washing with 10 volumes of diethyl ether.

Blockage of Aldehyde Groups. Ethanolamine in an amount of 0.35 ml per 1 g was added to the sorbent equilibrated in 0.1 M borate buffer, pH 8.5. This led to blockage of the remaining free aldehyde groups of the support. The excess of ethanolamine was eliminated by washing the sorbent with the buffer solution.

Determination of Phosphorus. A solution of 5.3 mg of anhydrous KH_2PO_4 in 100 ml of distilled water was prepared; it contained 53 μg of KH_2PO_4 or 13 μg of P_i in 1 ml. To each of six test-tubes were added the KH_2PO_4 solution [7], 0.2 ml of perchloric acid ($HClO_4$), and 1.3 ml of the Vas'kovskii reagent II. The tubes were heated in a water bath at 100°C for 30 min. After cooling, the intensity of absorption of each solution was measured on a SF-410 spectrophotometer at a wavelength of 750 nm and a calibration curve was plotted [7].

Determination of Protein. Protein concentrations were estimated spectrophotometrically from the optical densities of solutions at 280 and 260 nm.

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