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SPECIFIC INTERACTION OF CHROMATIN NON-HISTONE PROTEINS WITH DNA

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Summary

The interaction of different preparations of chromatin non-histone proteins of rat liver and thymus with homologous and heterologous DNA was studied. It is shown by the method of fixation of non-histone proteins—DNA complexes on nitrocellulose filters that: (1) all the non-histone proteins preparations studied form complexes with DNA in 0.02 M Tris—HCl (pH 7.5)—3 mM $MgCl_2$; (2) the main part of non-histone proteins interacting with DNA binds to it non-specifically; (3) a small part of non-histone proteins interacts specifically with the homologous native DNA in 5 M urea; (4) both homologous and heterologous denatured DNA binds non-histone proteins more effectively than the native one; (5) the specific interaction of non-histone proteins with the homologous denatured DNA is observed both without urea and in its presence. The specific interaction of a small part of non-histone proteins with the homologous native and denatured DNA is also shown by the method of non-histone proteins chromatography on polyacrylamide—agarose columns containing DNA. The data obtained are discussed in the light of the possible non-histone proteins role in the specific regulation of the transcription process.

Introduction

Recent investigations indicate the important role of the chromatin non-histone proteins in the specific regulation of transcription in eukaryotic cells [1—4]. In order to perform the specific regulation of the template activity of DNA the recognition of certain nucleotide sequences in DNA by corresponding protein regulators is apparently necessary. It was shown in a number of works that non-histone proteins are capable of interacting with DNA [5—10], and some authors observed a specific interaction of non-histone proteins with the homologous DNA [5—7]. At the same time this problem cannot be considered to be conclusively settled, since the data of different authors on the amount of non-histone proteins binding to DNA and on the degree of specificity of such

interaction are discrepant. It can be related to the fact that different authors use different methods for both non-histone proteins isolation and the studies of their interaction with DNA. Besides, we have not found any data in the literature concerning complexing with denatured DNA.

This paper presents data on the interaction of different non-histone proteins preparations with homologous and heterologous (native and denatured) DNA.

Methods

DNA isolation

Non-labelled animal DNA was isolated from rat thymus. The whole isolation was performed at 2—4°C. The tissue was homogenized in 0.1 M NaCl—0.005 M EDTA—0.01 M Tris—HCl (pH 7.5), 10% sodium dodecylsulphate was added to 1% and the mixture was shaken for 20 min. Then dry NaCl was added to 1 M, the mixture was shaken for 10 min, an equal volume of water-saturated phenol was added and 20 min later the mixture was centrifuged for 20 min at 5000 × *g*. The water phase was twice deproteinized by the mixture chloroform—isopentanol (24 : 1, v/v), DNA was precipitated by an equal volume of ethanol, dissolved in 0.15 M NaCl—0.015 M sodium citrate (pH 7.0) and digested with previously heated (10 min at 80°C) pancreatic RNAase (40 μg/ml) for 30 min at 37°C. After successive deproteinizations by 0.5% sodium dodecylsulphate, phenol and chloroform with isopentanol, DNA was precipitated by 2 vol. of ethanol. The protein content in the DNA preparations was not greater than 1%.

In order to obtain labelled DNA, rats with Zajdela ascites hepatoma were injected intraperitoneally with 50 μCi of [^{14}C]thymidine four times at 6-h intervals. 6 h after the last injection the rats were killed and the DNA was isolated from ascites cells, as described above. The specific activity of DNA was $1.7 \cdot 10^6$ cpm/mg. The non-labelled DNA from *Escherichia coli* and *Bacillus subtillis* were isolated by Marmur's method [11]. DNA from phage T4 was kindly given to us by Dr A.I. Gasiev. Low molecular weight DNA (about $2 \cdot 10^5$ from the data obtained from analytical ultracentrifugation) was obtained by sonication of the DNA solutions at 15 kHz, 20 times for 15 s with 45-s intervals in an ice bath.

In order to obtain a denatured DNA, the DNA solution in 0.01 M Tris—HCl (pH 7.5) in the concentration 200 μg/ml was heated on the boiling water bath and rapidly cooled in ice.

Isolation of chromatin

Nuclei from rat liver were isolated by a somewhat modified method of Shauveav et al. [12]. The livers were homogenized in 4 vol. of 0.32 M sucrose—3mM $MgCl_2$—0.01 M Tris—HCl (pH 7.2). The nuclei were pelletted by centrifugation (10 min at 700 × *g*) and suspended in 2.2 M sucrose—1 mM $MgCl_2$—0.01 M Tris—HCl (pH 7.2). The suspension obtained was layered on 5 ml of the same solution in centrifuge tubes of the SW 25 rotor and centrifuged for 1 h at 100 000 × *g*. The nuclear pellet was successively washed with 0.14 M NaCl—0.01 M Tris—HCl (pH 7.5), 0.075 M NaCl—0.024 M EDTA—0.01 M Tris—HCl

(pH 7.5) and three times with 0.01 M Tris—HCl (pH 7.5). The pellet obtained after the last washing was thought to be purified chromatin.

