
Protein dissociation from DNA in model systems and chromatin

Mitchell L.Shiffman, Rose A.Maciewicz, Ali W.Hu, James C.Howard and Hsueh Jei Li*

Division of Cell and Molecular Biology, State University of New York at Buffalo, Buffalo, NY 14260, USA

Received 8 June 1978

ABSTRACT

Salt induced dissociation of protamine, poly(L-lysine) and poly(L-arginine) from DNA was measured by relative light scattering at $\theta = 90^\circ$ and/or centrifugation. Dissociation of histones from DNA was studied using relative light scattering and intrinsic tyrosine fluorescence. Protamine was dissociated from DNA at 0.15 M $MgCl_2$ (ionic strength $\mu = 0.45$) or 0.53 M NaCl ($\mu = 0.53$) based on light scattering data and at approximately 0.2 M $MgCl_2$ ($\mu = 0.6$) or 0.6 M NaCl based on centrifugation data. NaCl induced dissociation of poly(Lys) or poly(Arg) from natural DNAs measured by light scattering did not depend on the guanine plus cytosine content. To dissociate poly(Arg) from DNA higher ionic strength using NaCl, $MgCl_2$, or $CaCl_2$, similar ionic strength using $NaClO_4$, and lower ionic strength using Na_2SO_4 was needed than to dissociate poly(Lys).

Both the decrease in light scattering and the enhancement of tyrosine fluorescence of chromatin occurred between 0.5 and 1.5 M NaCl when histones were dissociated.

INTRODUCTION

Protein-DNA interactions, such as repressor-DNA, polymerase-DNA, nonhistone protein-DNA and histone-DNA, play fundamental roles in controlling both the structure and functions of chromosomes. Progress in both biophysical and biochemical research on histones and chromatin in the past fifteen years has generated great interest among scientists in the investigation of histone assembly, histone-DNA interaction, and both secondary and tertiary structure of histone-bound regions in chromatin. Relating histone binding in chromatin to biological function inevitably will lead to questions of thermodynamic interactions between these chromosomal macromolecules. Similar questions can be asked about other protein-DNA complexes. Thermodynamic interactions between proteins and DNA are expected to be influenced greatly by ions in solution (both cations and anions) because both proteins and DNA contain ionic residues. To elucidate these properties, the effects of both cations and anions on the association of proteins to DNA in solution should be investigated.

Previously, physical chemical studies of protein dissociation from DNA have not been made seriously; because the methods available in the past generally needed a large amount of sample, these studies have been less attractive. Two methods have been used to probe salt dissociation of histones from DNA in chromatin: centrifugation^{1,2} and column chromatography³ followed by chemical determination of the histones in partially dehistonized chromatin. Both methods require a large quantity of chromatin and time to examine histone dissociation at one salt concentration. In fact, the above two methods were used primarily for the preparation of partially dehistonized chromatin, rather than for obtaining dissociation curves of histone from DNA in chromatin. Howard et al⁴ found that the increased light scattering intensity for protein-DNA complexes could be reduced to that for free DNA when the ionic strength was sufficiently high. They showed⁴ using ¹H NMR that the observed decrease in light scattering intensity was due to dissociation of the protein from the DNA. For example, polylysine proton resonances were observed at the same intensity and line width of a control solution of polylysine for a solution of a polylysine DNA complex that was 1.2 M in NaCl but no such resonances were observed when the solution was 1.0 M in NaCl. This report presents the results of light scattering studies of dissociation of poly(L-lysine) and poly(L-arginine) from DNA of varied G + C contents, as well as dissociations of protamine and histones from DNA by different cations and/or anions.

MATERIALS AND METHODS

The materials and methods used for this report were described in a previous report⁴ except as described below.

Purified chromatin was prepared according to the method of Marushige and Bonner⁵ as modified by Seligy and Miyagi.⁶ It was dialyzed finally to 0.25 mM EDTA, pH 8.0 (EDTA buffer). Various amounts of 4.0 M NaCl in EDTA buffer were added slowly to chromatin. The final NaCl concentrations were recorded. For measurements of both fluorescence and light scattering intensity, the total chromatin concentration at each NaCl concentration was measured by absorption ($\epsilon_{260} = 6,500 \text{ M}^{-1} \text{ cm}^{-1}$).

For measuring precipitation curves of protamine-DNA complexes at various salt concentrations, the complexes were first made at zero salt (in zero buffer without additional salt). Then various amounts of a stock solution of salt and/or buffer were added to obtain the appropriate salt concentration (NaCl or MgCl₂) and maintain a constant DNA concentration. The samples were centrifuged at 10,000 rpm in a Sorvall SS-34 rotor for 10 min. The super-

natants were collected and their absorbances at 260 nm measured.

To measure salt induced dissociation of model proteins from DNA, each complex was made in EDTA buffer followed by addition of an appropriate amount of a stock salt and/or buffer solution. Then the relative light scattering intensity of each complex at each salt concentration was measured as described by Howard et al.⁴

To measure the tyrosine fluorescence of chromatin in salt solutions, the excitation wavelength was fixed at 280 nm. Because of stray light from the excitation monochromator,⁴ a bandpass filter (Baird-Atomic, Inc.) with the central wavelength at 280.6 nm and a bandwidth of 13.6 nm at half maximum transmittance, was placed in front of the sample cell.

RESULTS

SALT INDUCED DISSOCIATION OF PROTAMINE FROM DNA

Fig. 1 shows precipitation curves of protamine-DNA complexes at various salt concentrations. When r was greater than 1.4 amino acids/nucleotide, nearly complete precipitation occurred at zero salt, while only partial precipitation occurred at 0.6 M NaCl or 0.2 M MgCl₂. No precipitation was observed at 0.8 M NaCl or 0.3 M MgCl₂. Therefore, using precipitation as a criterion to judge protamine dissociation from DNA, one would conclude that protamine was dissociated from DNA around 0.6 M NaCl or 0.2 M MgCl₂.

