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HETEROGENEITY OF DEPROTEINIZATION-RESISTANT PROTEINS FOR FIRMNESS OF BINDING TO DNA

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Heterogeneity of proteins of the nuclear matrix for firmness of DNA—protein interaction has been investigated for several years. Two groups of proteins have been found: firmly bound, for dissociation of which from DNA a high temperature is required, and weakly bound, dissociating in a solution of salt and urea. Firmly bound proteins are evidently located in the replicative complex, whereas the weakly bound type are evidently involved in transcription [1, 3, 5]. The polypeptide composition of firmly and weakly bound proteins has been shown to be very similar and to be characterized by the presence of components with mol. wt. of 54-68 kD [6]. Polypeptides with this molecular weight and entering into the composition of the nuclear matrix preserve their binding with DNA after deproteinizing procedures: stronger — treatment with proteinase K and phenol [9], or milder — treatment with sarcolysin combined with high ionic strength [13]. It has been shown that a certain proportion of proteins resistant to deproteinization are noncovalently bound to DNA, whereas some are covalently bound, by a phosphodiester bond [7, 11]. Data on the functional role of the firmly bound proteins are rather contradictory. By some workers they have been ascribed the role of constant "fixer" of DNA with the nuclear matrix, and a role in both replication and transcription [10, 12], whereas others postulate a transient association with the nuclear matrix, depending on the type of differentiation [13]. Their binding predominantly with satellite DNA [12] or with unique sequences [13] has been reported. Thus, on the one hand, firmly bound proteins similar in polypeptide composition are liberated by procedures of differing strength [7, 10, 11], but on the other hand the nucleoprotein complexes of the nuclear matrix, similar in their polypeptide spectrum with one another and with the firmly bound proteins, differ in stability and in their functional role [1, 3, 5, 6]. It has consequently been suggested that firmly bound proteins may also be heterogeneous for the strength of the DNA—protein bond. The aim of this investigation was to study the strength of DNA—protein interactions in complexes of DNA with firmly bound proteins by methods of nucleoprotein-celite chromatography (NPC) and elution from nitrocellulose.

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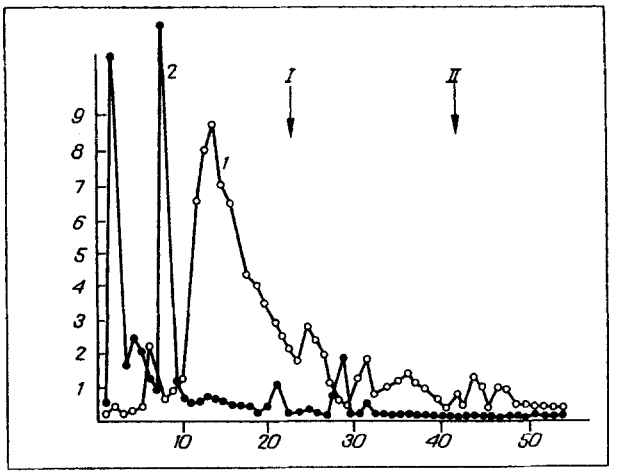

Fig. 1. NPC chromatography of DNA from starving Ehrlich's amcites carcinoma cells labeled by the DNA-polymerase reaction. 1) DNA with firmly bound proteins applied to filter in 0.5 M KCl, 2) pure DNA. Abscissa, Nos. of fractions; ordinate, radioactivity, $^3H \cdot 10^{-3}$ cpm. Arrows: I) end of NaCl gradient; II) end of LiCl and urea gradient, beginning of temperature gradient.

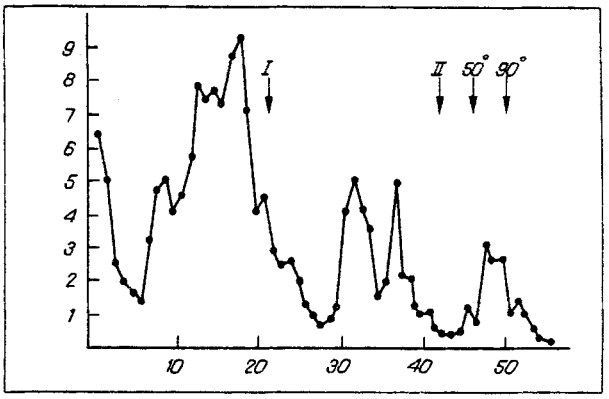

Fig. 2. NPC chromatogram of DNA complex with firmly bound proteins applied to nitrocellulose filter in 2 M KCl. Arrows: I) end of NaCl gradient; II) end of LiCl and urea gradient. Remainder of legend as to Fig. 1.