Rat thymuses were homogenized in a 10-fold volume of 0.25 M sucrose—3 mM $MgCl_2$—0.01 M Tris—HCl (pH 7.2), the nuclei were pelleted by centrifugation and washed two more times by the homogenization medium. Chromatin was isolated from the nuclei obtained, as described above for liver chromatin.

Isolation of chromatin non-histone proteins

The isolation of different non-histone proteins preparations from rat thymus and liver chromatins was performed in the same manner.

1. NHP_1, the total non-histone proteins of chromatin were isolated as described earlier [13]. Some modifications were used to avoid the action of denaturing agents as far as possible. The purified chromatin was dissociated in 2.5 M NaCl—0.01 M Tris—HCl (pH 8.2) and DNA was sedimented by ultracentrifugation (24 h at 180 000 × *g*). The supernatant containing chromatin proteins, was dialyzed against 0.5 M NaCl—0.01 M sodium phosphate buffer (pH 7.2) and passed through a column (2 × 18 cm) with Amberlite CG-50 type 1, equilibrated by the same buffer. Histones are retained by the column and the non-histone proteins are eluted by 0.5 M NaCl—0.01 M sodium phosphate buffer (pH 7.2). The non-histone proteins (NHP_1) obtained in this manner were dialyzed against 0.01 M Tris—HCl (pH 7.5) and kept frozen at —20° C.

2. NHP_2 were isolated by Wang's method [14]. The purified chromatin was dissociated in 1 M NaCl—0.01 M Tris—HCl (pH 8.0) (the final DNA concentration was about 400 μg/ml) and dialyzed against 6 vol. of 0.01 M Tris—HCl (pH 8.0). The precipitate was separated by centrifugation (20 min at 5000 × *g*) and used for the isolation of NHP_3 and the supernatant proteins were dialyzed against 0.5 M NaCl—0.01 M sodium phosphate buffer (pH 7.2) and then chromatographed on Amberlite CG-50 (for removing admixtures of histones) and kept as described above for NHP_1.

3. NHP_3 were isolated by the hydroxyapatite method [8,15], but 2.5 M NaCl was used instead of 2 M NaCl—5 M urea. The precipitate of the reconstructed chromatin obtained when isolating NHP_2, was dissociated in 2.5 M NaCl—0.001 M sodium phosphate buffer (pH 7.0) and passed through a column (3 × 30 cm) with hydroxyapatite. Histones were eluated by the same buffer, and non-histone proteins with some admixture of histones by 2.5 M NaCl—0.05 M sodium phosphate buffer (pH 7.0). NHP_3 were dialyzed against 0.5 M NaCl—0.01 M sodium phosphate buffer (pH 7.2), purified on Amberlite and kept as described above.

To obtain labelled non-histone proteins the rats were injected with 50 μCi [^{14}C]leucine intraperitoneally four times at 6-h intervals. 6 h after the last injection 200 μCi [^{14}C]leucine were injected and 2 h later the rats were killed. Non-histone proteins were isolated as described above. The specific activity of different non-histone proteins preparations from one organ was approximately the same: $7.4 \cdot 10^4$ cpm/mg and $5.2 \cdot 10^4$ cpm/mg for non-histone proteins of liver and thymus, respectively.

Non-histone protein interaction with DNA

The sample with the total volume of 0.5 ml contained 4 μg [^{14}C]DNA

(6800 cpm), 0.02 M Tris—HCl (pH 7.2), 3 mM $MgCl_2$, 25 μg of bovine serum albumin and varying amounts of non-histone proteins. The sample was incubated for 10 min at 37°C and passed through a nitrocellulose filter (d = 1 cm). Usually Czech filters Synpor 6 were used, but experiments with the HA (Millipore) and Synpor 8 filters gave similar results. The filtering rate of all the samples was the same (0.5 ml in 12 s), due to the control over the value of negative pressure in the system created by means of a water stream pump. After the sample has passed through, the filter was washed with 1 ml of 0.02 M Tris—HCl (pH 7.2)—3 mM $MgCl_2$ at the same filtering rate, dried and the radioactivity was counted in a liquid scintillation counter SL-30. The background i.e. the radioactivity of filters through which DNA without non-histone proteins had been passed was subtracted. The background was not more than 2%. In the experiments concerning the competition of non-labelled DNA with [^{14}C]DNA for binding non-histone proteins, the non-labelled DNA competitor was added to the sample before the non-histone proteins. Both polymer and sonicated DNA were used as competitors. In both cases similar results were obtained. It should be noted that adding high molecular weight DNA competitors to the sample lowers the filtering rate which results in the increase of background. Therefore, using polymer DNA competitors either the corresponding control was put ([^{14}C]DNA + DNA competitor without non-histone proteins) and the background was subtracted or the pressure was changed so that the filtering rate remained constant.

