Essential Role of Arginine Residues in the Folding of Deoxyribonucleic Acid into Nucleosome Cores†

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ABSTRACT: The kinetic studies of chemical modifications of the lysine and arginine residues of the nucleosome core particle from chicken erythrocytes with large molar excesses of 2,4,6-trinitrobenzenesulfonic acid and 2,3-butanedione, respectively, were performed over 20 mM–2.0 M NaCl. Each modification reaction was divided into the two, a rapid and an extremely slow, pseudo-first-order processes, and the numbers of the exposed and buried residues of the respective amino acids were determined. At very low ionic strength (20 mM boric buffer) all residues were inaccessible to the reagents. Between 0.3 and 0.6 M NaCl the basic amino acid residues in a nucleosome core particle were classified into the following three groups: (1) 42 arginine and 78 lysine residues in the N-terminal and C-terminal regions of the histones that are exposed to solvent, (2) a small number of arginine residues ($\lesssim$14 Arg) that are strongly bound to the DNA phosphates, and (3) 48 arginine and 38 lysine residues buried in the globular region of the histone octamer. The results suggest that a small number of arginine residues play an essential role in the folding of DNA into a nucleosome core particle.

In eukaryotic nuclei DNA is packed into the elementary subunit called nucleosomes. It is established that the nucleosome core is the stable disk-shaped particle (Langmore & Wooley, 1975; Finch et al., 1977). However, little is known about the detailed mechanisms of the wrapping of DNA around the histone octamer or of the stabilization of the globular structures of histones in the nucleosome core particle.

The DNA of a core particle has 290 phosphate groups whereas the core histone octamer has a net positive charge of ~150, and there is a potential that one negative DNA phosphate charge per DNA base pair is neutralized. Thus, charge–charge interactions between the DNA phosphates and the basic amino acid residues of histones would be essential to fold DNA into the nucleosome core particle. In fact histone molecules are dissociated from DNA by salt or acid but not by urea (Olins et al., 1977; Woodcok & Frado, 1978). In this respect, it has been suggested that only a small fraction of the DNA phosphates may be involved in the charge–charge interactions (McGhee & Felsenfeld, 1980a).

This paper attempts to determine the numbers of the exposed and buried arginine and lysine residues in a nucleosome core particle as a function of ionic strength over 20 mM–2.0 M NaCl by chemical modification methods to get detailed information about the interactions between the basic amino acids and the DNA phosphates.

Experimental Procedures

Preparation of Nucleosome Core Particles. Nucleosome core particles were prepared by micrococcal nuclease digestion of H1- and H5-depleted chicken erythrocyte chromatin as described in Tatchell & Van Holde (1977). A 5–20% linear sucrose density gradient ultracentrifugation was used to isolate core particles from chromatin digests. The core particles contained equimolar quantities of the histones H2A, H2B, H3, and H4, as demonstrated by electrophoresis on polyacrylamide gels containing sodium dodecyl sulfate. The DNA of the core particles moved as a single band on polyacrylamide gels for double-stranded DNA and single-stranded DNA. The length of DNA was estimated as 145 ± 5 base pairs by comparison with ϕX 174 RF DNA *Hae*III digest (New England Biolaboratories, Inc.).

Modification of Lysine Residues in Nucleosome Core Particles. The lysine residues in nucleosome cores were modified with a large molar excess of 2,4,6-trinitrobenzenesulfonic acid (TNBS) (Wako Pure Chemicals, Ltd.) (Fields, 1971). Nucleosome cores dissolved in 20 mM boric buffer/20% ethylene glycol, pH 8.0, or in 0.1 M borax/20% ethylene glycol containing various amounts of NaCl (0.3–2.0 M), pH 8.0, were preincubated at 30 °C for 20 min and then 0.01 volume of TNBS solution (0.1 M, freshly prepared before use) was mixed. The final concentrations of nucleosome cores and TNBS in the reaction mixture were 0.42 μM (~50 μM lysine) and 1 mM, respectively. The modification reaction was allowed to proceed at 30 °C, and the reaction was terminated at desired periods of incubation time by adding 6 N HCl to the reaction mixture to make the final concentration of 1 N in HCl. After termination of the reaction the unreacted TNBS was removed by an exhaustive dialysis of the sample solution against 1 mM HCl and then against redistilled water at 4 °C. Spectropor dialysis tubing was used throughout. During the dialysis the sample was completely precipitated, and the precipitates, collected by a centrifugation at 2000 rpm for 15 min, were dried by rotary evaporation. Unreacted histones were not detected by absorption in the supernatant of the dialyzed sample solution. The extent of modification of the lysine residues in nucleosome cores with TNBS was determined by amino acid analysis of the hydrolyzed samples with a Hitachi KLA-5 amino acid analyzer.

