Conformational Changes in the Nucleosome Followed by the Selective Accessibility of Histone Glutamines in the Transglutaminase Reaction: Effects of Ionic Strength†

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ABSTRACT: Transglutaminases, the enzymes that catalyze the acyl-transfer reaction between glutamine and primary amines, have been used to introduce probes into proteins in order to perform structural studies using physical techniques. Here we use an original approach in which the increasing accessibility of the glutamines of core histones to TGase is used to monitor the salt-induced conformational changes of the nucleosome. The rationale of this strategy is that the accessibility of a glutamine to transglutaminase depends on the blockage due to the presence of either other histones or DNA. At low ionic strength, only glutamines on the N-terminal tails of H2B and H3 are labeled with monodansylcadaverine when core particles are incubated with transglutaminase. The partial unfolding that occurs when going to higher ionic strength values results in an increase in the number of reactive glutamines up to a maximum value of 16 per nucleosome. Labeling of some residues (e.g., Gln^{104} and Gln^{112} of H2A) requires the unwinding of DNA and the dissociation of the H2A−H2B dimers. Gln^{76} of H3 is labeled in the H3−H4 tetramer only when the H2A−H2B dimers are dissociated. Interestingly, the labeling of Gln^{95} of H2B exclusively depends on the unwinding of DNA. The accurate analysis of these results indicates that the ionic-dependent unwinding of the DNA may occur following a two-state model.

The past 20 years have witnessed considerable efforts in the road to the high-resolution unraveling of the structure of the nucleosome core particle, the highly conserved, basic component of the nucleosome, formed by 146 bp of DNA wrapped around a histone octamer. This effort first culminated in the description of the structure of the nucleosome core particle at 7 Å resolution after the work of Klug and his colleagues (*1*). In 1991, the histone octamer was resolved at 3.1 Å (*2*). Six years later, the group of Richmond described the structure of the nucleosome core at 2.8 Å resolution (*3*). This work confirmed that the overall structure of the isolated histone octamer is conserved when wrapped by DNA to form the core particle, it added some details to the known structure of histones, and it allowed them to trace the exact path of DNA around the histones.

It is generally accepted that the nucleosome structure is not static, and that alternative conformations are adopted in response to several stimuli associated with the different nuclear functions (*4*). Unfortunately, the accurate nature of these alternative conformations is not known. In most cases, structural changes are subtle and transitory, and the chromatin-remodeling machinery is able to facilitate the structural transitions of nucleosomes in both senses (*5*, *6*). Thereby, the reversibility of the natural remodeling processes makes it difficult to isolate altered nucleosomes in a form suitable for structural studies.

In connection with this, it has been recently noted that the structure of nucleosomes modified under nonphysiological conditions, e.g., by changes in pH or ionic strength, may mimic that resulting from the action of remodeling complexes or by histone acetylation (*4*). The study of the changes in the nucleosome structure by modifying ionic strength is of particular interest, because the stability of the nucleosome is mainly governed by the electrostatic interactions established between the DNA and the histone octamer. Therefore, the systematic variation of the type and concentration of the salt in the medium has provided a method to examine the interactions that stabilize the nucleosome.

Many studies have been conducted to define the nature of the ionic strength-dependent structural changes of the nucleosome. Even before the discovery of the ATP-dependent nucleosome-remodeling mechanisms, it was recognized that the increase in the salt concentration induces changes that may be related to those occurring during transcription (*7*).

A possible approach to study conformational changes in the nucleosome could be provided by the use of an enzymatically directed modification of core histones. As proposed by Folk (*8*), the reaction catalyzed by TGase[1] could be used to analyze protein structure and organization. TGases catalyze an acyl-transfer reaction in which the γ-carboxamide group of a glutamine in a protein acts as acyl donor. The ϵ-amino groups of lysine residues as well as some polyamines are the physiological amine donors, but some nonphysi-

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[1] Abbreviations: TGase, transglutaminase; DNC, monodansylcadaverine; CD, circular dichroism; HPLC, high-performance liquid chromatography; RP-HPLC, reversed-phase HPLC; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

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ological amines, such as fluorescent amines, can also be used by the enzyme. The fluorescent derivatives have proved to be useful and sensitive tools to study the interactions between the peptides or proteins and their physiological counterparts in complex systems (see, for instance, ref *9*).

We have recently proposed that conformational changes of nucleosomes can be monitored by studying their modification by the reaction catalyzed by TGase, a possibility based on the fact that core histones are extremely good glutaminyl substrates for that enzyme (*10*). Our initial experiments showed a dramatic change in the introduction of the fluorescent amine DNC into histones by TGase when the ability of free histones and nucleosome cores as substrates is compared. At low ionic strength, only Gln^{22} of H2B and Gln^{5} and Gln^{19} of H3 become modified in nucleosomes (*10*). Seven additional glutaminyl residues that are modified in free histones are not substrates in the nucleosome due to structural reasons, and Gln^{22}, which is not recognized by the enzyme in free H2B, is readily modified in the nucleosome (*10*). The reasons to explain the latter result are related to the interaction of the N-terminal tail of H2B with DNA, and this allowed us to use the TGase reaction to study the ionic strength-induced dissociation of the H2B and H3 tails from DNA (*11*). In the present paper, we describe the use of this enzymatic reaction, by studying changes in the accessibility of glutamines, to monitor conformational transitions in the nucleosome. The results not only provide evidence as to the applicability of the method but also allowed us to propose a model for the salt-induced DNA unwinding in the nucleosome core, based on the changes in accessibility of histone glutamines.

