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Modification of Chromatin by Trypsin. The Role of Proteins in Maintainance of Deoxyribonucleic Acid Conformation†

Robert T. Simpson

ABSTRACT: Limited tryptic digestion of chromatin from rabbit liver or calf thymus leads to cleavage of *ca.* 55 peptide bonds per 100 DNA base pairs, equal to the number of lysyl + arginyl residues which are freely titratable in the native nucleoprotein complex. Nearly all the proteins of chromatin possess regions which are not bound to DNA, and hence are susceptible to tryptic proteolysis. The peptides which remain bound to DNA in the residual complex, tryptic chromatin, appear to occupy the same binding sites that they occupied in the native complex, and amount to 45–70% of the total protein initially present. Consequent to tryptic digestion, chromatin exhibits only slight alterations in thermal denaturation, but has an increased flow dichroism, increased specific viscosity, and a circular dichroism pattern apparently identical with that of protein-free DNA. These findings are interpreted as resulting from loss of the specific conformation which characterized DNA in chromatin, a conformation thought to be formation of a supercoil. In conjunction with recent results demonstrating the binding of histones to DNA at their amino and carboxyl termini, but not in their central regions, these results are discussed as indicating stabilization of the DNA supercoil in chromatin through the formation of histone bridges.

The conformation of DNA in chromatin, the nucleoprotein complex in eukaryotic cells, differs from the conformation of the protein-free nucleic acid in the B form in aqueous solution. Measurements of circular dichroism (Permogorov *et al.*, 1970; Shih and Fasman, 1970; Simpson and Sober, 1970), flow birefringence, flow dichroism (Ohba, 1966), light scattering, and viscosity (Zubay and Doty, 1959) have all suggested that the DNA of chromatin is shortened in overall length, and/or that there is a reduced perfection of base stacking for nucleic acid. These findings have been interpreted to be consistent with the formation of a supercoiled structure for DNA when it is complexed with proteins in chromatin; and this postulated structure has been supported by electron microscopic (Ris, 1967; Itzhaki and Rowe, 1969) and X-ray diffraction (Pardon *et al.*, 1967; Richards and Pardon, 1970) studies. Although protein is important in the stabilization of the supercoiled conformation, little evidence has been presented to indicate the mechanism through which the histone fractions maintain the native conformation of DNA in chromatin.

Recently, Boublik and collaborators (1970) have obtained evidence by nuclear magnetic resonance study of histone–DNA complexes that the central region of the slightly lysine-rich histone, FII, and the lysine-rich histone FI, are in a helical conformation, and are not bound to the nucleic acid. In contrast, the more highly basic amino-terminal and carboxyl-terminal regions of these two histones appear to be bound to DNA firmly. We concurrently have approached the binding of histones to DNA in chromatin by acetylation of the free lysyl residues of the histones in chromatin (Simpson, 1971) and localization of the modified residues in the sequence of the histone fractions. Preliminary results are consistent with the binding mode proposed for the FII histone by Boublik *et al.* (1971), so far.

The current investigation has attempted to ascertain the possible role of histones which possess nonbound central regions in the conformational stabilization of DNA in chromatin. Since trypsin requires a free basic amino acid for hydrolysis of a polypeptide, tryptic digestion of proteins bound

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to nucleic acids would be expected to occur only at those sites where electrostatic linkage of lysine or arginine to nucleic acid phosphate does not occur. Hence, it should be possible by digesting whole native chromatin with trypsin to selectively remove the nonbound regions of the proteins of chromatin, leaving those regions initially attached to the nucleic acid *in situ*. If the nonbound regions of the histones are merely looped out from a localized region of the complex, then little or no alterations in the physical properties of chromatin would be expected consequent to the digestion. However, if the nonbound regions of the histones serve to maintain the supercoil conformation of DNA, for example, as bridges between adjacent segments, then their removal should lead to alterations in the physicochemical properties which reflect supercoiling. The results of this study demonstrate such alterations in chromatin after limited tryptic proteolysis, and thus suggest that histone bridging contributes to the stabilization of supercoiled DNA in chromatin.