By parallel experiments the modification reaction was allowed to proceed at 30 °C in a quartz cuvette (10 mm × 10 mm), and the reaction was continuously followed by monitoring the absorbance increase at 420 nm of the reaction mixture by a Cary 17D spectrophotometer. The absorbance at 420 nm of free TNBS, which is almost undetectable at zero reaction time and exhibits a slight increase with reaction time, was subtracted as a background. The time course of the reaction thus obtained were in excellent agreement with those obtained by amino acid analysis and were calibrated quantitatively by using the results of amino acid analysis.

Modification of Arginine Residues in Nucleosome Core Particles. The arginine residues in nucleosome cores were

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modified with a large molar excess of 2,3-butanedione (Mita et al., 1981). 2,3-Butanedione was distilled and stored in sealed ampules at −20 °C. Nucleosome cores dissolved in 20 mM boric buffer, pH 7.6, containing various amounts of NaCl (0–2.0 M NaCl) were preincubated at 30 °C for 30 min, and then 0.01 volume of 2,3-butanedione was added to give the final concentrations of nucleosome cores and 2,3-butanedione of 10 μM (~1 mM arginine) and ~0.1 M, respectively. The modification reaction was allowed to proceed at 30 °C, and at each desired incubation time a part of the sample solution was pipetted off and 0.4 volume of 6 N HCl was added to the solution to terminate the reaction and to inhibit the regeneration of arginine. After termination of the reaction the sample solution was dialyzed exhaustively against redistilled water at 4 °C to remove the unreacted 2,3-butanedione and was dried by rotary evaporation. So that possible regeneration of arginine due to exposure to light could be prevented, the sample solution was kept in the dark throughout the modification reaction. The extent of the arginine modification was determined by amino acid analysis of the hydrolyzed samples as described above for the modified lysines.

Modification of Lysine and Arginine Residues of Core Histone Mixtures. The equimolar mixture of acid-extracted core histones was obtained by removing the F1 fraction with 5% $HClO_4$ from whole histone, which had been extracted from chicken erythrocyte nuclei with 0.25 N HCl (Johns, 1964). The chemical modifications of the lysine and arginine residues of the acid-extracted core histone mixture were performed by the same procedures as those of nucleosome cores.

Sucrose Density Gradient Sedimentation of Nucleosome Core Particles. Changes in the sedimentation profiles of nucleosome core particles as a function of salt concentration were examined as follows. A total of 0.8 mL of 300 μg/mL nucleosome cores, dissolved in 10 mM Tris-HCl, pH 7.1, containing various amounts of NaCl, was layered onto 5–20% (w/v) linear sucrose density gradients containing the same buffer and corresponding amounts of NaCl, followed by centrifugation in an International SB-283 rotor for 44 h at 40000 rpm at 3 °C. Ovalbumin (3.6 S) and bovine serum albumin (4.5 S) were run in parallel gradients as sedimentation markers. The sedimentation profiles were obtained by measuring the absorbances at 230 and 260 nm with a Hitachi 034 UV–vis effluent monitor.

