COTTONSEED GLOBULINS

V. 11S GLOBULIN

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Cottonseed globulins have been isolated by a number of workers [1–4]. It has been established that the total globulin fraction consists mainly of arginine and histidine components. The quaternary structure of the arginine component has been studied [4]. The molecular weight of the histidine globulin, according to Ibragimov et al. is 140,000, and according to Ovchinnikova et al. it is about 300,000. This difference may be a consequence of a change in the quaternary structure of the protein during the isolation process.

We have isolated the histidine globulin [1] in order to study its structure. Immediately after purification, the protein was dissolved in 8 M urea solution with pH 7.4. The mixture obtained was deposited on a column of DEAE-cellulose equilibrated with 8 M urea, pH 7.4. The results of chromatography are shown in Fig. 1a.

On gel filtration through a column of Sephadex G-75 (fine) equilibrated with a 0.1% solution of NaSDS fraction I gave two peaks (Fig. 1b). After chromatography, two protein fractions were obtained which were homogeneous on disk phoresis in the presence of 0.1% of NaSDS. Thus, we isolated three subunits. We determined the molecular weights of the subunits by disk phoresis: I' – 28,000; II' – 24,000, II – 12,000.

The amino-acid compositions of the subunits were as follows (mol. %) (see following page).

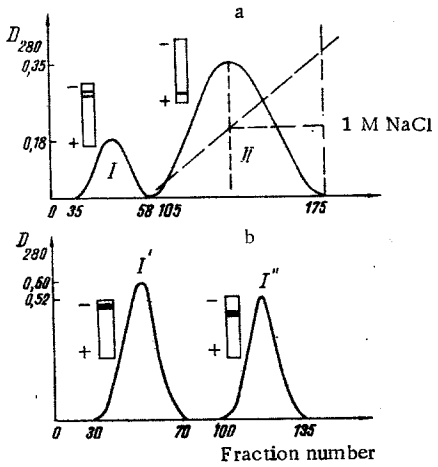

Fig. 1. Chromatography of the histidine globulin on DEAE-cellulose (a) and gel filtration of fraction I on Sephadex G-25.

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Amino acid	I'	I"	II
Asp	10.3	10.0	10.9
Tre	5.0	8.5	4.7
Ser	8.7	6.1	8.7
Glu	18.6	14.8	25.8
Pro	6.3	Tr	4.9
Gly	8.5	14.5	8.2
Ala	8.5	9.4	6.3
Val	8.5	10.2	7.5
Met	0.6	Tr	Tr
Ile	5.5	5.1	3.6
Leu	6.3	7.3	4.4
Tyr	1.0	1.5	2.0
Phe	4.0	3.6	4.9
His	2.1	2.2	2.6
Lys	3.3	6.5	2.5
Arg	11.9	4.0	9.4
Carbohydrates	–	–	3%
Trp (number of moles per mole of protein)	+	4	1

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THE PREPARATION OF BIOSPECIFIC SORBENTS BY THE ACTIVATION OF SEPHAROSE WITH p-BENZOQUINONE

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The method usually used for obtaining biospecific sorbents is the activation of Sepharose by cyanogen bromide followed by the addition of a ligand at the amino group [1], this method being associated with the use of an extremely toxic reagent. Recently, Porath et al. [2] have proposed a method of immobilizing enzymes which is based on the activation of Sepharose with p-benzoquinones and the addition of the protein to the activated Sepharose. It appeared important to study of the possibility of using this method for adding amines and obtaining biospecific sorbents.

The activation of Sepharose was performed in accordance with Porath's procedure: 10 g of washed and settled Sepharose 4B in 6 ml of 0.1 M $NaHCO_3$ (pH 8) was mixed with a solution of 0.108 g (1 mmole) of p-benzoquinone in 4 ml of ethanol. The suspension was stirred at room temperature for 1 h, and the activated Sepharose was washed with 20% ethanol, water, 1 M NaCl, and water again. The ligands used were N-2,4-

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