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INTERACTION OF RNA POLYMERASE II WITH ACETYLATED NUCLEOSOMAL CORE PARTICLES

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SUMMARY. Chemical acetylation of nucleosomal cores is accompanied by an increase in their efficiency as *in vitro* transcription templates. Low amounts of acetic anhydride cause preferential modification of the amino-terminal tails of core histones. Modification of these domains, which causes moderate structural effects, is apparently correlated with the observed stimulation of RNA synthesis. In contrast, extensive modification of the globular regions of core histones, which is accompanied by a large structural relaxation of the particle, causes little additional effect on transcription. Acetylation of the amino-terminal domains of histones might stimulate transcription by changing the interaction of the histone tails with components of the transcriptional machinery.

In previous work from our laboratory (1-3), a simple *in vitro* transcription assay has been used to evaluate the contribution of different structural features of the nucleosomal particle, individually or in combination, to its capacity to act as a transcription template. A better understanding of the interaction between RNA polymerase and mononucleosomal particles incorporating different structural alterations might help to elucidate the basic mechanism of transcription and the possible roles played by physiological modifications of the nucleosomal particles. The purpose of the present work is to determine the effects of the progressive weakening of the interactions between DNA and the lysine residues present in the globular and amino-terminal domains of core histones on the efficiency of nucleosomal cores as transcription templates. The results shows that a moderate acetylation of histones, which is mainly located on their amino-terminal domains, substantially improves the particle efficiency in transcription. Conversely, extensive acetyla-

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tion of the histone globular domains, which is accompanied by a large structural relaxation, does not seem to produce any additional changes in the transcriptional properties of the particle.

MATERIALS AND METHODS

Preparation of Nucleosomal Particles. Nucleosomal cores were prepared from chicken erythrocyte nuclei, isolated after lysis of the cells in the presence of Nonidet P40 (4). Nuclei were digested with micrococcal nuclease and extracted with 0.25 mM EDTA (pH 8.0)(5). Nucleosomal particles containing H1 and H5 were precipitated from the extract with 100 mM KCl, and the nucleosomal cores were isolated from the supernatant (1). Acetylation took place at room temperature by treatment of the nucleosomal particles (0.1 mg of DNA/ml), in 200 mM N-(tris-(hydroxymethyl)-methyl)glycine (Tricine)(pH 8.2), with the required amount of acetic anhydride disolved in dioxane (200 mg of acetic anhydride/ml). Reaction took place in the absence of base addition (6). To separate the acetylated nucleosomal particles, the modified preparations were centrifuged in 5-20% sucrose gradients containing 10 mM Tris-HCl (pH 8.2), 5 mM EDTA and 0.1 mM phenylmethanesulphonyl fluoride, in a Beckman SW40 rotor at 34,000 rev./min for 22 h at 4ºC. The sedimentation patterns show no release of free DNA for any of the treatments employed (not shown). The isolated nucleosomal particles were dialyzed at 4º C against 10 mM Tris-HCl (pH 8.2) and 5 mM EDTA.

Complex Formation. Free DNA (146 bp), labeled at its 5' ends with polynucleotide kinase and [γ-^{32}P]ATP, was incubated with RNA polymerase II in the presence and absence of competing unlabeled nucleosomal particles, the mixture was centrifuged in glycerol gradients, and the radioactivity was measured in the isolated fractions (1).

Transcription Assays. Transcription was estimated by measuring the radioactivity from [^{3}H]UTP incorporated into acid-insoluble RNA as previously described (2). The nucleosomal particles or free DNA were preincubated at 30ºC for 5 min with ATP, CTP, GTP and [^{3}H]UTP, in 10 mM Tris-HCl (pH 7.9), 10 mM $(NH_4)_2SO_4$, 2 mM $MnCl_2$, 2 mM dithioerythrytol, 5% (w/v) glycerol and 0.2 mg of bovine serum albumin/ml. Transcription was initiated by addition of RNA polymerase II from calf thymus. The size of the transcription products was determined by electrophoresis of the purified RNA synthesized in the presence of [^{32}P]CTP, in gels containing 7 M urea (7).