EXPERIMENTAL PROCEDURES

Preparation of Nucleosomes, Histones, and Histone Complexes. Chicken erythrocyte nucleosomes and free histones were obtained as previously described (*10*). Histone octamers, H2A−H2B heterodimers, and $(H3-H4)_2$ tetramers were isolated by hydroxyapatite chromatography (*12*).

Incorporation of DNC into Nucleosomes and Histone Complexes. The incorporation of the probe into core histones was carried out by incubating native core particles with DNC and TGase as previously described (*10*), except that the incubation medium was supplemented with NaCl to obtain the desired ionic strength. The reaction was stopped by adding H_2SO_4 to a final concentration of 0.1 M. The samples were stirred for 4 h at 4 °C and centrifuged. After acid extraction, histones were recovered from the supernatant by acetone precipitation and separated by reversed-phase HPLC, on a Deltapak C-18 column (Waters). Elution was carried out with an acetonitrile gradient (20−60%) in 0.3% trifluoroacetic acid (*13*). The dansyl group fluorescence was continuously monitored (excitation wavelength, 330 nm; emission wavelength, 510 nm). The same procedure was used to incorporate DNC into isolated histone octamers, dimers, and tetramers, except that the incubation medium contained, respectively, 2.4, 0.6, or 2.0 M NaCl. The reaction was stopped by adding trichloroacetic acid to a final concentration of 25% (w/v). The preparation was left on ice 2 h, and the precipitate was washed once in acetone−6 M HC1 (70:1) and twice in dry acetone and finally dried under vacuum. In some experiments, [^{14}C]methylamine or [^{14}C]putrescine was used, instead of DNC, as amine donor. The conditions for amine incorporation and detection have been described elsewhere (*10*).

TGase Assay. The dependence of TGase activity on ionic strength was determined by using CBZ-Gln-Gly as substrate. The incubation with TGase and DNC was carried out under the same conditions described for nucleosome core particles, and the analysis was done on a Deltapak C-18 column (15 × 0.78 cm) eluted with a linear gradient of acetonitrile (0−50%) in 0.1% trifluoroacetic acid. Dansyl fluorescence was monitored as above.

Identification of Glutamine Substrates in Histones. To identify the modified glutamine residues, the fluorescent histone peaks were pooled, and the histones were digested with trypsin at pH 8.1. Tryptic peptides were separated by reversed-phase HPLC, and the peptides were identified by comparing their retention times with our previous data (*10*). In some instances, sequencing was necessary to identify the modified glutamine-containing peptide. The relative usage of each glutamine residue of a given histone was quantified by integrating the area of the corresponding peak in the fluorescence profile of the chromatogram of tryptic peptides. The values were normalized to account for the small differences in sample loading.

Spectroscopic Monitoring of Ionic Strength-Induced Conformational Changes in Nucleosomes. The structure of DNA in the nucleosome core particles was studied by circular dichroism. Spectra were recorded between 250 and 300 nm in a CD-6 dichrograph (Jobin-Yvon), calibrated with isoandrosterone. The scan speed was 0.5 nm/s, and the integration constant was set at 1 s. The average of at least three runs was expressed in molar ellipticities. Fluorescence spectra were recorded in an LS-50 spectrofluorometer (Perkin-Elmer) at a scan speed of 200 nm/min. To determine the intrinsic fluorescence of tyrosyl residues in nucleosome cores at several ionic strength values, the excitation wavelength was 274 nm and the emission was measured at 303 nm.

Other Procedures. SDS−PAGE, photography and scanning of the gels, and DNase I digestion of core particles were carried out as described elsewhere (*10*).

RESULTS

Ionic Strength-Induced Dissociation of Nucleosomes. The ionic strength-dependent dissociation of nucleosomes has been monitored in the past by recording changes in the fluorescence of the histone tyrosines and molar ellipticity of DNA against NaCl concentration (*14*−*18*). Since the exact position and shape of the curves depend on several factors, including the ionic composition and pH of the initial buffer in which core particles are dissolved, it was judged convenient to include these controls using the precise conditions in which the accessibility experiments were performed (Figure 1)

Ionic Strength-Dependent Changes in the Reactivity of Nucleosomal Histones. The next step was the study of the influence of increasing salt concentration on the incorporation of DNC to glutamines in order to evaluate the effect of the ionic strength on the accessibility of glutamine residues. After incubation of core particles with TGase, as described under Experimental Procedures, histones were acid-extracted and separated by RP-HPLC. The results of a representative

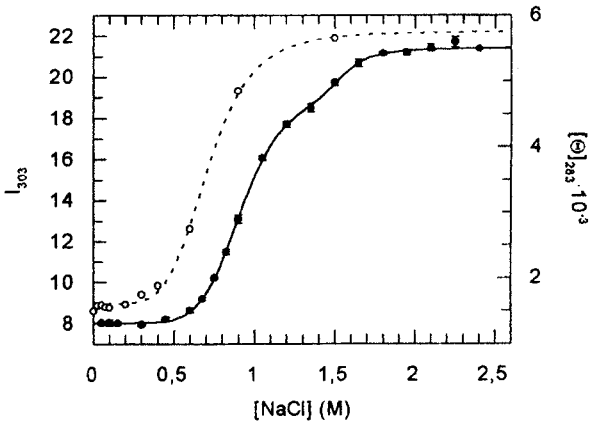

FIGURE 1: Ionic strength-dependent structural changes of the core particle. Changes induced in nucleosome core particles by increasing ionic strength were monitored by measuring molar ellipticity at 283 nm (○) and the intrinsic fluorescence intensity of histone tyrosyl residues (●). The lines represent the best fit to the equations described in the text under Discussion.