Experimental Section

Chromatin was isolated from mature New Zealand White rabbit liver as previously described (Simpson and Sober, 1970). The mass ratio of protein to DNA for the samples employed in the current studies was 2.1–2.5:1. Chromatin from calf thymus was prepared using the method of Zubay and Doty (1959) and possessed a mass ratio of protein to DNA of 1.7–1.9:1. DNA was isolated by dissociating chromatin at a DNA concentration of 0.5–1.0 mg/ml in 5 M guanidine hydrochloride and sedimentation of the nucleic acid for 36 hr at 100,000*g* and 15°. DNA was further purified from the pelleted material by three cycles of chloroform–isoamyl alcohol extraction and ethanol precipitation as described by Marmur (1961). Tritium-labeled calf thymus DNA was prepared by exchange with tritiated water at 85° for 4 hr in 2.5 M NaCl (Doppler-Bernardi and Felsenfeld, 1969).

Trypsin, treated with tosylphenylalanyl chloromethyl ketone to inactivate any contaminating chymotrypsin, was obtained from the Worthington Biochemical Corp. The enzyme was stored at −5° in 1 mM HCl. The trypsin lacked any detectable nuclease activity toward DNA, as measured by lack of release of 5% perchloric acid soluble nucleotides following incubation of a 1-mg/ml solution of DNA with 10% (w/w) trypsin at 37° and pH 8.5 for 90 min.

Titrations of chromatin were carried out in a microtitration cell under nitrogen atmosphere at 25°. A Radiometer PHM 27 pH meter equipped with a Beckman microcombination electrode was employed. Titrant was added with a syringe-type microburet. Blank titrations were made for all conditions, and appropriate corrections were made for the sodium ion error of the electrode and the apparent activity coefficient of hydroxyl ion. Tryptic digestion of chromatin was also monitored titrimetrically, employing in addition the Radiometer TTT-11 titrator and SBR2c recorder and syringe buret.

Determinations of protein and DNA, polyacrylamide gel electrophoresis in 0.1% sodium dodecyl sulfate, thermal denaturation, and spectral and circular dichroism measurements were all carried out as previously described (Simpson and Sober, 1970; Simpson, 1971).

Viscometric measurements utilized a three-bulb, low-shear, Ostwald-type, capillary viscometer with shear gradients of 26, 68, and 120 sec^{-1}. The viscometer (Union Carbide Corporation, Nuclear Division) is equipped with fiber optic connected photocells and light source, allowing automatic timing of flow times for the three bulbs. All measurements were extrapolated to zero shear gradient. Physical measurements on chromatin and trypsin-digested chromatin were performed in 1 mM sodium phosphate buffer, pH 7.3; while those on DNA were performed in 0.15 M NaCl–0.015 M sodium citrate.

Flow dichroism was measured in the Shimadzu instrument, as modified for use in a Cary Model 15 recording spectrophotometer. Determinations were made at DNA concentrations of about 0.5 mg/ml, and are corrected for the small flow dichroism observed with buffer solutions. The author is indebted to Dr. Martin Gellert for the use of this instrument.

Results

When studied by both potentiometric titration and chemical modification with acetic anhydride, about 25–30% of the lysyl and arginyl residues of the proteins of native chromatin appear to be free to interact with solution components (Walker, 1965; Simpson, 1971). The remaining basic residues of the proteins of the complex are presumably involved in salt linkage with DNA phosphate, the presence of electrostatic linkages between proteins being precluded by the full availability of the protein carboxyl groups for titration in the native complex (Walker, 1965). Thus, expressing all numbers as protein residues per 100 DNA base pairs in chromatin, the total lysine + arginine content is about 180 residues, while the tyrosine content is about 20 residues. Titration of native chromatin in low salt to the alkaline side of neutrality leads to the reversible titration of 70 groups between pH 9 and 12 (Figure 1C; Walker, 1965). These include the 20 tyrosyl residues previously shown to be free (Walker, 1965) and 50 of the 180 lysyl and arginyl residues. In contrast, when chromatin is titrated in 2 M NaCl, a concentration sufficient to dissociate the majority of the proteins from DNA (Ohlenbusch *et al.*, 1967), 200 basic groups are titrated, in agreement with the total analytical content of lysine, arginine, and tyrosine (Figure 1D; Walker, 1965).

