Use of the Transglutaminase Reaction To Study the Dissociation of Histone N-Terminal Tails from DNA in Nucleosome Core Particles†

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Received October 23, 1996; Revised Manuscript Received February 26, 1997⊗

ABSTRACT: We have recently shown that core histones are glutaminyl substrates for transglutaminase (TGase) and that when native nucleosome cores are incubated with monodansylcadaverine (DNC) as donor amine, this fluorescent probe is incorporated into Gln^5 and Gln^{19} of H3 and in Gln^{22} of H2B [Ballestar et al. (1996) *J. Biol. Chem. 271*, 18817−18825]. In the present paper, we report that the cause by which Gln^{22} of H2B is modified in nucleosomes but not in the free histone is the interaction of the region containing that glutamine with DNA. We have used the specificity of the TGase reaction to study the changes induced by increasing ionic strength in the interaction between the histone N-terminal tails and nucleosome DNA by two different approaches. First, the reactivity of the histone tail glutamines was employed to monitor changes in the interactions between the regions containing these residues and DNA. Second, by using reconstituted nucleosome core particles containing either H2B modified with DNC by the TGase reaction at Gln^{22} or H3 modified with the same procedure at Gln^5 and Gln^{19}, the dissociation of the histone tails was followed by the decrease of the fluorescence anisotropy of the probe. These methods allowed us to describe two ionic strength dependent structural transitions of the histone tails not reported to date. In the case of H2B, the first one occurs at very low ionic strength, and it can be assigned to an increase in the mobility of Gln^{22}. The second one results in the cooperative release of a region of the tail that includes lysine residues next to Gln^{22}, and it is followed by the overall release of the entire tail, described by other workers.

The discovery of the nucleosome, the basic structural subunit of chromatin, was founded on the original proposal of Kornberg (1974), which represented a historical landmark in the research in molecular biology and, especially, in the field of chromatin. For the next decade, there was rapid progress in the understanding of the organization of this particle, from the electron microscopic "beads-on-a-string" model of Olins and Olins (1974) to the low-resolution structure (27−7 Å) of the nucleosome core particle, derived from the diffraction studies of the Medical Research Council group (Finch et al., 1977; Klug et al., 1980; Richmond et al., 1984). Most of the structural data obtained in these years [reviewed in Van Holde (1988)] unconsciously gave rise to a predominantly static view of the nucleosome, which was often referred to as an obstacle that the transcriptional machinery had to circumvent. In this view, the N-terminal tails of the histones, due to the high density of basic amino acid residues, were firm candidates to interact with DNA. Since these tails are the target sites for histone acetylation [see Loidl (1994) for a recent review], it had long been suspected that this posttranslational modification of histones, which results in partial charge neutralization, may affect the structure of the nucleosome. Present data, however, do not provide clear evidence to confirm the above hypothesis [for a review, see Ausio (1992)].

A few years ago, a new, dynamic view of the nucleosome emerged, in which the histone N-terminal tails are thought to play a key role in their function. The first evidence on the specific interactions of histone tails with some protein factors was obtained in the course of studies on the repression of the yeast silent mating loci [reviewed by Grunstein (1990)]. Although the early models have been considerably developed to include a general model for heterochromatin formation (Hecht et al., 1995), the early ideas on the role of H3 and H4 tails are essentially unchanged (Thompson et al., 1994). Another series of experiments suggested that the N-terminal tails of histones are decisive to modulate chromatin structure (Durrin et al., 1991), and in this context, nucleosomes and, by extension, histones are no longer regarded as passive obstacles, but rather as regulators of transcription (Svaren & Hörz, 1993; Wolffe, 1994).

In parallel with the above-mentioned emerging emphasis on the dynamic aspects of histone and nucleosome structure, a new, high-resolution set of structural information became available. This fundamentally came from the work of the group of Moudrianakis, who unravelled the structure of histone octamers at 3.1 Å resolution (Arents et al., 1991), thus allowing the definition of the histone−DNA contacts in the nucleosome (Arents & Moudrianakis, 1993) and of the properties and evolutionary significance of an architectural motif conserved among the core histones, the histone fold (Arents & Moudrianakis, 1995). Unfortunately, the N-terminal tails of histones could not be imaged in nucleosome core crystals (Richmond et al., 1984); neither could they in the histone octamers (Arents et al., 1991). This may be obviously explained if the tails are unstructured, which in all probability occurs in the histone octamers, due to the high density of positively charged residues as well as of other

† This work was supported in part by Grant PB91-0328 from the Dirección General de Investigación Científica y Técnica (DGICYT), Spain. E.B. is the recipient of a fellowship from the Conselleria de Educació i Ciència (Valencia)

⊗ Abstract published in *Advance ACS Abstracts,* May 1, 1997.

