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Acetylated nucleosome assembly on telomeric DNAs

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Abstract

The role of histone N-terminal domains on the thermodynamic stability of nucleosomes assembled on several different telomeric DNAs as well as on 'average' sequence DNA and on strong nucleosome positioning sequences, has been studied by competitive reconstitution. We find that histone tails hyperacetylation favors nucleosome formation, in a similar extent for all the examined sequences. On the contrary, removal of histone terminal domains by selective trypsinization causes a decrease of nucleosome stability which is smaller for telomeres compared to the other sequences examined, suggesting that telomeric sequences have only minor interactions with histone tails. Micrococcal nuclease kinetics shows enhanced accessibility of acetylated nucleosomes formed both on telomeric and 'average' sequence DNAs. These results suggest a more complex role for histone acetylation than the decrease of electrostatic interactions between DNA and histones.

Keywords: Telomeres; Nucleosome; Histone acetylation; Thermodynamic stability; Nucleosome positioning

1. Introduction

Telomeres, the specialized structures located at the ends of eukaryotic chromosomes, generally consist of tandemly repeated units 5–8 bp long, rich in GT on the 3′ ending strands [1,2]. In higher eukaryotes, telomeres are organized in regular nucleosomal arrays with unusually short repeat length [3–6]. In yeast telomeres, nucleosomes seem to be absent [7], whereas in *Tetrahymena termophila* a nucleosome organization is present in 3–10% of telomeres, suggesting that the difference in telomeric chromatin between higher and lower eukaryotes could be quantitative, rather than qualitative [8].

We previously found that several telomeric DNAs from different organisms form the less stable nucleosomes among the DNA sequences so far studied [9–11]. The values of free energy of nucleosome formation of telomeric sequences with respect to an 'average' sequence DNA, obtained by the competitive reconstitution method [12], range from +1.80 to +0.40 kcal/mol of nucleosome. These values are in fairly good agreement with those obtained adopting a theoretical method recently developed by De Santis and co-workers [11,13–15], which derives the free energy of

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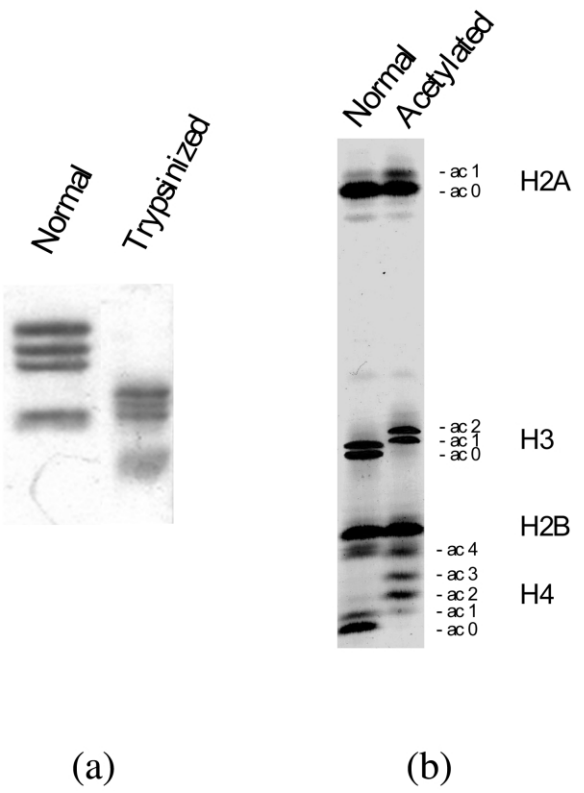

Fig. 1. Characterization of nucleosomes and histone octamers. (a) Analysis of normal and trypsinized histones by SDS–PAGE. (b) Acetic acid–urea–Triton gel analysis of normal and acetylated histones.

nucleosome formation from the sequence-dependent DNA elasticity.

Many research efforts have been recently spent in studying how histone modifications influence both the physico-chemical properties of the nucleosome and its role in biological processes. In the cell, nucleosome N-terminal domains undergo several post-translational modifications, such as acetylation, phosphorylation, methylation, ADP-ribosylation, etc. [16,17]. Acetylation of specific lysines is the best characterized among modifications of histone N-terminal tails. Strong evidence correlates gene expression to histone hyperacetylation [17–19]. Telomeres are heterochromatic regions and are generally hypoacetylated, although specific acetylation patterns have been reported [20–22]. However, during replication newly synthesized histones show a specific acetylation pattern for all chromatin domains [23,24]. Newly synthesized histone H4 is deposited in a diacetylated isoform in a wide variety of organisms. In *Tetrahymena thermophila*, *Drosophila melanogaster* and HeLa cells, H4 is acetylated at lysines 5 and 12, suggesting that the K5/K12 pattern of acetylation could be a hallmark property of new H4 in all organisms. Recently, Jasencakova et al. [25] have shown that histone H4 acetylation of euchromatin and heterochromatin is cell cycle dependent and correlated with replication rather than with transcription. These results indicate a relevant role of histone acetylation on chromatin structure also in chromosomal domains, such as telomeres, not involved in transcriptional processes.