Trypsin possesses a strict specificity for cleavage of polypeptides adjacent to basic protein residues. Hence, the stoichiometry of tryptic digestion of chromatin should be similar to that of the alkaline titration, if only the nonbound regions of the chromatin proteins are susceptible to tryptic hydrolysis. Native chromatin was digested with 2% (w/w protein) trypsin at pH 7.85 and 25° at a DNA concentration of 1 mg/ml, and the number of proteolytic splits calculated by maintaining the pH by addition of dilute NaOH and assumption of a pK for the α-amino group of 7.85. Digestion of the native nucleoprotein is essentially complete within 20 min, and proceeds to the extent of cleavage of 55 bonds/100 DNA base pairs (Figure 1A), in very good agreement with the titration of stoichiometry of similar chromatin samples (Figure 1C). Doubling or halving the trypsin concentration led to appropriate alterations in the rate of hydrolysis, but did not affect the limiting stoichiometry of the digestion. Similarly the stoichiometry was unaltered when digestions were performed at several pH values between 7.85 and 9.5, demonstrating that the assumed pK for the α-amino groups is valid for the current conditions.

Chromatin proteins free of DNA were obtained by dissociation of the complex in 5 M guanidine hydrochloride, sedimentation of the DNA, and removal of the denaturant by dialysis. Digestions of these proteins, free of nucleic acid, leads to the base uptake shown in Figure 1B, corresponding to the cleavage of 175 bonds/100 DNA base pairs in the original sample. Again the stoichiometry of digestion agrees adequately with the total number of titratable groups in the proteins of chromatin and with the analytical content of lysine and arginine.

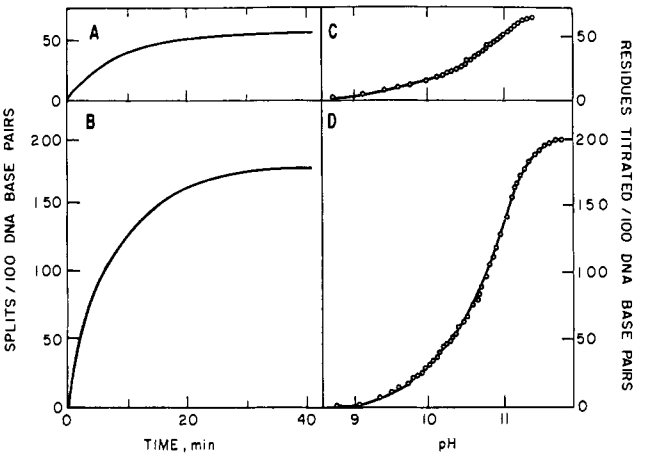

FIGURE 1: Kinetics of tryptic digestion (A and B) and titration (C and D) of native chromatin (A and C) and dissociated chromatin (B and D). (A) Native chromatin was digested with 2% (w/w protein) trypsin at 25° and pH 7.85, with digestion being monitored by pH-Stat assay. (B) Chromatin proteins dissociated from DNA were digested and monitored as in part A. (C) Native chromatin was titrated at a DNA concentration of 1 mg/ml in 1 mM NaCl. (D) Chromatin was dissociated in 2 M NaCl and titrated as in part C.

