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DNA–Protein Interaction Studied in Model Systems

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Abstract—Conditions for the formation of interpolymer complexes of DNA with insulin, cortexin, and cytochrome C in the neutral pH range were studied by UV spectroscopy, gel-permeation chromatography, and membrane diffusion techniques. The selected proteins differ in their acid-base properties and do not belong to natural DNA ligands. The DNA–protein complexes remained stable when the ionic strength of solution was increased up to 0.5 mol/l. The isotherms of DNA binding to proteins in solutions with an ionic strength of 0.3 mol/l and pH 7.8–8.2 exhibit a cooperative character. The intrinsic viscosity of DNA in the presence of proteins studied decreases proportionally to the amount of bound protein.

INTRODUCTION

Nucleic acids are natural high-molecular-mass rigid-chain polyelectrolytes capable of various intermolecular interactions. However, large molecular masses (10^6–10^8) and high viscosity hinder investigation of the features of such interactions in solution.

As is known, the intermolecular reactions between proteins and nucleic acids are involved in all stages of DNA replication and expression, as well as in the course of the endogenous regulation. Nevertheless, our knowledge of the physicochemical mechanisms of these interactions is still incomplete. This direction was extensively explored by the school of academician V.A. Kabanov, which studied the physicochemical features of DNA binding to synthetic polyelectrolytes [1, 2] and determined prospects for using the appropriate complexes for delivering the genetic material to target cells [3, 4]. These works included investigation of the features of interpolymer complex formation between protein macromolecules and synthetic or natural polymers containing sulfo and carboxy groups [5–7]. However, the laws of nucleic acid binding to proteins and the stability of complexes between nucleic acids and proteins are still not studied in sufficient detail.

The complexes of nucleic acid with proteins play an important role in regulation of the organism functioning: protein molecules with various acid-base properties enter into the chromatin composition of eucaryotic cells [8]. As is known, chromatin (the chromosome substance) comprises approximately equal amounts of DNA and protein components. The normal ratio between dividing and resting cells—the myotic equilibrium in permanently functioning organic and tissues—is maintained by special DNA-bound proteins performing regulatory functions (nuclear protein regulators) [9]. Therefore, the study of the features of complex formation between DNA and proteins is of considerable practical interest from the standpoint of new delayed-action drugs capable of correcting functional activity of the organism on the molecular level.

The purpose of this work was to study the process of complex formation between DNA and a series of globular proteins, including insulin, cortexin, and cytochrome C, in order to establish quantitative relationships governing the binding of these components in solution, evaluate stability of the resulting complexes, and determine a change in the DNA conformation upon binding to these proteins.

EXPERIMENTAL

The experiments were performed with sodium salt of DNA from bovine spleen (Olain Chemical Plant, Biokhimreaktiv Co., Russia) and bovine insulin, cortexin, and cytochrome C (Medicinal Chemicals Plant, Samson Company, St. Petersburg). Cortexin is a new preparation isolated from bovine brain, containing linear peptides including neuropeptide Y and neurokinins A and B containing homologic segments of the type Asp-Ser-Phen-Val-Gly-Leu-Met. These peptides, possessing neurotropic activity, are characterized by molecular masses not exceeding 1×10^4 [10].

The DNA solutions of a preset concentration were prepared using the following procedure. First, a weighted amount of DNA was put in a small volume of distilled water in cold. The swelling and solvation of native DNA continued for 16–20 h, after which the swelled DNA was dissolved in a phosphate buffer (pH 7.8–8.0) with sodium chloride added to provide for an ionic strength of 0.3 mol/l. The DNA concentration in

working solutions did not exceed 1.0 mg/cm^3. The native state of DNA was checked by a considerable hyperchromic effect observed upon boiling DNA followed by rapid cooling on ice [11]. The DNA concentration was monitored by measuring the absorbance (optical density) of solutions at a wavelength of 260 nm. The protein concentrations were determined by the Lowry method (calibrated with respect to bovine serum albumin). The concentration of cytochrome C was determined with the help of a calibration plot upon measuring the solution absorbance at a wavelength of 410 nm. The calibration was performed using commercial cytochrome C (Serva, Germany).