Since acetylation of lysine residues reduces the positive charge of the histone octamer, it has been proposed (and generally accepted) that this modification could weaken electrostatic histone–DNA interactions leading to a more ‘open’ chromatin conformation. This representation is probably too simple [26], and does not fit with all experimental data. In vitro studies, using selectively trypsinized chicken erythrocytes nucleosomes or hyperacetylated nucleosomes from HeLa cells, have shown that histone tail acetylation or removal do not affect salt-dependent nucleosome stability, whereas they play a role in determining nucleosome thermal stability [27,28]. Moreover, higher-order chromatin structure and DNA topology are influenced by acetylation or selective removal of histone N-terminal tails [29–31]. Recently, Widlund et al. [32] analyzed the role of histone N-terminal domains on nucleosome assembly on four synthetic DNAs and the mouse major satellite DNA. The removal of histone N-terminal domains causes the decrease of nucleosome thermodynamic stability with a sequence-dependent pattern. Surprisingly, the acetylation of histone N-terminal domains causes a slight increase of nucleosome stability in all cases. These data suggest that histone tails may play a role in nucleosome formation on specific DNA sequences, taking also into consideration that they do not contribute to ionic strength-dependent stability of nucleosomes organized on

heterogeneous sequence genomic DNA (bulk nucleosomes) [27,28].

In this study we analyze the influence of histone N-terminal domains on thermodynamic stability of telomeric nucleosomes compared to nucleosomes formed on DNA sequences with high affinity for the histone octamer such as 5S rDNA and TG pentamer [12], and on the sequence TAND-1, which has the same free energy of nucleosome formation as 'average' sequence DNA [11]. Telomeric sequences are straight, due to the regular repetition of short nucleotide sequences out of phase with the B-DNA helical period. They fold around the histone octamer in a sequence-dependent way with lower affinity with respect to bulk nucleosomes. Therefore, they represent an interesting structurally homogeneous set of sequences to study the role of histone N-terminal domains on nucleosome thermodynamic stability. We find that hyperacetylation favors nucleosome formation, to an extent that is substantially similar in all examined sequences. On the contrary, the enzymatic removal of the histone tails causes a decrease of nucleosome stability, which is smaller in the case of telomeres. In apparent contrast with the results on thermodynamic stability, micrococcal nuclease (MNase) digestion studies show an increased accessibility of the enzyme to hyperacetylated nucleosomes.

2. Materials and methods

2.1. DNA and histone octamer preparation

TAND-1 was a gift from A. Travers. The sequence TG was a gift from D. Crothers. Esa1p/pRSET plasmid was kindly provided by C.D. Allis. Telomeric DNAs and TAND-1 were prepared as previously described [8,10]. *Kluyveromyces lactis* telomeric DNA $(GGTATGTGGTGTACGGATTTGATTA)_6$ was multimerized as previously described [9] and cloned in the *Sma*I site of pBluescript II KS(−). 5S rDNAs were prepared by partially digesting the plasmid pPOL 208–12 [33] containing 12 copies of 5S rRNA gene from *Lytechinus variegatus* (a gift from R. Sendra) with *Ava*I. Fragments corresponding to the monomer and the dimer bands of 5S rDNA were gel extracted and cloned in the *Eco*RV site of pSTBlue1 (Novagen) after filling in protruding ends. H1-free polynucleosomal particles were prepared from chicken erythrocytes as previously described [9]. Purified histone octamers were obtained from polynucleosomes by hydroxyapatite chromatography. Trypsinized nucleosomes were obtained by digesting chicken erythrocytes nucleosomes with immobilized trypsin according to Ausio et al. [28]. The extent of trypsin digestion was assessed on standard SDS–18% polyacrylamide gel.

Recombinant enzyme rEsa1p was expressed in *Escherichia coli* BL21-DE3 cells and purified by affinity chromatography on Ni^{2+}–agarose as described by Smith et al. [34]. A stoichiometric quantity (500 μg) of chicken erythrocyte core histones was mixed in a final reaction volume of 2.5 ml, with approximately10 μg of recombinant enzyme, in the presence of 100 mM acetyl-CoA (or absence in case of the controls), and proteases inhibitors [1 mM PMSF and 10 μM *trans*-epoxysuccinyl-L-leucylamido-(4-guanidino)butane]. The mixture was incubated at 25 °C for 2 h and the reaction was stopped by 0.25 M HCl. After centrifugation at $10\,000 \times g$ for 10 min, histones were recovered in the supernatant and the core histones octamers were reconstituted by consecutive dialyses following the procedure of Marvin et al. [35]. The extent of histone acetylation was checked by acetic acid–urea–Triton polyacrylamide gel electrophoresis [36] (Fig. 1).