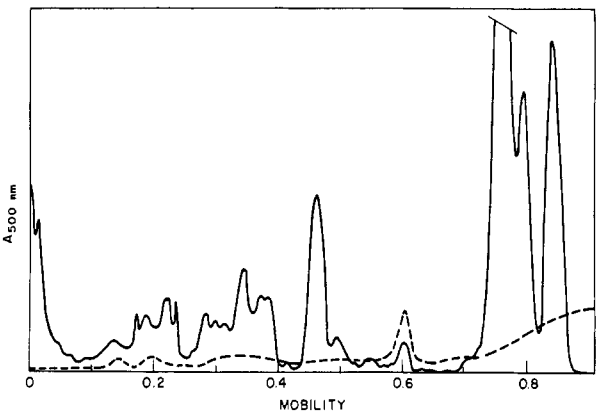

FIGURE 2: Polyacrylamide gel electrophoresis of native chromatin proteins (—) and the proteins bound to DNA after tryptic proteolysis of native chromatin (---). Electrophoresis was carried out in 0.1% sodium dodecyl sulfate. Gels were stained with Aniline Blue Black, and after destaining were scanned in a Zeiss chromatogram scanning spectrophotometer. Mobilities are expressed relative to the mobility of Bromophenol Blue tracking dye. The load of protein applied to the column in the case of the tryptic chromatin was twice that for the native chromatin sample.

For further studies, chromatin was routinely digested at pH 7.85, either in the absence of buffer on a pH-Stat, or in the presence of 0.05 M NH_4HCO_3, with 2% trypsin at 25° (w/w protein) for 25 min. The preceding data suggest that under these conditions only peptide bonds adjacent to lysyl or arginyl residues in the nonbound regions of the proteins are digested, and that the regions presumably bound to DNA remain undigested.

Consequent to tryptic digestion, 30–35% of the protein of rabbit liver chromatin, and 45–55% of the protein of calf thymus chromatin become dialyzable through an Amicon XM-300 ultrafiltration membrane in 0.02 M NH_4HCO_3. The amount of protein remaining bound to DNA is 1.7 (rabbit liver) or 0.9 (calf thymus) the mass of the nucleic acid. Polyacrylamide gel electrophoresis in 0.1% sodium dodecyl sulfate was utilized to evaluate the susceptibility of the various classes of chromatin proteins to tryptic hydrolysis. The protein remaining bound to DNA after tryptic digestion was compared with the total proteins of chromatin dissociable by 1% sodium dodecyl sulfate (Figure 2). The main histone fractions (mobilities of 0.45, 0.77, 0.80, and 0.83) are all digested by trypsin. Similarly, all the nonhistone proteins, with one apparent exception (at 0.6 mobility) are susceptible to tryptic hydrolysis in the native chromatin complex, leading to a virtual absence of discrete protein bands on electrophoresis of the proteins of tryptic-digested chromatin. These results indicate that a limited amount of protein is rendered dialyzable by tryptic digestion of chromatin, and demonstrate that all histone fractions are cleaved. They contrast with earlier observations in which removal of most of the proteins of chromatin, with the exception of proteins with compositions similar to those of arginine-rich histone, was observed (Marks and Schumaker, 1968). This previous work was performed with more vigorous digestion conditions and with trypsin which was not proven to be free of enzymatic contamination.

The bound peptides remaining in tryptic-digested chromatin appear to be attached to the same sites that they occupied in the original nucleoprotein, as demonstrated by the results of the test procedure for dissociation of proteins from chromatin suggested by Clark and Felsenfeld (1971). Tritium-labeled, calf thymus DNA was added to calf thymus chromatin to obtain final concentrations of both DNA species of 0.75 mg/ml, and the mixture was digested with trypsin and dialyzed. A duplicate sample was incubated in the absence of trypsin and similarly dialyzed. Then both samples were digested with pancreatic deoxyribonuclease I. The time course and eventual release of 5% perchloric acid soluble ^3H-labeled nucleotide were identical for the two samples, indicating that peptide interchange from digested chromatin to exogenous DNA had not occurred.

Digestion of native chromatin with trypsin under the described conditions thus appears to lead to selective hydrolysis of about 25–30% of the potentially available peptide bonds, converting nearly all the proteins of chromatin into smaller peptides, while leaving about half the peptides still firmly bound to their original binding sites on the DNA molecule. Trypsin has thus apparently removed the nonbound portions of the histone and nonhistone proteins of chromatin, and the physicochemical properties of the resultant complex pertain to the question of the forces responsible for the maintainance of the conformation of DNA in the nucleoprotein complex.