EXPERIMENTAL METHOD

Cells of an Ehrlich's ascites carcinoma (tetraploid strain) were subjected to passage through noninbred mice. DNA–protein interactions were studied by means of two approaches.

1. **NPC chromatography** of DNA, labeled in the DNA-polymerase reaction. Cells were incubated in ascites fluid with 1 mg/ml of heparin for 4 h at 37°C in order to make breaches in the DNA [14]. The ascites fluid was washed out with physiological saline, the cells were suspended in 50 mM Tris-HCl, pH 6.8, and 25 mM EDTA, and SDS was added to 0.5% and pronase P to 1 mg/ml. The samples were incubated overnight at 37°C. The SDS concentration was increased to 1 M and NaCl was added to 1 M. Extraction was then carried out with an equal volume of a mixture (24:1) of chloroform and isoamyl alcohol. Nucleic acids were preciptated from the aqueous medium with ethanol, dissolved in 10 mM Tris-HCl, pH 7.6, 10 mM EDTA, and treated with pancreatic RNase (100 μg/ml, 30 min, 37°C). Extraction with chloroform was repeated and the DNA precipitated with ethanol. The DNA was dissolved in 10 mM Tris-HCl, pH 7.6, 5 mM EDTA with 0.5 M or 2 M KCl, and the DNA with bound proteins was separated from the free DNA by adsorption on type HA nitrocellulose filters (Millipore, USA) with a pore diameter of 0.45 μ [10]. The DNA–protein complexes were removed from the filters by a solution containing 10 mM Tris-HCl, pH 7.6, and 5 mM EDTA. The optical density ratio at 260/280 nm was 1.8 in the original DNA, 1.8-2.0 in free DNA, and 1.6-1.7

in the DNA–protein complexes. DNA was labeled by a nick translation kit (Amersham, England). To a mixture of triphosphates were added 10 μg DNA, 5 units of DNA-polymerase I (Ferment, Vilnius), and 0.8 mBq of ^{3}H-thymidine triphosphate. The reaction was carried out at 15°C for 10 min and the material mixed with a suspension of celite, when proteins were irreversibly adsorbed on the carrier. DNA was released from the bond with protein by NaCl and LiCl-urea gradients and temperature gradients as described previously [5].

2. **Elution from Nitrocellulose [5].** Biosynthetic labeling of DNA was carried out by an injection of 4 mBq of ^{3}H-thymidine 1-2 days before sacrifice of the animals. DNA was isolated by extraction with phenol:chloroform [2] and fragmented by restriction endonucleases EcoRI, BamHI, and BCN I (100 units/ml, 1 h, 37°C), which was followed by further deproteinization by phenol:chloroform and dissolving in 4 M LiCl, 8 M urea, 25 mM Tris-HCl, pH 7.6, and 5 mM $MgCl_2$. The DNA–protein complexes were adsorbed on nitrocellulose [10]. The filter was placed in a thermostated cell through which 4 M LiCl with 8 M urea was passed, the temperature being raised gradually from room to 100°C. Fractions 3 ml in volume were collected and their ^{3}H-thymidine activity was determined.

EXPERIMENTAL RESULTS

NPC chromatography of biosynthetically labeled DNA did not give reproducible results, possibly on account of the low specific radioactivity of the material. The DNA was therefore labeled by the DNA-polymerase reaction. Nicking by DNase I causes a sharp change in the character of chromatography [6], and to avoid this, breaks in DNA were induced by starving the cells [4]. The NPC chromatograms of the cell lysates were not changed by introducing breaks in this way (not shown here). NPC chromatograms of pure DNA and of a complex of DNA with firmly bound proteins are known in Fig. 1. Most of the label (over 90%) in pure DNA was not bound with celite, and the rest gave two peaks: at the beginning of the NaCl gradient and at the beginning of the LiCl-urea gradient. These DNA–protein complexes can be formed by DNA-polymerase I, for we know that this enzyme forms ionic (ruptured in an NaCl gradient) and hydrogen (dissociates in the presence of urea) bonds [8]. The NPC chromatogram of DNA with proteins resistant to deproteinization unexpectedly was very reminiscent of the chromatogram of cellular DNA after chromatin fragmentation [6]. Much of the DNA was eluted by an NaCl gradient (DNA 0), there were peaks in LiCl-urea gradients (DNA I) and temperature gradients (DNA II), and about 80% of the DNA did not bind with the column. Both DNA I and DNA II are considered to be retained by proteins of the nuclear matrix [6], and for that reason the presence of these types of bond in firmly bound proteins is not surprising [10, 13]. Binding of DNA 0 has been ascribed to chromatin proteins [6]. During isolation the DNA was subjected to the action of a high ionic strength, and it is therefore not clear how proteins of this class could remain bound with DNA. Increasing the ionic strength of the solution in which the complexes were adsorbed on nitrocellulose from 0.5 to 2.0 M KCl did not change the chromatogram significantly (Fig. 2). Perhaps the conditions of dissociation of DNA and protein on celite are different from those in solution. It was suggested that proteins forming a bond sensitive to NaCl are not of the type resistant to deproteinization, and they may contaminate the preparation because of inadequate deproteinization. In that case the fraction eluted by NaCl ought to disappear on additional deproteinization. Incubation of DNA in 1% SDS with 1 mg/ml pronase P at 37°C actually increased the fraction of DNA not bound with celite, but the character of the chromatogram was unchanged, evidence that proteins forming all three types of DNA–protein bond are equally resistant to deproteinization (not shown here). Thus proteins firmly bound with DNA are heterogeneous for DNA–protein interaction and preserve all types of DNA–protein binding possessed by the intact nucleus. Firm and weak bonds of DNA with the nuclear matrix may perhaps be formed by proteins resistant to deproteinization.