We have studied the formation of complexes between native DNA and insulin (isoelectric point pI 5.4), cortexin (pI 9.5), and cytochrome C (pI 10.6) [12]. DNA was considered as a natural polyelectrolyte containing functional monobase phosphate groups and charged nucleotide groups [13]. The total positive and negative charges on the molecules of proteins were determined based on the data concerning the amino acid compositions and dissociation constants of proteins [14–16]. For the neutral pH range selected, these charges were as follows: insulin, 2^- (4^+, 6^-); cytochrome C 4^+ (24^+, 20^-); cortexin, 2^+ (6^+,4^-). Thus, the selected range of pH 7.8–8.3 determined excess positive charge for cortexin and cytochrome C, while the insulin molecule possessed an excess negative charge.

The reaction of complex formation was effected by mixing DNA and protein solutions in the same buffer, followed by incubation for 16–20 h in a refrigerator at 4°C. The UV spectra of the solutions of DNA, insulin, cortexin, cytochrome C and their mixtures were measured on an SF-26 spectrophotometer. Stability of the DNA–protein complexes in solutions of various ionic strengths was studied by gel-permeation chromatography (GPC) on a 2.0 × 60 cm column filled with Sephadex G-200 Superfine. A sample of the DNA–protein complex in 1 ml of the corresponding buffer solution was applied onto the column and eluted with a phosphate buffer (pH 8.0) with NaCl added to an ionic strength of 0.1, 0.3, or 0.5 mol/l. The protein content corresponding to GPC peaks was determined by the Lowry method.

The DNA–protein binding was studied by the method of membrane dialysis [17]. The kinetics of protein diffusion through porous membranes was measured in a two-compartment cell with a compartment volume of 50 cm^3 and a window diameter of 2.65 cm. The samples were dialyzed through double-folded 10-μm-thick Lavsan (Dacron analog) track membranes with a pore size of 0.46 μm. This porosity ensures free passage of the unbound proteins, while DNA and its complexes are fully retained. The permeation of proteins was checked in preliminary control experiments. A protein solution was placed in the source compartment, and the receiver compartment was filled with a buffer solution. The diffusion was determined by monitoring the protein concentration in the receiver, which was performed by taking samples at a fixed time interval and measuring the solution absorbance. The coefficient of protein permeation P (cm^2/s) through the membrane was calculated from the increase in the optical density of solution in the receiver measured at 260 or 280 nm [18]:

$$P = \frac{4c_t VL}{\pi D^2 t c_0}, \quad (1)$$

where c_t (mg/cm^3) is the protein concentration in the receiver at a current time instant t (s), V is the receiver volume (cm^3), D (cm) is the window diameter, L (cm) is the membrane thickness, and c_0 is the initial protein concentration in the source (mg/cm^3).

The same cell was used for the analogous experiments with a DNA–protein system placed in the source compartment. The free protein diffusion from the source was measured and the effective permeation coefficient P was used to calculate the free protein concentration in equilibrium with the complex. Then the bound protein concentration (mg/cm^3) in the complex was calculated by the formula

$$c_c = c_0 - c_{eq}, \quad (2)$$

where c_0 (mg/cm^3) is the initial protein concentration used in the complex formation reaction and c_{eq} (mg/cm^3) is the equilibrium concentration of protein not bound in the complex.

The intrinsic viscosity was determined by the method of serial dilutions. The measurements were performed at 20°C on the Ostwald viscometer using a capillary with a diameter of 0.73 mm. The plots of reduced viscosity versus the solution concentration were extrapolated to obtain the intrinsic viscosities of free DNA and its complexes with insulin and cytochrome C.

