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Yeast Nucleosomal Particles: Structural and Transcriptional Properties†

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ABSTRACT: Yeast nucleosomal core particles have been characterized by thermal denaturation, circular dichroism, and digestion with DNase I and with trypsin. Practically all nucleosomal DNA melts in one transition centered at 70 °C, and the circular dichroism spectrum is displaced to lower wavelengths as compared to that corresponding to chicken nucleosomal cores. The susceptibility of yeast nucleosomal particles to dissociation by salt is significantly higher than that of chicken nucleosomal cores, a substantial dissociation being observed at 0.5 M NaCl. Treatment of yeast nucleosomal particles with the amino group reagent dimethylmaleic anhydride is accompanied by selective release of histones H2A and H2B. The results indicate not large but significant structural differences between yeast and chicken nucleosomal cores. However, the in vitro transcription properties of complete and H2A·H2B-deficient nucleosomal cores are similar in the two kinds of particles: the histone octamer blocks RNA synthesis, this block being eliminated in part by the partial loss of histones H2A and H2B.

Yeast chromatin is of special interest to understand the relationships between structure and function in the nucleosome-complexed DNA. Important differences have been found between the chromatin of bakers' yeast and that of higher organisms. No typical condensed chromosomes have been observed in yeast (Gordon, 1977), which might be related to the apparent absence of histone H1 (Certa et al., 1984; Smith et al., 1984) and to the high transcriptional activity of yeast chromatin. The large proportion of DNA (about 40%) transcribed under normal growth conditions (Hereford & Rosbash, 1977) and the low level of repetitive sequences are properties which make yeast an attractive organism to study transcriptionally active chromatin.

Although yeast chromatin contains nucleosomal core particles basically similar to those found in higher eukaryotes, their shorter linker (Lohr & Van Holde, 1975; Thomas & Furber, 1976; Nelson et al., 1977), the apparent absence of histone H1, and the sequence changes present in the conservative core histones (Van Holde, 1988) might significantly affect its transcription-related dynamics. In spite of the high interest of yeast nucleosomal particles, there is a paucity of relevant structural information, probably because of the difficulty in obtaining suitable nucleosomal particles. The published data seem to indicate a relaxed structure of yeast nucleosomal particles as compared with those from chicken or calf thymus (Lee et al., 1982; Morse et al., 1987).

The purpose of the present work is the structural characterization of isolated nucleosomal particles from yeast and the evaluation of their transcriptional efficiency. By use of a simple in vitro transcription system, previous work from our laboratory shows that the loss of one H2A·H2B dimer from the chicken nucleosomal core is accompanied by partial elimination of the block to transcription and a substantial increase in the level of RNA synthesis (González et al., 1987; González & Palacián, 1989). The results described in the pesent paper show small but significant structural differences between yeast and chicken nucleosomal particles, which indicate a slight relaxation of those from yeast. Moreover, the yeast nucleosomal particles, like chicken nucleosomal cores, are inefficient

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in vitro transcription templates which can be substantially improved by partial elimination of histones H2A and H2B.

