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ISOLATION OF PROTEIN-NUCLEIC COMPONENTS OF COTTON CHROMATIN AND INVESTIGATION OF THEIR INTERACTION WITH THE CYTOKININ-RECEPTOR COMPLEX

I. A. Arzanova, O. N. Veshkurova, and Sh. I. Salikhov UDC 547.577.112.582.796

Cotton chromatin was fractionated into its components. Fractions of histone proteins, nonhistone proteins (NHp-1, NHp-2, NHp-3), and DNA were obtained. Their interactions with ^{3}H-BAP and ^{3}H-BAP-CBP complex were investigated. The RNA-polymerase activities of the obtained fractions were investigated. Fraction NHp-2 was shown to bind specifically hormone and its complex with the receptor. RNA-polymerase was localized in fraction NHp-2.

Key words: chromatin, cytokinin-receptor protein, ^{3}H-benzylaminopurine, cotton.

The molecular mechanisms of hormone signal transduction are related to the interaction of the hormone-receptor complex (HRC) with chromatin. It was previously demonstrated that a complex of steroidal hormones with the receptor is capable of recognizing specific DNA sequences and binding to them [1]. In order to elucidate the role of plant chromatin in HRC acceptance, we isolated chromatin from nuclei of 3-day cotton sprouts and fractionated it into structural components. We obtained one histone and three nonhistone fractions (differing in degree of DNA binding) and one DNA fraction. We fractionated the chromatin on Ultragel HA hydroxyapatite using a concentration gradient of sodium phosphate (Fig. 1). This is one of several one-step methods that has been applied previously to separate chromatin of animal cells into its components [2].

Histone proteins (Hp), which are most weakly bound to chromatin, are eluted from the column without binding to the sorbent. Nonhistone proteins (NHp-1) were eluted by 50 mM phosphate buffer. A fraction of nonhistone proteins (NHp-2) that was eluted by more concentrated salt (200 mM) contains the bulk of chromosomal RNA. A fraction of strongly bound nonhistone proteins (NHp-3) was eluted by 2 M guanidinium chloride; the DNA fraction, by 500 mM phosphate buffer.

We then studied the ability of each fraction to bind to the cytokinin receptor complex and the synthetic phytohormone ^{3}H-BAP (benzylaminopurine). The affinity was estimated from the amount of bound radioactivity. The binding constant was determined by the Scatchard method [3] (Fig. 2).

We studied the localization of RNA-polymerase in the isolated fractions because the stimulating effect of the HRC is related to activation of RNA-polymerase localized in chromatin. Table 1 presents data for the specificity of HRC binding and ^{3}H-UTP incorporation into the fractions isolated from chromatin. The specificity of the binding was determined from the difference in the binding of fractions with the HRC and the hormone.

The binding ability of the HRC with chromatin components was estimated by performing six series of experiments. At first, the binding of ^{3}H-BAP to chromatin fractions was estimated. Then, this same experiment was performed in the presence of unlabeled BAP at a concentration of 1 mM to determine the nonspecific binding. Then, binding of ^{3}H-BAP to chromatin fractions in the presence of CBP and also in the presence of the HRC (BAP-CBP) was estimated. The ability of ^{3}H-BAP to bind only to CBP was estimated. Finally, binding of labeled BAP to CBP in the presence of unlabeled BAP was determined.

Table 1 shows that fraction NHp-2 has the highest affinity as an acceptor. This agrees with data for steroidal hormones [1]. RNA-polymerase was localized mainly in fraction NHp-3.

A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 162 70 71. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 421-422, September-October, 2000. Original article submitted November 13, 2000.

