Modification of the lysine residues of histones H1 and H5:

Effects on structure and on the binding to chromatin

J. Jordano, J. L. Barbero*, F. Montero* & E. Palacián

*Centro de Biología Molecular, Consejo Superior de Investigaciones Científicas and Universidad Autónoma de Madrid, Canto Blanco, Madrid-34, and *Departamento de Bioquímica, Facultad de Ciencias Químicas, Universidad Complutense, Madrid-3, Spain*

Abstract

The extensive modification of histone H1 from calf thymus with the amino-group reagent dimethylmaleic anhydride (over 35 lysine residues modified per molecule) produces no effect on its secondary structure detectable by circular dichroism (far UV). Fluorescence and circular dichroism (near-UV) of the modified histone show variations in the local environment of its sole tyrosine residue. These changes are reversed on regeneration of the modified amino groups. While histone H1 is easily dissociated with this reagent from calf thymus or chicken erythrocyte chromatin, a much stronger treatment is needed to liberate histone H5 from erythrocyte chromatin. This difference appears to be related to the higher arginine content of histone H5.

Introduction

Modification of protein amino groups with the acylating reagent dimethylmaleic anhydride introduces, for each modified amino group, a residue that is negatively charged at pH values higher than 5 (17). When protein-containing particles are treated with this reagent, the extensive modification of amino groups can be accompanied by dissociation from the particles of individual proteins (17). Since the modified amino groups can be regenerated under gentle conditions (7, 19), this reagent has been used to dissociate protein-containing structures in a reversible way (11, 12, 20, 21, 23). Therefore, it was interesting to determine the structural effects produced by this reagent on a protein rich in lysine residues, such as histone H1.

Histone H1 is associated to the DNA stretch that links adjacent nucleosome cores, and seems to play an important role in the condensation of chromatin to form higher-order structures (16, 22). This protein has a molecular weight of 22 000 and a very high lysine content (over 25% of all amino acid components are lysine residues) (24). Three differ-

ent domains can be distinguished in the molecule (8): a globular central region of approximately 80 residues, and the amino-terminal and carboxy-terminal portions, which show no fixed structure in solution, although their interaction with the globular domain appears to modulate its precise tertiary structure (3). Histone H5, a variant of H1, is specific of certain vertebrate erythrocytes. Both proteins are structurally and functionally related (24, 26).

The present work studies the structural changes produced by modification with dimethylmaleic anhydride of histone H1, and the effect of modification on the binding to chromatin of the two homologous histones H1 and H5, which have a different composition in basic amino acids.

Materials and methods

Preparation, modification and regeneration of histone H1

Histone H1 from calf thymus was purified as previously described (9, 10). Histone concentration

Fig. 1. Circular dichroism spectra of different preparations of histone H1 from calf thymus. Spectra were obtained in 0.3 M NaCl and 50 mM phosphate buffer (pH 8.7). (A) Dimethylmaleic anhydride-modified histone and untreated control (—). (B) Dimethylmaleic anhydride-modified histone (---), the same preparation after regeneration of the modified amino groups (.....), and the untreated control (—). Ellipticity is expressed as $\text{deg} \cdot \text{cm}^2 \cdot (\text{dmol protein})^{-1}$. The spectrum of the modified preparation in the near-UV region is not shown for wavelengths lower than 270 nm because of the interference caused by the reagent moieties.

no substantial alteration in secondary structure. This stability of the secondary structure appears to be the result of the preferential location of lysine residues at the unstructured tails of the molecule. However, the globular region, although not as rich in lysines as the amino- and carboxy-terminal portions, has a lysine content of 14.5% (4), which is high as compared with normal globular proteins. Therefore, the observed results suggest that modification of globular proteins with this reagent is accompanied by little disruption of the secondary structure.

In the near-UV region, the circular dichroism spectrum of the modified histone is qualitatively similar to that of the native protein (Fig. 1B). The decrease in absolute value of the negative ellipticity on modification indicates a loss of anisotropy of the sole tyrosine residue. The quantitative changes observed are practically eliminated upon regeneration of amino groups at acid pH. The recovery of the optical anisotropy at 283 nm on regeneration at pH 6.0 and room temperature was measured with time. This process follows first-order kinetics, with a determined value for the corresponding kinetic constant of $1.5 \times 10^{-3} \text{ s}^{-1}$, which is of the same order of magnitude as the rate constant for the regeneration

of the modified amino groups ($2.7 \times 10^{-3} \text{ s}^{-1}$ for dimethylmaleic anhydride-modified chicken ovalbumin, determined at pH 6.0 and 20°C) (19).

The modified histone shows a change in fluorescence emission (Fig. 2). The wavelength of maximal emission is not affected by modification. However, quenching is produced, which reaches 42% at the wavelength of maximal emission. Regeneration of the modified amino groups at acid pH brings about a complete recovery of the original spectrum. The excitation maximum of the modified preparation is the same (280 nm) as that of the native histone, and does not depend on the wavelength at which emission is observed. The quenching of fluorescence emission and the change in the near-UV spectrum are consistent with a change in the local environment of the tyrosine residue.

Treatment with dimethylmaleic anhydride of calf thymus chromatin is accompanied by release of histones H1, H2A and H2B (Fig. 3), H1 being the one most easily dissociated. Dissociation of H2A and H2B from chromatin and nucleosomes is discussed elsewhere (11–13). As shown by the results already described, modification of histone H1 appears to take place in the absence of extensive structural changes. Therefore, the dissociation from

Fig. 2. Fluorescence spectra of different preparation of histone H1 from calf thymus. Spectra were obtained in 0.3 M NaCl and 50 mM phosphate buffer (pH 8.7). Excitation took place at 270 nm. Dimethylmaleic anhydride-modified histone (---), the same preparation after regeneration of the modified amino groups (-.-), and the untreated control (—). The spectra of the untreated control and the regenerated preparation are coincident.

chromatin of this histone is most likely produced by the drastic change in the electrostatic interactions which maintain H1 bound to chromatin, rather than by an extensive structural change of the protein. Histone H1 is also easily released from chicken erythrocyte chromatin on dimethylmaleic anhydride treatment, while the homologous histone H5 is more resistant to dissociation than H2A and H2B. This difference between H1 and H5 appears to be related to their different amino acid composition. Both proteins have a high lysine content, but while H1 has very few arginines (2 per molecule), H5 has a large number (22 per molecule) (26). Since arginine residues are known to interact strongly with phosphate groups (6, 25), a larger level of lysine modification would be required in the case of histone H5 to overcome the attractive forces due to arginine. Our results are in agreement with the higher affinity of H5 for DNA shown previously (2, 14, 18). This property of histone H5 appears to be of functional significance, since accumulation of this histone in chromatin is thought to be correlated

Fig. 3. Densitometric tracings from histone electrophoretic patterns of different chromatin preparations. Untreated controls (C); residual chromatin obtained after modification with dimethylmaleic anhydride at the following ratios of reagent to DNA (mg/mg): 1.0 (thymus RC1), 1.5 (chicken RC1), and 2.0 (chicken RC2); and released fraction corresponding to thymus RC1 (RF1). The released fractions not shown give electrophoretic patterns complementary to those shown in the Figure.

with the chromatin condensation and transcriptional inactivation observed in mature erythrocyte cells (1, 5). Because of the interacting properties of arginine with phosphate groups, this residue might be used whenever strong binding of a protein with DNA or RNA is required, while lysine residues would be employed to afford a more flexible binding.

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