Materials and Methods

Nucleosomal Particles. Yeast nucleosomal particles were prepared from *Saccharomyces cerevisiae* strain ABY-S66 grown in YEP medium at 30 °C. Cells were collected in the logarithmic phase (OD_{660} = 0.8–1.0) and washed with 0.9 M sorbitol/0.1 M EDTA (pH 7.5). Protoplasts were obtained by incubation of the cells (OD_{660} = 50–65) in the sorbitol/EDTA solution with 0.1 mg of zymolyase/mL, at 37 °C for 1 h. Protoplasts were collected by centrifugation, washed with 1 M sorbitol, and resuspended in 5 mL of this solution for each liter of culture medium. This suspension was mixed with 1 volume of 20 mM Tris-HCl (pH 7.4), 20 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, 2 mM phenylmethanesulfonyl fluoride (PMSF),[1] 2 mM iodoacetic acid, and 1% Nonidet P-40 and incubated at 4 °C for 15 min with mild mechanical stirring. The nuclei were collected by centrifugation, and after several washings with 15 mM Tris-HCl (pH 8.0), 50 mM NaCl, 1.4 mM $CaCl_2$, 0.4 mM EDTA, 1 mM PMSF, and 1 mM iodoacetic acid, the nuclei were suspended in this same solution at a concentration of 1 mg of DNA/mL, and 1 mM fresh PMSF was added to the suspension. This preparation was digested with micrococcal nuclease (80 units/mg of DNA) at 37 °C for 15 min. Digestion was ended by addition of 0.25 M EDTA (pH 7.4) to a final concentration of 15 mM, and cooling to 0–4 °C. After overnight dialysis at 4 °C against 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, and 1 mM PMSF, the preparation was centrifuged in an SS34 rotor at 10000 rpm for 10 min, the sediment being discarded. To the supernatant 2 M $MgCl_2$ was added with mild mechanical stirring to a final concentration of 5 mM. The precipitate was collected by centrifugation in the same rotor at 8000 rpm for 10 min and suspended in 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, and 1 mM PMSF to a final DNA concentration of approximately 1 mg/mL. The preparation thus obtained was dialyzed for 6 h at 4 °C against the same solution, and the nucleosomal particles (Figure 1A, fraction I) were isolated by centrifugation in a 5–20% sucrose gradient containing the components of the dialysis buffer solution. Centrifugation took place in an SW40 Ti rotor at 35000 rpm and 4 °C for 21 h. DNA from nucleosomal particles has an average length of 145 base pairs (Figure 1B) and a histone composition similar to that of chicken nucleosomal cores (Figure 1C). In most preparations, substantial amounts of a proteolytic component are found, this product being located between the electrophoretic bands corresponding to histones H2A and H4. It originated from the moderately lysine-rich histones and is also present in comparable amounts in the preparations of histones directly isolated from nuclei by acid extraction (not shown).

Chicken nucleosomal cores were obtained from chicken erythrocytes as previously described (González et al., 1987).

Trypsin Digestion. Yeast nucleosomal particles (125 μg of DNA/mL), in 10 mM Tris-HCl (pH 7.5) and 0.7 mM EDTA, were treated at 4 °C with 30 μg of trypsin/mL (trypsin from bovine pancreas, Sigma) for 40 min. Digestion was ended by addition of trypsin inhibitor (from soybean, Sigma) at a final concentration of 0.14 mg/mL. After digestion, the mixture was dialyzed at 4 °C against 10 mM Tris-HCl (pH 8.2) and 5 mM EDTA, and the digested particles were isolated by centrifugation in a 5–20% sucrose gradient. Trypsin digestion of yeast nucleosomal particles, under the indicated conditions, yields the limit histone fragments shown in Figure 1C (lane t). Increase of the trypsin concentration to 60 μg/mL does not affect the electrophoretic pattern of digested histones obtained with 30 μg/mL (not shown). Chicken nucleosomal cores were digested under the conditions indicated for yeast nucleosomal particles, but using 60 μg of trypsin/mL (González & Palacián, 1989).