experiment are given in Figure 2. A general ionic strength-dependent enhancement of DNC incorporation, related to the disassembly of the core particle, is clearly observed. On the other hand, the increase in ionic strength also induces a qualitative change in the distribution of the fluorescent label among the different histones. At low ionic strength (10 mM NaC1), H2B and H3 are the only histones accessible to the modification reaction in the nucleosome (Figure 2). As previously shown (*10*), the modified residues are Gln^{22} of H2B and Gln^{5} and Gln^{19} of H3, which lie in the accessible N-terminal tails of the histones (*13*). Labeling of Gln^{22} of H2B requires the neutralization of the positive charges of adjacent lysyl residues by the interaction of the H2B tail with DNA (*11*). At higher ionic strength (300 mM NaCl), H2B loses its ability to act as TGase substrate, and the labeling of H3 is diminished (Figure 2). Both effects are related to the dissociation of the N-terminal tails from the DNA (*11*). When ionic strength was further increased, a nucleosome unfolding-related enhancement of the substrate capability of the histones is observed (Figure 2). Surprisingly enough, H2B becomes once more a substrate for TGase at 600 mM NaCl, and H2A, which was not labeled at lower ionic strength, also incorporates the label. At still higher ionic strength values (900 or 1400 mM NaC1), two additional peaks, referred to as D and 3′ in Figure 2, were present in the chromatograms. The protein in peak 3′ migrates as H3 in SDS−PAGE, and its profile of tryptic peptides was typical of H3, although an additional fluorescent peptide was found (data not shown). It seems that the increase in hydrophobicity that results from the incorporation of additional DNC probably alters the interaction of the histone with the hydrophobic matrix of the HPLC column. The additional fluorescent peptide was recovered and analyzed, yielding the sequence Leu-Pro-Phe-X-Arg-Leu-Val, where X stands for an unidentified amino acid derivative. This sequence matches that of H3 residues 73−79, containing Gln^{76}, which is not a TGase substrate in free histone (*10*). Core histones require their heterodimeric partner to maintain their native structure (*19*). This may explain the differential labeling of Gln^{76}, which, on the other hand, becomes exposed in the core particle once the H2A−H2B dimers begin to be released (as at 900 mM NaCl).

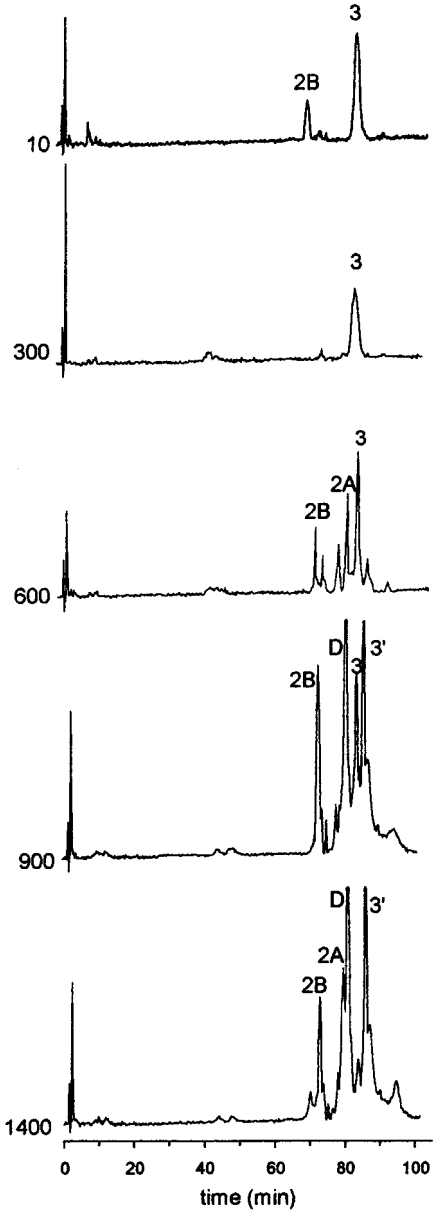

FIGURE 2: Incorporation of DNC into nucleosomal histones as a function of ionic strength. Core particles were incubated with DNC in the presence of TGase and of NaCl added to obtain the desired ionic strength as indicated on the left (in a mM scale). After incubation, histones were isolated and resolved by RP-HPLC. Only the fluorescence of the dansyl group (in arbitrary units) is plotted. Peaks corresponding to H2A, H2B, and H3 are indicated in the profiles. Peaks labeled D and 3′ correspond, respectively, to a cross-linked H2A−H2B dimer and to a tridansylated H3 species (see the text).

The mobility in SDS−PAGE of the material isolated from peak D is similar to that of histone dimers (*20*), and, based on several facts, we think that it is an H2A−H2B dimer cross-linked by the reaction of TGase. First, the HPLC profile of the tryptic peptides derived from the material recovered from peak D has many common peptides to that of H2A. Moreover, the formation of D is accompanied by a concomitant decrease in the concentration of H2A and H2B, as judged by the diminution both of their peaks in an HPLC absorbance profile and of their bands in SDS−PAGE (data not shown). On the other hand, the mobility of the protein recovered from peak D in a high-resolution SDS−PAGE is intermediate between those of TGase-cross-linked H2A and

H2B homodimers (data not shown; see Figure 1 in ref *10* for the H2A and H2B homodimers). The residues involved in the cross-linking reaction have not been examined, but a plausible hypothesis will be given under Discussion.