RESULTS

Acetylation of nucleosomal particles. The nucleosomal cores were chemically acetylated with acetic anhydride. Fig. 1 shows the extent of modification of nucleosomal cores for different treatments with acetic anhydride. The amino groups present in the amino-terminal tails of histones are those preferentially modified with low amounts of reagent. With 10 µmol of acetic anhydride/ml, maximal modification of the amino-terminal tails is

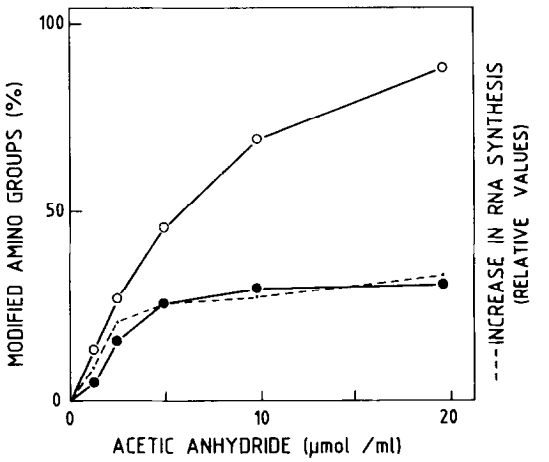

Fig. 1. Extent of acetylation of histones and of their amino-terminal domains in acetic anhydride-treated nucleosomal cores. The figure shows the extent of modification of the total amino groups of histones (○), and that of the amino groups present in the amino-terminal domains (●). Modification is expressed as the percentage of modified groups relative to the total number of histone amino groups present in the nucleosomal core. The degree of acetylation of nucleosomal cores was determined, before and after trypsin digestion (2), by measuring the radioactivity from [^{14}C]acetic anhydride incorporated into nucleosomal particles. The extent of modification of the amino-terminal tails was obtained by substracting from the modification of the whole nucleosomal core that corresponding to the trypsin-digested residual particle. The broken line shows the relative increase in RNA synthesis obtained with the corresponding preparations of nucleosomal cores used as *in vitro* transcription templates (see Fig. 3).

practically obtained. A correlation appears to exist between modification of the amino-terminal domains and stimulation of RNA synthesis using as *in vitro* transcription templates the acetylated nucleosomal cores (Fig. 1).

Structural properties of the acetylated particles. Modification of nucleosomal cores with acetic anhydride is accompanied by a decrease in the temperature at which denaturation of nucleosomal DNA takes place (Table I), in agreement with previous results using a crude mononucleosomal preparation containing dinucleosomes and trinucleosomes, and histone H1 (8). Acetylation causes a progressive decrease in the midpoint temperatures (T_m) corresponding to the two observed transitions (a and b). At the same time, the amount of DNA melting in the lower transition increases with acetylation, except for the highest treatment where the proportion of DNA melting in this transition is the same as that in the untreated nucleosomal cores. Changes in the globular domains of histones appear to be mainly responsible for the observed denaturation, since the extent of acetylation of these

TABLE I

Thermal Denaturation and Circular Dichroism Parameters of Different Acetylated Nucleosomal Cores

Treatment (μmol of AA/ml)	T_ma (ºC)	Ha (%)	T_mb (ºC)	Hb (%)	F
0	61	31	75	69	1.00
1.25	60	37	74	63	----
2.5	60	41	72	59	0.79
5.0	57	43	70	57	----
10	53	49	65	51	0.58
20	50	31	60	69	----

The thermal denaturation profiles and circular dichroism spectra were obtained in 0.25 mM EDTA (pH 8.0) as previously described (3). T_m, transcription midpoint, is the temperature of maximum dH/dt. H, hyperchromicity. F, fraction of altered DNA structure contributing to the circular dichroism difference band (3).

regions is directly proportional to the decrease in the temperature midpoint of the two transitions. The circular dichroism properties of the acetylated nucleosomal cores are indicative of a relaxation of the asymmetric tertiary structure of nucleosomal DNA (Table I).