Results

Effect of NaCl on the Dissociation of Nucleosome Core Particles. Figure 1 shows the sucrose density gradient sedimentation patterns of nucleosome cores obtained at different salt concentrations. The absorbances at 260 and 230 nm refer mainly to DNA and histones, respectively. In the presence of NaCl up to 0.6 M, all the loaded material reached near the bottom of the gradients under the present conditions of centrifugation and no peak of released histones or dissociated DNA was detected. The fact indicates that, up to 0.6 M NaCl, the octameric histone complex remains bound to DNA (Cary et al., 1978; Wilhelm & Wilhelm, 1980). With increasing salt concentration above 0.6 M NaCl a new peak (peak II) appears at 0.8 M NaCl and another slower sedimentating peak (peak I) appears at 1.0 M NaCl. At above 1.5 M NaCl the peak II disappears and the peak I is predominant. From our electrophoretic analysis of the peak fractions (data not shown) the peaks II and I were assigned to the (H3–H4)–DNA complex and to the free nucleosome core DNA, respectively. The facts demonstrate that histones are fully released from DNA at above 1.5 M NaCl. These observations are consistent with the results reported previously by other authors that

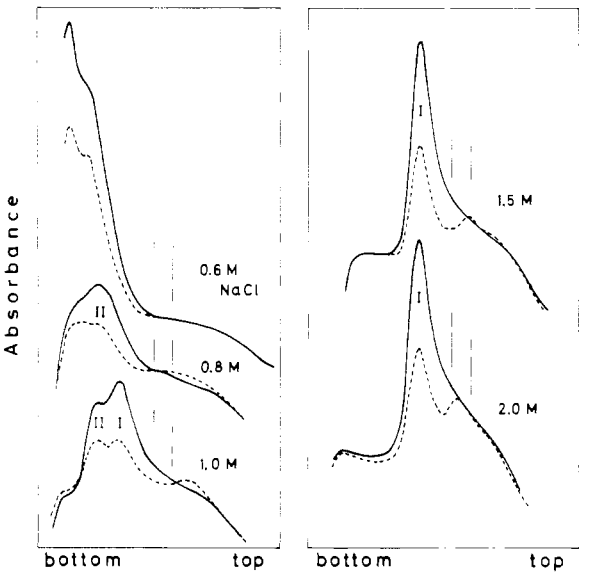


FIGURE 1: Sucrose density gradient sedimentation patterns of nucleosome core particles at various salt concentrations: (—) 260 nm; (---) 230 nm. The vertical lines mark the positions at which the sedimentation velocity markers, ovalbumin (3.6 S) and bovine serum albumin (4.5 S), sediment. Nucleosome cores, dissolved in 10 mM Tris-HCl, pH 7.1, containing various amounts of NaCl, were layered onto 5–20% (w/v) linear sucrose density gradients containing the same buffer and corresponding amounts of NaCl. Centrifugation was in an International SB-283 rotor for 44 h at 40000 rpm at 3 °C.

histones H2A and H2B are eluted together between 0.8 and 1.2 M NaCl while histones H3 and H4 are eluted together between 0.9 and 1.6 M NaCl (Ohlenbush et al., 1967) and that the fractions of histones bound in the course of the reconstitution of nucleohistone from DNA and core histones are 90% at 0.6 M NaCl and 5% at 1.4 M NaCl (Jorcano & Ruiz-Carrillo, 1979).

The released histones at above 0.6 M NaCl exhibit the broad bands of the absorbance at 230 nm having the sedimentation velocity peaks at ~4.0 S in 2.0 M NaCl, at ~3.6 S in 1.5 M NaCl, and at the positions smaller than 3.6 S in 0.6–1.0 M NaCl. By the reported sedimentation velocity values of the histone octamer (4.8 S), the H3–H4 tetramer (3.2 S) and the H2A–H2B dimer (2.3 S) (Ruiz-Carrillo & Jorcano, 1979), the released histones at 2.0 M NaCl in the present sedimentation experiments are estimated to be the mixture of the histone hexamer and the lower histone assemblies. It should be pointed out here that, when compared at the same ionic strength, the degree of assemblies of the released histones from nucleosome cores in the sedimentation and in the chemical modifications experiments might be different, since the two experiments have been performed under different temperatures and nucleosome concentrations (see Experimental Procedures) (Eickbusch & Moudrianakis, 1978).

Modification of Lysine Residues in Nucleosome Core Particles. The lysine residues in nucleosome core particles were modified with a large molar excess of TNBS at various salt concentrations. Because the solubility of the TNBS-reacted nucleosome cores decreased considerably, ethylene glycol was added to the reaction mixture to give the final concentration of 20% (v/v). The presence of 20% ethylene glycol has little effect on the nucleosome core conformation (Zama et al., 1978).