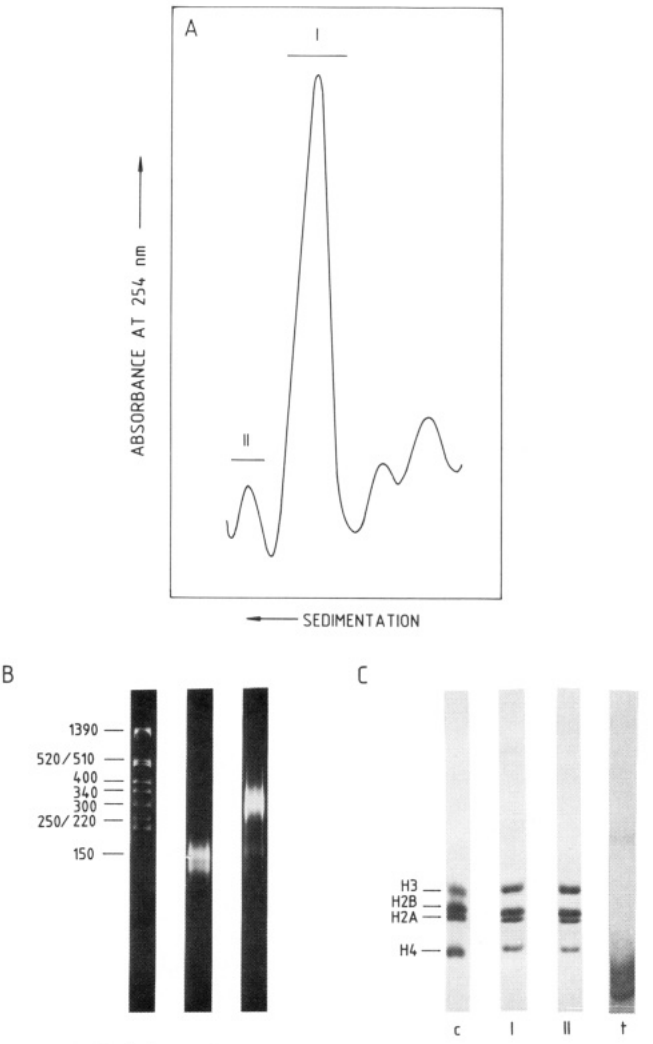

FIGURE 1: Isolation and characterization of yeast nucleosomal particles. (A) Fractionation of the preparative sedimentation gradient. The bars (I and II) show the fractions isolated. (B) Electrophoresis of the DNA present in fractions I and II. Plasmid pBR322 digested with nucleases *Pst*I and *Hin*fI is included as a size marker. The numbers refer to the lengths of DNA fragments expressed as base pairs. (C) Electrophoresis of the histones present in fractions I and II, and after trypsin digestion of fraction I (t). Core histones from chicken erythrocytes were also included (c).

Treatment of Nucleosomal Particles with Dimethylmaleic Anhydride. Nucleosomal particles, in 200 mM Tricine (pH 8.2), were treated at room temperature with different amounts of dimethylmaleic anhydride (De la Escalera et al., 1988). To eliminate the hydrolyzed reagent, the modified preparation was dialyzed at 4 °C against 10 mM Tris-HCl (pH 8.2), 5 mM EDTA, and 0.1 mM PMSF. The residual nucleosomal particles were separated from the released proteins by centrifugation in sucrose gradients as described under Analytical Procedures. To regenerate the modified amino groups, the residual particles and the released proteins were dialyzed at 4 °C against a solution at pH 6.0 (De la Escalera et al., 1988) and subjected to a second dialysis against 10 mM Tris-HCl (pH 8.2), 5 mM EDTA, and 0.1 mM PMSF.

Analytical Procedures. Thermal denaturation profiles and circular dichroism spectra were obtained as previously de-

[1] Abbreviation: PMSF, phenylmethanesulfonyl fluoride.

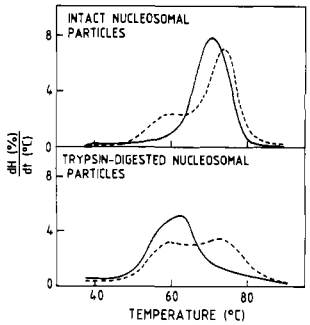

FIGURE 2: Thermal denaturation profiles of yeast (—) and chicken (---) nucleosomal particles. Denaturation of the nucleosomal particles took place in 0.25 mM EDTA (pH 8.0).

scribed (González & Palacián, 1990). To fractionate the dissociation products of nucleosomal particles, after modification with dimethylmaleic anhydride or salt treatment, preparations were centrifuged in linear 5–20% sucrose gradients containing 10 mM Tris–HCl (pH 8.2), 5 mM EDTA, 0.1 mM PMSF, and the indicated NaCl concentration. Centrifugation took place in a Beckman SW40 rotor at 4 °C, and either at 33 000 rpm for 21 h (modification with dimethylmaleic anhydride) or at 39 000 rpm for 24 h (NaCl treatments). The distribution along the gradient of materials absorbing at 254 nm was determined with an ISCO density fractionator, which was also used for the separation of fractions. Electrophoretic analysis of histones was conducted in a 10–20% linear gradient polyacrylamide gel containing 0.1% sodium dodecyl sulfate (Laemmli, 1970), using gel lengths of 19 and 35 cm. DNA was analyzed by electrophoresis in 8% polyacrylamide gels (Maniatis et al., 1982). Protein concentration was determined according to Bradford (1976) or to Lowry et al. (1951). The concentration of the nucleosomal particles was evaluated spectrophotometrically in 0.1 M NaOH, taking A_{260} = 26.0 for a solution containing 1.0 mg of DNA/mL.