Interaction of the acetylated particles with RNA polymerase. The relative affinities for RNA polymerase II of different acetylated nucleosomal cores were evaluated from the decrease in the amount of polymerase.[^{32}P]DNA complex formed when the corresponding unlabeled particles were present in the incubation mixture (1). Fig. 2 shows that acetylation of nucleosomal cores is accompanied by a substantial increase in their affinity for RNA polymerase II. Similar results were obtained with RNA polymerase from Escherichia coli (not shown). In addition to their increased affinity for RNA polymerase, the acetylated nucleosomal cores are more efficient in vitro transcription templates than the non-modified particles. Fig. 3 shows the levels of RNA synthesis obtained with acetylated nucleosomal cores as transcription templates and RNA polymerase II. Acetylation is accompanied by increased RNA synthesis. When the transcription assay is supplemented with 0.15 M NaCl, RNA synthesis is increased 2-3 times, but no change is detected in the relative levels of synthesis obtained with free DNA, untreated nucleosomal cores and nucleosomal cores modified with 2.5 and 20 μmol of acetic anhydride/ml (not shown). The absence of any relative increase in

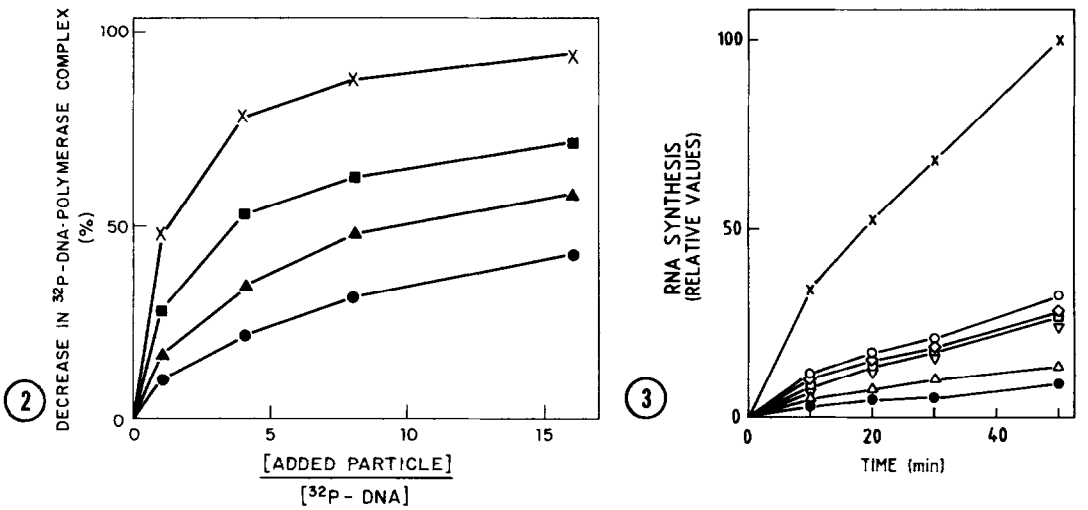

Fig. 2. Relative affinities of acetylated nucleosomal particles for RNA polymerase II. Affinity was estimated from the effect of the unlabeled particles on the amount of complex formed between polymerase and free DNA. RNA polymerase II was incubated with [^{32}P]DNA (146 bp) (2.5 µg/ml), at a molar ratio of polymerase to [^{32}P]DNA equal to 2, in the presence of the following unlabeled particles: free DNA (146 bp) (x), intact nucleosomal cores (●), and nucleosomal cores modified with 2.5 (▲) and 10 (■) µmol of acetic anhydride/ml. In the absence of added unlabeled particles, 41% of [^{32}P]DNA was bound to polymerase.

Fig. 3. RNA synthesized by RNA polymerase II using different acetylated nucleosomal templates. The template concentration was 4 µg of DNA/ml, and the molar ratio of RNA polymerase II to particle was 2. The nucleosomal cores were treated with the following amounts of acetic anhydride (µmol/ml): 0 (●), 1.25 (△), 2.5 (▽), 5.0 (□), 10 (◇), and 20 (○). A control template of free DNA (146 base pairs) (x) was also included for each series of particles. After 50 min of incubation 7 UTP molecules were incorporated per molecule of free DNA (x).