Figure 2 presents the time courses of the modification reaction at various salt concentrations. In 20 mM boric buffer the lysine residues are modified at an extremely slow rate, indicating that the residues are hardly accessible to TNBS

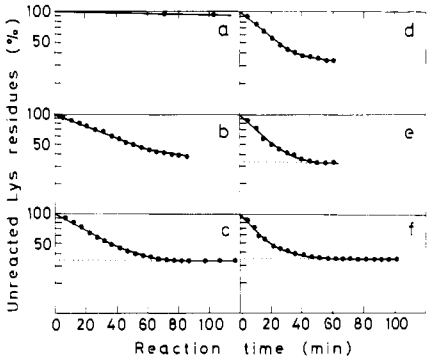


FIGURE 2: Time courses of the chemical modification reaction of the lysine residues in nucleosome core particles with a large molar excess of TNBS at 30 °C. Reaction mixtures contained 0.42 μM nucleosome cores (~50 μM lysine residues) and 1 mM TNBS. Buffer: (a) 20 mM boric buffer/20% ethylene glycol, pH 8.0; (b–f) 0.1 M borax/20% ethylene glycol containing various amounts of NaCl, pH 8.0. NaCl concentration: (a) 0 M; (b) 0.3 M; (c) 0.5 M; (d) 0.7 M; (e) 1.0 M; (f) 2.0 M.

(Figure 2a). With increasing salt concentration nucleosome core particles become partially insoluble at above 0.1 M NaCl and again become completely soluble at above 0.3 M NaCl. In soluble core particles at 0.3 M NaCl a considerable fraction of the lysine residues becomes reactive with TNBS (Figure 2b). At 0.5 M NaCl, the semilogarithmic plot of the percentage of the unreacted lysine residues in the nucleosome core against reaction time consists of two straight lines, indicating the existence of fast and extremely slow processes of the pseudo-first-order modification reaction (Figure 2c). When extrapolated to zero reaction time, the straight line of the slow process intersects the ordinate at 33%. The fact that 67% of the lysine residues (78 Lys per histone octamer) of the nucleosome core particle react rapidly with TNBS and the remaining 33% of the lysine residues (38 Lys per histone octamer) are shielded from the modification suggests that the former residues are exposed to solvent while the latter residues are scarcely accessible to solvent. The latter residues would be buried in interior regions of the core particle. The time courses of the modification reaction of the lysine residues with TNBS, which are similar to that at 0.5 M NaCl, are seen at 0.3–2.0 M NaCl (Figure 2b–f).

From the data in Figure 2 the fraction of the exposed lysine residues is plotted against NaCl concentration (Figure 4). With increasing salt concentrations above 0.6 M NaCl, histones are gradually released from the core particle as mentioned above (Figure 1); nonetheless, the fraction of the exposed lysine residues is independent of NaCl concentration and remains constant at 67% up to 2.0 M NaCl where histone molecules are completely released from DNA.

Whereas histone molecules are assembled into the octameric complex bound to DNA below 0.6 M NaCl, the released histones at higher salt concentrations would be highly dissociated one another, particularly at 0.6–1.0 M NaCl, in the present reaction mixture containing 47 μg/mL core histones at 30 °C (Eickbusch & Moudrianakis, 1978). In this respect it is interesting to note that no lysine residue is newly exposed to solvent in spite of the dissociation of histones from the octameric complex to lower order assemblies.

Modification of Arginine Residues in Nucleosome Core Particles. The arginine residues in nucleosome core particles were modified with a large molar excess of 2,3-butanedione at various salt concentrations. Figure 3 presents the time courses of the arginine modification reaction at different NaCl concentrations. Only an extremely slow modification process