Elution directly from the nitrocellulose filter has proved to be a more convenient method of studying interaction between DNA and firmly bound proteins [6]. At ionic Strength above 0.1 M proteins bind with nitrocellulose and retain DNA until rupture of the DNA–protein bond. In the present investigation only a temperature gradient was used. If DNA-protein complexes were applied to the filter in 4 M LiCl + 8 M urea peaks were obtained in the 80-90°C region. The method proved to be more sensitive than NPC chromatography, for it enabled several subfractions of DNA II to be distinguished. If the material was applied to the filter in 1 M HCl, a considerable fraction of the DNA was eluted at room temperature (corresponding to the sum of DNA 0 + DNA I). This confirms the conclusion regarding heterogeneity of bonds between DNA and proteins resistant to deproteinization (Fig. 3, curves 1 and 2). Some DNA remained on the filter after the end of the temperature gradient and it may have been retained by proteins covalently bound with the DNA [7, 11].

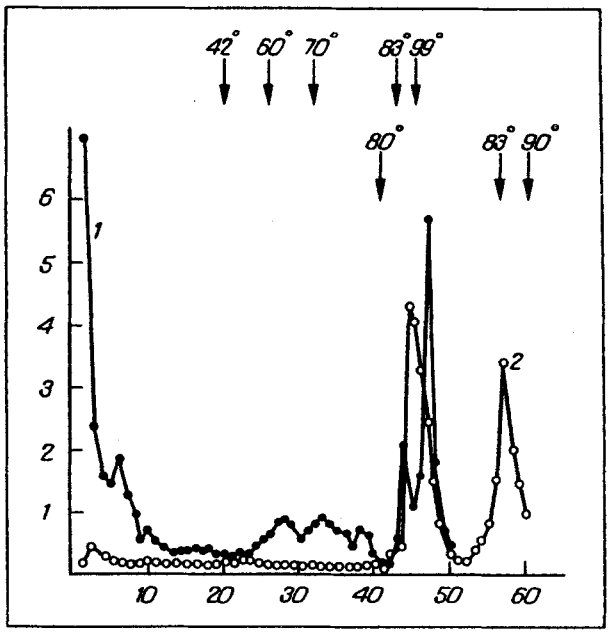

Fig. 3. Temperature elution of DNA complexes with firmly bound proteins from nitrocellulose. Applied to filter: 1) in 1 M KCl, 2) in 4 M LiCl and 8 M urea. Arrows indicate stages of temperature gradient, top row for 1, bottom row for 2. Separate points indicate level of radioactivity of filter after end of temperature gradient. Abscissa, Nos. of fractions; ordinate, radioactivity, $^3H \cdot 10^{-4}$ cpm.

Proteins resistant to deproteinization are thus heterogeneous for strength of DNA–protein interactions and they form at least four types of DNA–protein complexes: 1) destroyed at high ionic strength (DNA 0); 2) destroyed in a solution of salt and urea (DNA I); 3) dissociated at a high temperature (DNA II); 4) covalent complexes resistant to all conditions listed above. The heterogeneity of these proteins for strength of binding with DNA may explain some of the contradictory data about them, for example, differences in the functions of proteins which are similar in polypeptide composition but resistant to the action of detergent and high ionic strength [13] and to deproteinization by phenol [9-12].

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