S0006-2960(96)02662-1 CCC: $14.00

polar amino acids. On the other hand, histone acetylation may modify the capability of their tails to interact with protein factors (Lee et al., 1993), perhaps by releasing them from DNA. Therefore, it would be interesting to experimentally discern the conditions in which the histone N-terminal tails are bound to DNA from those in which they are released. Several approaches have been used to address this issue (Cary et al., 1978; Walker, 1984; Ausio et al., 1984, 1989; Smith & Rill, 1989; Dong et al., 1990; Hill & Thomas, 1990). Although there are some conflicting results, especially when trying to elucidate the fate of each individual histone tail, it seems clear that they are bound to DNA in low salt, but they become released on increasing ionic strength.

We have recently found that core histones are substrates for tissue transglutaminase (TGase),[1] an enzyme that catalyzes the reaction of the side chain of glutaminyl residues in proteins with primary amines, the latter becoming covalently bound to the protein by an isopeptide bond. The fluorescent amine DNC may be used to label the various glutaminyl residues that act as TGase substrates in free histones, namely, Gln^{95} of H2B; Gln^{5}, Gln^{19}, and Gln^{125} of H3; Gln^{27} and Gln^{93} of H4; and Gln^{24}, Gln^{104}, and Gln^{112} of H2A (Ballestar et al., 1996). When intact nucleosome cores were used as substrates for the TGase reaction, only two of the above residues, Gln^{5} and Gln^{19} of H3, are accessible but an additional residue, Gln^{22} of H2B, also becomes modified (Ballestar et al., 1996).

We describe here research in which we take advantage of the specificity of the TGase reaction to study the dissociation of histone N-terminal tails from DNA in nucleosome cores. To this end, two different approaches have been used. First, the labeling of Gln^{22} of H2B, and to a lesser extent that of Gln^{5} and Gln^{19} of H3 with DNC, depends on the neutralization of the charges of adjacent lysines, and thus it can be used to probe the interaction of the neighboring residues with DNA. Second, nucleosome cores reconstituted from H2B or H3, modified with DNC in their tails, have been used to study by fluorescence anisotropy the mobility of these regions.

EXPERIMENTAL PROCEDURES

Materials. Chicken erythrocyte nucleosomes and histones were obtained as previously described (Ballestar et al., 1996). Peptides NQ22 (AVTKTQKKGDK) and MQ22 (AVTKTQTAGDA) were custom-synthesized by Genosys. The sequence of the former corresponds to that of the residues 17−27 of chicken H2B. In the second peptide, neutral amino acids substitute for the lysines at the C-terminal side of glutamine.

Amino Group Blocking by Acid Anhydrides. H2B (7.5 mg/mL) was treated with a 100-fold excess of dimethylmaleic or monomethylmaleic anhydride at room temperature. The reagent was added from a dioxane solution (300 mg/mL) so that the final concentration of dioxane in the modification mixture was always below 5%. The pH was kept at 8.2 by adding 3 M NaOH with a pH-stat microTT 2050 (Crison). Reaction was completed in less than 2 h. The hydrolyzed reagent was removed by dialysis against the appropriate buffer or on PD-10 columns.

Chemical acetylation of amino groups in the synthetic peptide NQ22 was carried out as described by Mingarro et al. (1993). The modified peptide was then loaded onto a C-18 Sep-Pak cartridge previously equilibrated with 0.1% trifluoroacetic acid. After washing the cartridge with the same solvent, the peptide was eluted with a mixture of water/acetonitrile (40:60) containing 0.1% trifluoroacetic acid and freeze-dried.

TGase Assay. The dependence of TGase activity on ionic strength was measured following the colorimetric assay of Folk and Chung (1985) with CBZ-Gln-Gly as glutaminyl substrate and hydroxylamine as amine donor, except that the incubation medium was supplemented with NaCl to obtain the desired ionic strength.

Incorporation of DNC into Nucleosomes, Histones, or Peptides. The incorporation of the fluorescent probe into nucleosome core particles was carried out as described by Ballestar et al. (1996). The same conditions were employed to incorporate DNC into synthetic peptides (final concentration 1 mg/mL). In the latter instance, nucleosomal DNA (final concentration 1 mg/mL) was added to the incubation mixtures in some cases. To prepare H2B-labeled with DNC at Gln^{22}, advantage was taken of the fact that this is the only H2B reactive residue in nucleosome cores at low ionic strength (Ballestar et al., 1996). Therefore, core particles were labeled under these conditions, and H2B was recovered as described elsewhere (Ballestar et al., 1996).