The Incorporation of DNC Does Not Result in Gross Changes in the Structure of the Core Particle. The validity of the above results requires that neither the enzymatic reaction itself nor the successive introduction of DNC groups alters the structure of the core particle. The first question was evaluated by incubating nucleosome core particles in the presence of TGase and DNC at low ionic strength. The structure of the DNC-labeled particles was analyzed by CD and DNase I digestion. In both instances, the results were indistinguishable from those obtained with native core particles (data not shown). Therefore, it can be concluded that the general features of nucleosome conformation are preserved after incubation with TGase.

It can be objected that a high number of DNC-modified residues, as occurs when modification is carried out at high ionic strength, could significantly distort the nucleosome structure. If this were true, a possible artifact would arise: a distorted nucleosomal structure may have artifactually exposed glutamines, which would be otherwise buried and could also become labeled. Should this occur, the TGase-catalyzed reaction would not be an appropriate strategy to analyze conformational changes. To discard this possible artifact, the experiments were repeated, but smaller amines, namely, [^{14}C]methylamine and [^{14}C]putrescine, were used instead of DNC. The introduction of these amines into histones does not alter the hydrophobicity as DNC does, and their size, especially in the case of methylamine, is so small that an artifactual alteration of nucleosomal structure by steric hindrance may be disregarded. The results (not shown) were similar to those obtained with DNC, and the behavior of nucleosomes in the incorporation of the radioactive probe was similar to that described in Figure 2, although no peak equivalent to 3′ was detected. Obviously, the binding of additional putrescine or methylamine to H3 does not result in a significant change in the hydrophobicity. Since the results were similar regardless of the type of polyamine utilized, one can conclude that the only determinant in the increase of accessibility is the ionic strength-dependent unfolding of the nucleosome.

Identification of the Reactive Glutamines at Different Ionic Strength Values. The peaks from the chromatograms in Figure 2 were recovered, freeze-dried, and digested with trypsin to identify the TGase glutaminyl substrates. Most of the peptides were easily identified by comparison of their retention times with those of peptides identified in previous work (*10*, *11*), and peptide sequencing was used when necessary. Table 1 summarizes the use of glutamines at different concentrations of NaCl.

Once the glutamines labeled at each ionic strength were identified, their reactivity was quantified (Figure 3). These results are corrected for the ionic strength-dependent changes of TGase activity (see Experimental Procedures). Therefore, the changes in reactivity shown in Figure 3 correspond only to changes in the accessibility of glutamines, which in turn depend on the changes in the conformation of the core particle.

Incorporation of DNC into DNA-Free Histone Complexes. It can be wondered whether the presence of released DNA in the high-salt samples may alter the labeling pattern of the histones. To check this possibility, we also incubated isolated histone octamers in 2.4 M NaCl in the presence of DNC and TGase as described under Experimental Procedures. Although the ionic strength is significantly higher than the one used with core particles, these conditions guarantee the integrity of the histone octamer in the absence of DNA. The analysis of the glutamine usage in histone octamers showed that, under these conditions, no glutamines, other than those reactive in nucleosomes at 1.4 M NaCl, were modified.

The reaction of H2A−H2B heterodimers at 0.6 M NaCl with DNC in the presence of TGase resulted in an effective incorporation of the fluorescent probe into both histones. The analysis of tryptic peptides allowed us to identify the reactive residues in H2A as Gln^{24}, Gln^{104}, and Gln^{112}. H2B was mainly labeled in Gln^{95}, but the probe was also incorporated, though to a much lower extent, into Gln^{22}. Moreover, a peak coincident with the one labeled D in Figure 2 also appeared, confirming the above assumptions on the composition of the dimeric material. In a similar experiment carried out with H3−H4 tetramers at 2.0 M NaCl, the HPLC analysis of

Table 1: Glutamine Substrates for TGase in the Nucleosome Core Particle as a Function of Salt Concentration

histone	[NaCl] (M)	glutamine usage
H2A	≥0.6	Gln^{24}, Gln^{104}
	≥0.9	Gln^{24}, Gln^{104}, Gln^{112}
H2B	0	Gln^{22}
	0.3	none
	≥0.6	Gln^{22}, Gln^{95}
H3	<0.9	Gln^{5}, Gln^{19}
	≥0.9	Gln^{5}, Gln^{19}, Gln^{76}
H4		nd[a]

[a] Incorporation of DNC to H4 was below the limit of detection.

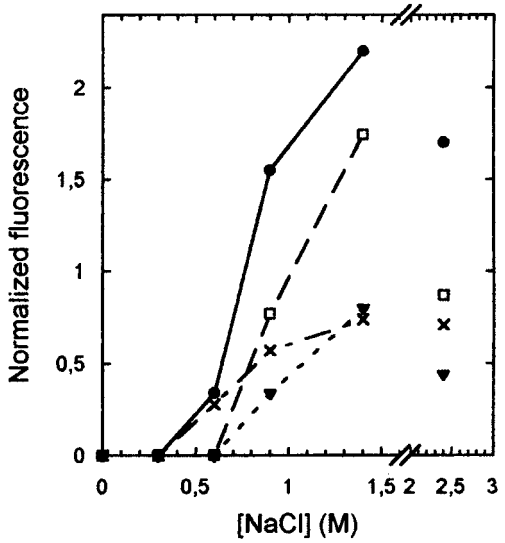

FIGURE 3: Ionic strength-dependent changes in the reactivity of the different glutamine substrates in nucleosome cores. The individual dansylated histones were recovered from RP-HPLC chromatograms as those of Figure 3 and trypsinized, and the tryptic peptides were resolved by RP-HPLC. The reactivity of each glutamine substrate was determined from the area of the fluorescence intensity of the tryptic peptide containing the corresponding glutamine and normalized according to the amount of sample loaded into the column. Finally, the resulting values were corrected to account for the dependence of TGase activity on ionic strength, and plotted against [NaCl]. (●) Gln^{104} of H2A; (□) Gln^{112} of H2A; (×) Gln^{95} of H2B; (▼) Gln^{76} of H3.

histones revealed the presence of two fluorescent peaks, with retention times similar to those labeled 3 and 3′ in Figure 2. Although the samples were not further analyzed in this instance, the appearance of both peaks suggests that the three reactive glutamines found in core particles, namely, Gln^{5}, Gln^{19}, and Gln^{76}, are also labeled in the DNA-free tetramer.