Visually, tryptic-digested chromatin is slightly less turbid than untreated chromatin, and shows less tendency to form aggregates, even at moderate (*e.g.*, 0.15) ionic strengths. Thermal denaturation of tryptic chromatin led to the same hyperchromicity as was obtained for the native material (Figure 3). Further, the melting profiles for the two chromatin samples were both of a broad nature, in contrast to the much more highly cooperative melting of protein-free DNA (Figure 3). The melting temperature for tryptic chromatin, 69–70°, is somewhat lower than that for native chromatin, 73°, and well above that for free DNA, 52° in this solvent system. The similarities in melting behavior between native and tryptic chromatins suggest that the degree and localization of charge neutralization by basic groups bound to DNA phosphate must be quite similar for the two species.

The viscosity of chromatin is markedly reduced when compared to that of free DNA of the same contour length (Zubay and Doty, 1959), a finding thought to reflect an alteration of the structure of DNA in chromatin, becoming more like a

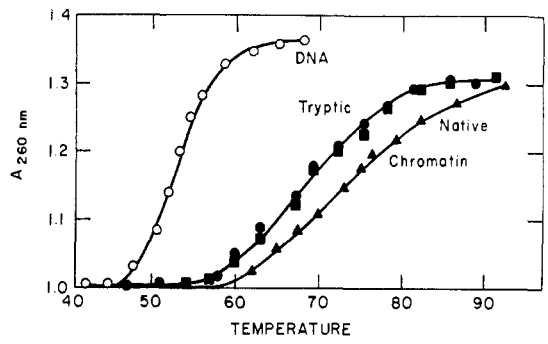

FIGURE 3: Thermal denaturation of DNA (○), native chromatin (▲), and two samples of tryptic chromatin (●, ■). Melting was performed in 1 mM Tris-chloride (pH 7.8) at about 50 μg of DNA/ml.

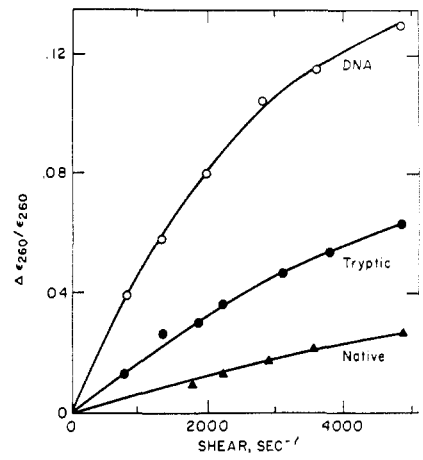

FIGURE 5: Flow dichroism of DNA (○) and native (▲) and tryptic (○) chromatins. Measurements were performed at DNA concentrations of about 0.5 mg/ml in 1 mM Tris-chloride (pH 7.8) at 23°.

prolate ellipsoid than the extended flexible rod configuration of free DNA in solution. The concentration dependence of the viscosities of native and tryptic chromatins, and of free DNA, are shown in Figure 4. All measurements were extrapolated to zero shear gradient and are expressed as reduced specific viscosities per mass of DNA. The intrinsic viscosity of native chromatin in the solvent employed, 13 dl/g of DNA, is increased to 19 dl/g of DNA consequent to the tryptic digestion. Due to loss of protein, the molecular weight of the particle is actually decreased by tryptic digestion, while the molecular weight of the DNA is presumably unchanged. Consequently the increase in viscosity accompanying the digestion must result from an increase in the axial ratio of the particle, consistent with relaxation of the DNA supercoil.

Similarly, flow dichroism measurements also indicate that removal of the nonbound regions of the chromatin proteins alters the structure of the chromatin complex to become more like that of DNA, As initially noted by Ohba (1966), the flow dichroism of chromatin at 260 nm is markedly reduced when compared to that of free DNA, reflecting both decreased orientation of the particle in the flow stream and an altered angle of the bases from the perpendicular to the particle axis. Similar results for chromatin and DNA were obtained in this investigation (Figure 5). Tryptic digestion of chromatin leads to nearly a trebling of the observed flow dichroism, from a value for $\Delta\epsilon/\epsilon$ of 0.025 to 0.07 at shear values of 4400 sec^{-1}.