Incubation of maleylated H2B was carried out under the standard conditions described in Ballestar et al. (1996) except that the pH was 8.2 (adjusted with HEPES buffer) instead of 6.0 (see below). In some instances, DNC was incorporated into unmodified H2B in the presence of poly(L-glutamic acid) [histone/poly(L-glutamic acid) 1:1, w/w].

Analysis of the Glutamine Substrates in Histones and Peptides. DNC-labeled histones were digested with trypsin in 200 mM *N*-ethylmorpholine buffer, pH 8.1, at an enzyme:histone ratio of 1:40 (w/w). Hydrolysis was carried out at 37 °C for 4 h. Reaction was stopped by placing the tubes in boiling water and freeze-drying. The isolation and analysis of the composition of the fluorescent tryptic peptides were performed as described by Ballestar et al. (1996). Prior to digestion with trypsin, maleylated H2B was incubated in 10 mM HCl at 4 °C for 6 h to reverse the blocking of lysines. When working with synthetic peptides, the analysis of the labeling was followed by HPLC using a UHG-120 column. The solvent employed was 80 mM sodium acetate, pH 4.4, 20% acetonitrile under isocratic conditions at a flow rate of 1 mL/min. Fluorescence was monitored (excitation wavelength, 330 nm; and emission wavelength, 510 nm) with an F-1050 fluorescence spectrophotometer (Merck-Hitachi).

Nucleosome Reconstitution and Analysis. Nucleosome cores were reconstituted from either unmodified or DNC-modified core histones and DNA of core size following the procedure of Marvin et al. (1990). Prior to reconstitution, DNC-modified histones were purified by HPLC as previously described (Ballestar et al., 1996). The reconstituted particles were routinely analyzed by native gel electrophoresis and DNase I digestion as previously described (Ballestar et al., 1996). Ion-exchange HPLC on a DEAE-5PW column (Richmond, 1988) was used to purify reconstituted nucleosome cores.

[1] Abbreviations: TGase, transglutaminase; DNC, monodansylcadaverine; HPLC, high-performance liquid chromatography; CD, circular dichroism; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid.

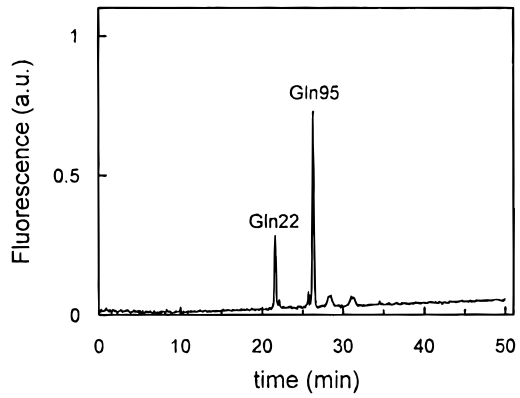

FIGURE 1: Fluorescent tryptic peptides obtained after DNC modification of maleylated H2B. DNC was incorporated into maleylated H2B by the TGase-catalyzed reaction, and prior to digestion with trypsin, the maleyl groups were removed by incubating the histone at acidic pH. DNC-modified H2B was then trypsinized, and the peptides were resolved by reversed-phase HPLC.

Spectroscopic Data. Circular dichroism of reconstituted nucleosomes (in the near-UV region) and of peptides (in the far-UV region) was recorded in the conditions described elsewhere (Ballestar et al., 1996). Fluorescence anisotropy was measured in an LS-50 luminescence spectrometer (Perkin Elmer) at an excitation wavelength of 330 nm and an emission wavelength of 500 nm in cells of 1 cm optical path. Slit widths of both monochromators were set at 10 nm. The sample compartment was maintained at 22 °C, and nucleosome concentration was in the range 0.030−0.035 mg/mL. The figures given are an average of 20 measurements.

RESULTS

We have previously shown that Gln^{22} of H2B, which is not a TGase substrate in the free histone, becomes modified with amines when the reaction was carried out with nucleosome cores instead of free histones (Ballestar et al., 1996). At the same time, we advanced the hypothesis that the positive charges of the lysyl residues close to Gln^{22}, especially those of Lys^{23} and Lys^{24}, are an obstacle to the action of the enzyme, but that these charges are neutralized by the interaction of the N-terminal tail of the histone with DNA in the nucleosome. If this assumption proved to be correct, the reactivity of Gln^{22} would provide an indirect way of measuring the interaction of N-terminal tails of histones and DNA.