DISCUSSION

We describe in this paper a sensitive method, based on the behavior of the histone glutaminyl residues in the reaction catalyzed by TGase, to study the conformation of nucleosome core particles and their ionic strength-induced conformational changes. The possibility of using TGase to analyze protein structure and organization was foreseen by Folk (*8*), although he reported that the failure of many proteins to act as TGase substrates can lead to disappointing results. Fortunately enough, this is not the case with histones, and we have already reported the use of the TGase reaction as a novel approach to study the interactions of histone tails with DNA in the nucleosome (*11*).

The use of a given glutamine as TGase substrate depends on several factors, and its accessibility may often be the determinant one. The mechanism of TGases involves an acyl-enzyme intermediate formed by the carboxyl moiety of the carboxamide group of the glutaminyl donor and the thiol group of a cysteine of the active site (*21*), and this results in a severe limit to the accessibility of the enzyme to the potential glutaminyl substrates. The actual three-dimensional structure of tissue TGase is not known, but it can be inferred from the structure of Factor XIII (*22*, *23*). The relative sizes of the monomer tissue TGase, which represents the physiological form of the enzyme (*24*), and a nucleosome core are shown in Figure 4a, in which the potential glutamine substrates are marked to emphasize the resulting steric hindrance. In view of this figure, it seems obvious that the ionic strength-dependent increment in DNC incorporation observed in the experiment of Figure 2 reflects an increase in glutamine accessibility as the nucleosome unfolds.

The accessibility of a particular glutamine residue may be hindered by the presence of either other histones or DNA. Histone−DNA interactions are relaxed for [NaCl] > 0.3 M (*25*), the heterodimers H2A−H2B are released from chromatin between 0.8 and 1.0 M, and the dissociation of H3 and H4 requires higher ionic strength values (*26*). The results of Figure 1 inform of the precise salt concentration range in which the above events take place under our experimental conditions. The increase of DNA ellipticity adopts the form of a sigmoidal curve, indicating that the unwinding of the nucleosomal DNA superhelix is a cooperative process, which may be described by the equation:

$$y = \frac{y_0 + (y_\infty - y_0)C^n}{K + C^n} \qquad \text{(I)}$$

where y stands for the variable represented on the y axis (molar ellipticity in the CD curve), the subscripts 0 and ∞ indicate, respectively, the initial and final values of the variable, C represents the salt concentration, and K is the equilibrium dissociation constant for the binding of saline ions to the nucleosome. The best fitting of experimental data to eq I was achieved for $K = 0.172 \pm 0.032$ M and $n = 5.17 \pm 0.37$. By analogy with the Hill treatment for cooperative binding, the latter parameter may be considered as a cooperativity index, which is related, but not necessarily equal, to the number of saline ions bound per nucleosome. The fitting of experimental data to eq I is reasonably good (Figure 1).

On the other hand, the increase in tyrosine fluorescence cannot be described as a single cooperative process, but rather adopts a biphasic profile, in close agreement with the results of other workers (*14*, *15*, *18*). The existence of a two-step increase in fluorescence intensity may indicate that there are two processes resulting in the elimination of fluorescence quenching of tyrosine residues, and that the second one is only noticeable once a given threshold in salt concentration is surpassed. In other words, the following equation would apply:

$$y = \begin{cases} y_0 + \dfrac{f(y_\infty - y_0)C^n}{K_1 - C^n} + \dfrac{(1-f)(y_\infty - y_0)(C-t)^m}{K_2 + (C-t)^m} & \text{if } C > t \\ y_0 + \dfrac{f(y_\infty - y_0)C^n}{K_1 + C^n} & \text{if } C \le t \end{cases} \qquad \text{(II)}$$

where f is the fraction of total change accounted for by the first process, with cooperativity index n and dissociation constant K_1, t stands for the concentration threshold necessary for the second process—with cooperativity index m and dissociation constant K_2—to be displayed, and the remaining symbols are as above, except that y now represents the fluorescence intensity. Experimental data fit remarkably well eq II (Figure 1), with $K_1 = 0.568 \pm 0.035$ M, $K_2 = 0.053 \pm 0.050$ M, $n = 6.93 \pm 0.17$, $m = 8.15 \pm 1.72$, $f = 0.837 \pm 0.015$, and $t = 0.843 \pm 0.101$ M. The first step of fluorescence increase, which roughly occurs between 0.6 and 1.4 M (Figure 1), is clearly different from the process observed by CD, which takes place at lower ionic strength (see Figure 1 and the above fitting data). It would represent the removal of fluorescence quenching as the tyrosines buried by the interactions between the $(H3-H4)_2$ tetramer and the two H2A−H2B dimers are broken. Actually, Tyr^{83} of H2B, and Tyr^{72} and Tyr^{88} of H4 in both copies of the histones form a hydrophobic cluster at the H2B−H4 four-helix bundles; Tyr^{98} of H4 is close to this hydrophobic cluster, and Tyr^{39} of H2A lies in the interface with the other H2A molecule of the nucleosome (*3*). Obviously, the environments and/or interactions of all these tyrosines are altered when the H2A−H2B dimers are released, and this fact supports our assumption on the origin of the first step of the tyrosine fluorescence curve. The release of H2A−H2B heterodimers, in turn, probably requires a loosening of the DNA wrapping (*18*), and this explains why the increase in fluorescence intensity is preceded by that of DNA ellipticity (Figure 1). This interpretation is also in agreement with the results of Zweidler (*27*), who described that the chemical modification of the tyrosines of the hydrophobic cluster with *p*-nitroben-