2.2. Competitive reconstitutions

When normal or selectively trypsinized polynucleosomes were used as the histone donor, reconstitutions were carried out by the salt dilution method [12], as described by Filesi et al. [11]. For reconstitutions starting from histone octamers, 2 μg of purified histone octamer (hypo- or hyperacetylated) was mixed with various amounts of sonicated calf thymus DNA and 30 ng of radiolabeled DNA in 10 μl of dilution buffer [10 mM Tris–HCl (pH 8), 1 mM EDTA, 0.1% Nonidet-P40, 0.1 mg/ml BSA] plus 1.0 M NaCl, or in 15 μl of dilution buffer plus 0.65 M NaCl. Samples at 1.0 M NaCl were incubated for 1 h at 25 °C,

Table 1
Free energies of nucleosome reconstitution with respect to the sequence TAND-1: comparison among different competitive reconstitution methods

DNA	Stepwise dilutions 1.00–0.1 M NaCl Nucleosome $\Delta\Delta G$ Sample/TAND-1 (kcal/mol)[a]	Stepwise dilutions 0.65–0.1 M NaCl Histone octamer $\Delta\Delta G$ Sample/TAND-1 (kcal/mol)[a]	Stepwise dilutions 1.00–0.1 M NaCl Histone octamer $\Delta\Delta G$ Sample/TAND-1 (kcal/mol)[a]	Dialysis 2.0–0.1 M NaCl Histone octamer $\Delta\Delta G$ Sample/TAND-1 (kcal/mol)[a]
Arabidopsis thaliana telomere (195 bp)[b]	0.75 ± 0.25	0.65 ± 0.25	0.65 ± 0.25	0.70 ± 0.20
Bombyx mori telomere (171 bp)[b]	0.70 ± 0.20	0.65 ± 0.15	0.61 ± 0.20	
Chlamydomonas reinhardtii telomere (198 bp)[b]	0.80 ± 0.15	0.75 ± 0.20		0.95 ± 0.20
Human telomere (192 bp)[b]	1.20 ± 0.05	1.05 ± 0.20	1.00 ± 0.20	1.15 ± 0.15
Human telomere (254 bp)[b]	0.80 ± 0.20	0.75 ± 0.25		
Kluyveromyces lactis telomere (172 bp)[c]	0.20 ± 0.10	0.20 ± 0.15		
TAND-1 (159 bp)[b]	0.00	0.00	0.00	0.00

[a] Values are given as mean ± S.D.
[b] DNA sequence reported in Filesi et al. [11].
[c] GATCCCCC (GGTATGTGGTGTACGGATTTGATTA)$_6$ GGGCTGCAGGAATT.

and then diluted by adding three 30-μl aliquots of dilution buffer (at 20-min intervals). Samples dissolved in the 0.65 M NaCl buffer were incubated overnight at 37 °C, followed by three additions of dilution buffer (at 20-min intervals) to a final concentration of 0.1 M NaCl. For reconstitution by dialysis [37], 2 μg of purified histone octamer samples were mixed with various amounts of sonicated calf thymus DNA and 30 ng of radiolabeled DNA in 50 μl of starting buffer [10 mM Tris–HCl (pH 8), 1 mM EDTA, 0.1% Nonidet-P40, 0.1 mg/ml BSA, 2 M NaCl]. Samples were loaded on QuixSep microdialyzers (MFPI), and placed inside a dialysis bag containing 200 ml of starting buffer, in order to obtain a slow lowering of NaCl molarity. Samples were dialyzed for 2 h in the starting buffer at 4 °C, followed by two dialyses against 2 l of 10 mM Tris–HCl (pH 8), 1 mM EDTA, 0.1% Nonidet-P40, 0.1 mg/ml BSA, 50 mM NaCl, each at 4 °C for more than 12 h. Reconstituted samples were resolved on 5% polyacrylamide gels. Gels were dried and analyzed by Instant Imager (Packard). The free energy difference for a given sequence (seq) with respect to a reference DNA (ref) was calculated from the equation $\Delta\Delta G(\text{seq}) = RT\ln[\alpha(\text{ref})] - RT\ln[\alpha(\text{seq})]$, where α(ref) is the ratio of labeled reconstituted nucleosome to labeled free DNA for the reference sequence TAND-1, and α(seq) is the analogous ratio for the examined sequences (Table 1). To calculate the free energy differences due to histone tails hyperacetylation or removal, for every sequence the ratio of acetylated (trypsinized) nucleosome to free DNA was compared to the ratio of normal nucleosome to free DNA (Tables 2 and 3). Since it was previously shown that acetylation or removal of histone N-terminal domains do not influence ionic strength-dependent stability of bulk nucleosomes [27,28], the affinity of competitor DNA (sonicated calf thymus DNA) for normal or acetylated (trypsinized) histone octamer does not vary.