Interaction of non-histone proteins with DNA on DNA-containing polyacrylamide—agarose columns

Polyacrylamide—agarose columns containing native or denatured DNA, was prepared by the method of Cavalieri et al. [16]. The quantity of DNA available for the interaction with proteins, in 1 ml of "packed" gel was determined in the following manner. The small column filled with DNA-containing gel was washed by 0.01 M Tris—HCl (pH 7.5) to zero absorbance at 260 nm. Then the buffer was lowered onto the upper surface of the gel, 5 ml 0.01 M Tris—HCl (pH 7.5)—0.01 M $MgCl_2$ containing 50 μg/ml of DNAase 1, was introduced into the column, a layer of gel was filled with this solution and the column was closed. After 1 h of incubation at 24°C the column was washed with 0.01 M Tris—HCl (pH 7.5) and the DNA content was determined in the eluate. On the basis of the data obtained, the DNA content per 1 ml of "packed" gel was calculated. This value was usually 150—200 μg DNA/ml.

Labelled non-histone proteins (50—100 μg/ml) in 0.01 M Tris—HCl (pH 7.5) were passed through a column with 3 or 5 mg of DNA at the rate of 10 ml/h. The column was washed free from unbound proteins by 0.01 M Tris—HCl (pH 7.5) and the bound non-histone proteins were eluted by 0.15 M, 0.6 M and 2 M NaCl in the same buffer. From the columns containing denatured DNA some part of the protein was additionally eluted by 2 M NaCl—5 M urea—0.01 M Tris—HCl. The determination of radioactivity in the eluate fractions was performed in the following manner. In the course of column loading with proteins the void volume of the column was determined (it was usually 30—40% of the whole volume of the packed gel). For each salt solution practically all radioactivity from the columns with 5 mg of DNA (the volume was

25—30 ml) was eluted in 30—35 ml, and from the columns with 3 mg of DNA (the volume was 15—20 ml) in 20 ml of the solution. Therefore, after introducing into the column of the new salt solution the fraction corresponding to the void volume of the column was discarded and 50 ml and 30 ml from the columns with 5 mg and 3 mg of DNA, respectively, were collected. Bovine serum albumin was added to the eluate to a final concentration of 100 μg/ml and 50% trichloroacetic acid to 10%. The precipitate was collected on nitrocellulose filters Synpor 6.

To determine the percent of proteins, eluted at the corresponding salt concentration, it is necessary to take into account the self-absorption caused by albumin. Therefore, the non-histone protein aliquot (0.5—1 ml) before passing through the column, was diluted to 30 or 50 ml, albumin was added (100 μg/ml), the mixture was treated as the eluate fractions and the total radioactivity of proteins introduced on the column was calculated. Radioactivity on the filters was counted on the liquid scintillation counter Sl-30.

Results

Characterization of different non-histone protein preparations

When isolating non-histone proteins by the first method, the total yield of NHP_1 is 60% of their content in purified chromatin. The major losses are related to the fact that a part of non-histone proteins sediments with DNA in the course of ultracentrifugation. The yield can be increased to 90% if chromatin dissociation and DNA separation are performed in the presence of 5 M urea [13]. Bornkamm et al. [17] have shown that non-histone proteins are capable of aggregation in solutions of high ionic strength and are sedimented by ultracentrifugation even in the absence of DNA, sedimentating non-histone proteins not being different from the supernatant ones. Dissociation of non-histone proteins aggregates by 5 M urea was also shown earlier [13]. Thus, the increase of the non-histone proteins yield in the presence of urea is related not to the dissociation of an additional quantity of proteins from DNA, but to the decrease of their aggregation and also to the increase of solution density at ultracentrifugation. Thus, NHP_1 can be thought to represent the total non-histone proteins. It should be also noted that NHP_1 conserve their native structure, which is indicated by the activity of DNA ligase, an extremely labile enzyme, found in them [18]. When isolating non-histone proteins by the second method, the total yield (NHP_2 + NHP_3) is 90—92% of the overall content of non-histone proteins in purified chromatin, 83% and 77% being due to NHP_2 and 17% and 23% to NHP_3 for liver and thymus, respectively. The amount of non-histone proteins eluated from hydroxyapatite together with DNA in 2.5 M NaCl—0.5 sodium phosphate buffer (pH 7.2), constitute not more than 5—6% by weight and 7% by radioactivity of the total amount of non-histone proteins.

After chromatography on Amberlite CG-50 non-histone proteins were not contaminated with histones as was shown by amino analysis [13] and by disc electrophoresis in polyacrylamide gel (Umansky and Zotova, unpublished).

Non-histone proteins retain their properties for a long time when stored in 0.01 M Tris—HCl (pH 7.5) at —20° C.

Interaction of non-histone proteins with homologous native DNA by the membrane filter technique

The method of retention of nucleic acids with proteins complexes on nitrocellulose filters was used by different authors for the analysis of DNA interaction with *lac* repressor [19], RNA polymerase [20], antibodies [21], and tRNA with aminoacyl-tRNA synthetase [22]. The method is based on the fact that the free native DNA is not bound with filters, and proteins and their complexes with DNA are retained by filters. The method is simple and it makes it possible to perform a great number of analyses for a short time and to compare the protein interaction with different DNA preparations.