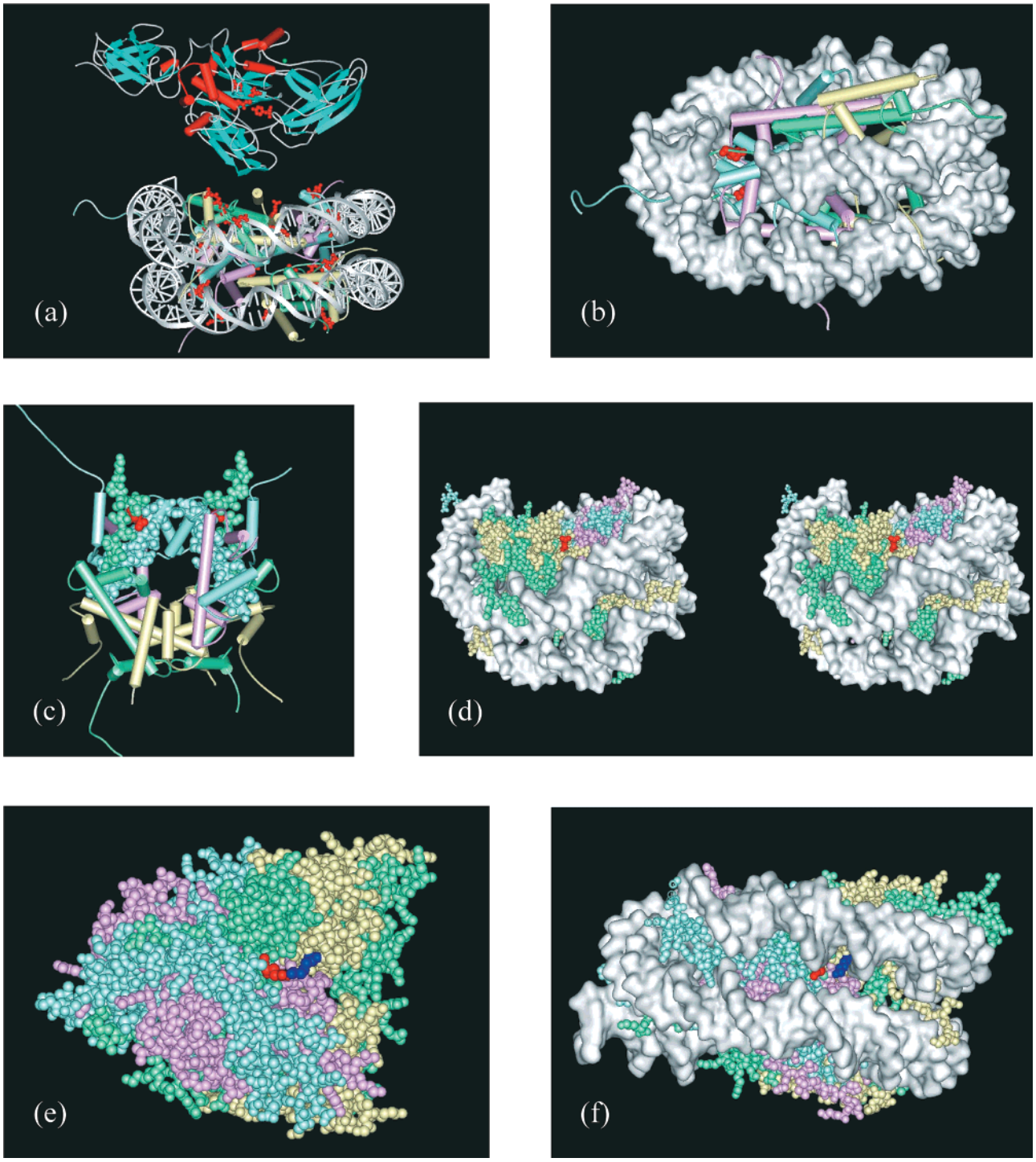

FIGURE 4: Localization of several glutamine substrates in the nucleosome. The figures were constructed with the coordinates of the core particle (*3*) or the histone octamer (*2*) as required. DNA is white, and histones are colored: green (H2A), yellow (H2B), light blue (H3), and pink (H4). Histone glutamines modified by TGase are shown in red in a space-filling mode. (a) Comparative sizes of the nucleosome core particle and TGase [according to Yee et al. (*22*)]. The catalytic triad of the enzyme is also shown in red in a ball-and-stick mode. (b) DNA limits the accessibility of the enzyme to Gln^{112} of H2A (red, space-filling). The dyad axis of the nucleosome runs from left to right, and the superhelical axis forms an angle of ~15° with the vertical. DNA between sites 6 and 7 has been omitted to show how Gln^{112} is hindered. (c) Gln^{112} of H2A is also hindered by the α2 helix of histone H3′. The histone octamer is viewed down the superhelical axis; the DNA has been omitted to show how the α2 helix of histone H3′ also is an obstacle to the accessibility of Gln^{112} of H2A (red). The pertinent regions of both H2B and H3′ are represented in a space-filling mode. (d) Stereoview of the cavity holding Gln^{95} of H2B. Nucleosome core particle viewed from the exit of the dyad axis, and the superhelical axis forms an angle of ~25° with the vertical to show how the proximity of DNA limits the accessibility to the residue. (e) The pair H2A Gln^{104} (red)−H2B Lys^{57} (blue) forms a hydrogen bridge in the isolated octamer. The figure was drawn with the coordinates of the isolated histone octamer (*2*). The plane of the paper contains the 2-fold and the superhelical axes, with the former running from left to right. (f) The pair Gln^{104} (H2A)−Lys^{57}(H2B) in the nucleosome core. Same orientation as in panel e.

zenesulfonyl fluoride does not occur for [NaCl] < 0.8 M, in contrast with those located close to DNA, such as Tyr^{41} of H3, whose modification is virtually completed at this salt concentration.