Precipitation is a measurement of the formation of large aggregates

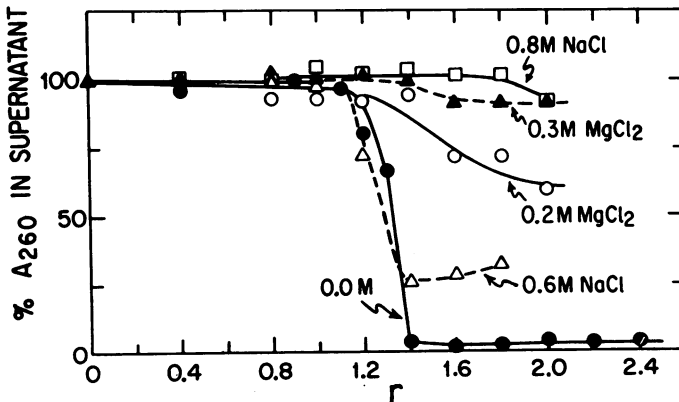


Fig. 1. Precipitation curves of protamine-DNA complexes at various salt concentrations. Salt concentrations (NaCl or MgCl₂) in the solution of complex are given in the figure.

which may or may not be directly related to tight binding of protein to nucleic acids. To measure dissociation of protamine from its complex with DNA the relative light scattering intensity⁴ was used and the results are shown in Fig. 2. Based upon the criterion of light scattering, protamine was dissociated from DNA at 0.53 M NaCl and 0.15 M MgCl₂ which are close to the 0.6 M NaCl and 0.2 M MgCl₂ determined by centrifugation (Fig. 1).

DISSOCIATION OF POLYLYSINE OR POLYARGININE FROM COMPLEXES WITH DNA OF VARIED G + C CONTENTS

When DNAs of various (G + C) content competed for protein binding at high ionic strength or during reconstitution from high salt to low salt, (A+T)-rich DNA was favored by polylysine^{7,8} lysine-rich histones H5⁹ and H1,^{10,11,12} and slightly lysine-rich histones H2A and H2B.¹³ Therefore, it is interesting to examine the dependence of salt induced dissociation of polylysine or polyarginine from the complexes on the G + C content of DNA.

Fig. 3 shows NaCl induced dissociation curves of polylysine-DNA complexes with varied G + C contents. For natural DNAs with G + C content varied from 31 to 70%, dissociation occurred in the narrow range of 1.0-1.2 M NaCl.

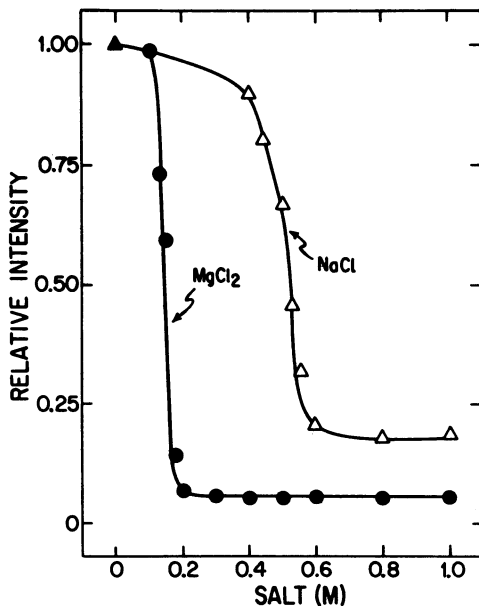


Fig. 2. Dependence of scattering intensity at 305 nm of protamine-DNA complex (r = 0.8 amino acid residues/nucleotide) on salt concentration.

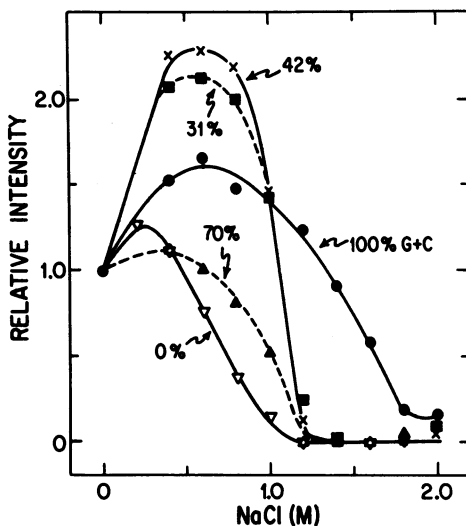


Fig. 3. Dependence of scattering intensity at 320 nm of polylysine-DNA complexes with varied G + C content on NaCl concentration. G + C content in each DNA is given in the figure. $r = 0.9$ lysine/nucleotide.

There is no apparent (G + C) dependence of NaCl induced dissociation. However in the complexes with synthetic DNAs of 100% G + C, e.g., poly(dG)·poly(dC), or 100% A + T, e.g., poly(dA-dT), the NaCl induced dissociation curves differed greatly between themselves as well as from those of natural DNAs. Polylysine was dissociated from poly(dG)·poly(dC) in 1.3-1.8 M NaCl and from poly(dA-dT) in 0.5-1.0 M NaCl. These results cannot be attributed only to differences in G + C content, because there was no consistent increase or decrease in the range of NaCl concentration required to dissociate polylysine. On the other hand, these unusual results for poly(dG)·poly(dC) and poly(dA-dT) could be related to the unique thermal denaturation and circular dichroism properties possessed by their complexes with polylysine, when compared with those of natural DNA.¹⁴

Judged by light scattering criterion, polyarginine was dissociated from DNA in a much broader range of NaCl concentration (Fig. 4) than was polylysine (Fig. 3). For instance, for calf thymus DNA with 42% G + C, polyarginine was dissociated in 1.0-1.8 M NaCl compared with 1.0-1.2 M NaCl for polylysine.