Fig. 1 gives the data of experiments on the complexing of different non-histone proteins of rat liver (Fig. 1a) and thymus (Fig. 1b) with homologous [^{14}C]DNA. With the ratio NHP_1/DNA equal to 10, about 40%, 23% and 8% DNA are retained on the filter for NHP_1, NHP_2 and NHP_3 of liver, respectively. For thymus NHP_1, NHP_2 and NHP_3 this value is 16%, 15% and 1—2%.

The results of these experiments point to the fact that all the non-histone proteins preparations studied form complexes with homologous native DNA.

The data of these experiments do not point to the number of non-histone proteins interacting with DNA, since the retention of DNA by filters depends not only on this parameter, but also on the character of the interaction, on the distribution of proteins between the DNA molecules and on the ability of different non-histone proteins to be retained by filters. Thus, after a 2-day storage of NHP_1 in 0.01 M Tris—HCl (pH 7.5) at 2°C their ability to retain DNA on filters sharply decreases (Fig. 2a). At the same time their interaction with DNA hardly changes quantitatively (according to the data of non-histone proteins chromatography on DNA-containing columns), but their ability to be

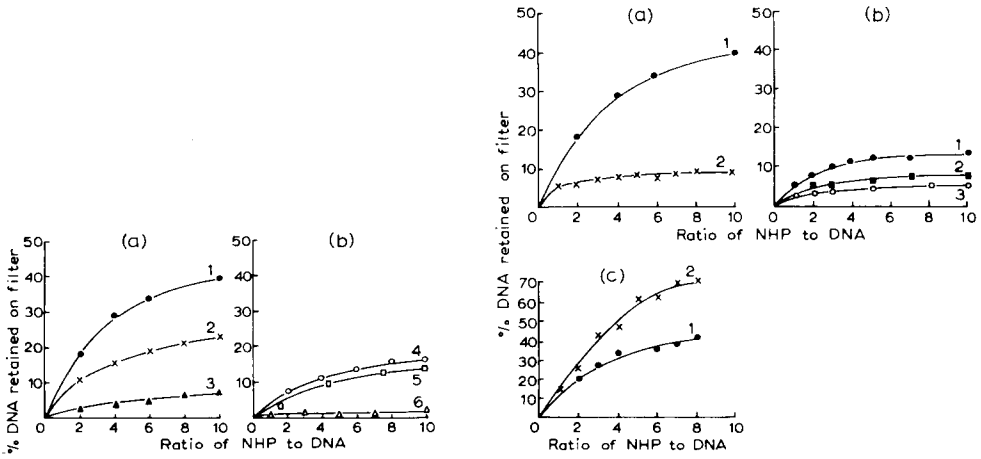


Fig. 1. Interaction of non-histone proteins of rat liver (a) and thymus (b) with the homologous [^{14}C]-DNA. 1 and 4, NHP_1; 2 and 5, NHP_2; 3 and 6, NHP_3.

Fig. 2. Effect of protein "aging" and DNA molecular weight on DNA fixation on filters. (a) Interaction of fresh (1) liver non-histone proteins and the ones kept for 2 days at 2°C (2) with the homologous DNA. (b) Interaction of liver NHP_1 (1) and NHP_2 (2) and thymus NHP_2 (3) with the sonicated homologous DNA. (c) Interaction of liver NHP_1 with [^{14}C]DNA of rat (1) and [^{3}H]DNA of the phage T4 (2).

retained on filters sharply decreases (to elucidate this problem labelled non-histone proteins were passed through nitrocellulose filters without DNA).

As will be shown below, NHP_3 interacts with DNA quantitatively most effectively, but retains it on nitrocellulose filters to the least extent.

Thus, comparison of the interaction of different non-histone proteins with DNA by this method should be done with great care. It is important, however, that in standard conditions different non-histone proteins preparations, isolated by one method, give reproducible results when studying their ability to retain DNA on filters.

The quantity of DNA retained on filters depends on its molecular weight. When adding liver NHP_1 and NHP_2 to the sonicated DNA, only 15% and 12% DNA, respectively, are retained by filters (Fig. 2b).

As will be shown below, the main part of non-histone proteins interacts with DNA non-specifically, therefore, any molecule of fragmented DNA is able to react with non-histone proteins. Thus, retention of DNA on filters really depends on its molecular weight and not on the specific distribution of non-histone proteins between the DNA molecules. Besides, DNA of the phage T4 with the molecular weight several times higher than the rat DNA isolated by us was retained by filters in the presence of liver NHP_1 more effectively than the homologous DNA (Fig. 2c). The reasons of this effect are not clear, but it is evident, however, that comparison of non-histone proteins interaction with different DNA on the basis of their retention on filters is impossible.

Comparison of non-histone proteins interaction with homologous and heterologous native DNA

In order to compare non-histone proteins interaction with homologous and heterologous DNA, the ability of non-labelled homologous and heterologous DNA to compete with homologous [^{14}C]DNA for non-histone proteins binding was studied. As competitors, DNA of rat thymus (homologous) and that of *E. coli*, *B. subtilis* and of the phage T4 (heterologous) were used. As to the ability to compete with [^{14}C]DNA, all the heterologous DNA were alike.