To test the above hypothesis, we blocked the lysyl residues of H2B with dimethylmaleic anhydride. Maleylated H2B was treated with DNC in the presence of TGase, and the blocking of lysines was reversed by lowering the pH. DNC-modified H2B was then digested with trypsin, and the tryptic peptides were resolved by HPLC. Apart from the fluorescent peptide containing Gln^{95}, which is the only reactive glutamine in the free unmodified histone, a second fluorescent peptide, with a retention time of 21 min, appeared in the chromatogram (Figure 1). The two fluorescent peptides recovered were identified as the peptide 21−23, containing Gln22, and the peptide 93−99, that contains Gln95, by comparison with the previously published data (Ballestar et al., 1996). Due to the instability of the maleyl derivative at lower pH values (Nieto & Palacián, 1983), the incubation of maleylated H2B with TGase was carried out at pH 8.2 in HEPES buffer, whereas free unmodified H2B was incubated at pH 6.0 in MES buffer. We therefore checked whether the change of pH and/or the buffer substance had any influence on the labeling pattern. To do this, we incubated free unmodified H2B with DNC in the presence of TGase at several pH values between 5.5 and 7.0 in MES buffer and between pH 7.0 and 8.5 in HEPES buffer. After trypsinizing, only one fluorescent peptide, identified as that containing Gln^{95} by its retention time, was obtained in all the cases. An obvious change in the yield of DNC incorporation was observed when the pH changed, due to changes in TGase activity, but this experiment discarded the possibility that labeling of Gln^{22} either in nucleosomes or in maleylated H2B was a result of a pH-induced change of TGase glutamine specificity. Similar results were obtained when H2B lysines were blocked with citraconic anhydride, whose derivatives, as reported by Nieto and Palacián (1983), are stable under the standard incubation conditions, *i.e.*, at pH 6.0 (data not shown).

Apart from chemical modification, a second possibility of masking the charges of lysine side chains is the interaction of the histone with polyanions. When H2B was incubated with DNC and TGase in the presence of poly(L-glutamic acid), the pattern of peptide labeling was essentially the same as that of Figure 1; *i.e.*, in addition to a large peak of the peptide containing Gln^{95}, a second minor fluorescent peak with a retention time of 21 min appeared (data not shown).

The above results support our hypothesis, but they do not prove it. It is clear that the reactions used for the chemical modification of lysine side chains result in the elimination of their positive charges, but the use of a glutamine residue as TGase substrate seems to depend on both sequence and conformational motifs (Aeschlimann & Paulsson, 1994; Aeschlimann et al., 1992). In the present instance, as negative charges substitute for the positive ones found in the unmodified histone, it cannot be discarded that the usage of Gln^{22} result from a substantial conformational alteration of H2B, which contains a large number of lysyl residues. To rule out this possibility we examine the synthetic peptides NQ22 and MQ22 for their capability to act as substrates for TGase. NQ22 matches the native sequence 17−27 of H2B, and the three lysines in the C-terminal side of Gln^{22} are replaced by neutral amino acids in MQ22. Their small size (11 residues) and sequence make it unlikely that they acquire any compact structure. The validity of this assumption was experimentally ascertained (see below).

Under the conditions described under Experimental Procedures, *i.e.*, in the presence of 10 mM NaCl, NQ22 was used as TGase substrate and incorporated DNC to a small extent. MQ22, which does not possess lysines at the C-terminal side of the glutaminyl residue, incorporated 2−3 times more DNC, depending on the batch of the enzyme used, indicating that the elimination of basic amino acids at positions +1 and +2 favors the usage of a glutaminyl residue as TGase substrate. But the most interesting result is that in the presence of DNA, the incorporation of DNC into NQ22 is always about 3-fold increased. Incorporation of DNC into MQ22, on the other hand, is little or not affected by the presence of DNA. The points at the lowest ionic strength in Figure 2 depict the results of a representative experiment.

Moreover, acetylated NQ22, in which the α-amino group of the N-terminal alanine and the ϵ-amino groups of lysines are blocked, was as good a substrate as MQ22 (data not

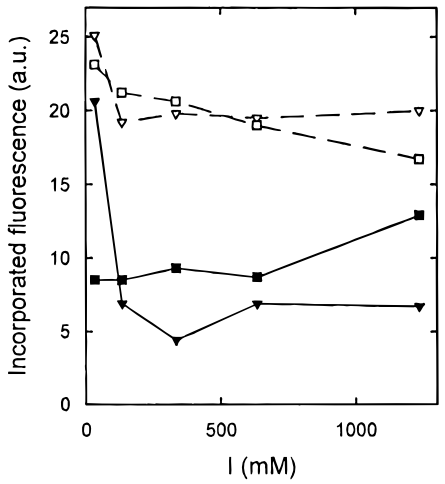

FIGURE 2: Influence of ionic strength and DNA on the reactivity of the single glutamine of the synthetic peptides NQ22 and MQ22 in the reaction catalyzed by TGase. The synthetic peptides NQ22 (filled symbols) and MQ22 (open symbols) were incubated for 90 min with DNC in the presence of TGase at various ionic strength values. Incubation was carried out with (triangles) or without (squares) added DNA. After incubating, the peptides were separated from the excess of free DNC and minor byproducts, and the fluorescence was quantified and corrected for the ionic strength influence on TGase activity.