2.3. Micrococcal nuclease digestion assay

DNAs for the MNase assay were internally labeled by 30-cycle PCR amplification in the presence of [α-^{32}P]ATP using primers which hybridize outside of the polylinker region of the plasmids containing telomeric and TAND-1 sequences [9]. The products of the amplification were then digested with *Eco*RI and *Bam*HI, and

Table 2
Differences in nucleosome formation free energy due to hyperacetylation of histone amino-terminal domains

DNA	Stepwise dilutions 0.65–0.1 M NaCl $\Delta\Delta G$ Acetyl./Nor.[a] (kcal/mol)[b]	Stepwise dilutions 1.00–0.1 M NaCl $\Delta\Delta G$ Acetyl./Nor.[a] (kcal/mol)[b]	Dialysis 2.0–0.1M NaCl $\Delta\Delta G$ Acetyl./Nor.[a] (kcal/mol)[b]
Arabidopsis thaliana telomere (195 bp)	-0.32 ± 0.12	-0.20 ± 0.18	-0.39 ± 0.05
Bombyx mori telomere (171 bp)	-0.46 ± 0.23	-0.38 ± 0.20	
Chlamydomonas reinhardtii telomere(198 bp)	-0.44 ± 0.19		-0.47 ± 0.20
Human telomere(192 bp)	-0.45 ± 0.27	-0.32 ± 0.20	-0.48 ± 0.15
Human telomere (254 bp)	-0.40 ± 0.14		
Human telomere (458 bp)[c]	-0.57 ± 0.35		
Kluyveromyces lactis telomere (172 bp)	-0.46 ± 0.14		
5S rDNA (251 bp)[d]	-0.66 ± 0.25		
5S rDNA dimer (459 bp)[d]	-0.64 ± 0.27		
TG pentamer (151 bp)[e]	-0.48 ± 0.24		-0.51 ± 0.10
TAND-1 (159 bp)	-0.30 ± 0.18	-0.24 ± 0.20	-0.29 ± 0.14

[a] For every sequence examined, $\Delta\Delta G(\text{seq}) = RT\ln[\alpha(\text{nor})] - RT\ln[\alpha(\text{acetyl})]$, where α(nor) is the ratio of normal nucleosome to free DNA, and α(acetyl) is the ratio of acetylated nucleosome to free DNA for the same sequence.
[b] Values are given as mean $\pm$ S.D.
[c] GATCCCCGGTTA $(GGGTTA)_{71}$ GGGGGTACCGAGCTCGAATT.
[d] DNA sequence reported in Georgel et al. [33].
[e] DNA sequence reported in Shrader and Crothers [12].

gel purified. The labeled DNAs were reconstituted into nucleosomes, lowering the salt concentration to 50 mM NaCl. $CaCl_2$ was added to a final concentration of 3 mM, and samples were preincubated for 5 min at 37 °C. MNase was added to a final concentration of 1 U/ml. Aliquots were removed at various times, stopped with an equal volume of 20 mM EDTA, 0.2% SDS, and phenol extracted. Mock reactions containing control DNA instead of reconstituted nucleosomes were performed in the same experimental conditions. Samples were separated on native 5% polyacrylamide gel.

3. Results

3.1. Acetylation of histone N-terminal domains increases the thermodynamic stability of telomeric nucleosomes

To determine to what extent acetylation of the histone tails could influence telomeric nucleosome

Table 3
Differences in nucleosome formation free energy due to removal of histone amino-terminal domains

DNA	$\Delta\Delta G$ Tryps./Nor.[a] (kcal/mol)[b]
Arabidopsis thaliana telomere (195 bp)	0.58 ± 0.08
Bombyx mori telomere (171 bp)	0.52 ± 0.30
Chlamydomonas reinhardtii telomere(198 bp)	0.55 ± 0.19
Human telomere(192 bp)	0.60 ± 0.15
Kluyveromyces lactis telomere (172 bp)	0.73 ± 0.20
TG pentamer (151 bp)	1.19 ± 0.40
TAND-1 (159 bp)	0.95 ± 0.32

[a] For every sequence examined, $\Delta\Delta G(\text{seq}) = RT\ln[\alpha(\text{nor})] - RT\ln[\alpha(\text{tryps})]$, where α(nor) is the ratio of normal nucleosome to free DNA, and α(tryps) is the ratio of trypsinized nucleosome to free DNA for the same sequence.
[b] Values are given as mean $\pm$ S.D.