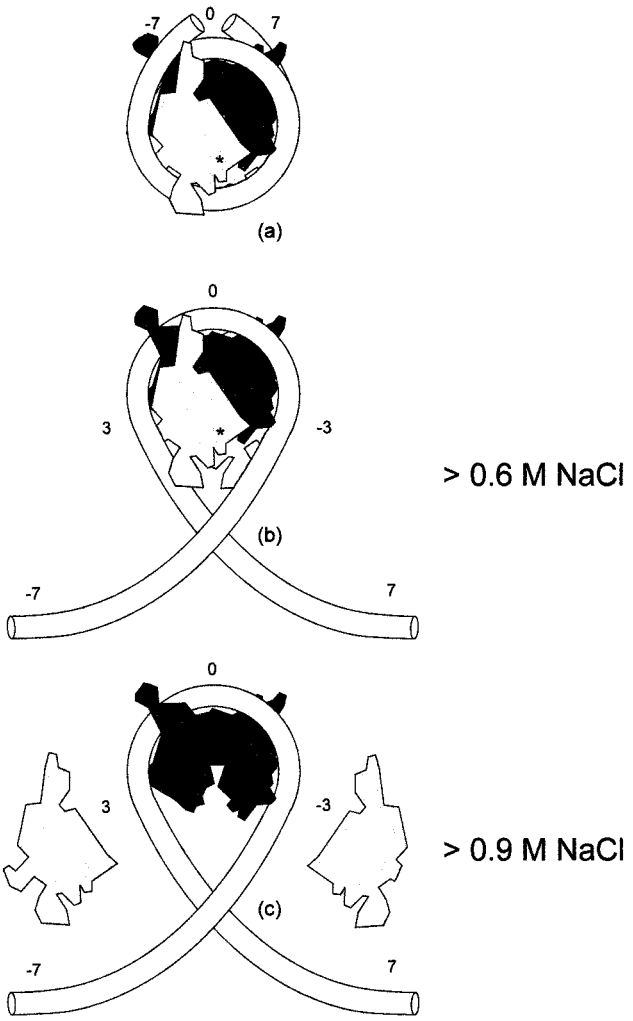

FIGURE 5: Model for ionic strength-induced disassembly of the nucleosome core. In (a), the core particle is viewed down the superhelical axis, with the dyad axis entering at site 0. The H2A−H2B dimers are shown in gray and the $(H3-H4)_2$ tetramer in black. The DNA is in white. The position of H2B Gln^{95} is indicated by an asterisk. DNA unwinding is proposed to occur following a two-state model, to yield (b), in which H2B Gln^{95} is fully accessible. Unwinding of DNA as in (b) is required to dissociate the H2A−H2B dimers, to yield the subnucleosomal particle $(H3-H4)_2$-DNA. The path of DNA in this particle may be different than in (b).

The second step in the increase of fluorescence intensity only takes place after passing a threshold around 0.85 M NaCl, and it is completed at around 2 M NaCl (Figure 1). It seems obvious that this transition represents the dissociation of the subnucleosomal particle formed by the $(H3-H4)_2$ tetramer and the already partially straightened DNA. The increase in fluorescence intensity would then be related to the diminution of fluorescence quenching, probably due to the release of DNA from the tetramer. Tyr^{54} and Tyr^{99} of H3, as well as Tyr^{51} of H4, which are somewhat distant from the H2A−H2B dimers, may be responsible for this effect. On the other hand, Tyr^{50} and Tyr^{57} of H2A would not contribute to the increase in fluorescence intensity, because they are placed in the interface with the associated H2B, which is not altered upon dimer release.

A consequence of the above interpretation is that the unwinding of the DNA superhelix affects roughly four turns of double helix at each side of the core particle (see the model of Figure 5), because the central contacts between DNA and the tetramer occur between positions −3 and +3 of nucleosomal DNA (*3*). Therefore, the ionic strength-induced unwinding of DNA between ~0.4 and ~1.3 M is probably more extensive than previously suspected.

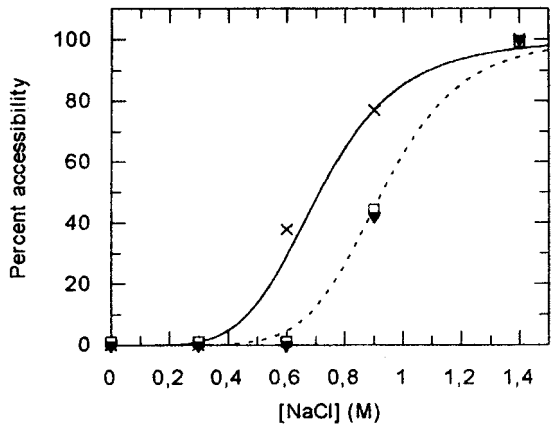

FIGURE 6: Relationships between the accessibility of several glutamines and the processes of salt-induced nucleosome dissociation. The reactivity of Gln^{104} of H2A, Gln^{76} of H3, and Gln^{95} of H2B is plotted in a percent scale against ionic strength. The symbols are as in Figure 4, and the lines drawn represent eq I (—) and the first component of eq II (- - -) with the parameters obtained from the fits of Figure 1 as given under Discussion.