Transcription Assay. Transcription was estimated by measuring the radioactivity from [^{3}H]UTP incorporated into acid-insoluble RNA, using RNA polymerase from *Escherichia coli* (Boehringer Mannheim) and different nucleosomal particles as templates. Transcription took place in the absence of rifampicin as described in a previous work (González et al., 1987), except that 2 mM $MnCl_2$ was included in the transcription mixture instead of 2 mM $MgCl_2$.

RESULTS

Structural Properties. The thermal denaturation profiles of yeast nucleosomal particles and chicken nucleosomal cores are shown in Figure 2. In contrast with chicken nucleosomal cores, which show two thermal transitions in agreement with previous results (Weischet et al., 1978; De la Escalera et al., 1988), yeast nucleosomal particles denature in one transition centered at 70 °C. The temperature at which half of the DNA is denatured is only slightly lower for yeast nucleosomal particles than for chicken nucleosomal cores, which reflects a similar overall stabilization of DNA. However, the absence in the yeast particles of a significant first transition, which in chicken corresponds to melting of the DNA ends, indicates structural differences between the two types of nucleosomal particles. The partial proteolysis of yeast histones does not appear to account for the differences in thermal denaturation observed between yeast and chicken nucleosomal particles, since extensive trypsin digestion of chicken nucleosomal cores does not produce effects related to those observed in yeast nucleosomal particles, such as the elimination of the first

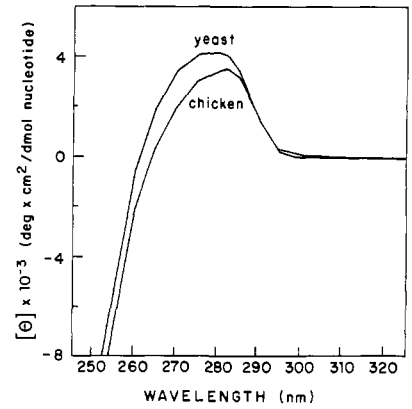

FIGURE 3: Circular dichroism spectra of yeast and chicken nucleosomal particles. Spectra were obtained in 0.25 mM EDTA (pH 8.0).

thermal transition (Figure 2).

Trypsin digestion of the amino-terminal tails of core histones is a probe of nucleosome structure which might increase our knowledge of the yeast particles. As with chicken nucleosomes (Díaz & Walker, 1983), a limit digestion particle seems to be produced in the case of yeast particles (Figure 1C, lane t). The difference between yeast and chicken particles observed by thermal denaturation is maintained in the corresponding trypsin-digested particles (Figure 2). Whereas two thermal transitions are observed in the digested chicken particles, only one is found with those from yeast. The results point to a similar overall structure of yeast and chicken nucleosomal particles, which allows digestion by trypsin of the amino-terminal histone tails but prevents that of the globular domains of core histones. However, thermal denaturation detects a structural difference between the two types of trypsin-digested particles.

The circular dichroism spectrum of yeast nucleosomal particles is also significantly different from that of chicken nucleosomal cores (Figure 3). The maximal value of ellipticity is slightly higher for yeast than for chicken particles, being located at a lower wavelength. In addition, the wavelength at which ellipticity changes from negative to positive values is lower for yeast nucleosomal particles. These results indicate that the negative ellipticity component, which in nucleosomal particles is superimposed to the positive spectrum of double-stranded DNA, and is assigned to the asymmetric DNA structure induced on DNA by the histone octamer (Cowman & Fasman, 1978, 1980), is altered in yeast nucleosomal particles as compared to chicken nucleosomal cores. This change suggests that in the yeast particles histones induce an asymmetric structure on DNA different from that in chicken.