RNA synthesis with the different nucleosomal preparations indicates that the raise in salt concentration does not promote the release of free DNA during the transcription assay, even with the highly acetylated preparation and after incubation for 50 min. Analysis of the transcription products shows that, like the loss of one H2A.H2B dimer (2), acetylation of nucleosomal cores partially eliminates the block present in intact nucleosomal cores, allowing transcription of the whole length of the nucleosomal DNA template (Fig. 4). Similar results were obtained with RNA polymerase from E. coli (not shown).

DISCUSSION

When nucleosomal cores are treated with acetic anhydride under the conditions used in the present work, the amino

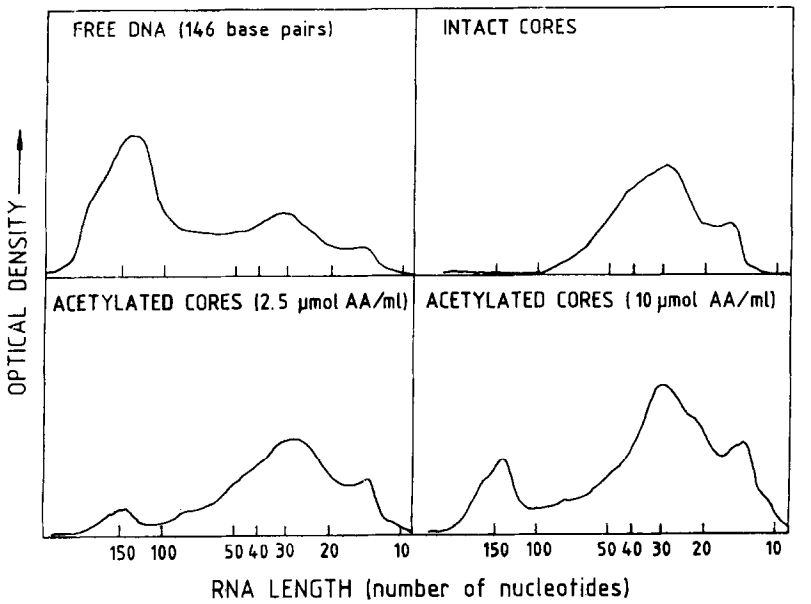

Fig. 4. Size distribution of the RNA molecules synthesized by RNA polymerase II using different acetylated nucleosomal templates. Densitometric tracings obtained after electrophoresis of the RNA molecules synthesized with different template particles. Template concentration was 4 µg of DNA/ml, and the molar ratio of polymerase to particle was equal to 2. Incubation took place for 50 min.

groups located in the amino-terminal tails of histones are preferentially modified as compared with those corresponding to the globular domains, in agreement with previous results (9). The thermal denaturation studies indicate that acetylation of histone amino-terminal domains causes only a small effect on the thermal stability of nucleosomal DNA, whereas that of the globular regions is accompanied by a large destabilization of the nucleosomal particle with a substantial decrease in the two transition midpoints (T_ma and T_mb) (Table I).

The stimulation of RNA synthesis induced by acetylation of nucleosomal cores appears to be correlated to modification of the histone amino-terminal tails (Fig. 1). Therefore, acetylation of these domains, which causes only a small structural effect as determined by thermal denaturation, might be accompanied by a substantial improvement in the particle efficiency as a transcription template. In contrast, extensive acetylation of the histone globular regions, which produces a large destabilization of the particle, causes little additional increase in transcription. These results point to a specific effect of the acetylation of histone amino-terminal domains. Since elimination

of the amino-terminal tails of histones, by trypsin digestion, does not significantly change the transcription properties (2), the observed stimulation appears even more remarkable. Apparently, deblocking of transcription is produced by a specific change in the interacting capabilities of the amino-terminal domains, not by the non-specific suppression of interactions which accompanies the loss of these regions.

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