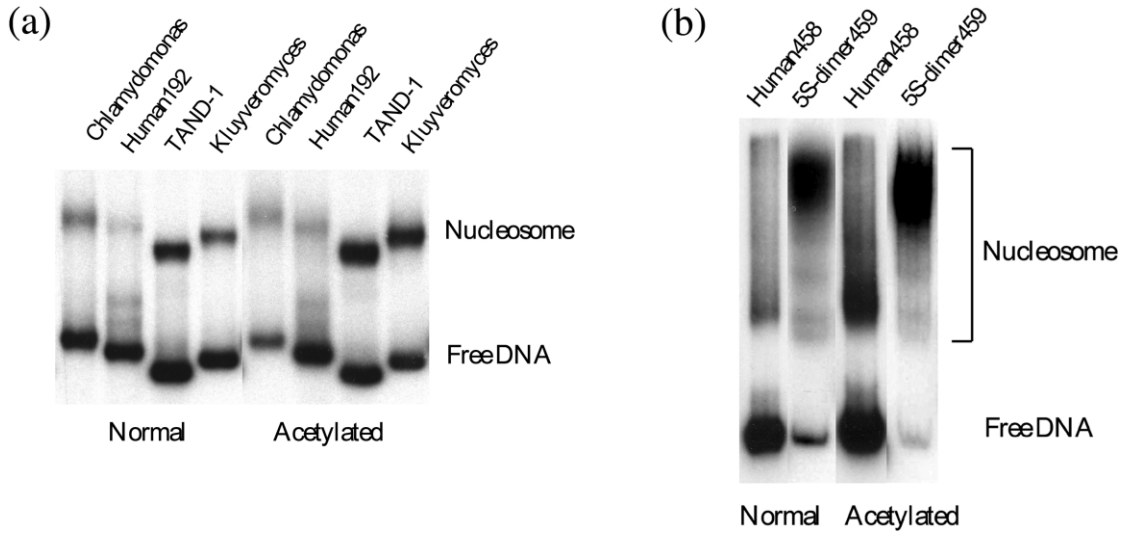

Fig. 2. Competitive nucleosome reconstitution assays with normal and acetylated histones. Products of competitive reconstitution of (a) some of the telomeric sequences reported in Table 2, resolved by 5% native polyacrylamide electrophoresis, and of (b) 458-bp-long human telomeric DNA and 459-bp-long 5S rDNA dimer.

thermodynamic stability, competitive reconstitution experiments were carried out using hyperacetylated and normal histone octamers (Fig. 2). Competitive reconstitutions allow to measure the difference of free energy of nucleosome formation relative to a reference sequence, by quantifying the relative amounts of labeled fragments in the nucleosomal and free DNA bands after separation on a 5% polyacrylamide gel. To be sure that what we observe is a real equilibrium, we have used three different reconstitution methods. Besides the classical salt dilution method starting from 1 M NaCl and by successive dilutions [12], we set up a salt dilution method incubating samples overnight at 37 °C at 0.65 M NaCl followed by dilutions up to 0.1 M NaCl. Since it has been reported some weakening of histone tails–DNA interactions at elevated ionic strengths, an equilibrium procedure at a lower ionic strength is likely to put in better evidence free energy differences due to tail interactions [38]. Finally, we used the double dialysis method starting from 2 M NaCl concentration [37], that assures a gradual achievement of equilibrium. The three series of data, obtained with the sequences shown in Fig. 2a and with other telomeric sequences, are in substantial agreement with $\Delta\Delta G$ values obtained with the standard competitive reconstitution method [11] and confirm that what we observe is a thermodynamic equilibrium and not a kinetic intermediate (Table 1). In addition to the previously studied telomeric sequences [9–11], here we report free energy values for the telomeric sequence of *Kluyveromyces lactis*, which is characterized by a repeating unit of 25 bp, one of the longest so far reported. We obtained a $\Delta\Delta G$ value of $+0.20$ kcal/mol of nucleosome with respect to the standard sequence TAND-1, very similar to that of bulk nucleosomes and noticeably lower than those of other telomeric sequences of the same length (Table 1).

Reconstitutions were performed with the same amounts of either normal or hyperacetylated purified histone octamer. In order to have the maximum difference of acetylated residues we have used histone octamers from chicken erythrocytes, generally known as hypoacetylated [39]. Hyperacetylated octamers were obtained by in vitro acetylation by rEsa1p (Fig. 1). The values of free energy differences of nucleosome formation using acetylated with respect to normal histone octamers

are shown in Table 2. Also when acetylated histone octamers are used, data obtained using the three different competitive reconstitution protocols are in substantial agreement, giving us confidence of the correctness of the experimental procedure.

We have studied acetylated nucleosome formation also on the sequence TAND-1, as a standard of nucleosome formed on 'average' sequence DNA, the sequence TG pentamer, a synthetic DNA known as a sequence with high affinity for the histone octamer and a standard for curved DNA [12], and the strong nucleosome positioning sequence 5S rRNA gene from *Lytechinus variegatus*, in monomeric and dimeric form.

It is evident that in all cases the acetylation of histone N-terminal domains increases the nucleosome thermodynamic stability. The increase is approximately 0.5 kcal/mol of formed nucleosome and it is almost equal in the case of all the examined sequences, indicating a sequence-independent effect of nucleosome stabilization.