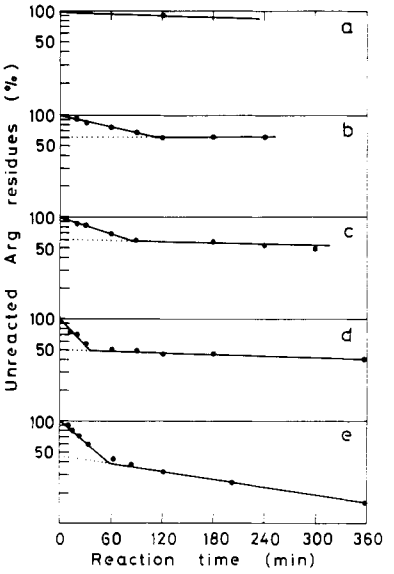


FIGURE 3: Time courses of the chemical modification reaction of the arginine residues in nucleosome core particles with a large molar excess of 2,3-butanedione at 30 °C. Reaction mixtures contained 10 μM nucleosome cores (~1 mM arginine residues), 0.1 M 2,3-butanedione, and various amounts of NaCl in 20 mM boric buffer, pH 7.6. NaCl concentration: (a) 0 M; (b) 0.3 M; (c) 0.5 M; (d) 1.0 M; (e) 2.0 M.

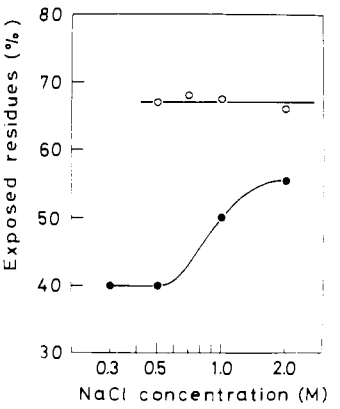


FIGURE 4: Changes in the percentage of the exposed lysine or arginine residues of nucleosome core particles as a function of NaCl concentration: (○) lysine residues; (●) arginine residues.

is observed in 20 mM boric buffer, while the two-step pseudo-first-order reaction processes are observed at above 0.3 M NaCl. The former observation indicates that none of the arginine residues in nucleosome core particles are accessible to 2,3-butanedione in 20 mM buffer. Figure 4 presents the plot of the percentage of the exposed arginine residues vs. NaCl concentration that was obtained from the data in Figure 3.

Between 0.3 and 0.6 M NaCl, 40% of the arginine residues (42 Arg per histone octamer) are exposed to solvent while the remaining 60% of the residues (62 Arg per histone octamer) are hardly accessible to solvent and probably are buried in interior regions of the core particle. At above 0.6 M NaCl, some additional arginine residues are newly modified with 2,3-butanedione, and at 2.0 M NaCl, where histones are fully released from DNA, 54% of the arginine residues (56 Arg per histone octamer) are available for the rapid modification. This shows that 14 arginine residues of a histone octamer are exposed, accompanied by the release of histones from DNA. These 14 arginine residues could be involved either in the strong binding to the DNA phosphate groups or in the histone–histone interactions to form the octameric histone

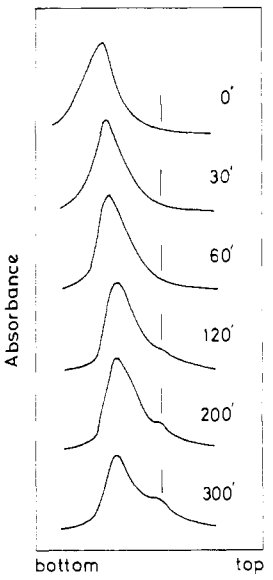


FIGURE 5: Sucrose density gradient sedimentation profiles of nucleosome core particles modified with 2,3-butanedione at 0.3 M NaCl for various periods of time at 30 °C. The absorbance was measured at 260 nm. The numbers indicate the reaction times (minutes). The vertical lines mark the position at which the 146-base-pair free core DNA sediments. The modified nucleosome cores in 0.3 M NaCl/20 mM boric buffer, pH 7.6, were layered onto 5–20%(w/v) linear sucrose density gradients containing 0.3 M NaCl and 20 mM boric buffer, pH 7.6. Centrifugation was in an International SB-283 rotor for 15 h at 40 000 rpm at 4 °C.

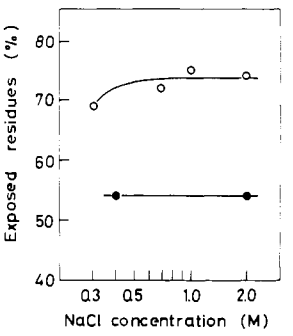


FIGURE 6: Changes in the percentage of the exposed lysine or arginine residues of core histone mixtures as a function of NaCl concentration: (○) lysine residues; (●) arginine residues.