As mentioned earlier, the accessibility of a given glutaminyl residue would be limited both by the structure of the octamer itself and by the path of DNA. For instance, if the accessibility of a glutaminyl residue of either H2A or H2B in the residual nucleosome at ~1.4 M NaCl (when the dimers are almost totally released) is similar to that in the isolated octamer, it can be concluded that the access of TGase to that particular glutamine is not limited by the H3−H4 tetramer. This is the case of Gln^{95} of H2B (Figure 3) and Gln^{24} of H2A (data not shown). Similarly, if the reactivity of a glutamine of H3 is the same in the isolated octamer and subnucleosomal particles at 1.4 M NaCl, neither the residual DNA nor the H2A−H2B dimers limit the accessibility of that residue. This is the case of the glutamines of the H3 N-terminal tail (data not shown). Conversely, the reactivity of Gln^{112} and, to a lesser extent, that of Gln^{104} of H2A are lower in the isolated octamer than in the released dimers at 1.4 M NaCl (Figure 3). This means that the accessibility to those residues is hindered by the presence of the H3−H4 tetramer.

Many of these conclusions are substantiated by observing the localization of those residues in the core particle (*3*). The accessibility of Gln^{112} of H2A is limited both by DNA (Figure 4b) and by the proximity of the α2 helix of H3′ (Figure 4c). This explains why this residue cannot be labeled at all by DNC in the nucleosome core, whereas it is readily accessible in the released dimers. Obviously, its reactivity in the octamer is halfway between both extreme situations (Figure 3). Gln^{95} of H2B, which is located in the α3 helix of its histone fold, lies in the bottom of a cavity that opens obliquely to the flat side of the core particle. The access of TGase is hindered by the extra helix of H2B and, especially, by DNA (Figure 4d). According to the reasons given above, the results of Figure 3 indicate that DNA represents the only obstacle to the accessibility of TGase to Gln^{95} of H2B. The validity of this assumption is substantiated by the plot of Figure 6, which shows that the increase in the accessibility of Gln^{95} of H2B fits the curve drawn with the parameters of

DNA unwinding. These results suggest that the cooperative, salt-induced, DNA unwinding, described by eq I, follows a two-state model (Figure 5) and not a sequential one, as the region of DNA closest to Gln^{95} of H2B is site ±3.5. Figure 6 also shows that the increase in accessibility of Gln^{112} of H2A and Gln^{76} of H3 fits the curve corresponding to the first step of the tyrosine fluorescence increase, which we have above interpreted as associated with the dissociation of H2A−H2B dimers from the residual subnucleosomal complex. The partial masking of these residues by the respective dimer or tetramer counterpart (see above) supports the coherence of our interpretation.

Finally, in the isolated octamer, Gln^{104} of H2A, which is a good glutaminyl donor in the free histone (*10*) and in nucleosomes at [NaCl] ≥0.6 M (Figure 3), forms a hydrogen bridge with Lys^{57} of H2B (*2*). The distance between the glutaminyl carboxamide group and the lysyl ϵ-amino group is only 1.95 Å, i.e., the optimum distance required for TGase to form an isopeptidic bond. This pair of residues are accessible in the isolated octamer (Figure 4e). However, in the nucleosome core, the distance between the reactive atoms is greater than 5.6 Å, as revealed by the data of Luger et al. (*3*), and they no longer form a hydrogen bridge (Figure 4f). Moreover, both residues are not accessible due to the presence of DNA (Figure 4f). It is, therefore, highly probable that the cross-linked dimer present in peak D results from the TGase-catalyzed formation of an isopeptide bond between the glutamyl moiety of Gln^{104} of H2A and Lys^{57} of H2B. The fluorescence of the cross-linked dimer is easily explained because Gln^{95} of H2B and/or Gln^{112} of H2A are susceptible to nucleophilic attack by DNC.

In summary, the reaction catalyzed by TGase has proved to be a useful tool to study conformational changes in the nucleosome. The information provided by the use of this novel approach is complementary to that supplied by chemical modification of histone residues (*27*), and its resolution is much higher than that achieved by other techniques, either physical (*28*) or immunological (*29*). Moreover, besides the specificity and absence of side reactions, the mild conditions in which the reaction is carried out minimize the occurrence of secondary effects. In some instances (e.g., in the analysis of the labeling of Gln^{95} of H2B), the approach proposed in the present paper has allowed us to obtain novel details on the mechanism of salt-induced dissociation of the core particle, which are included in the model of Figure 5. This model postulates that, on increasing ionic strength, DNA extensively unwinds in an all-or-none way and that this unwinding occurs prior to the dissociation of H2A−H2B dimers. The mechanistic possibility of this dissociation is particularly interesting. Hamiche et al. (*30*) and Langst et al. (*31*) have recently described that remodeling complexes NURF and CHRAC induce a cis sliding of the histone octamer. For this remodeling to occur, the interactions between DNA and the octamer have to be, at least partially, disrupted, and this involves the existence of a high-energy intermediate state (*6*). The model proposed in Figure 5 provides a basis for the nature of this transition state, which would be easily reached in the presence of remodeling complexes which lower the activation energy. The possibility of using the experimental strategy described in this work to follow some physiological conformational changes of nucleosomes is currently being studied in our laboratory.

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