Similar to the case of polylysine, there was no major difference in NaCl induced dissociation of polyarginine from complexes with natural DNA

of varied G + C content (31% to 70%). The dissociation of polyarginine from synthetic poly(dA-dT) was similar to that of natural DNAs but was very different from that of poly(dG).poly(dC). The results in Fig. 4 suggest that polyarginine was not dissociated from poly(dG).poly(dC) by NaCl concentrations as high as 3.0 M. It is noted that both thermal denaturation¹⁵ and circular dichroism properties¹⁶ of complexes of polyarginine with poly(dG).poly(dC) were very different from those of complexes with natural DNA or poly(dA-dT).

The above results indicate that the G + C content of a DNA is not directly a major factor which controls salt induced dissociation of polylysine or polyarginine from DNA. Instead, the different dissociation properties shown in Figs. 3 and 4 are more likely related to structural differences between natural and synthetic DNA, and thus, with differences in the mode of binding.

CATION AND ANION DEPENDENCE OF DISSOCIATION OF POLYLYSINE OR POLYARGININE FROM COMPLEXES WITH DNA

The ionic bonds formed between basic amino acid residues in proteins and phosphates in DNA can be broken resulting in dissociation of the protein from the DNA. To understand the breakage of these bonds one has to consider the competition among five groups: cations (lysine or arginine) and anions (glutamic or aspartic acid) in proteins, phosphates in DNA, and cations and

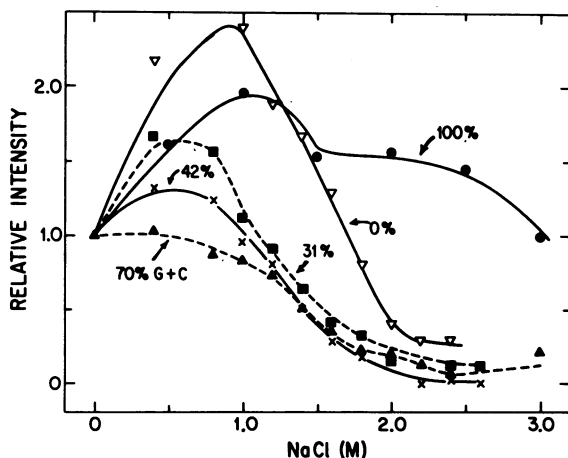


Fig. 4. Dependence of scattering intensity at 320 nm of polyarginine-DNA complexes with varied G + C content on NaCl concentration. G + C content in each DNA is given in the figure. $r = 0.8$ arginine/nucleotide.

anions in solution. To investigate this competition and its effects on protein dissociation from DNA, the light scattering intensity of polylysine-DNA and polyarginine-DNA complexes was measured as a function of the type and concentration of salt.

For polylysine-DNA complexes, there was always an initial increase of scattering intensity at lower salt concentration followed at higher salt concentration by a decrease to its initial value and then at still higher concentration by a further decrease to the level of DNA alone. As reported in the previous paper,⁴ the initial increase and following decrease of intensity was probably caused by salt-induced aggregation and disaggregation of the complexes while the last decrease of the intensity to the DNA level was caused by dissociation of polylysine from DNA. Therefore, these two phenomena have to be examined separately.

According to the results shown in Fig. 5, dissociation of polylysine from DNA occurred after the breakage of salt-induced aggregation in NaCl, Na₂SO₄ and MgCl₂. CaCl₂ and NaClO₄ behaved differently in this regard. For CaCl₂, there was a decrease of intensity at an ionic strength of 0.3-0.9 to about 75% of that at zero salt. Then between 1.1 and 1.2 ionic strength the intensity decreased to the level of DNA alone. For NaClO₄, the decrease due to breakage of the aggregates of complexes occurred between 0.2 and 0.4 and was followed by a broad dissociation between 0.4 and 1.0 ionic strength.

Polylysine was observed to dissociate from DNA when the ionic strength

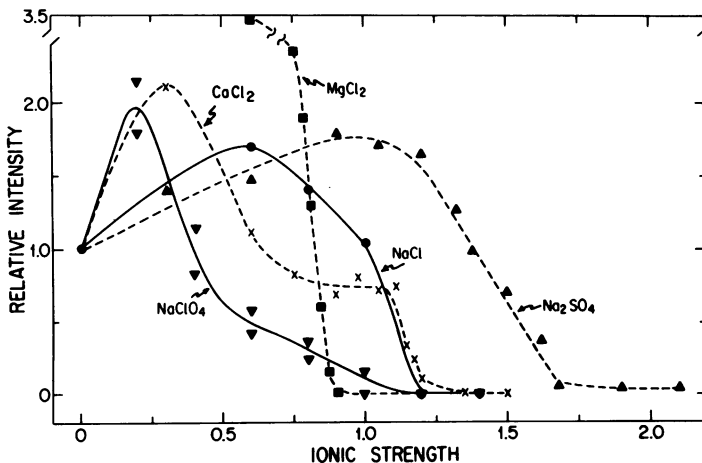


Fig. 5. Dependence of scattering intensity at 320 nm of polylysine-DNA complexes on ionic strength. Types of salt used in the solution are given in the figure. $r = 0.5$ lysine/nucleotide for NaCl, CaCl₂, and MgCl₂, and 0.66 lysine/nucleotide for Na₂SO₄ and NaClO₄.

was 1.0-1.2 for NaCl, 0.4-1.2 for NaClO₄, 1.35-1.65 for Na₂SO₄, 1.10-1.35 for CaCl₂ and 0.83-0.90 for MgCl₂. For the same anion, e.g., chloride, Mg⁺⁺ was more effective than Ca⁺⁺ which, in turn, was more effective than Na⁺. For the same cation, e.g., Na⁺, perchlorate was more effective than chloride in initiating the dissociation of polylysine from DNA but the end point was the same. Sulfate was not nearly as effective as chloride.