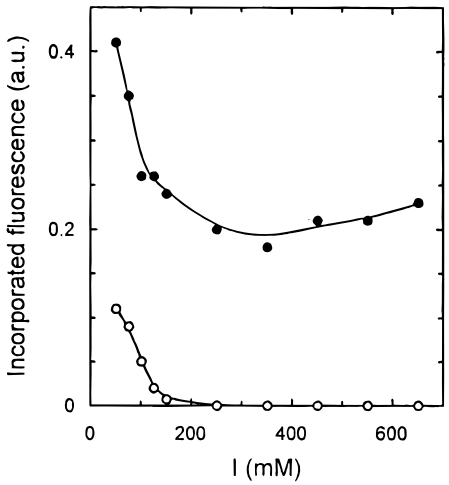

FIGURE 3: Incorporation of DNC into Gln^{22} of H2B, and Gln^{5} and Gln^{19} of H3 in nucleosome cores by the TGase-catalyzed reaction as a function of ionic strength. Nucleosome cores were incubated with DNC in the presence of TGase at various ionic strength values and the fluorescent H2B and H3 histones separated by HPLC. The fluorescence intensity of the histones, corrected for the ionic strength induced differences in TGase activity, is represented against NaCl concentration. (○) H2B, in which Gln^{22} is the only residue that incorporates the fluorescent probe. (●) H3, which contains modified Gln^{5} and Gln^{19} (see the text for further details).

shown). All these results seem to confirm the hypothesis that the cause of Gln^{22} dansylation in nucleosome cores is the neutralization of positive charges as a consequence of the interaction between the lysine-containing N-terminal tail of H2B and nucleosomal DNA.

Therefore, we next examined the ionic strength dependence of the substrate capability of glutamine in peptides NQ22 and MQ22, both in the absence and in the presence of DNA. To do this, we first determined the influence of ionic strength on TGase activity. We found that increasing the ionic strength up to 1.4 M results in a linear decrease of the enzymatic activity. The best fit was used to determine, at any given ionic strength value, the remaining activity relative to the theoretical extrapolated activity at $I = 0$ (data not shown), and these values were used to correct the DNC incorporation into peptides. The results are also shown in Figure 2, in which, as previously mentioned, the points at the lowest ionic strength value correspond to the experiment described just above. With the correction for enzymatic activity loss, the results given in Figure 2 represent the true effects of ionic strength on the substrate capability of the glutamine substrates. The behavior of NQ22 as TGase substrate is little affected by ionic strength in the absence of added DNA. However, the enhancement of Gln^{22} reactivity caused by DNA in NQ22 is virtually abolished at $I \simeq 150$ mM or higher. The curves for MQ22 are less dependent on ionic strength than those for NQ22. At any rate, the changes observed in Figure 2 are not due to ionic strength dependent conformational alterations of the peptides. Actually, the CD spectra of NQ22 and MQ22 are superimposable to each other in the range 0−1.2 M NaCl, and the conformation of the peptides is also independent of ionic strength in the presence of DNA (data not shown). On the other hand, the CD spectra are compatible with the idea that both peptides are predominantly unstructured, although the presence of some β-structure cannot be discarded. At any rate, the possibility that differences in conformation accounted for the changes observed in the substrate behavior of both peptides can be ruled out from the CD experiments.

If all the above reasoning is true, the labeling of Gln^{22} of H2B in nucleosomes with DNC in the absence of NaCl indicates that the N-terminal tail of the histone, or at least the lysyl residues close to Gln^{22}, is interacting with DNA. Most importantly, the degree of Gln^{22} labeling may measure the extent of this interaction. It is, therefore, conceivable that conditions that cause the releasing of the tail, for instance, the increase in ionic strength, result in the decrease of Gln^{22} reactivity in the presence of TGase. To check this possibility, we studied the TGase-catalyzed labeling of Gln^{22} of H2B with DNC when nucleosomes were incubated at various ionic strength values. The results show that the incorporation of the fluorescent probe decreases on increasing ionic strength, and it does no longer take place at $I \geq 150$ mM (Figure 3).