The data presented in Fig. 3, point to the fact that homologous and heterologous DNA compete equally with [^{14}C]DNA for binding liver NHP_1 (Figs 3a and 3b) and NHP_2 (Fig. 3b) and thymus NHP_2 (Fig. 3d). Similar data were also obtained with respect to NHP_3, but due to the fact that these proteins retain DNA on filters less effectively, the results proved to be less reliable.

Non-specific binding of the main part of non-histone proteins to DNA is not accounted for by the aggregation of non-histone proteins. Firstly, the non-histone proteins concentration was very low in our experiments. Secondly, aggregation of the proteins depends on temperature but the same quantity of [^{14}C]DNA was retained by non-histone proteins on filters at 0, 22 and 37°C (data are not presented). Thirdly, the most aggregating NHP_3 retain the smallest amount of DNA on filters. Fourthly, as it will be shown, specific interaction of non-histone proteins with denatured DNA can be observed in the same conditions.

In experiments on chromatin reconstruction, 5 M urea is often used, its presence being necessary for specific reconstruction [1,23]. Therefore, an at-

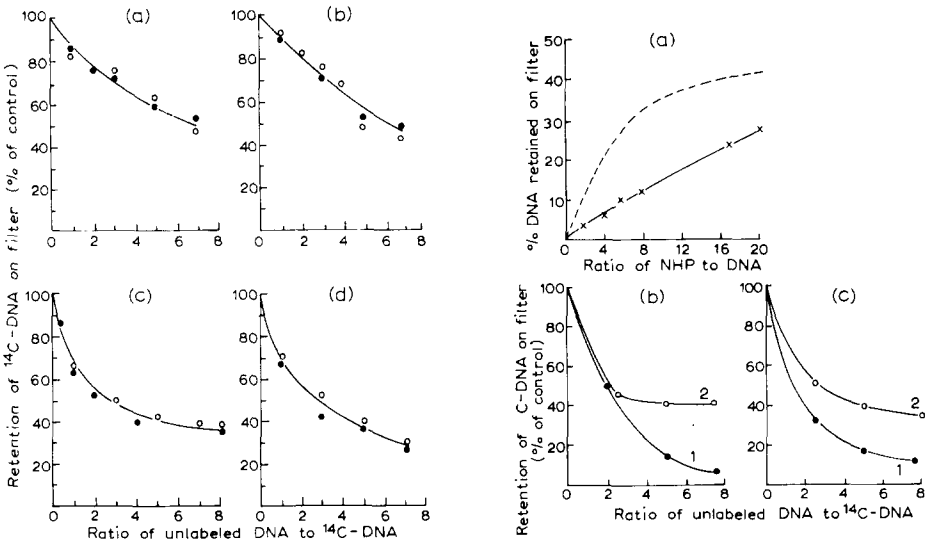


Fig. 3. Competition of the homologous and heterologous native DNA with the homologous [^{14}C]DNA for binding liver NHP_1 (a,b) and NHP_2 (c) and thymus NHP_2 (d). a,c,d are high molecular weight DNA competitors; b is a sonicated one; ●, is an homologous DNA competitor, ○, is an heterologous one. The ratio non-histone proteins/DNA in all the experiments is equal to 7.5.

Fig. 4. The competition of homologous and heterologous native DNA with the homologous [^{14}C]DNA for binding the thymus NHP_2 in 5 M urea. (a) Interaction of NHP_2 with DNA in 5 M urea—0.02 M Tris—HCl—3 mM $MgCl_2$—25 μg/ml of albumin. Dotted line, interaction in standard conditions. (b) High molecular weight DNA competitors. (c) Sonicated ones. 1, homologous DNA; 2, heterologous one. The ratio non-histone proteins/DNA in b and in c is equal to 20.

tempt was made to compare the non-histone proteins interaction with homologous and heterologous DNA in the presence of 5 M urea. It should be noted that 5 M urea weakens the non-histone proteins interaction with DNA (Fig. 4a). The data presented in Figs 4b and 4c show, that the native homologous DNA (polymer and sonicated) competes with the homologous [^{14}C]DNA for thymus NHP_2 binding better than the heterologous DNA. Similar data were also obtained for liver NHP_2. Even with a large excess of heterologous DNA, complete competition is not achieved. Thus, in the presence of 5 M urea a part of NHP_2 specifically interacts with the homologous DNA.

Comparison of non-histone proteins interaction with the homologous and heterologous denatured DNA

The study of the retention of the denatured [^{14}C]DNA with the non-histone protein complex on filters proved to be impossible, due to the high background binding of denatured DNA by filters even at low ionic strength. Therefore, in this series of experiments, the ability of homologous and heterologous denatured DNA to compete with the native homologous [^{14}C]DNA for binding non-histone proteins was compared. The data obtained are presented in Table I and Fig. 5.