The circular dichroism pattern of DNA in chromatin differs from that of free DNA in that the value of the maximal ellipticity at the positive maximum at 275 nm is reduced by about 50% when DNA is present in the nucleoprotein complex (Permogorov *et al.*, 1970; Shih and Fasman, 1970; Simpson and Sober, 1970). Digestion of chromatin with trypsin leads to total reversion of the circular dichroism spectrum to that of free DNA (Figure 6). Thus, consequent to the proteolytic modification, the ellipticity at 275 nm is increased from 5000 to 6000 deg per cm per dmole for chromatin to 9000 to 10,000 deg per cm per dmole for tryptic chromatin. The latter value is that obtained previously for DNA in the B form in aqueous solutions. Insofar as reflected by this spectroscopic measurement, the conformation of DNA in tryptic chromatin appears to be identical with that of protein-free DNA, and, hence, strikingly different from that of native chromatin.

Discussion

A plethora of experimental evidence has accumulated to indicate the conformation of DNA in the chromatin of eukaryotic cells differs from the conformation of isolated, protein-free DNA. The conclusion from hydrodynamic, spectroscopic, microscopic, and other measurements has been that DNA in chromatin is most probably supercoiled into a tertiary helix, likely with a pitch of 120 Å and a diameter of about 100 Å

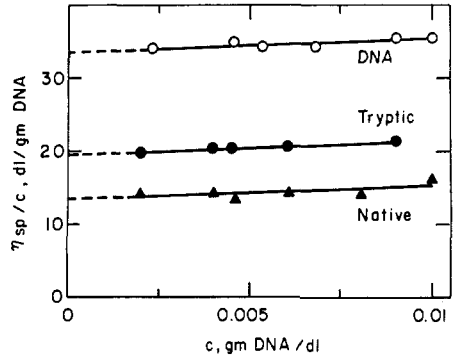

FIGURE 4: Viscosities of DNA (○), and native (▲), and tryptic (●) chromatin samples. Viscometric measurements were performed as in the Experimental Section, and extrapolated to zero shear gradient at each concentration.

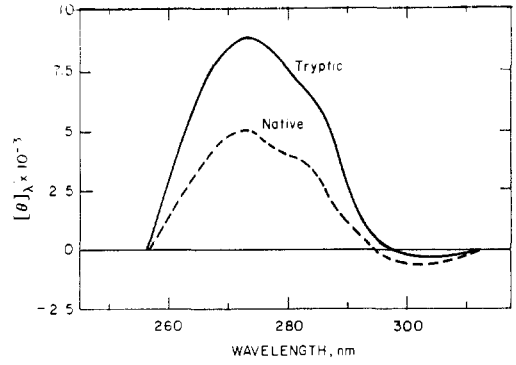

FIGURE 6: Circular dichroism of native (----) and tryptic (——) chromatins. Circular dichroism spectra were determined at 25° in 1 mM Tris-chloride buffer (pH 7.8). The DNA concentration was 100 μg/ml and the path length was 10 mm.

(*cf.* Richards and Pardon, 1970). The mechanism of stabilization of such a supercoil has not been apparent.