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TABLE 1. Specific Activity of Fractions Isolated from Cotton Chromatin

Chromatin fraction	K_d µM per mg protein	Specific binding (CBP-^{3}H-BAP) with fractions		^{3}H-UTP incorporation into fractions	
		counts/min	%	counts/min	%
1. Histone Hp	0.10	1257	84	1080	131
2. Nonhistone NHp-1	0.13	2016	135	991	120
3. Nonhistone NHp-2	0.10	3056	205	1168	142
4. Nonhistone NHp-3	0.14	964	65	1717	209
5. DNA	0.15	358	24	-	-

Note: Binding of ^{3}H-BAP-CBP with chromatin (1491 counts/min) is taken as 100%.

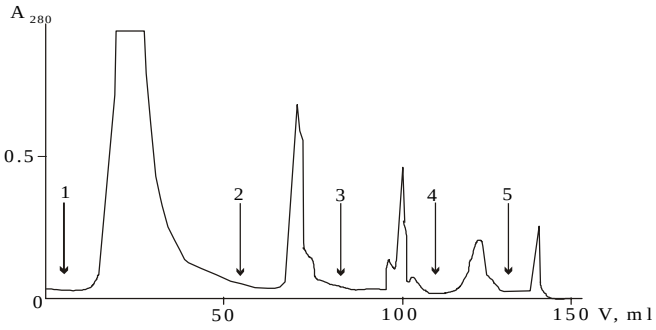

Fig. 1

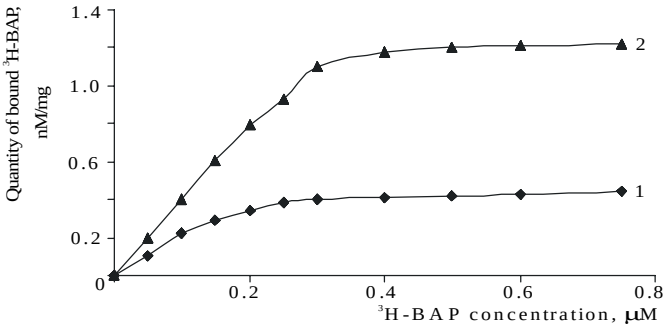

Fig. 2

Fig. 1. Chromatography of chromatin proteins on Ultragel HA hydroxyapatite column (2.9×8 cm). Elution rate 5 ml/h. Eluents: 2 M NaCl, 5 M urea, 1 mM sodium phosphate at pH 6.8, fraction Hp (1); 0.05 M sodium phosphate at pH 6.8, fraction NHp-1 (2); 0.2 M sodium phosphate at pH 6.8, fraction NHp-2 (3); 2 M guanidinium chloride, fraction NHp-3 (4); 0.5 M sodium phosphate at pH 6.8, fraction DNA (5).

Fig. 2. Specific binding of ^{3}H-BAP with fractions: DNA (1) and NHp-2 (2).

EXPERIMENTAL

We used 3-4-day cotton (*Gossypium hirsutum* L., variety 175-F) sprouts.

CBP was isolated by the literature method [4]. The fraction containing chromatin was prepared by the literature method [5].

Chromatin fractionation was performed by chromatography on hydroxyapatite using a sodium phosphate gradient [2].

Specific binding of the HRC to chromatin fractions was studied using filtration on Synpor (Czech Rep.) 24-cm nitrocellulose filters with a pore size of 0.4 µm. Aliquots (20 µl) of ^{3}H-BAP of specific activity 8 Ci/mmol that was synthesized by G. V. Sidorov (Institute of Molecular Genetics, Russian Academy of Sciences, Moscow) was placed in 200-µl microtubes. Binding of CBP and chromatin components was performed in 25 mM *Tris*-HCl (pH 7.6). The mixtures were incubated for 30 min at 20°C and placed on the rough side of a filter previously moistened with water. The mixtures were filtered under vacuum. The filter was washed with cold water (5 ml). The filters were dried after the filtration. The radioactivity on them was counted in a standard toluene scintillant on a β-analyzer. The standard deviation after four determinations for each sample was <10%.

RNA-polymerase activity was estimated according to the literature [6].

Protein was determined by coloration of coomassie Brilliant Blue G-250 according to Bradford. The standard was ovalbumin [7].

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