Dissociation of polyarginine from DNA occurred at an ionic strength of 1.0-1.8 for NaCl which was broader and the end point was at a higher value than that for polylysine (1.0-1.2 for NaCl). NaClO₄ also dissociated polyarginine in a broad range (0.4-1.2). However, MgCl₂ dissociated polyarginine between 1.2 & 1.3 and CaCl₂ between 1.6 & 1.75 which are slightly higher than the ionic strengths required for polylysine dissociation. For the same anion, e.g., chloride, divalent Mg⁺⁺ and Ca⁺⁺ dissociated both polyarginine and polylysine in a narrow range of ionic strength while monovalent Na⁺ dissociated both polylysine and polyarginine in a broad range of salt which indicates that dissociation of polylysine and polyarginine from DNA by Na⁺ is less cooperative than by Ca⁺⁺ and Mg⁺⁺.

Although sulfate of Na₂SO₄ was significantly less effective than chloride of NaCl in dissociating polylysine from DNA (Fig. 5), it was indeed much more effective than chloride in dissociating polyarginine from DNA (Fig. 6). It is noted that at higher Na₂SO₄, the scattering intensity of polyarginine-DNA complexes did not decrease to the DNA level. It was found that such residual scattering intensity was contributed by polyarginine because free polyarginine showed slightly increased scattering when the

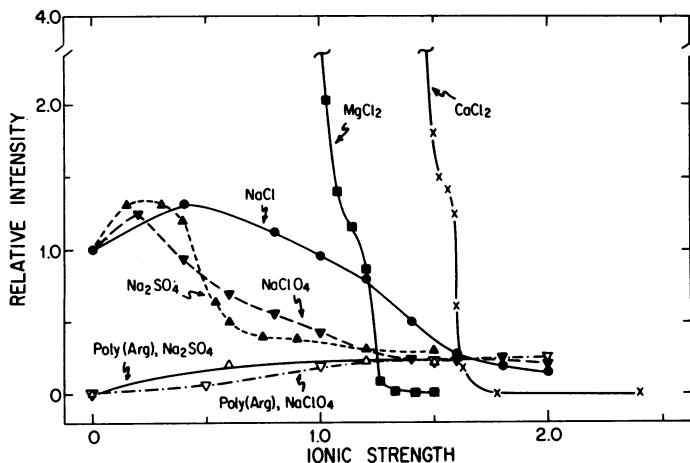


Fig. 6. Dependence of scattering intensity at 320 nm of polyarginine-DNA and polyarginine alone on ionic strength. Types of salt used in the solutions are given in the figure. $r = 0.65$ arginine/nucleotide.

concentration of Na_2SO_4 was increased (Fig. 6). Dissociation of polyarginine from DNA by K_2SO_4 also occurred at 0.45-0.75 ionic strength similar to that for Na_2SO_4 (data not shown).

It has been reported^{17,18} that the effectiveness of anions to induce conformational changes in arginine-rich histone H4 was as follows: divalent SO_4^{2-} , HPO_4^{2-} > monovalent anions H_2PO_4^- , ClO_4^- > Cl^- . Differential effectiveness of inducing conformational changes in histone H4 was explained to be due to different binding affinities of these anions to histone H4, presumably to the basic arginine and lysine residues. To determine whether or not all divalent anions are as effective in dissociating polyarginine from DNA as they were in inducing ordered secondary structures in histone H4, a mixture of Na_2HPO_4 and NaH_2PO_4 at various pHs was used to dissociate polyarginine-DNA complexes. The results (not shown) indicated that dissociation of polyarginine occurred at 0.7-1.0 M sodium phosphate for pH 5.5, 7.0 and 8.6, even though the concentration of the divalent species, HPO_4^{2-} , increased relative to the monovalent species H_2PO_4^- as the pH was raised. Apparently divalent HPO_4^{2-} was not more effective than monovalent H_2PO_4^- in dissociating polyarginine from the DNA phosphates. Therefore, sulfate was unique in its effectiveness in dissociating polyarginine from DNA.

A substantial light scattering (10-30% of that of the polyarginine-DNA complexes at zero salt) was detected when polyarginine without DNA was added to Na_2SO_4 , NaClO_4 or sodium phosphate solutions at the concentrations in the range that polyarginine dissociated from DNA complexes (Fig. 6). Perhaps these anions effectively caused structural changes or aggregation in polyarginine. No such induced scattering was found when free DNA or free polylysine was added to these salt solutions.

DEPENDENCE OF SALT-INDUCED DISSOCIATION OF PROTEIN ON ITS CHAIN LENGTH

At each ionic condition tested, direct complexing of oligolysine with DNA¹⁹ or trypsin digestion of polylysine-DNA or polyarginine-DNA²⁰ showed that oligopeptides did not induce biphasic melting. It had been suggested²¹ that biphasic melting can be induced if a protein binds to DNA so strongly that no appreciable amount of dissociation occurs during the melting experiment. For polylysine or polyarginine, the longer the chain length the more ionic bonds formed between each polypeptide molecule and DNA, and the greater the binding affinity. Biphasic melting can be generated if the chain length is longer than a certain limit, e.g., 14-18 lysine residues.¹⁹ Because biphasic melting of a protein DNA complex depends upon the chain length of the protein, one might expect some dependence of salt induced

dissociation of proteins from DNA on protein chain length. For instance, Fig. 2 shows that protamine of 30 amino acid residues with 67% arginine and 33% nonbasic residues was dissociated from DNA at 0.15 M $MgCl_2$ ($\mu = 0.45$) or 0.55 M NaCl ($\mu = 0.55$), which are, respectively, lower than the 0.4 M $MgCl_2$ ($\mu = 1.2$) or 1.0-1.8 M NaCl ($\mu = 1.0-1.8$) needed to dissociate polyarginine with an average chain length of 72 residues (Fig. 6). This difference could be due to the different chain lengths of protamine and polyarginine (respectively, 30 and 72, in amino acid residues or 20 and 72 in arginine residues), or possibly to a destabilization effect from the nonbasic residues in protamine.