RESULTS AND DISCUSSION

Figure 1 shows typical UV spectra of the solutions of DNA, insulin, and their mixture in comparison with the additive spectrum. The hyperchromic effect observed upon the DNA mixing with insulin (curve *4*) is evidence that the DNA conformation changes as a result of the interpolymer complex formation with the protein. Analogous data were obtained for the DNA mixtures with cortexin and cytochrome C. As is known the complexation of polyelectrolyte macromolecules proceeds by forming ionic bonds or hydrophobic contacts, or by the ion–dipole interactions [5]. In the systems involving DNA, the binding is provided by the interaction of the phosphate groups and amino groups of nucleotide bases from the DNA double helix with the acid and base side groups of the amino acid units of proteins [13]. The formation of DNA–cortexin, DNA–insulin, and DNA–cytochrome C complexes by means

of polar interactions of these polyampholytic macromolecules is quite natural.

In order to determine the character of intermolecular interactions involved in the DNA–insulin complex formation, we have studied stability of the resulting complexes in solutions of various ionic strength by the method of gel-permeation chromatography. Figure 2 shows typical GPC chromatograms. As is seen, the protein component (insulin) appears in the form of an intense peak adjacent to the peak of a high-molecular-mass DNA component. The concentration of protein corresponding to this peak intensity was the same independently of the ionic strength of solution. In other words, adding a low-molecular-mass electrolyte does not lead to dissociation of the DNA–insulin complex. Apparently, the interaction of DNA with this protein is provided not as much by simple electrostatic attraction, as by the intermolecular contacts of some other types. These contacts probably include the interaction of a polypeptide protein chain with the DNA double helix, whereby the β-layer builds into the minor groove of the DNA helix on the side of an O(2) oxygen atom of pyrimidine and an N(3) nitrogen atom of purine [13].

Taking into account that the complex formation in the systems studied involves a polyelectrolyte with $M \sim 1.9 \times 10^5$ (DNA) and a protein whose molecular mass is lower by two orders of magnitude, it was important to determine the molar ratio of components in the complex. For this purpose, the DNA complexation with insulin, cortexin, and cytochrome C taken in various ratios (by weight) was studied by membrane dialysis. Figure 3 shows data on the diffusion kinetics of free (not bound to DNA) insulin and cytochrome C through the track membranes. Analogous data were obtained for the DNA–cortexin system. The concentrations of free and DNA-bound proteins in the source compartment were calculated by equations (1) and (2). The permeation coefficients were calculated by the method described in [18], taking into account only the initial linear portions of the curves. As is seen, variation of the ratio of components in the DNA–protein system leads to a change in the free protein concentration in the source compartment, which is related to various degrees of the protein association with DNA. Using these data, it is possible to construct the isotherms of DNA binding to the proteins studied. The isotherms are plotted in terms of the degree of protein binding m determined as the ratio of the amount of bound protein to the amount of DNA. In all cases, the process of DNA–protein complex formation is described by a cooperative isotherm. The interaction of DNA with cytochrome C leads to a sharp increase in the degree of protein binding when the free protein concentration in solution reaches 0.5 mg/cm^3. At the same time, the isotherm describing the DNA binding to insulin exhibits a linear portion extending to a protein concentration as high as 3.5 mg/cm^3.