The altered conformation of DNA in chromatin might arise as a consequence of simple charge neutralization by bound histones, suggested by the circular dichroism studies of DNA at high salt concentrations (Permogorov *et al.*, 1970). Alternatively, the regions of the histones of chromatin which are bound to DNA might generate the supercoiled conformation of the nucleic acid as a thermodynamic consequence of their *direct* interactions with the polynucleotide phosphate backbone. A third possibility, suggested from recent studies of the mode of binding of histone molecules to DNA, is that some or all of the histones might form bridged structures which constrain DNA into a supercoiled conformation in the nucleoprotein complex. The studies of Boublik *et al.* (1971) indicate that for two of the main histone fractions the amino- and carboxyl-terminal regions of the protein are salt linked to DNA. The middle portion, comprising nearly half the mass of the molecule, relatively poor in basic residues, and containing most of the hydrophobic amino acid residues, is likely in a helical conformation, and appears not to be bound directly to nucleic acid. These observations, together with the previous demonstration that these same slightly lysine-rich and/or arginine-rich histones served primarily in maintainance of the altered conformation of DNA in chromatin (Ohba, 1966; Simpson and Sober, 1970; Richards and Pardon, 1970) have suggested to us that histones might bridge between adjacent segments of DNA in chromatin, and thereby induce and stabilize a supercoiled conformation for the nucleic acid.

The current study has attempted to examine that possibility. Since trypsin requires a free basic amino acid as the carboxyl residue for a susceptible polypeptide bond, cleavage would not be expected when the basic group is complexed to nucleic acid. Thus, proteolytic modification of chromatin with trypsin might be expected to remove only the regions of the proteins of the complex which are not firmly bound to DNA. This process would then leave a residual complex, containing the nucleic acid plus peptides corresponding to the regions of chromatin proteins which were initially tightly linked to the nucleic acid. The physicochemical properties of this residual complex should be identical with those of native chromatin, insofar as they are determined directly by the bound regions of the proteins. On the other hand, the physicochemical properties of tryptic chromatin should differ from those of its parent species if constraints imposed by nonbound protein regions, most simply, bridging, were involved in the structure of the nucleoprotein.

Under the conditions employed in the current study, tryptic digestion of native chromatin appears to be a selective, limited proteolytic degradation. In contrast to a previous study (Marks and Schumaker, 1968), where tryptic digestion led to a more nearly complete deproteinization of the nucleic acid, in our hands, the number of bonds split on digestion of native chromatin was almostly exactly equal to the number of lysyl + arginyl reisdues which can be freely and reversibly titrated in the native nucleoprotein complex (Figure 1). Equal stoichiometries for the two measurements, titration and digestion, strongly supports the contention that only the nonbound regions of the proteins of chromatin have been cleaved by this tryptic hydrolysis. Further support for this contention arises from the demonstration that the peptides which are not dissociated after tryptic digestion remain bound to the same DNA sites they occupied in the native complex, a fact demonstrated by the total nuclease susceptibility of exogenous tritium-labeled DNA carried through the digestion procedure. At one time, this type of experiment demonstrates both that the bound regions of chromatin proteins are not removed by tryptic proteolysis, and secondly, that the regions removed by trypsin are incapable of binding to DNA in a fashion which prevents its digestion by nucleases.

As would be expected from previous results on the acetylation of chromatin (Simpson, 1971), all histone fractions are susceptible to trypsin in native chromatin, providing further evidence that no particular histone is totally salt linked to DNA in the nucleoprotein complex. Nearly all the higher molecular weight nonhistone proteins of chromatin are also digested by trypsin. An interesting protein, of approximately 15,000 molecular weight, appears to be totally salt linked to DNA in rabbit liver chromatin, and is thus completely resistant to the action of trypsin. This observation suggests a potentially unique role for this protein, a suggestion currently under investigation. While nearly all the proteins of chromatin possess basic groups which are not salt linked to DNA phosphate, and hence are hydrolyzed by trypsin, only 30–55% of the total protein of chromatin is dissociated from the complex consequent to tryptic digestion. Apparently most of the proteins of chromatin possess regions which are firmly bound to DNA, and regions which are not bound—an extension of earlier hypotheses concerning the interaction of specific histone fractions with nucleic acids.

While we will discuss the results of the physical studies in terms of histone binding, it must be noted that nonhistone proteins, also digested by trypsin, could also contribute to stabilization of DNA conformation. We feel this latter possibility is made less likely by the following considerations. Similar results were obtained with chromatin of rabbit liver and calf thymus, tissues which differ both qualitatively and quantitatively in their nonhistone protein complement. Secondly, the amount of any single nonhistone protein detected by polyacrylamide gel electrophoresis is well below that of any of the five histone fractions (*cf.* Figure 2). It would seem likely that proteins involved in the highly repetitive interaction with DNA leading to formation of an altered conformation should be present in chromatin in relatively large amounts.