To elaborate the chain length question, dissociation of polylysine-DNA complexes using polylysine of several chain lengths was examined. Fig. 7 shows that a slight decrease of the NaCl concentration was needed for dissociating polylysine from DNA when the average chain length of polylysine was shortened from 857 to 147 residues. The NaCl concentration needed for dissociation decreased more significantly as the average polylysine chain length was reduced to 28 residues. The results suggest a chain length dependence of NaCl induced dissociation of polylysine from DNA. Such dependence becomes more significant when the chain length becomes small. These results also suggest that the chain length effect may be the reason that protamine dissociated from DNA at a salt concentration 2-3 times lower than that required for dissociating polyarginine.

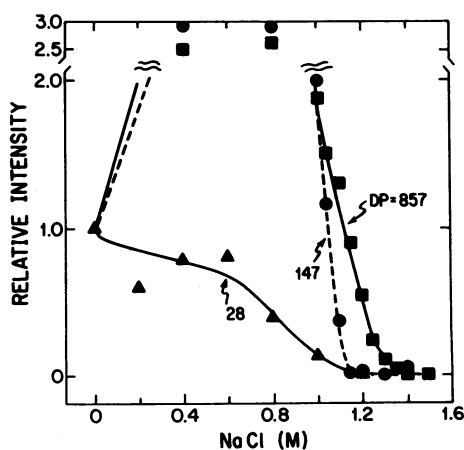


Fig. 7. Dependence of scattering intensity at 320 nm of polylysine-DNA complexes of varied polylysine chain length on NaCl concentration. Average chain length or degree of polymerization used was 857, 147 or 28.

HISTONE DISSOCIATION FROM CHROMATIN

Histones are basic proteins which, when bound to DNA in chromatin, induce multiphasic melting curves.²²⁻²⁸ Therefore, according to what has been observed in model systems, it should be expected that histone binding to DNA in chromatin will induce light scattering well above that of pure DNA. As the histones are dissociated from chromatin DNA, a decrease in light scattering should be observed and therefore relative light scattering could be used also for probing histone dissociation from chromatin.

Fig. 8 shows the dependence of scattering intensity of chromatin on NaCl concentration. No scattering data were measured between zero salt concentration (EDTA buffer alone) and 0.5 M NaCl because of a substantial reduction of the concentration of the soluble fraction due to both aggregation and adhesion of some chromatin to the test tube and/or the curvette in this salt range. Such an effect becomes less significant above 0.5 M NaCl. The absorbance, A_{260} , of chromatin in NaCl solution relative to that at zero salt concentration was increased from 80% at 0.5 M NaCl to 100% at 2.0 M NaCl. No correction of this absorbance difference was made on salt-induced dissociation curves of histones from chromatin measured either by relative light scattering (Fig. 8) or by fluorescence (Fig. 10).

Fig. 8 shows that a substantial reduction in the relative light scatter-

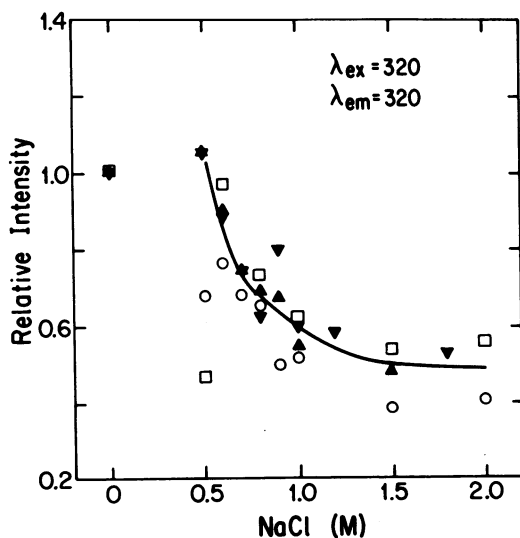


Fig. 8. The light scattering intensity at 320 nm of calf thymus chromatin at various NaCl concentrations. Each symbol represents one set of data.

ing intensity occurred between 0.5 and 1.0 M NaCl, followed by a more gradual reduction between 1.0 and 1.5 M NaCl. These results are similar to NaCl-induced dissociation curves of histones from chromatin measured by a combination of centrifugation and biochemical determination of the histone content in NaCl-treated chromatin² except that the decrease of scattering intensity after 1.0 M NaCl was smaller than should be expected from the amount of histones still associated with DNA at this salt concentration. There are at least three possible explanations for this difference: (a) According to centrifugation criterion, arginine-rich histones and some slightly lysine-rich histones were not fully dissociated from DNA at NaCl = 1.0 M, but portions of these molecules may have been partially dissociated; such partially dissociated histones might cause a reduction of scattering intensity before they were fully dissociated by salt, because the report of Howard et al.⁴ indicated a proportionality of the light scattering intensity to the number of base pairs tightly bound by proteins. (b) The reduction of intensity due to histone dissociation after 1.0 M NaCl may be partially compensated by an increase of light scattering of dissociated histones at higher salt concentration, a phenomenon similar to polyarginine in Na₂SO₄ and phosphate buffer; this explanation receives support from the observation of histone aggregation at high salt concentration.^{17,18,29-31} (c) those histones dissociated before 1.0 M NaCl (lysine-rich histones and some slightly lysine-rich histones), when bound to DNA, induce greater scattering intensity than the others.