First of all attention is drawn to the fact that even with the ratio DNA competitor/[^{14}C]DNA, equal to 2—3, only a small quantity of [^{14}C]DNA is retained on the filters. It points to the fact that all non-histone proteins inter-

TABLE I

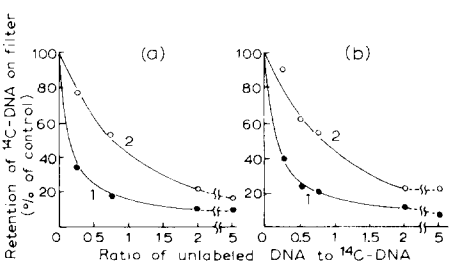
RETENTION OF THE HOMOLOGOUS NATIVE [^{14}C]DNA (IN % ON THE CONTROL) BY LIVER NON-HISTONE PROTEINS ON FILTERS IN THE PRESENCE OF DIFFERENT QUANTITIES OF DENATURED DNA COMPETITORS

Ratio of DNA competitor to [^{14}C]DNA	NHP_1		NHP_2		NHP_3	
	Homologous DNA	Heterologous DNA	Homologous DNA	Heterologous DNA	Homologous DNA	Heterologous DNA
0.5	—	—	20.0	27.0	3.0	50.0
1.0	—	—	13.0	20.5	0	3.0
2.0	24.0	43.0	5.0	13.0	0	0
3.0	11.0	19.0	—	—	0	0
4.0	10.0	18.0	4.0	7.0	0	0

acting with the native DNA, are also bound with the denatured DNA, this binding being much more effective. NHP_3 interacts with the denatured DNA most effectively. Almost complete competition is achieved both with homologous and heterologous DNA. But with small concentrations of the DNA competitor, the homologous denatured DNA competes better than the heterologous one (Fig. 5, Table I).

It undoubtedly points to the fact that at least some part of non-histone proteins interacts with the homologous denatured DNA more effectively. It should be also be borne in mind that while performing such an experiment interaction with the denatured DNA is analysed, but only of those non-histone proteins which are bound with the native DNA.

Fig. 6 presents the data of the experiments on the competition of denatured DNA with the homologous [^{14}C]DNA for the binding of thymus NHP_2 in the presence of 5 M urea. In these conditions the homologous denatured DNA competes better than the heterologous one.



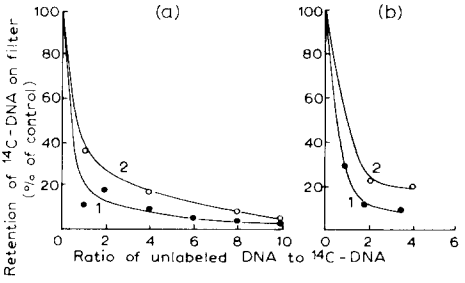


Fig. 5. Competition of homologous and heterologous denatured DNA with the homologous [^{14}C]DNA for binding the thymus NHP_2. (a) High molecular weight DNA competitors. (b) Sonicated ones. 1, homologous DNA; 2, heterologous one. The rato non-histone proteins/DNA is equal to 7.5.

Fig. 6. Competition of homologous and heterologous denatured DNA with the homologous [^{14}C]DNA for the binding NHP_2 of thymus in 5 M urea. (a) High molecular weight DNA competitors. (b) Sonicated ones. 1, homologous DNA, 2, heterologous one. The ratio non-histone proteins/DNA is equal to 20.

TABLE II

INTERACTION OF NHP_1 WITH HOMOLOGOUS AND HETEROLOGOUS NATIVE DNA

0.5 mg of liver [^{14}C] NHP_1 (50 μg/ml) were passed through the column with 3 mg of rat and *E. coli* DNA. The amount of proteins eluted at different concentrations of NaCl is tabulated as the percentage of the amount of proteins introduced into the column.

DNA	Bound with DNA (in %)	Elution by NaCl (M)		
		0.15	0.6	2.0
Homologous	19.54	8.46	7.78	3.3
Heterologous	19.9	8.4	8.2	3.3

Non-histone proteins interaction with DNA on DNA-containing polyacrylamide—agarose columns

In the first series of experiments the liver NHP_1 were passed through columns, containing homologous and heterologous native DNA and the bound proteins were eluted as described in Methods. It is clear from the data presented in Table II, that the same quantity of NHP_1 is bound with the homologous and heterologous DNA and an approximately equal quantity of non-histone proteins is eluted from both columns by different concentrations of sodium chloride.

In another series of experiments, non-histone proteins were previously passed through a column containing the heterologous DNA, the unbound proteins were collected, divided in half, put on the columns with homologous and heterologous DNA and eluted by different NaCl concentrations. In Table III data on the interaction of thymus NHP_2 and NHP_3 with native DNA are presented. First of all it should be noted that a much larger quantity of NHP_3

TABLE III

INTERACTION OF NHP_2 AND NHP_3 WITH THE HOMOLOGOUS AND HETEROLOGOUS NATIVE DNA

1 mg of ^{14}C labelled non-histone proteins (70 μg/ml) was passed through a column with 5 mg of the *E. coli* DNA (herologous I). Proteins not bound with the column, were eluted by 0.01 M Tris—HCl (pH 7.5), divided in half and passed through columns with 3 mg of the homologous or heterologous (II) DNA. The amount of proteins eluted from all the columns at different NaCl concentrations as a percentage of the amount of proteins introduced into the column is tabulated.