Digestion of yeast nucleosomal particles with DNase I produces the typical electrophoretic ladder of single-stranded DNA fragments, with a length difference between consecutive bands of approximately 10 nucleotides (not shown), in agreement with previous work (Thomas & Furber, 1976). The susceptibility of yeast nucleosomal particles to DNase I digestion appears to be higher than that of chicken nucleosomal cores.

Response to Salt. Yeast nucleosomal particles are more susceptible to salt than chicken nucleosomal cores. Treatment with 0.5 M NaCl, which causes no effect on the integrity of chicken nucleosomal cores, is accompanied by substantial dissociation of yeast nucleosomal particles. Figure 4 shows the sedimentation patterns of yeast and chicken nucleosomal particles in the presence of 0.5 and 0.75 M NaCl, and the histone composition of the corresponding isolated particles and released fractions. With the gel length used in this experiment

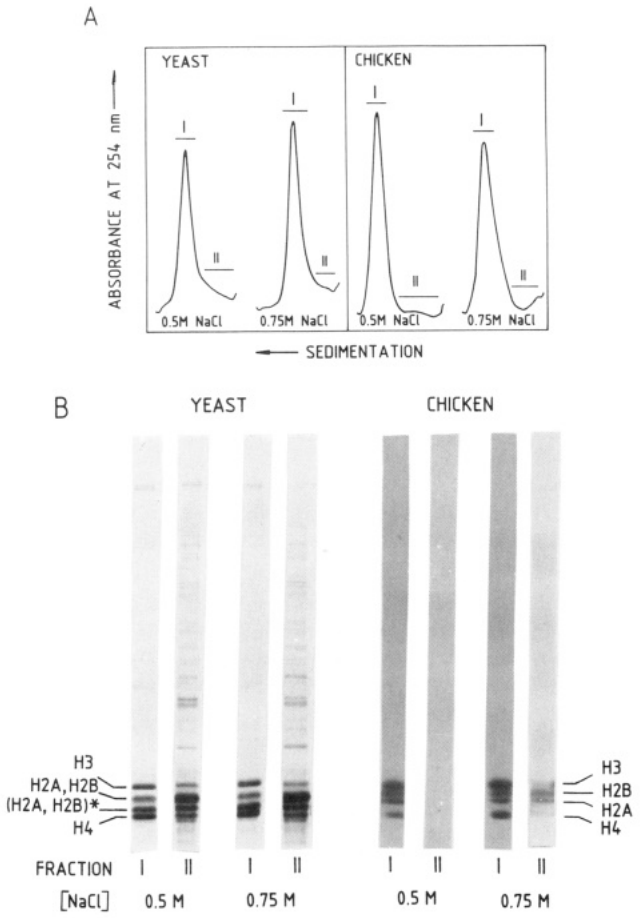

FIGURE 4: Dissociation of yeast nucleosomal particles by salt. Nucleosomal particles were dialyzed overnight against 10 mM Tris-HCl (pH 8.2), 5 mM EDTA, 0.1 mM PMSF, and the indicated NaCl concentration and centrifuged in linear 5–20% sucrose gradients containing the components of the corresponding dialysis solution. (A) Sedimentation patterns. The bars (I and II) show the fractions isolated. (B) Electrophoresis of the histones released (fractions II) and of those present in the residual particles (fractions I). The amounts of fractions II charged in the gel were twice as large as those complementary to the corresponding fractions I. Band (H2A,H2B)* is derived from band H2A,H2B by proteolysis.

(19 cm), no clear separation of H2A and H2B is achieved, the band close to that of H4 being produced by proteolysis of H2A, H2B, or both, as indicated under Materials and Methods. Whereas no histone liberation from chicken nucleosomal cores is observed at 0.5 M NaCl, a substantial fraction of histones (28%) is separated from yeast nucleosomal particles under the same conditions, the released fraction being enriched in histones H2A and H2B. In the absence of NaCl, practically no dissociation of yeast nucleosomal particles is observed on sucrose gradient centrifugation (see Figure 5). At 0.75 M NaCl, only a small amount of histones, mainly H2A and H2B, is released from chicken nucleosomal cores, as compared to the large dissociation obtained with yeast nucleosomal particles. In the yeast-released fractions (II), the ratio of proteolyzed (H2A,H2B)* to intact H2A,H2B is lower than in the residual particles (I), indicating that the nucleosomal particles containing proteolyzed histones are not preferentially dissociated by salt. These results indicate that yeast nucleosomal particles are more susceptible to dissociation by salt than those from chicken, probably because of weaker electrostatic interactions between the components of the yeast nucleosomal particles.