Since it has been reported that histone tails could be involved in nucleosome–nucleosome interactions [40], we have studied how histone octamer acetylation influences the thermodynamic stability of nucleosomes formed on human telomeric DNA and 5S rDNA approximately 450 bp long, capable of accommodating two nucleosomes (Table 2 and Fig. 2b). Long human telomeric DNA and 5S rDNA show an increase in nucleosome stability due to histone acetylation similar to that of shorter fragments with the same sequence. These results suggest that acetylation does not modify dinucleosome thermodynamic stability, at least in our experimental system.

It is worth noting that besides the dinucleosomal band, several bands due to the different mononucleosomal positions are present in competitive reconstitution experiments. These bands are discrete in the case of 5S rDNA and almost continuous in the case of human telomeric DNA (Fig. 2b). These features are in agreement with theoretical nucleosome positioning according to a method developed in our research group [14], which predicts a discrete number of energetically preferred positions in the case of 5S rDNA dimer (Fig. 3a), and multiple isoenergetic nucleosome positions for human telomeric DNA (Fig. 3b). In this latter case, the gel matrix is unable to separate the multiple positions spaced every 6 bp [11], giving rise to a unique smeared band. Footprinting studies to clearly assess these features are in progress in our laboratory.

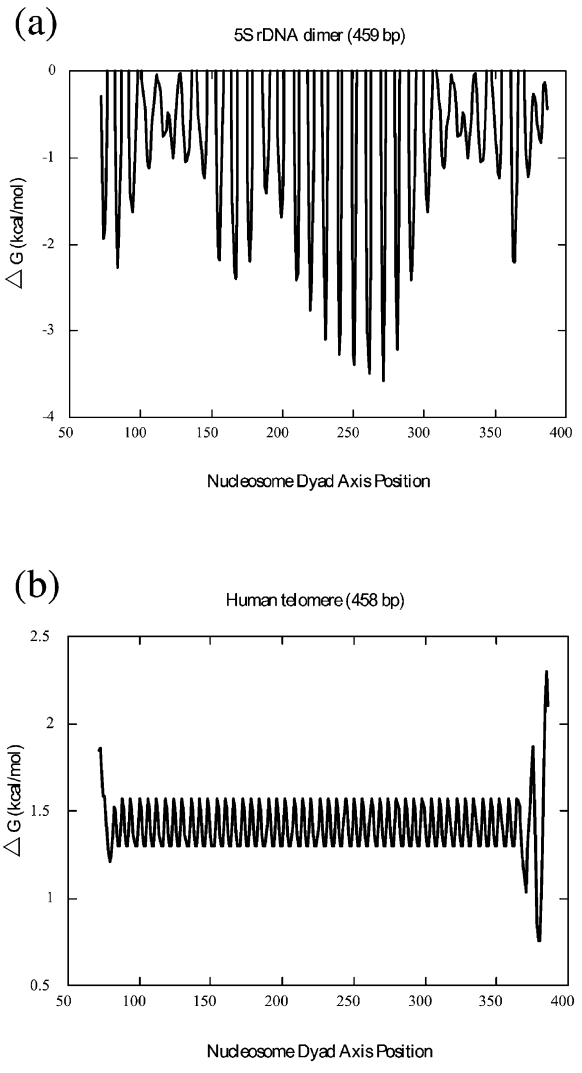

Fig. 3. Theoretical nucleosome dyad axis positioning on (a) 458-bp-long human telomeric DNA and on (b) 459-bp-long 5S rDNA dimer. Minima correspond to the energetically most favored nucleosome dyad axis positions.

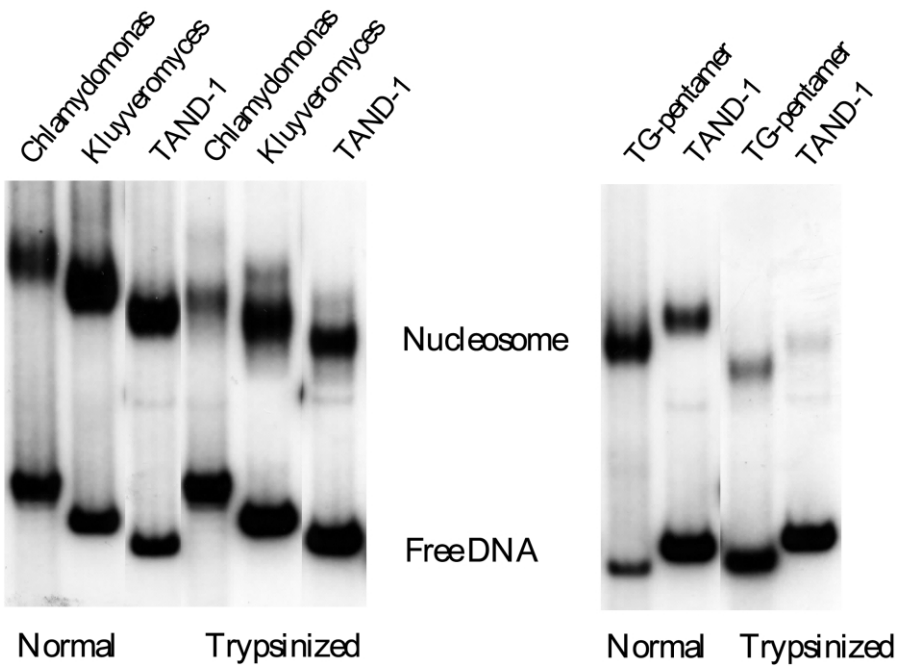

Fig. 4. Polyacrylamide gel electrophoresis of competitive nucleosome reconstitution experiments with normal and trypsinized histones.