Table I: Numbers of Exposed and Buried Lysine and Arginine Residues of a Nucleosome Core Particle from Chicken Erythrocytes as a Function of Salt Concentration[a]

	20 mM boric buffer		0.3–0.6 M NaCl		2.0 M NaCl	
	Lys	Arg	Lys	Arg	Lys	Arg
exposed residues	0	0	78	42	78	56
buried residues	116	104	38	62	38	48

[a] It is assumed that the total numbers of the basic amino acid residues of a nucleosome core particle from chicken erythrocytes are the same as those of a nucleosome core particle from calf thymus, i.e., 116 lysines and 104 arginines.

complex. It is noticeable that a surprisingly small number of arginine residues, smaller than 14 residues, and none of the lysine residues contribute to the strong binding of the histone octamer to DNA.

For examination of the structure of the core particles after the reaction with 2,3-butanedione, the core particles modified at 0.3 M NaCl were centrifuged in a 5–20% sucrose density gradient containing 0.3 M NaCl. The sedimentation profiles are shown in Figure 5. The core particles modified for various periods of time up to 120 min, where arginines are rapidly modified (see Figure 3b), have a sharp sedimentation peak, although the position of the peak shifts slightly toward the top of the gradient with reaction time, in comparison with that of unmodified core particles. The $NaDodSO_4$–polyacrylamide gel electrophoresis showed that the peak fractions contain equimolar quantities of the core histones. Interestingly, in the sedimentation profiles of the core particles modified for longer than 120 min, where the buried arginine residues begin to be modified by an extremely slow reaction process (see Figure 3b), a shoulder begins to appear at the position where the 146-base-pair free core DNA sediments, in addition to the sharp peak that no longer shifts with reaction time. These observations demonstrate that the modified nucleosome core retains its folded structure for the maximum extent of the rapid reaction, while the DNA begins to fall off the histones, leading to complete unfolding of the core particle, when the slow reaction process proceeds. The facts confirm the conclusion of the present study that a small number of arginine residues are strongly bound to the DNA phosphates at 0.3–0.6 M NaCl.

Modification of Lysine and Arginine Residues of Core Histone Mixtures. The chemical modifications of the lysine and arginine residues with TNBS and 2,3-butanedione, respectively, of the equimolar mixture of core histones without DNA were performed at different salt concentrations. These reactions consisted of the fast and slow processes, similar to the case of the modifications of nucleosome cores. Figure 6 shows the fractions of the rapidly reacted lysine and arginine residues as a function of salt concentration. The fractions of the lysine residues rapidly reacted are unchanged above 0.6 M NaCl. The result is essentially identical with that obtained on the core particle. In the case of the arginine modification, however, the result that there is no difference in the fractions of the rapidly reacted residues at 0.4 and 2.0 M NaCl is in marked contrast to the result on the core particle that the fraction of the rapidly modified arginines at 0.3–0.6 M NaCl is smaller than that at 2.0 M NaCl. These facts are consistent with the conclusion drawn above by the experiments on the nucleosome core particles that arginines are involved in the strong binding of the histones to DNA.

Discussion

By chemical modifications of lysine and arginine residues with TNBS and 2,3-butanedione, respectively, we have determined the numbers of the exposed and buried lysine and arginine residues of a nucleosome core particle as a function of salt concentration. The results are summarized in Table I.

At very low ionic strength (20 mM boric buffer) all residues are inaccessible to the reagents. In this respect Cary et al. (1978) have shown by their NMR study that there is mobility of a considerable number of lysines in a nucleosome core even at low ionic strength. These mobile lysine residues might be buried to such an extent that they are scarcely attacked by TNBS.