Treatment with Dimethylmaleic Anhydride. Modification of chicken nucleosomal cores with dimethylmaleic anhydride,

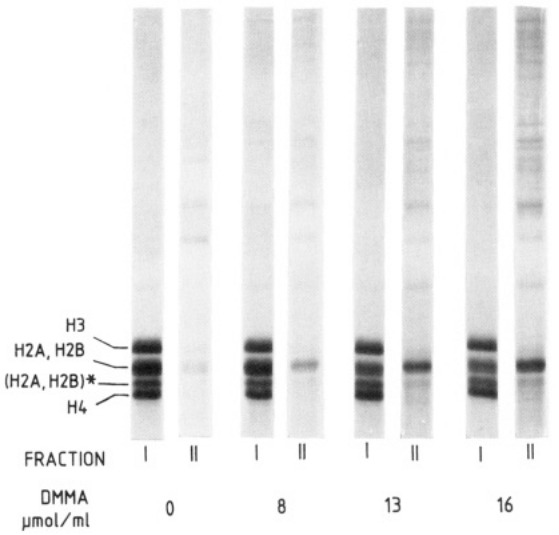

FIGURE 5: Release of histones H2A and H2B from yeast nucleosomal particles by modification with dimethylmaleic anhydride (DMMA). Electrophoresis of the histones released and of those present in the residual particles. Nucleosomal particles were treated with the indicated amounts of reagent and centrifuged in a sucrose gradient. Fractions I (residual particles) and II (released protein) were isolated as indicated in Figure 4A. The amounts of fractions II charged in the gel were twice as large as those complementary to the corresponding fractions I. Band (H2A,H2B)* is derived from band H2A,H2B by proteolysis.

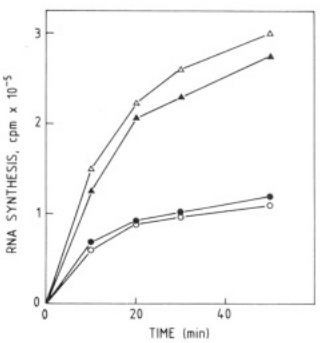

FIGURE 6: RNA synthesis with yeast nucleosomal templates. The template concentration was 4 μg of DNA/mL, and the molar ratio of polymerase to particle was equal to 2. (●) Chicken nucleosomal cores; (○) yeast nucleosomal particles; (▲) chicken H2A·H2B-deficient nucleosomal cores; (△) yeast H2A·H2B-deficient nucleosomal particles. Both yeast and chicken deficient particles were obtained by treatment with 13 μmol of dimethylmaleic anhydride/mL. They were deprived of 35% (yeast) and 40% (chicken) of the normal complement of histones H2A and H2B. The gel electrophoretic patterns of the employed yeast particles are shown in Figure 5 (fractions I obtained with 0 and 13 μmol of dimethylmaleic anhydride/mL). The level of RNA synthesis with free DNA (146 bp) was 2.5 times higher than that obtained with chicken H2A·H2B-deficient nucleosomal cores. Approximately 10 molecules of UTP were incorporated into RNA per molecule of free DNA template in 50-min incubation.

a reagent specific for protein amino groups (Palacián et al., 1990), is accompanied by specific release of H2A·H2B dimers (Nieto & Palacián, 1988). Since this behavior is dependent on the structure of chicken nucleosomal cores, it is interesting to know the way in which yeast nucleosomal particles respond to modification by this reagent. Figure 5 shows that treatment of yeast nucleosomal particles with different amounts of dimethylmaleic anhydride is accompanied by a progressive specific release of histones H2A and H2B. Densitometric analysis of the gel shows a total decrease of histones H2A and H2B, bands H2A,H2B and (H2A,H2B)*, of 35% in the yeast preparation treated with 13 μmol of dimethylmaleic anhydride/mL. Therefore, nonmodified yeast particles deficient

in histones H2A and H2B can be obtained after regeneration of the modified amino groups in the residual particles.