Proteins	NaCl (M)	DNA			B/A
		Hetero-logous I	Hetero-logous II (A)	Homo-logous (B)	
NHP_2	0.15	3.64	1.03	1.55	1.5
NHP_2	0.6	2.74	0.71	0.87	1.22
NHP_2	2.0	1.48	1.06	1.06	1.0
Total:		7.86	2.80	3.48	1.24
NHP_3	0.15	7.1	3.12	7.3	2.35
NHP_3	0.6	15.0	1.96	2.82	1.44
NHP_3	2.0	4.05	1.5	1.43	0.95
Total:		26.15	6.58	11.55	1.75

TABLE IV

INTERACTION OF THYMUS NHP_1 WITH THE HOMOLOGOUS AND HETEROLOGOUS DENATURED DNA

The procedure of the experiment is the same as in Table III.

Proteins	NaCl (M)	DNA			B/A
		Hetero-logous I	Hetero-logous II (A)	Homo-logous (B)	
NHP_1	0.15	5.08	2.2	1.8	0.82
	0.6	1.91	0.99	1.59	1.6
	2.0	0.55	0.74	1.5	2.1
	2 M NaCl + 5 M Urea	0.92	0.81	1.4	1.72
	Total	8.46	4.64	6.29	1.36

is bound with all the columns when compared with NHP_2 binding. It is not surprising, since NHP_3 are a fraction of the non-histone proteins, which remain bound with DNA at the chromatin dissociation in 1 M NaCl or interact with it when the ionic strength decreases to 0.14 M NaCl. When passing non-histone proteins which have not reacted with the heterologous DNA, through the DNA-containing columns for the second time, some specificity in interaction of non-histone proteins with DNA is found. The quantities of NHP_2 and NHP_3, binding with the homologous DNA, are 1.2—1.5 and 1.4—2.3 times larger, respectively, than those binding with the heterologous DNA. Specificity is found for proteins eluted by 0.15 M and 0.6 M NaCl and more pronounced for NHP_3. The most firmly bound proteins (eluted by 2 M NaCl) interact with the homologous and heterologous DNA with equal effectiveness.

In a similar experiment the interaction of thymus NHP_1 with the denatured DNA (Table IV) was studied. In this case, also after a preliminary passing of non-histone proteins through the column with the heterologous DNA, during the repeat chromatography of non-histone proteins on DNA-containing columns, a larger quantity of non-histone proteins is bound with the homologous DNA than with the heterologous one. Unlike the native DNA, the specificity is more pronounced for non-histone proteins eluted at high NaCl concentration and in 2 M NaCl—5 M urea.

Discussion

The data presented in the given work point to the fact that all the non-histone proteins preparations studied interact with DNA. This conclusion agrees well with the results of works of others authors [5—10].

It should be noted that experiments represented in the figures and Table I were repeated four to six times at a different ratio of non-histone proteins to DNA with quite similar results. Experiments represented in the Tables II—IV were also repeated two to three times with qualitatively similar results.

Both data obtained by the membrane filter technique (Fig. 3) and the experiments on non-histone proteins chromatography on DNA-containing col-

umns (Tables II and III) point to the fact that the main part of non-histone proteins, interacting with DNA, is bound with it non-specifically. Similar data were also obtained in other authors' works [5,8,10]. It is not surprising if the fact is taken into account that a number of enzymes which do not possess a pronounced species specificity are found in non-histone proteins: DNA polymerase [24], polynucleotide ligase [18], DNAase [25], phosphokinase, acetyl- and methyltransferase [26,27] etc.

Specific interaction of non-histone proteins with the native DNA is revealed by chromatography on DNA-containing columns of non-histone proteins, previously passed through the column with the heterologous DNA. Specificity is manifested by the fact that a larger quantity of proteins is bound with the homologous DNA than with the heterologous one. This effect is most distinctly revealed for NHP_3. It is not clear what it is due to: either the non-histone proteins specifically interacting with the homologous DNA are able to react, though in a lesser degree, with the heterologous DNA; or the passing of 1 mg of non-histone proteins through the column with 5 mg of heterologous DNA is not sufficient to remove all the non-specifically interacting non-histone proteins.

It is also shown that at least a part of non-histone proteins specifically interact with the denatured DNA. This result was obtained by both methods used to study non-histone proteins binding with DNA (Figs 5 and 6, Tables I and IV). It should be also noted that non-histone proteins interact with the denatured DNA much more effectively than with the native one (a considerable competition of denatured DNA with [^{14}C]DNA is achieved at a low concentration of the competitor, and in experiments on DNA-containing columns non-histone proteins are eluted from the denatured DNA at higher ionic strength, than from the native one).

Therefore, it is highly interesting that by the membrane filter technique the specific interaction of non-histone proteins with DNA is found only in 5 M urea. One can suggest that the effect is due to destabilization of DNA in these conditions. Then also the specific reconstruction of chromatin by gradient dialysis in the presence of urea, but not without it, can be accounted for at least partially by the destabilizing effect of urea on DNA which makes the interaction non-histone proteins—DNA easier.