Figure 3 also shows that the use of Gln^{5} and/or Gln^{19} of H3 as TGase substrates in nucleosomes depends on ionic strength. In the range of NaCl concentration used in this study, the maximum incorporation of DNC takes place in the absence of NaCl ($I = 51$ mM), and a broad minimum is observed between $I = 250$ and 450 mM. Anyway, even at the minimum, the incorporation of the probe takes place to a substantial degree, as it does in the free histone (Ballestar et al., 1996). It can be wondered if these changes are also related to charge neutralization, but some data suggestive of changes in the interaction of the H3 tail with DNA will be provided later on this paper.

The aforementioned results strongly point to the idea that a change in the interactions between the H2B N-terminal tail and DNA occurs in the ionic strength interval 0−200 mM. A different line of evidence is provided by the experiment of Figure 4. Nucleosome cores were reconstituted from DNA and a mixture of histones containing either H2B modified with DNC at Gln^{22} or H3 modified at Gln^{5} and Gln^{19}. We have previously shown that these nucleo-

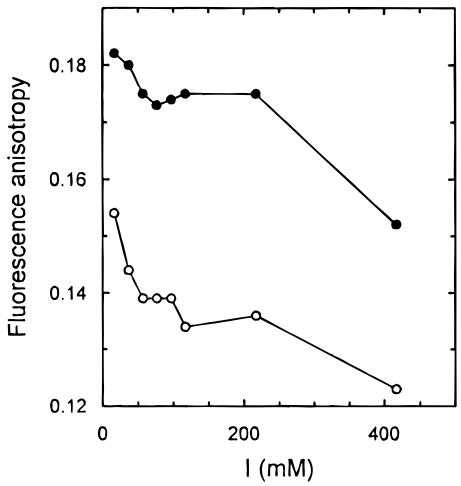

FIGURE 4: Influence of ionic strength on the fluorescence anisotropy of nucleosomes containing DNC-modified histones. Nucleosome core particles were reconstituted from nucleosome-size DNA and a mixture of histones containing either DNC-modified H2B at Gln^{22} (○) or DNC-modified H3 at Gln^{5} and Gln^{19} (●). The fluorescence anisotropy of the dansyl group was measured as a function of ionic strength. Each point is the average of 20 measurements.

somes were indistinguishable from the native ones by several criteria (Ballestar et al., 1996). The reconstituted nucleosomes were purified by ion exchange HPLC to eliminate excess of free DNC-modified histones, and the ionic strength dependence of fluorescence anisotropy was measured. The curves of Figure 4 show that the fluorescence anisotropy diminishes with increasing ionic strength. Both curves roughly run in parallel; *i.e.*, after an initial drop in anisotropy, there is a plateau up to 250 mM, followed by a further decrease. Statistical analysis (not shown) indicated that both anisotropy decreases for H2B and H3 are significant.

The ionic strength induced decrease in fluorescence anisotropy shown in Figure 4 is not an intrinsic property of the probe. We have carried out two additional control experiments to rule out that possibility. In the first one, nucleosomes were reconstituted from H2B modified with DNC at Gln^{95} as previously described (Ballestar et al., 1996). According to the data of the Moudrianakis' group (Arents et al., 1991; Arents & Moudrianakis, 1995), Gln^{95} is located near the flat surface of the core, somewhat apart from DNA. When an experiment similar to that of Figure 4 was carried out with these nucleosomes, no significant changes in the fluorescence anisotropy of the DNC probe were detected up to 0.3 M NaCl, and a small decrease appeared at higher ionic strength values (data not shown). In the second control experiment, the anisotropy of free H2B and H3, both labeled at the Gln residues of the N-terminal tails, was measured as a function of ionic strength. While H2B modified with DNC at Gln^{22} showed a gentle increase in anisotropy, free H3 modified at Gln^{5} and Gln^{19} showed an abrupt increase between 0 and 0.15 M NaCl (data not shown). This is obviously due to the well-known self-association of free H3 in salt solution, which also results in the increase of intrinsic anisotropy fluorescence of tyrosyl residues (D'Anna & Isenberg, 1974).

At any rate, the control experiments indicate that the decrease in fluorescence anisotropy observed in Figure 4 is a property characteristic of the DNC probe attached to the N-terminal tails of nucleosome-bound histones. The anisotropy data may then indicate that the histone tails, or at least the probe-containing regions of the histone tails, gain mobility on increasing ionic strength in a two-phase process.