Transcriptional Properties. When yeast intact and H2A·H2B-deficient nucleosomal particles were used as in vitro transcription templates for bacterial RNA polymerase, the results shown in Figure 6 were obtained. The levels of RNA synthesis are similar to those found with the corresponding chicken nucleosomal particles. The presence of intact histone octamers makes the nucleosomal particles poor transcription templates, while the loss of histones H2A and H2B causes a substantial increase in transcriptional efficiency, in agreement with results previously obtained with chicken nucleosomal cores (González et al., 1987; González & Palacián, 1989). Therefore, no differences were found in transcriptional properties between yeast and chicken nucleosomal cores.

Discussion

The structural studies of the yeast nucleosomal particles detect significant differences with chicken nucleosomal cores. The thermal denaturation profile of yeast nucleosomal particles does not allow the identification of two structural domains, as is the case with chicken nucleosomal cores (Weischet et al., 1978; De la Escalera et al., 1988). At temperatures which cause denaturation of the DNA ends of the chicken particles, little DNA melting is observed with the yeast particles. On the other hand, the central DNA of chicken nucleosomal cores melts at temperatures higher than those corresponding to overall yeast DNA. In spite of these differences, the temperatures at which half of the total DNA is denatured are not significantly different for the two types of particles. In agreement with these results, the two corresponding circular dichroism spectra are clearly different. Not only is the ellipticity at 275 nm larger for the yeast particles, although not as large as previously reported (Lee et al., 1982), but also the whole spectrum appears to be displaced to lower wavelengths. These results suggest that differences may exist between the two particles in the asymmetric condensation of the DNA helix around the histone core.

The observed proteolysis of the slightly lysine-rich histones from yeast is also found when histones are acid-extracted from nuclei (not shown). Therefore, at least part of the proteolysis detected in the isolated nucleosomal particles might be present in vivo, and be related to the described specific proteolysis of histone H2A (Elia & Moudriankis, 1988). The H2A-specific protease would act on physiologically acetylated nucleosomes, being probably involved in facilitating transcription and replication.

The partial dissociation of yeast nucleosomal particles at 0.5 M NaCl, under conditions which do not affect the chicken nucleosomal cores, indicates a higher susceptibility to ionic strength of the yeast particles. The core histone octamer of yeast is less basic than that of chicken: there are 10 more basic residues in the chicken than in the yeast histone octamer, but the same number of acid residues (Van Holde, 1988). Therefore, the electrostatic forces holding together the nucleosomal particle might be smaller in yeast than in chicken, and the ionic strength required for dissociation would be lower for the yeast particles, in agreement with the experimental results.

Modification of chicken nucleosomal cores with dimethylmaleic anhydride is accompanied by a biphasic release of the two H2A·H2B dimers from the particle, the first dimer being more easily liberated than the second (Jordano et al., 1985; Nieto & Palacián, 1988). This release of H2A·H2B dimers upon weakening of the electrostatic forces between histones and DNA is a structure-dependent property of chicken nucleosomal particles probably related to their structural dynamics in vivo. The partial loss of histones H2A and H2B from yeast nucleosomal particles upon modification with dimethylmaleic anhydride suggests the presence of similar electrostatic interactions among the components of the nucleosomal particles in the two kinds of particles.