Aromatic amino acids in histones, e.g., tyrosine and phenylalanine, possess intrinsic fluorescence. In particular, tyrosine residues have a greater quantum yield than phenylalanine residues.³² It is likely that binding of histones to DNA in chromatin changes the electronic environment surrounding tyrosine chromophores as well as the quantum yield of these residues. Thus, fluorescence might be used as another convenient tool for probing histone dissociation from chromatin.

Fig. 9 shows uncorrected emission spectra of chromatin at three salt concentrations when excited at 280 nm. Because of the stray light in our instrument,⁴ a bandpass filter centered at 280.6 nm was used before these measurements. Chromatin at zero salt concentration had an emission maximum at 307 nm, which is the same as that of poly(Lys⁵⁰ Tyr⁵⁰) either free or complexed with DNA³³ and free tyrosine.³² At 0.6 M NaCl, there was a slight decrease of the fluorescence intensity, while at 2.0 M NaCl, the intensity was much greater than that of chromatin at zero salt concentration. The

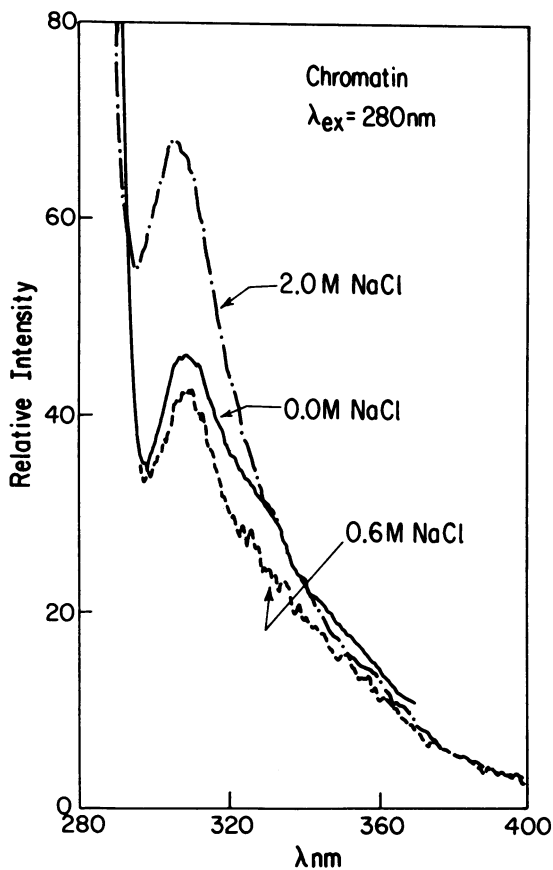


Fig. 9. Uncorrected tyrosine fluorescence spectra of chromatin at various NaCl concentrations. $\lambda_{ex} = 280 \text{ nm}$.

dependence of the fluorescence intensity at 307 nm of chromatin on NaCl concentration is shown in Fig. 10. It is noted that dissociation of histone H1 by 0.6 M NaCl¹ caused a reduction of the fluorescence intensity while dissociation of other histones by higher NaCl concentrations caused an increase of the intensity. This difference could be due to a much lower tyrosine content in histone H1 (0.5%) than in other histones (2.1 to 3.8%).³⁴

The histone dissociation curve measured by fluorescence (Fig. 10) is similar to that measured by light scattering (Fig. 8), namely the main change in intensity occurred between 0.5 and 1.0 M NaCl. Perhaps the three possible explanations suggested above for light scattering also can be applied here.

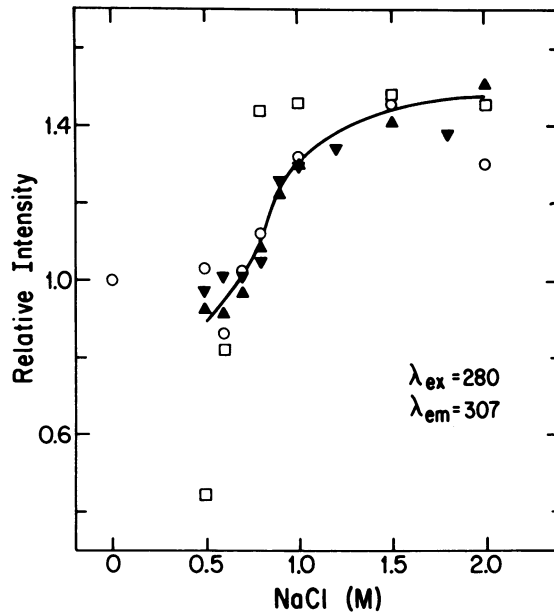


Fig. 10. Dependence of tyrosine emission intensity of chromatin on NaCl concentration. $\lambda_{ex} = 280$ nm and $\lambda_{em} = 307$ nm.

DISCUSSION

Protein and DNA are macromolecules whose interactions are expected to depend greatly upon solution conditions, because the latter control not only the conformations of these two macromolecules but also the type and affinity of bonding involved in the interactions. So far most of the studies of protein-DNA interactions, especially of histone-DNA and model protein-DNA, have been made at low ionic strength on complexes prepared either by direct mixing or by reconstitution. One of the main reasons for using low ionic strength is to separate the protein-free and protein-bound regions in the thermal denaturation profile of the complex. At higher ionic strength such separation is diminished. However, the disadvantage of using low ionic strength is that the interactions are dominated by ionic bonding between amino acid residues in the proteins and phosphates in the DNA. Other types of bonding, nonpolar interactions and hydrogen bonds, for example, which presumably play more important roles in binding of proteins to specific DNA sequences, may not be as important as they are in natural complexes. Partly for this reason, reconstitution which is essentially salt gradient dialysis from high salt concentration (in which

proteins and DNA are dissociated) to low salt concentration (in which protein and DNA are complexed) commonly has been adopted. Nevertheless, using reconstituted complexes, one can examine only the final complexes at low ionic strength and then speculate what has happened in interactions at an unknown salt range wherein proteins and DNA interact reversibly. Therefore, in order to improve our understanding of protein-DNA interactions, it is desirable to examine the complexes in the salt ranges in which reversible interactions occur. It also is desirable to examine the effects of solution cations and anions on ionic bonds formed between proteins and DNA.