Regarding the specific question posed in these studies, that is, the potential role of nonbound regions of chromatin proteins in maintainance of the supercoiled conformation of DNA in chromatin, the physicochemical studies of tryptic chromatin are of particular interest. That property of chromatin which probably relates most closely to the presence of the appropriate basic groups directly linked to the nucleic acid backbone, thermal denaturation, is only slightly altered by tryptic digestion. Unlike DNA, the melting of tryptic chromatin is broad and the midpoint of the thermal transition only slightly lower than that for native chromatin (Figure 3). This observation is consistent with the hypothesis that tryptic digestion has removed only the nonbound regions of chromatin proteins, and suggests that these regions are of little importance in stabilization of the direct base–base interactions of DNA in chromatin. The slight alterations in melting behavior contrast with the far more striking alterations in melting profiles observed when any single class of histone is totally removed from the chromatin complex (Ohlenbusch *et al.*, 1967).

In contrast with the relatively similar melting properties of native and tryptic chromatin, those hydrodynamic and spectroscopic properties of DNA which have been thought to be associated with supercoiling of the nucleic acid are all altered significantly consequent to tryptic proteolysis. In all cases, the changes are in the direction of the structural properties of protein-free DNA, suggesting that relaxation of the DNA super-

coil has occurred as a result of removal of the nonbound regions of chromatin proteins by trypsin.

The conformation of DNA, as reflected by its circular dichroism spectrum, appears to be identical in isolated free DNA and in tryptic chromatin, and differs markedly from the conformation generating the circular dichroism spectrum of native chromatin (Figure 6). The overall structure of the chromatin particle is also altered by tryptic proteolysis, becoming a more rodlike structure, evidenced by an increased intrinsic viscosity, in the face of a decrease in particle mass (Figure 4). Similarly, the bases of DNA are more nearly oriented in a shear gradient to be perpendicular to the direction of the particle axis as a consequence of tryptic digestion of chromatin (Figure 5). Indeed, the orientation of the bases may be quite similar in free DNA and in tryptic chromatin, since the degree of orientation of the particles of tryptic chromatin, since the degree of orientation of the particles of tryptic chromatin would be expected to be less than for protein-free DNA.

Finally, in a report appearing during the course of these studies, Toczko and Jazwinski (1971) observed the transformation of chromatin from a spherical to a fibrillar form following tryptic digestion, using scanning electron microscopy to evaluate the structure of the chromatin particle. All these findings are consistent with the third alternative detailed above for the role of histones in the maintainance of the proposed supercoil structure for the DNA of chromatin. That is, that bridged structures, formed through the binding to DNA of histones at their amino and carboxyl ends, but with the centers inbound, are responsible for the structure of DNA which characterizes chromatin.

Such bridging could involve either direct binding of the ends of a given histone molecule to different segments of the DNA supercoil; or alternatively, both ends of a histone molecule could be bound to a segment of DNA, and the looped out portion could form a bridge through (probably) hydrophobic interaction with a similarly bound histone on an adjacent segment of DNA. The latter alternative is favored by the observation that the supercoil rings observed by X-ray diffraction with native chromatin can be reversibly destroyed by stretching and shrinking the nucleohistone fiber (Richards and Pardon, 1970).

The potential biological ramifications of DNA supercoil with histone bridges are of interest. Johns (1969) has suggested that the supercoiled conformation of DNA in chromatin might preclude its transcription. Specifically it might be thought that this conformation of DNA is not a suitable substrate for the binding of RNA polymerase. Consistent with this suggestion, preliminary studies of the template properties of tryptic chromatin indicate that it is transcribed at a far greater rate than native chromatin, indeed, nearly the rate of free DNA (R. T. Simpson, unpublished observations).

Acknowledgment

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