The high transcription levels of yeast cells in exponential growth might be related to the presence in yeast chromatin of nucleosomal particles more efficient as transcription templates than those of higher eukaryotes. The structural peculiarities observed in yeast nucleosomal particles would also be in favor of this possibility. However, the results obtained by using an in vitro transcription system indicate that the isolated yeast nucleosomal particles are no better transcription templates than those from chicken erythrocytes. This result does not appear to be a consequence of the proteolysis observed in the preparation of yeast particles, since extensive trypsin digestion of chicken nucleosomal cores does not affect RNA synthesis by bacterial RNA polymerase (González & Palacián, 1989). In our previous work describing the transcriptional properties of different nucleosomal particles from chicken erythrocytes (González & Palacián, 1989, 1990), no significant differences were observed between RNA polymerase from *Escherichia coli*, the enzyme used in the present work, and the RNA polymerase II from calf thymus. The partial release from yeast nucleosomal particles of histones H2A and H2B (35% of all H2A plus H2B histones were lost) is accompanied by a substantial increase in transcription similar to the one observed in chicken H2A·H2B-deficient nucleosomal cores (preparation lacking 40% of H2A·H2B dimers). Therefore, our results do not detect any significant difference between the transcriptional properties of yeast and chicken nucleosomal particles. Moreover, the mechanism responsible for transcriptional deblocking of the nucleosomal template might be similar in the two organisms, the release of one H2A·H2B dimer being the probable central step (González & Palacián, 1989).

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Proline for Alanine Substitutions in the C-Peptide Helix of Ribonuclease A†

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ABSTRACT: The effect on overall α-helix content of substituting proline for alanine has been determined at 5 positions (1, 2, 4, 5, and 13) of a 13-residue peptide related in sequence to residues 1–13 of ribonuclease A. The helix content falls off rapidly as proline is moved inward, and the proline residue effectively truncates the helix. No helix-stabilizing effect of proline is found at positions 2 or 4 within the first turn of the helix. Proline substitution at either end position (1, 13) has little effect on overall helix content, in agreement with an earlier study of glycine for alanine substitutions. The two end residues of the helix appear to be strongly frayed.

The proline ring is not easily accommodated in the interior of an α-helix, and proline lacks the NH group needed to maintain the H-bonding pattern of the α-helix backbone. Thus, it is not surprising that proline is the most strongly helix-breaking amino acid when helix propensity is measured either by host–guest studies using random copolymers (Altmann et al., 1990) or by single proline substitutions in a coiled-coil helix (O'Neil & DeGrado, 1990) or in an isolated α-helix (Merutka et al., 1990). Nevertheless, proline is found fairly often within α-helices in proteins: it occurs in 8% of the helices analyzed by Richardson and Richardson (1988). The proline ring can be accommodated comfortably within the first turn of an α-helix, and since the NH groups of the first four residues of an α-helix are not H-bonded, proline's lack of a free NH group presents no problem at positions 1–4. Indeed, proline is found more frequently in the first four residues of protein α-helices than in their interiors and strikingly so at the first helical position (N1, or N-cap plus one) (Richardson & Richardson, 1988). A free energy simulation study has found that proline is actually helix-stabilizing at N1 (Yun et al., 1991).

The basic aim of our study is to determine how the helix content of an isolated α-helix is affected by substituting proline for alanine at different positions. These substitutions are made singly in individual peptides. The reference peptide is related to the C-peptide (residues 1–13) of RNase A. The reference peptide, Suc-AATAAAKYLAAHA-NH_2, differs only by the substitution F8 → Y from a peptide studied earlier, RN 44, which is known to be a strong helix-former and which was used in a study of the Glu-2^-–Arg-10^+ salt bridge of C-peptide (Fairman et al., 1990). Both Glu-2 and Arg-10 have been replaced by Ala in RN 44; the purpose here of eliminating the Glu-2^-–Arg-10^+ salt bridge is to avoid steric hindrance between the salt bridge and a substituted proline residue. The substitution F8 → Y allows the peptide concentration to be determined by tyrosine absorbance (Marqusee et al., 1989), which is needed for accurate measurement of the mean residue ellipticity. The reason for using a unique-sequence peptide rather than one of the repeating sequence peptides studied by Marqusee and Baldwin (1987), or by Marqusee et al. (1989), is to facilitate NMR analysis of the system.

Our second aim is to investigate fraying of the end residues (1,13) of the helix. An earlier study of glycine for alanine substitutions (Strehlow & Baldwin, 1989) revealed that glycine substitution at either end position has little or no effect on the

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