Since the main model proteins studied in this report are polylysine and polyarginine, and since the main ionic bonds in protein-DNA complexes occur between lysine ϵ -amino groups and phosphates or between arginine guanidinium groups and phosphates, the discussion to be presented here will emphasize the following two major questions: (a) What is the relationship between selective binding of proteins to a specific DNA sequence and salt induced dissociation of these proteins from the DNA? In other words, is there any dependence of the ionic bonds formed between proteins and DNA on its G + C content? (b) how do solution cations and anions dissociate proteins from DNA?

It is known for conditions of reversible binding that (A+T)-richer DNA is selectively bound by polylysine^{7,8} histone H5,⁹ histone H1,^{10,11,12} and histone H2A or H2B.¹³ Presumably the explanation is that, under these conditions, these proteins bind (A+T)-richer DNA more strongly than the (G+C)-richer DNA. One might then expect that a complex with greater binding affinity would require higher salt concentration to break the bonds and cause dissociation. However, the results in Figs. 3 and 4 indicate that this is not the case. Perhaps, the relationship between selectivity of a DNA sequence for protein binding and salt induced dissociation of proteins from DNA should be examined more critically.

Previously, it was shown that the circular dichroism properties of polyarginine-DNA¹⁶ and polylysine-DNA¹⁴ complexes were essentially the same for natural DNAs of varied G + C contents. The melting properties of these complexes vary but could be explained as a result of higher thermal stability of a G-C pair than an A-T pair either in a free or complexed state. The CD and melting properties, however, differ significantly in complexes with synthetic DNAs, e.g., poly(dA-dT) and poly(dG).poly(dC), more so in the latter.^{14,15,16} These differences had been explained as being a result of different chemical and structural features in these synthetic DNAs such

that the modes of binding between these DNAs and polylysine (or polyarginine) were different from those of natural DNAs. The results of Figs. 3 and 4 also indicate that NaCl induced dissociation of polylysine or polyarginine from synthetic and from natural DNAs was quite different. Since a high concentration of NaCl primarily breaks the bonds formed between phosphates and lysine or arginine residues, the present results suggest that the ionic bonds formed with natural DNAs are independent of the G + C content, while those formed with synthetic DNAs depend upon both the chemical and the structural nature of the particular synthetic DNA. Because of these differences in complexes with natural and with synthetic DNA, one should be cautious about using synthetic DNAs as models for A·T or G·C pairs in natural DNA.

For natural DNAs, there seems to be no difference in the ionic bonds formed between basic amino acid residues and phosphates linked to G·C or A·T pairs. Therefore the greater affinity of polylysine for an (A+T)-rich DNA seems to be determined by factors other than ionic bonding, which can include hydrophobic and/or hydrogen bonding between methylene groups as well as neutralized amino groups of polylysine and the groove of DNA which has both a chemical and structural nature unique for A·T pairs. Cooperativity of protein binding to DNA can also play some role in selectivity of DNA molecules for binding. For instance, there has been a consistently observed correlation between these two phenomena, i.e., selectivity and cooperativity of protein binding to (A+T)-rich DNA, such as for polylysine,^{7,8,35,36} histone H5,⁹ histone H2B or H2A¹³ and histone H1.^{11,12} Consequently, these factors have to be examined more extensively if the selectivity of a specific DNA sequence for protein binding is to be understood.

For the discussion of the roles played by solution cations and anions in dissociating proteins from DNA, we define the following items:

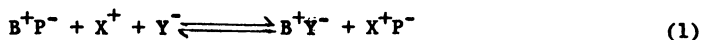
B^+ : a cation in a basic amino acid residue (lysine or arginine) of a protein

P^- : an anion in the phosphate of a DNA

X^+ : a solution cation which can be monovalent, e.g., Na^+ , K^+ , or divalent, e.g., Ca^{++} , Mg^{++}

Y^- : a solution anion which can be monovalent, e.g., Cl^- , ClO_4^- or $H_2PO_4^-$, or divalent, e.g., SO_4^{--} or HPO_4^- .

Dissociation of a protein from DNA by salt can be described by the following reaction:



When a protein is held by DNA primarily through ionic bonds, dissociation

tion can happen either through a strong binding of solution cations to DNA phosphates or through strong binding of solution anions to basic amino acid residues in the protein or through a combination of both.

According to the dissociation results of Figs. 5 and 6, it always requires more chloride to completely dissociate polyarginine from DNA when the same cation is used, e.g., Na^+ , Mg^{++} or Ca^{++} . It is deduced that a chloride anion binds the ϵ -amino group of lysine more strongly than the guanidinium group of arginine. The above preference is insignificant for perchlorate and is reversed for sulfate. These results could be related to a similar chemical structure in the sulfate and perchlorate ions. It is possible that the guanidinium group of arginine binds these anions more strongly than chloride anion and that these anions facilitate polyarginine dissociation from DNA equally well or even better than polylysine dissociation.