At 0.3–0.6 M NaCl 40% of the arginine residues (42 Arg) and 67% of the lysine residues (78 Lys) of a histone octamer are rapidly modified. Among the remaining basic amino acid residues that are prevented from the chemical modifications, only 14% of the arginine residues (14 Arg) are additionally exposed accompanied by the release of histones from DNA between 0.6 and 2.0 M NaCl. The newly exposed 14 arginine

Table II: Numbers of Basic and Acidic Amino Acid Residues in the N-Terminal and C-Terminal Regions or in the Globular Region of a Histone Octamer Obtained from the Schematic Representation of Core Histones from Calf Thymus (McGhee & Felsenfeld, 1980b)

	Lys	Arg	Glu plus Asp
N- and C-terminal regions	78	44	14
globular region	38	60	60

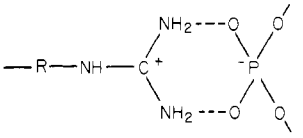
residues could be involved either in histone–DNA or in histone–histone interactions. It is noteworthy that a surprisingly small number of arginine residues (maximally 14 Arg) are involved in the strong DNA–histone octamer binding, which is stable below 0.6 M NaCl, in a nucleosome core particle. The basic amino acid residues in a nucleosome core particle at 0.3–0.6 M NaCl are classified into the following three groups: (1) the residues that are exposed to solvent (42 Arg and 78 Lys), (2) the residues strongly bound to the core DNA (maximally 14 Arg but no Lys), and (3) the residues buried in the globular region of a histone octamer (48 Arg and 38 Lys).

Since the NMR studies (Cary et al., 1978) have shown that the basic N-terminal and C-terminal regions of the octameric histones are released by increasing ionic strength of nucleosome core particle solutions to 0.6 M NaCl, the exposed basic amino acid residues at 0.3–0.6 M NaCl are estimated to be located on the basic histone tails. As listed in Table II, the schematic representation of the four histones from calf thymus nucleosome core particles (McGhee & Felsenfeld, 1980b) gives 44 arginine and 78 lysine residues as the numbers of the basic amino acid residues located in the terminal ends of histones in an octameric complex. Interestingly, these numbers are in excellent agreement with the experimentally obtained numbers of the exposed basic amino acid residues at 0.3–0.6 M NaCl, 42 arginine and 78 lysine residues (Table I). Thus, it is plausible to conclude that these exposed amino acid residues are in the released N-terminal and C-terminal regions of histones in the octameric complex bound to DNA.

The most striking finding of the present study is that the strong DNA binding sites of the octameric histones within a nucleosome core particle between 0.3 and 0.6 M NaCl contain maximally 14 arginine residues but no lysine residues. The small number of the arginine residues ($\lesssim$14 Arg) involved in the strong binding sites with DNA would be located on the surface of the globular region of the octameric histones and would play an essential role in folding the core DNA into a nucleosome core particle.

These results are consistent with some evidence supporting the existence of the DNA-folding sites of nucleosome core particles on the globular region of the histone octamer. Cary et al. (1978) reported that the structured, apolar regions of the four histones in nucleosome core particles are not free below 0.6 M NaCl and are released by increasing the ionic strength of the core particle solutions from 0.6 to 2.0 M NaCl. Weintraub & Van Lente (1974) and Lilley & Tatchell (1977) showed that the nucleosome core particle maintains essentially its original folded structure after removal of the N-terminal ends of histones by trypsin hydrolysis. Whitlock & Stein (1978) have demonstrated that nucleosome core particles can be reconstituted using trypsinized histones. Palter & Alberts (1979) have measured the elution of core histones reconstituted onto high molecular weight DNA cellulose and have found that both intact histones and trypsinized histones elute over the same salt range as found in native chromatin.

The reason why only the arginine residues in the core particle participate in the strong binding with the DNA phosphates, while none of the lysine residues do, might be explained by the characteristic strong binding mechanisms of arginine residues with tetrahedral-type anions that we have designated as "ringed-structure" salt bridges in our previous works (Ichimura et al., 1978; Mita et al., 1978, 1980). Since the PO_2^- group of DNA is a tetrahedral-type anion, we propose here that the arginine residues involved in the strong binding with the nucleosome core DNA might be interacting with the DNA phosphate groups by forming the following ringed-structure salt bridges:



The existence of the same type of salt bridges between PO_2^- and the guanidinium ions of arginine residues has been proposed by the X-ray crystallography study of staphylococcal nuclease to explain the mechanism of action of the enzyme (Cotton et al., 1979). Phosphorylated serine residues also form salt bridges with arginine residues (Sprang & Fletterick, 1980).

