ISOLATION OF CYTOKININ-BINDING PROTEINS FROM COTTON-PLANT SEEDLINGS

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In this paper we consider the isolation of a protein factor present in cotton-plant seedlings and possessing specific affinity for highly labeled cytokinin – [^{3}H]-benzyladenine (^{3}H–BA).

Three-day etiolated seedlings of the cotton plant Gossypium hirsutum, variety 48-80 in an amount of 10 g were homogenized in buffer A (50 mM Tris-HCl, 5 mM EDTA, 2.5 mM $MgCl_2$, 50 mM KCl, pH 7.4). Homogenization and all the following operations were performed strictly at 2-4°C. Large cell organelles (nuclei, chloroplasts, and mitochondria) were eliminated by centrifugation at 18,000 × g for 40 min. The proteins were salted out from the supernatant with ammonium sulfate at 100% saturation and, after centrifugation at 18,000 × g for 30 min, the solution was dialyzed against water. An affinity column (1.8 × 7.0 cm) containing BA immobilized on iodoacetamidohexyl-Sepharose 4B was equilibrated with buffer B (50 mM Tris-HCl, pH 7.6), and 15 ml of the dialysate was deposited on it. The unbound proteins were eluted by the same buffer until the base line at 280 nm was reached, and then the cytokinin-binding proteins (CBPs) were eluted with 1 M NaCl (Fig. 1). The amount of proteins in peak II after dialysis was 4.8 mg.

To determine cytokinin-binding activity, ^{3}H–BA with a specific radioactivity of 2-8 TBq/mmole, which we obtained by the method of heterogeneous catalytic exchange in gaseous tritium over $Pd/BaSO_4$, was used.

Aliquots containing from 0.5 to 5 nM ^{3}H–Ba were incubated in buffer B with 10 ml of protein solution (150 μg/ml) for an hour. Nonspecific binding was determined in the presence of a thousandfold excess of unlabeled BA. After incubation, the ^{3}H–BA–CBP complex that had

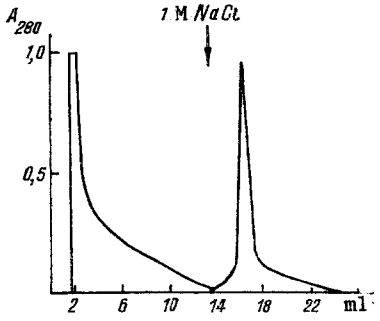

Fig. 1

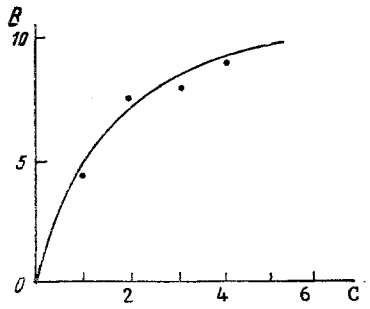

Fig. 2

Fig. 1. Affinity chromatography of the CBPs from benzyladenine-Sepharose 4B (along the axis of abscissas: the concentration of protein expressed as $A_{280\ nm}$; along the axis of ordinates: fraction numbers).

Fig. 2. Binding of ^{3}H–Ba with the protein fraction (peak II). C: concentration of ^{3}H–Ba, nM; B: bound ^{3}H-BA in pmole/mg of protein.

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been formed was separated by filtration on Synpor filters with a pore size of 0.4 μm. The filters were dried and the radioactivity of the deposits on them was counted in toluene scintillator (ZhS-1) in a Beta-1 counter.

As follows from Fig. 2, ^{3}H-BA specifically and reversibly binds to the CBPs. The high affinity of ^{3}H-BA for the isolated site of the proteins shows that they belong to the CBPs.

BIOSPECIFIC SORBENTS FOR OBTAINING AND PURIFYING ACTIVE FRAGMENTS OF ANTIBODIES TO α-LATROTOXIN

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The most effective and specific agent for the treatment and prophylaxis of poisonings by the venom of the spider Latrodectus tredecimguttatus consists of antivenom sera (Tashkent Scientific Research Institute of Vaccines and Sera) obtained against the whole venom. However, the quality of the immunoglobulin preparations made deteriorates considerably as the result of the aggregation of the protein components of the serum and also of the cleavage taking place during the fractionation process.

At the present time, enzymatic hydrolysis, treatment with β-propiolactone, and other methods are used for breaking down the aggregates [1]. When any of the methods mentioned are used, the immunoglobulin preparations become contaminated with these compounds. To prevent this it is desirable to synthesize ligand-immobilized sorbents to isolate the active $F(ab')_2$ fragments of the antibodies which retain the properties of the immunoglobulins and are free from the possibility of aggregation.

In order to isolate the $F(ab')_2$ fragments of antibodies for the hydrolysis of the immunoglobulins, various enzymes are used in the form of their aqueous solutions [2]. Here, difficulties arise that are connected with the additional purification of the hydrolysis products. We have attempted to convert pepsin, which cleaves the antibody molecules into $F(ab')_2$ and F_C fragments, into an insoluble form by immobilizing it on a cellulose sorbent [3].

Protein A from Staphylococcus aureus has affinity for the F_C fragment of certain types of immunoglobulins and forms a noncovalent bond with them [4]. We have used this property of protein A for separating the $F(ab')_2$ fragments from the F_C fragments of antibodies obtained previously [5]. Pepsin (Worthington) and protein A (Pharmacia) were immobilized on the cellulose sorbent Tsellopor as described in [3]. The amount of proteins bound to the Tsellopor was determined form the binding of Bromophenol Blue [6].

A solution of 10 mg of antibodies in 0.15 M sodium chloride solution was transferred by dialysis into 0.1 M acetate buffer, pH 4.5, and was incubated with pepsin immobilized on the sorbent at 37°C for 10 h.. The hydrolysate was subjected to gel chromatography on a column of TSK HW-55 equilibrated with 0.15 M NaCl. On a comparison of chromatograms of the native antibodies and their peptic hydrolysate, peaks of low-molecular-mass components belonging to the $F(ab')_2$ and F_C fragments were observed. The peptic hydrolysate was then incubated for an hour with the protein A-Tsellopor sorbent. On gel chromatography of the hydrolysate, the peaks corresponding to the unhydrolyzed molecules of the immunoglobulins and the F_C fragments practically disappeared through binding with the protein A-Tsellopor sorbent, which can be regenerated and used repeatedly.

Thus, the biospecific sorbents synthesized can be used repeatedly for obtaining and purifying active fragments of antibodies free from a capacity for aggregation. The isolated

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