It is noted that, when the effectiveness in dissociating polyarginine from DNA phosphates by anions is compared, one has to consider the affinity of complexing guanidinium groups with solution anions, e.g., perchlorate or sulfate, as compared to that with phosphates, PO_4^- , in DNA. The results of figs. 5 & 6, therefore, indicate that sulfate competes much better for arginine residues than perchlorate or chloride when the competition is against DNA phosphates. Possibly this competition is the reason for the observation that divalent phosphate, HPO_4^{--} , is not better than monovalent phosphate, H_2PO_4^- , in dissociating polyarginine from DNA, although HPO_4^{--} induces conformational changes in arginine-rich histone H4 as effectively as does sulfate¹⁸ and more effectively than does H_2PO_4^- .¹⁷

The above discussion has emphasized the role of anions in dissociating polylysine or polyarginine from DNA. Cations also play a role in these dissociation processes. For instance, for polyarginine-DNA complexes, MgCl_2 and CaCl_2 give a much sharper transition than does NaCl (Fig. 6).

Comparing MgCl_2 and CaCl_2 , the former appears to be more effective than the latter in dissociating polylysine or polyarginine from DNA. This difference could be due to a lower activity coefficient for Ca^{++} than for Mg^{++} ,³⁷ so that at the same total concentration, the Mg^{++} activity is higher than the Ca^{++} activity.

Interactions between proteins and DNA are very complex. Our studies of salt induced dissociation of proteins from DNA in model systems and in chromatin are preliminary to more extensive studies in chromatin and in nuclease resistant particles.

REFERENCES

- 1 Ohlenbusch, H.H., Olivera, B.M., Tuan, D. and Davidson, N. (1967) *J. Mol. Biol.* 25, 299-315.
- 2 Fambrough, D. and Bonner, J. (1968) *Biochim. Biophys. Acta* 154, 601-602.
- 3 Paoletti, R.A. and Huang, R.C.C. (1969) *Biochemistry* 8, 1615-1625.
- 4 Howard, J.C., Shiffman, M.L., Santella, R.M. and Li, H.J. *Biochemistry* (submitted).
- 5 Marushige, K. and Bonner, J. (1966) *J. Mol. Biol.* 15, 160-174.
- 6 Seligy, V. and Miyagi, M. (1969) *Exp. Cell Res.* 58, 27-34.
- 7 Leng, M. and Felsenfeld, G. (1966) *Proc. Nat. Acad. Sci. U.S.A.* 56, 1325-1332.
- 8 Li, H.J., Brand, B. and Rotter, A. (1974) *Nucl. Acids Res.* 1, 257-265.
- 9 Hwan, J.C., Leffak, I.M., Li, H.J., Huang, P.C. and Mura, C. (1975) *Biochemistry* 14, 1390-1396.
- 10 Sponar, J. and Sormova, Z. (1972) *Eur. J. Biochem.* 29, 99-103.
- 11 Renz, M. and Day, L.A. (1976) *Biochemistry* 15, 3220-3228.
- 12 Polacow, I., Cabasso, L. and Li, H.J. (1976) *Biochemistry* 15, 4559-4565.
- 13 Leffak, I.M. and Li, H.J. (manuscript in preparation)
- 14 Li, H.J., Herlands, L., Santella, R. and Epstein, P. (1975) *Biopolymers* 14, 2401-2415.
- 15 Epstein, P., Yu, S.S. and Li, H.J. (1974) *Biochemistry* 13, 3706-3712.
- 16 Yu, S.S., Epstein, P. and Li, H.J. (1974) *Biochemistry* 13, 3713-3717.
- 17 Li, H.J., Wickett, R., Craig, A.M. and Isenberg, I. (1972) *Biopolymers* 11, 375-397.
- 18 Wickett, R.R., Li, H.J. and Isenberg, I. (1972) *Biochemistry* 11, 2952-2957.
- 19 Olins, D.E., Olins, A.L. and von Hippel, P.H. (1968) *J. Mol. Biol.* 33, 265-281.
- 20 Li, H.J., Rothman, R., Hu, A. and Pinkston, M. (manuscript in preparation).
- 21 Li, H.J. (1973) *Biopolymers* 12, 287-296.
- 22 Li, H.J. and Bonner, J. (1971) *Biochemistry* 10, 1461-1470.
- 23 Ansevin, A.T., Hnilica, L.S., Spelsberg, T.C. and Kehm, S.L. (1971) *Biochemistry* 10, 4793-4803.
- 24 Hanlon, S., Johnson, R.S., Wolf, B. and Chan, A. (1972) *Proc. Nat. Acad. Sci. USA* 69, 3263-3267.
- 25 Li, H.J., Chang, C. and Weiskopf, M. (1973) *Biochemistry* 12, 1763-1772.
- 26 Subirana, J.A. (1973) *J. Mol. Biol.* 74, 363-386.
- 27 Hjelm, R.P., Jr. and Huang, R.C.C. (1974) *Biochemistry* 13, 5275-5283.
- 28 Tsai, Y.H., Ansevin, A.T. and Hnilica, L.S. (1975) *Biochemistry* 14, 1257-1265.
- 29 Edwards, P.A. and Shooter, K.V. (1969) *Biochem. J.* 114, 227-235.
- 30 Boublik, M., Bradbury, E.M. and Crane-Robinson, C. (1970) *Eur. J. Biochem.* 14, 486-497.
- 31 Smerdon, M.J. and Isenberg, I. (1974) *Biochemistry* 13, 4046-4049.
- 32 Teale, F.W.J. and Weber, G. (1957) *Biochem. J.* 65, 476-482.
- 33 Santella, R.M. and Li, H.J. (1974) *Biopolymers* 13, 1909-1926.
- 34 Hnilica, L.S. (1972) "The Structure and Biological Functions of Histones," CRC Press, Cleveland, Ohio.
- 35 Olins, D.E., Olins, A.L. and von Hippel, P.H. (1967) *J. Mol. Biol.* 24, 157-176.
- 36 Weiskopf, M. and Li, H.J. (1977) *Biopolymers* 16, 669-684.
- 37 Lang, N.A. (1967) "Handbook of Chemistry," McGraw-Hill Book Co. New York, p. 1216.