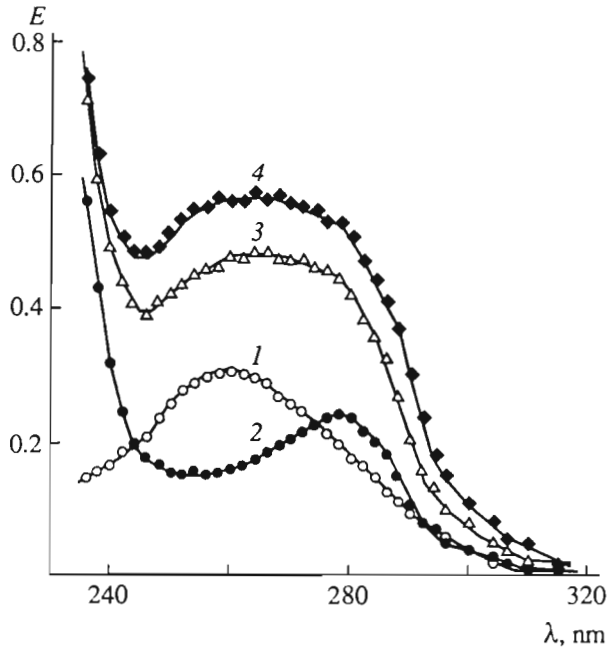

Fig. 1. UV spectra of (*1*) DNA, (*2*) insulin, (*3*) DNA + insulin mixture (additive spectrum), and (*4*) DNA–insulin complex. E is the absorbance of solutions.

The values of the protein binding characteristics calculated from the initial portions of the binding isotherms are summarized in the table. The DNA–protein weight ratio in the complexes was recalculated so as to determine the number of nucleotide units per bound protein molecule. Under the experimental conditions studied (ionic strength 0.3 mol/l, pH 8.0) insulin occurs in solution in the form of dimers, which was confirmed by GPC of pure insulin. The selectivity of complexation was characterized by the DNA binding coefficient k determined as

$$k = \left(\frac{dc_c}{dc_{eq}}\right)_{c_{eq} \to 0}.$$

As seen from the table, the coefficient of DNA binding to proteins (insulin, cytochrome C) not capable of

Characteristics of DNA–protein interpolymer complexes

Protein	$M \times 10^{-4}$	Binding coefficient k	n*
Insulin	1.2	0.6 ± 0.1	36.9
Cortexin	1.0	2.8 ± 0.1	7.5
Cytochrome C	1.3	0.6 ± 0.1	36.3

* Number of DNA chain units per protein molecule in the complex.

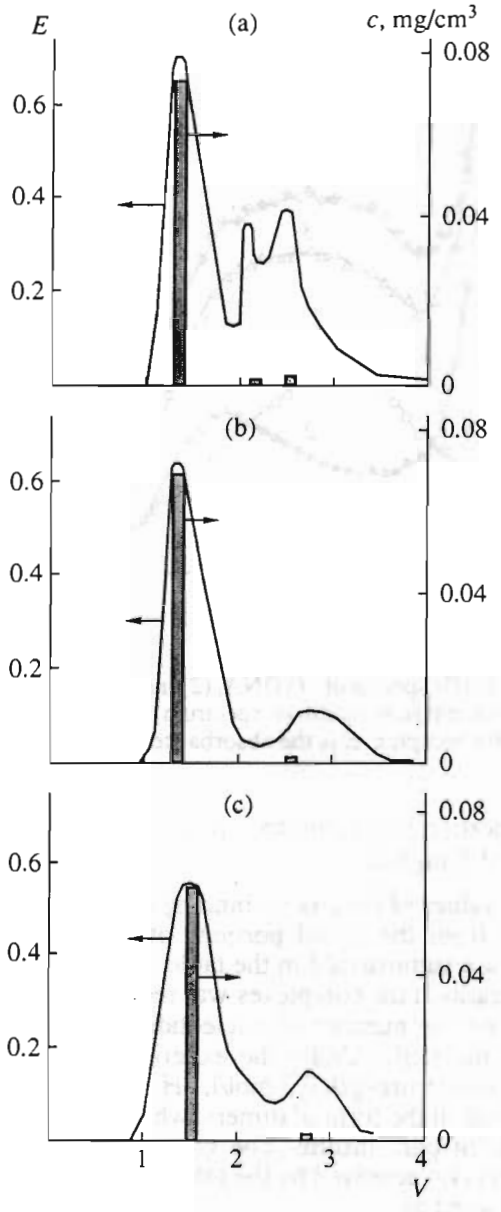

Fig. 2. GPC chromatograms of DNA–insulin complexes in solutions of various ionic strength (mol/l): (a) 0.1; (b) 0.3; (c) 0.5. V is the relative output volume (the ratio of the sample output volume to the retention volume); E is the absorbance of solutions at λ = 260 nm; c is the protein concentration.