DISCUSSION

The causes for the glutamine substrate specificity of TGase have long been uncertain. As pointed out above, the selection of a given glutamine by the enzyme seems to depend on both the conformation and the sequence around the residue (Aeschlimann & Paulsson, 1994; Aeschlimann et al., 1992). No consensus sequence has ever been described, although Coussons et al. (1992) found that some charged residues around a glutamine may act as "discouraging" sequence features for the TGase reaction. In a previous work (Ballestar et al., 1996), we suggested that the presence of positively charged amino acids at positions +1 and/or +2 relative to glutamine may also represent a discouraging effect. If it were so, the fact that Gln^{22}, which is not a TGase substrate in chicken-free H2B, is readily used by the enzyme in nucleosomes (Ballestar et al., 1996) could be explained by the charge neutralization caused by an interaction of the histone N-terminal tail with DNA.

The experiments carried out with the synthetic peptides NQ22 and MQ22 show that the presence of positive charges at the C-terminal side of the glutaminyl residue makes the TGase reaction more difficult. The fact that MQ22 (Figure 2) and acetylated NQ22, in which those positive charges are absent, are better substrates than unmodified NQ22 strongly supports that idea. Actually, CD data showed that the conformation of NQ22 is identical to that of MQ22, thus ruling out the possibility that conformational differences are decisive for the substrate behavior of both peptides.

The effects of DNA on NQ22 labeling at low ionic strength are also very clear (Figure 2). Although the actual existence of a physical interaction between the peptide and nucleosome DNA has not been proved by the CD experiments nor by a gel retardation assay (unpublished results), it would be difficult to explain how the presence of DNA at low ionic strength, but not at $I \geq 200$ mM (Figure 2), enhances the substrate capability of NQ22. Within the same context, these results allowed us to explain the behavior of whole H2B either chemically modified to block its lysyl residues (Figure 1) or in the presence of poly(L-glutamic acid). The experiments with the synthetic peptides were necessary because conformational changes following maleylation of the free histone add another difficulty to the interpretation of the results of Figure 1. Moreover, the experiments with synthetic peptides seem to confirm our previous hypothesis as to the reason by which Gln^{22} of H2B is labeled in nucleosomes.

In contrast with the labeling experiments discussed hitherto, a simple, direct interpretation can be drawn from the fluorescence anisotropy data of Figure 4. In view of the control experiments carried out, the decrease in fluorescence anisotropy can only be explained in terms of an increase in the mobility of the histone tail portion containing the probe. By proton NMR spectroscopy of H1-depleted chromatin, Walker (1984) was able to detect a cooperative all-or-none dissociation of the histone tails in the interval 200−600 mM NaCl. The second drop in fluorescence anisotropy of the H2B or H3 tails detected in our experiments (Figure 4) occurs for $I > 200$ mM, and, therefore, it may correspond to the general increase in histone tail mobility observed by Walker (1984). The first decrease in anisotropy,

however, takes place at lower ionic strength values and must, therefore, correspond to a different process. We wondered whether this unknown process, which involves an increase in the histone tail mobility, is responsible for the changes in the reactivity of Gln^{22} in both NQ22−DNA complexes (Figure 2) and nucleosomes (Figure 3). The latter data describe the transition from a state in which Gln^{22} is labeled to a second one in which DNC is not at all incorporated. This transition, induced by increasing salt concentration, occurs in an ionic strength interval sharp enough to suggest a highly cooperative two-state process. We therefore analyzed the curve of H2B in Figure 3 according to the scheme:

$$R + \beta AX \rightleftharpoons N \quad (1)$$

in which R and N stand, respectively, for the H2B tails in the reactive and nonreactive states and β is the number of moles of a monovalent salt, AX, per mole of histone tail required to convert a reactive species to a nonreactive one. A similar analysis was first proposed by Walker (1984), to describe the ionic strength dissociation of tails monitored by NMR. In our case, apart from NaCl, there are calcium ions, required by the enzyme, so that the apparent equilibrium dissociation constant for eq 1 may be written

$$K = \frac{[N]}{[R]I^{\beta}} \quad (2)$$

where I stands for the ionic strength. Assuming that the concentration of the reactive species is proportional to the amount of DNC incorporated, eq 2 may be rearranged to give

$$R = \frac{X}{KI^{\beta} + 1} \quad (3)$$

where X stands for the amount of probe incorporated at $I = 0$. The units in which X and R are expressed cancel in eq 3, so it can be used for the cooperative decrease of any property of the tails induced by increasing ionic strength.