3.2. Removal of histone N-terminal domains decreases the thermodynamic stability of telomeric nucleosomes

The results obtained in the case of hyperacetylated nucleosomes suggest that the decrease of electrostatic interactions between DNA and histone side chains does not necessarily give rise to less stable nucleosomes. Since nucleosomes lacking histone N-terminal domains have been often considered as a suitable model for hyperacetylated histones, we carried out competitive reconstitution experiments using selectively trypsinized nucleosomes in comparison with normal nucleosomes from chicken erythrocytes (Fig. 4, Table 3). We measured the free energies of nucleosome formation on the telomeric sequences, together with the sequence TAND-1, and the sequence TG pentamer. Tail removal causes a decrease of nucleosome stability for all the examined sequences, but the decrease is significatively smaller for telomeric sequences (approx. 0.5 kcal/mol of nucleosome) with respect to average sequence and curved DNAs (approx. 1 kcal/mol of nucleosome). These results indicate that the interactions of histone tails with telomeric sequences are less strong than with TAND-1 and TG pentamer.

3.3. Acetylated nucleosomes show higher accessibility to MNase digestion than normal nucleosomes

MNase was used as a probe of acetylated nucleosome structure. We have previously found that MNase and DNAse I digestion patterns of telomeric nucleosomes are typical of canonical nucleosomes [9,10]. In fact MNase digestion gives rise to a digest limit corresponding to 146 ± 2 bp, while DNAse I footprinting is characterized by the canonical 10 base periodicity. The finding that nucleosome organized onto hyperacetylated histone octamers are thermodynamically more stable than those organized onto normal histone octamers suggests that this histone modification should not increase nucleosome accessibility to MNase. In Fig. 5 MNase digestion kinetics of nucleosomes reconstituted on *Chlamydomonas* telomeric DNA

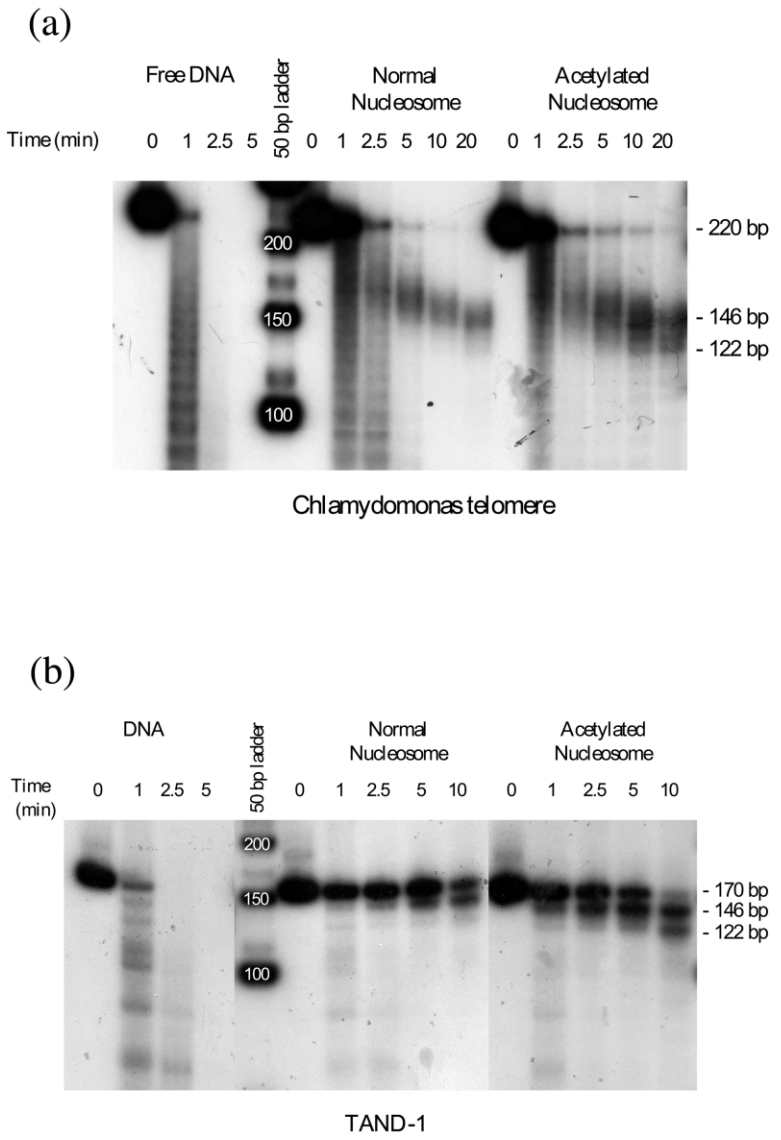

Fig. 5. Micrococcal nuclease digestion kinetics of nucleosomes reconstituted on (a) *Chlamydomonas* telomeric DNA and (b) on TAND-1 DNA.