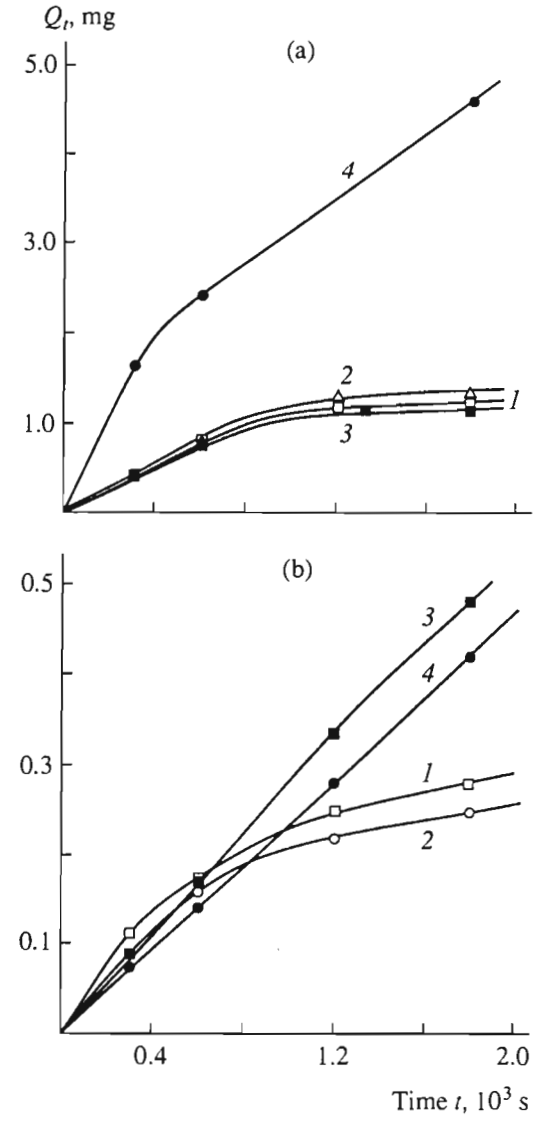

Fig. 3. Diffusion Q_t of (a) free insulin and (b) cytochrome C from the protein solutions and from the DNA–protein complex solutions through a track membrane. (a): Insulin (*1*) and complexes with the ratio (w/w) DNA/insulin = 1 : 3 (*2*), 1 : 7 (*3*), 1 : 16 (*4*); (b): cytochrome C (*1*) and complexes with the ratio (w/w) DNA/cytochrome C = 1 : 3 (*2*), 1 : 6 (*3*), 1 : 16 (*4*).

specific binding to nucleic acids is markedly lower as compared to that of cortexin containing neurohormones possessing certain selectivity with respect to DNA. Data in the table indicate that the selective interaction of cortexin with DNA corresponds to a lower number of nucleotide units per bound protein molecule in the initial stage of complexation.

Since the substances studied represent macromolecular compounds, a considerable role in their interactions must belong to steric factors and conformational changes involved in the complex formation. As is known, a change in the DNA structure in solution is accompanied by increasing optical anisotropy [19] and decreasing intrinsic viscosity [20].

Figure 5 shows a plot of the intrinsic viscosity of DNA [η] versus the number n of nucleotide units per bound protein molecule (i.e., versus the density of DNA coverage by protein molecules). Since insulin is an acid globular protein, the change in the DNA to protein weight ratio from 1 : 3 to 1 : 16 in the DNA–insulin complex (line *1*), which implies a decrease in the number of DNA units binding the protein, the intrinsic vis-