In the histone complexes released completely from DNA at 2.0 M NaCl, 48 arginine and 38 lysine residues are buried per histone octamer (Table I). On the other hand, 60 out of a total of 74 acidic amino acids of a histone octamer are involved in the histone globular regions (Table II). Therefore, it is very likely that the buried basic amino acid residues might extensively form salt bridges with acidic amino acid residues to stabilize the globular structures of individual histones or of histone complexes. Here, again, the strong ringed-structure salt bridges formed between arginine residues and glutamic acid or aspartic acid residues (Mita et al., 1978) would be important and play a prominent role. The validity of this idea is supported by our preceding paper (Mita et al., 1981), which suggested that arginine residues are important to stabilize the globular structure of histone H1. Nicola et al. (1978) reported that the structured regions of histone complexes are rich in hydrophobic amino acids, as well as arginine and some acidic amino acids. The existence and the importance of the salt bridges between arginine residues and glutamic acid or aspartic acid residues in inter- or intrasubunit interactions of proteins have recently been noticed with several proteins, though not with histones, by X-ray crystallography analysis (Sprang & Fletterick, 1980; Bloomer et al., 1978; Hendrickson & Teeter, 1981).

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Compositional and Structural Heterogeneity of Avidin Glycopeptides†

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ABSTRACT: Avidin, purified to apparent homogeneity by cation-exchange chromatography, was resolved into three incompletely separated fractions on carboxymethylcellulose columns at pH 9. These fractions were indistinguishable by specific biotin-binding activity, N-terminal analysis, carbohydrate composition, and polyacrylamide gel electrophoresis at acid pH and in sodium dodecyl sulfate. Although some heterogeneity was detected by discontinuous gel electrophoresis at basic pH, no definitive source for the chromatographic heterogeneity of purified avidin was identified. Commonly encountered sources of charge heterogeneity such as variable phosphorylation and sialylation were shown by specific analyses to be not responsible for the apparent charge heterogeneity of avidin. Purified avidin (780 mg) was reduced, carboxymethylated, and exhaustively digested with Pronase. The resulting neutral glycopeptides were fractionated by Dowex 50 chromatography into five major components containing asparagine as the only amino acid. After purification of the glycopeptides by gel filtration, compositional analysis by gas-liquid chromatography showed that the glycopeptide fractions ranged from 1.2 to 2.0 in the ratio of mannose to *N*-acetylglucosamine. Examination of the glycopeptides by 250-MHz proton nuclear magnetic resonance (NMR) spectroscopy showed that all of these fractions were heterogeneous. Oligosaccharides were prepared by cleavage of the glycopeptides with endoglycosidase H, followed by high-resolution gel filtration, and major oligosaccharide fractions were characterized by NMR spectroscopy. Some samples were also examined by two-dimensional *J*-resolved proton NMR. Comparison of the spectra of avidin samples with those of ovalbumin glycopeptides and oligosaccharides prepared by the same methods showed that the avidin carbohydrate contains at least three distinct oligosaccharide structural types of similar composition and size. In addition to oligomannosidic and bisected hybrid components like those of ovalbumin, the avidin carbohydrate also contains nonbisected hybrid structures similar to those of bovine rhodopsin.

Avidin, a glycoprotein synthesized in the oviduct and deposited in the albumen fraction of eggs, binds the water-soluble vitamin biotin very tightly and specifically with a dissociation constant of $\sim 10^{-15}$ M (Green, 1975). The protein has been extensively characterized due to its remarkable ligand-binding activity and unusual solution stability [see Green (1975) for review]. Avidin is a tetrameric protein, composed of subunits of identical amino acid composition and sequence (DeLange & Huang, 1971). Of the ten asparagine residues of each subunit, only Asn_{17} is glycosylated. It occurs in the general tripeptide sequence -Asn(CHO)-X-Ser(Thr)-, where in the case of avidin, X is methionine, followed by threonine (DeLange, 1970). The remaining nine asparagine residues in each subunit neither occur in the general tripeptide sequence nor are glycosylated (DeLange & Huang, 1971), consistent with the conclusion that the general tripeptide sequence is a prerequisite for glycosylation (Hart et al., 1979). Carbohydrate accounts for about 10% of the avidin molecular weight of 68 000 (Green, 1975). Previous analyses have shown that the carbohydrate units of avidin are composed of an average of four to five mannose and three *N*-acetylglucosamine residues

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