and on TAND-1 are shown. In both cases the accessibility of acetylated nucleosomes to MNase is higher than that of normal nucleosomes. In fact, while in both cases MNase digestion of normal nucleosomes gives rise to the canonical limit digest of 146 ± 2 bp length, in the case of acetylated nucleosome the enzyme surpass the 146-bp limit, since a band of 122 ± 2 bp is visible. These findings raise the interesting question of how the increase in nucleosome thermodynamic stability could correlate with the increase in nuclease accessibility.

4. Discussion

Although the influence of histone N-terminal domains acetylation on structural and functional

properties of nucleosomes has been extensively studied, acetylated nucleosome structural and functional features have yet to be adequately established [26]. In this research, we studied the effect of histone acetylation and DNA length on the thermodynamic stability of nucleosomes formed on a set of several different telomeric sequences, on DNAs with high affinity for the histone octamer (5S rDNA and TG pentamer), and on the sequence TAND-1, having the same free energy of nucleosome formation as bulk DNA. We have previously shown that telomeric DNAs are straight and organize nucleosomes with the lowest stability among all the DNA sequences so far studied [10,11].

We find that acetylation increases the stability of nucleosomes formed on all the examined sequences by approximately 0.5 kcal/mol, making the assembly of nucleosome twice more probable with acetylated than with normal histones (see Table 2). These findings suggest the possibility that in nucleosome assembly histone acetylation, besides favoring the interactions with assembly factors such as CAF [23], could have an intrinsic role in making more probable the nucleosome formation. These features would be relevant in heterochromatic as well as in euchromatic domains. Since these findings could be considered in apparent contrast with the hypothesis that one of the main effects of acetylation should be the decrease of electrostatic interactions between histones and DNA, we have studied the influence of the removal of histone N-terminal on nucleosome thermodynamic stability. The removal of histone amino terminal domains causes a decrease of nucleosome thermodynamic stability for all the examined sequences. However, telomeric sequences are less affected by tail removal than 'average' nucleosomal DNA (TAND-1) and curved DNA (TG pentamer). These results indicate that telomeric sequences should interact less tightly with histone tails than the other examined DNA sequences, and suggest that the extent of tail interactions is sequence-specific.

The enhanced stability of acetylated nucleosomes is in apparent contrast with the view of more 'open' conformation with respect to normal nucleosomes. Since evidences on facilitated binding of specific proteins to DNA organized on acetylated nucleosomes is still very controversial [26], we studied the accessibility of nucleosomal DNA to MNase, a probe with low sequence-specificity that preferably digests internucleosomal DNA. MNase digestion kinetics shows an enhanced accessibility of nucleosomal DNA when the histone octamer is hyperacetylated, both in the case of telomeric and 'average' sequence DNA, with a protection from digestion of approximately 120 bp instead of the canonical 146 bp.

At first sight our results might appear as contradictory, because we have found that hyperacetylated nucleosomes are thermodynamically more stable than hypoacetylated nucleosomes, and, at the same time, they show an enhanced accessibility to MNase as well as to other probes [41]. However, the recent resolution of the nucleosome structure at 2.8 Å [42] and at 1.9 Å [43] suggests interesting remarks. Although several histone N-terminal residues interact with nucleosomal DNA, acetylatable lysines seem not involved in histone–DNA contacts [43]. Consequently, histone acetylation is likely not to cause any binding loss between N-terminal tails and nucleosomal DNA. The increased stability of acetylated nucleosomes could derive from more favorable local interactions or from conformational changes influencing the overall nucleosome structure. It has been reported that histone acetylation is able to reduce the linking number change per nucleosome in reconstituted minichromosomes [44]. More recently Ausio and co-workers showed that acetylation of histones results in a significant increase of the α-helical content of their tails [45]. This increase could cause an increased hydrophobicity of the tails allowing better interactions with DNA grooves. Moreover, it has been proposed that the increase of the α-helical content could result in shortening of histone N-terminal domains [45]. This last observation could contribute to explain the enhanced accessibility of hyperacetylated nucleosomes to enzymatic probes, since from the nucleosome structure at 1.9 Å resolution [43] it emerges that the terminal 13 bp of the DNA superhelix interacts with H3 N-terminal tail. However, it is likely that a definite answer to this issue could come only from the resolution of the structure of

an acetylated nucleosome by crystallografic analysis.

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