Equation 3 was fitted to the data from H2B in Figure 3 by using the GraFit software (Leatherbarrow, 1992). The best fitted line, shown in Figure 5, was obtained with $K = 4.96 \times 10^5$ and $\beta = 5.6$. Experimental points corresponding to the labeling of NQ22 in the presence of DNA (Figure 2) also fit this line (Figure 5). This means that the dissociation of Lys^{23} and Lys^{24} from DNA, with the concomitant decrease in Gln^{22} labeling, takes place in the complex NQ22−DNA under the same conditions as in the native nucleosome. However, the increase in the mobility of the DNC probe attached to Gln^{22} occurs in a lower ionic strength interval, as suggested by the position of the experimental points of the first anisotropy decrease, when replotted in Figure 5.

Therefore, the present data suggest that two structural transitions, unnoticed to date, occur in the ionic strength interval 0−200 mM. These transitions are not related to the folding of the ends of nucleosome DNA, which occurs in going from very low ionic strength to $I \simeq 100$ mM (Garcia-Ramirez et al., 1992). Actually, the length of DNA involved in this change is low enough to affect the positions at which the tails of H3 and H2B are thought to interact. According to the available data from different experimental approaches (Ausio et al., 1989; Mirzabekov et al., 1978; Bavykin et al., 1985; Arents & Moudrianakis, 1993), the N-terminal tail of H2B interacts with DNA between positions ±5 and ±6, while the tail of H3 interacts with DNA between the dyad axis and positions ±1 [reviewed in Pruss et al. (1995)]. We suggest that the transition observed in the anisotropy experiments represents a widening of the cone of rotation of the fluorescent probe bound to Gln^{22}. It has to be noted that for this residue to be a substrate of TGase, it has to project somewhat outside the DNA major groove. An increase in the oscillation of histone tails suggested by Shick et al. (1980) may well account for that widening of the cone of rotation. As for the second transition described, by the curve drawn in Figure 5, a partial dissociation of a region of the H2B tail affecting the environments of Gln^{22}, for instance, the region 17−25, may result in the inhibition of the TGase reaction. This region may be easier to dissociate than the remainder of the tail, as it contains two nonpolar amino acids, Ala^{17} and Val^{18}, and an acidic residue, Asp^{25}. A similar analysis of the behavior of the H3 tail is more difficult because there are two glutaminyl substrates for TGase.

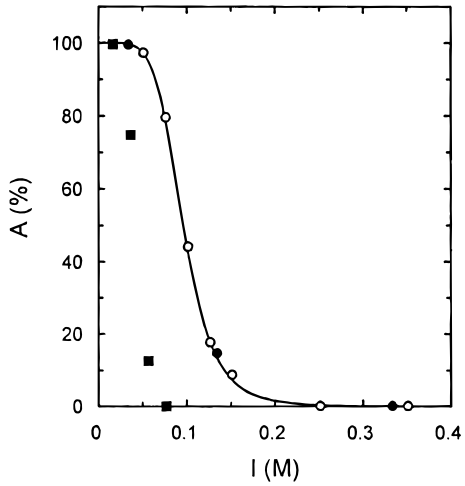

FIGURE 5: Comparison of the ionic strength-dependent changes in the various physical or biochemical parameters related to the mobility of the H2B tail. Equation 3 was fitted to the experimental data corresponding to the incorporation of DNC into Gln^{22} of H2B (Figure 3). Points were then normalized as percent of the maximum value of the ionic strength-dependent property (A) and plotted (○). The line shown is the best fit, with the parameters given in the main text. In the figure were also plotted: (●) the normalized fluorescence incorporated into NQ22 in the presence of DNA (see Figure 2); (■) the fluorescence anisotropy of nucleosomes reconstituted from DNC-modified H2B. To normalize the latter data, it was assumed that the plateau in Figure 4 corresponds to the end of the structural transition under study (0% *A*).

At any rate, our system constitutes a novel approach to study conformational changes of the histone tails, and we intend to also use this approach to investigate the effects of specific histone acetylation on the interactions between tails and DNA. Several histone acetyltransferases have been partially purified in our laboratory (López-Rodas et al., 1989), but neither of them is specific for H2B in nucleosomes. On the other hand, yeast histone acetyltransferase A2 is highly specific for H3 in nucleosomes (López-Rodas et al., 1991), but unfortunately the yield with which acetyl groups are transferred from acetyl-CoA to nucleosomes is so low that no conformational changes can reasonably be expected from this acetylation reaction. Experiments to circumvent these difficulties are being carried out in our laboratory.

ACKNOWLEDGMENT

We are very indebted to Juan Ausio, Joaquín Moreno, and Evangelos N. Moudrianakis for their valuable advice and many helpful suggestions.

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BI9626620