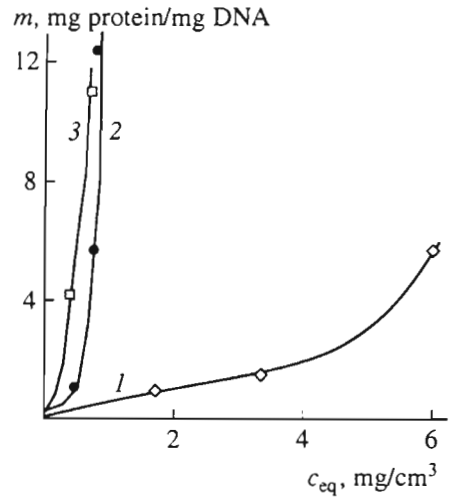

Fig. 4. DNA–protein binding isotherms for (*1*) insulin, (*2*) cytochrome C, and (*3*) cortexin; m is the degree of binding, c_{eq} is the equilibrium concentration of free protein.

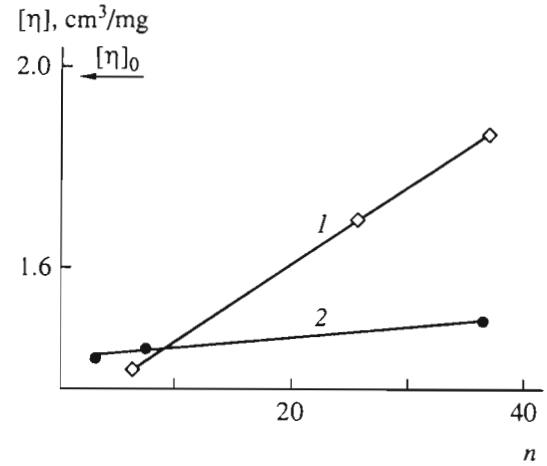

Fig. 5. The plot of intrinsic viscosity [η] vs. the number n of DNA chain units per bound protein molecule for DNA complexes with (*1*) insulin and (*2*) cytochrome C; $[\eta]_0$ indicates the intrinsic viscosity of free DNA.

cosity of DNA decreases from 1.90 ± 0.15 to 1.40 ± 0.15 cm^3/mg (against 2.0 ± 0.15 cm^3/mg for the native DNA). This result indicates that the DNA binding to globular proteins is not only determined by the electrostatic interaction with positive charges on the protein molecule, but involves some additional contacts as well. In particular, the α and β regions of protein may build into the minor groove of the DNA helix as a result of binding between negative charges of the protein molecule and positive charges of the nucleotide chain.

As is known, the interaction of DNA with alkaline proteins of the histone group results in compactization of the linear macromolecules [21]. Cytochrome C is a protein possessing acid-base properties close to those of histones, while the excess positive charge renders this protein analogous to protamines. The intrinsic viscosity of the DNA–cytochrome C complex (Fig. 5, line *2*) is as low as 1.5 ± 0.15 cm^3/mg even for a DNA to protein weight ratio of 1 : 3 and then decreases to 1.5 ± 0.15 cm^3/mg for the 1 : 16 ratio.

These data indicate that the process of DNA compactization during the interaction with proteins may be different for the proteins possessing dissimilar acid-base and physiological properties. The observed pattern of changes in the intrinsic viscosity suggests that the interaction with insulin leads to gradual densification of the DNA structure, followed by the formation of a "necklace" configuration and, eventually, by superspiralization of the complex, as was demonstrated for the DNA interaction with histones [20]. This course of compactization is consistent with the absence of dissociation of the DNA–insulin complex in solutions with increasing ionic strength. The compactization process proceeds much faster in the course of DNA binding to cytochrome C. Here, the available protein is virtually completely bound to DNA with the formation of necklace structures and subsequent superspiralization. Note that the limit of compactization of the interpolymer DNA complexes with various proteins is approximately the same, irrespective of their physical properties.

Thus, we have established that the DNA complex formation with insulin, cortexin and cytochrome C is determined by the acid-base properties of these proteins, the ratio of components in the complex, the presence of specific interactions between macromolecules, with the corresponding changes of the DNA conformation.

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