The non-histone proteins interaction with DNA in the presence of heterologous DNA competitor with the subsequent complex fixation on nitrocellulose filters is in our opinion extremely promising, since this method in principle enables us to isolate DNA specifically interacting with non-histone proteins. Such experiments are being carried out at present.

From the available data it is not clear so far whether the same of different non-histone proteins are bound specifically with the native and denatured DNA.

At present it is not clear whether in vivo non-histone proteins are bound with the native DNA or at least some part of them interacts only with the denatured DNA. The specific interaction of protein regulators with the locally denatured DNA was earlier postulated by Crick [28], who suggested the model of a chromosome, in which histones maintain regulatory parts of DNA in a denatured state. In our opinion there is in cells another possibility to provide

an interaction between regulator proteins and denatured DNA. Thus, if reprogramming of DNA occurs in dividing cells, the complexing of protein regulators with denatured sites of DNA can occur in the S-period. The presence of denatured sites in DNA during its synthesis is shown in a number of works [29,30].

At least, the data on specific interaction of non-histone proteins with the native and denatured DNA support the point of view that some part of non-histone proteins participates in the specific regulation of the transcription process in the cells of eukaryotes.

References

1 Gilmour, R.S. and Paul, J. (1969) J. Mol. Biol. 40, 137–140
2 Spelsberg, T.C. and Hnilica, L.S. (1970) Biochem. J. 120, 435–437
3 Kostraba, N.C. and Wang, T.Y. (1972) Biochim. Biophys. Acta 262, 169–180
4 Shelton, K.S. and Allfrey, V.G. (1970) Nature 228, 132–134
5 Kleinsmith, L.J., Heidema, J. and Carrol, A. (1970) Nature 226, 1025–1026
6 Teng, C.S., Teng, C.T. and Allfrey, V.G. (1971) J. Biol. Chem. 246, 3597–3609
7 Wakabayashi, K., Wang, S., Hord, G. and Hnilica, L.S. (1973) FEBS Lett. 32, 46–48
8 Patel, G.L. and Thomas, T.L. (1973) Proc. Natl. Acad. Sci. U.S. 70, 2524–2528
9 Chaudhuri, S., Stein, G. and Baserga, R. (1972) Proc. Soc. Exp. Biol. Med. 139, 1363–1366
10 Van den Brock, H.W.J., Nooden, L.D., Sevall, J.S. and Bonner, J. (1973) Biochemistry 12, 229–236
11 Marmur, J. (1961) J. Mol. Biol. 3, 208–218
12 Shauveav, J., Moule, J. and Roueller, C. (1956) Exp. Cell. Res. 11, 317–321
13 Umansky, S.R., Tokarskaya, V.I., Zotova, R.N. and Migushina, V.L. (1971) Mol. Biol. U.S.S.R. 5, 270–279
14 Wang, T.Y. (1967) J. Biol. Chem. 242, 1220–1226
15 MacGillivray, A.J., Cameron, A., Krauze, R.J., Rickwood, D. and Paul, J. (1972) Biochim. Biophys. Acta 277, 384–402
16 Cavalieri, L. and Carrol, E. (1970) Proc. Natl. Acad. Sci. U.S. 67, 807–812
17 Bornkamm, G.V., Nobis, P. and Sonnenbichler, J. (1972) Biochim. Biophys. Acta 278, 258–265
18 Gasiev, A.J., Zakrzhevskaya, D.T., Umansky, S.R., Strazhevskaya, N.B., Struchkov, V.A. and Kuzin, A.M. (1971) Proc. Acad. Sci. U.S.S.R. 199, 470–473
19 Riggs, A.D., Bourgeois, S., Newby, R.F. and Cohn, M. (1968) J. Mol. Biol. 34, 365–368
20 Johns, O.W. and Berg, P. (1966) J. Mol. Biol. 22, 199–209
21 Mikoyan, V.S. and Zamchuk, L.A. (1973) Mol. Biol. U.S.S.R. 7, 714–719
22 Yarus, M. and Berg, P. (1967) J. Mol. Biol. 28, 479–490
23 Bekhor, I., Kung, G.M. and Bonner, J. (1969) J. Mol. Biol. 39, 351–364
24 Patel, G., Howk, R. and Wang, T.Y. (1967) Nature 215, 1488–1489
25 O'Connor, P.J. (1969) Biochem. Biophys. Res. Commun. 35, 805–810
26 Kish, M. and Kleinsmith, L.Y. (1972) J. Cell. Biol. 55, 138a
27 Kamiyama, M., Dastugue, B., Defer, N. and Kruh, J. (1972) Biochim. Biophys. Acta 277, 576–583
28 Crick, F. (1971) Nature 234, 25–27
29 Mizuno, N.S., Stoops, C.E. and Peiffer, R.L. (1971) J. Mol. Biol. 59, 517–525
30 Dashkevich, V.S., Dimshitz, T.M., Salganik, R.I. and Ulanov, B.P. (1972) Mol. Biol. U.S.S.R. 6, 689–697