THE JOURNAL OF BIOLOGICAL CHEMISTRY
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Vol. 260, No. 16, Issue of August, 5, pp. 9382-9384, 1985
Printed in U.S.A.

Dissociation of Nucleosomal Particles by Chemical Modification

EQUIVALENCE OF THE TWO BINDING SITES FOR H2A.H2B DIMERS*

(Received for publication, February 4, 1985)

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Treatment of nucleosomal particles with dimethylmaleic anhydride, a reagent for protein amino groups, is accompanied by a biphasic release of histones H2A plus H2B; one H2A.H2B dimer is more easily released than the other. This behavior allows the preparation of nucleosomal particles containing only one H2A.H2B dimer, which were complemented with ^{125}I-labeled H2A.H2B. These reconstituted particles, which contain one labeled and one unlabeled H2A.H2B dimer, were treated with the amount of reagent needed to release one of the two H2A.H2B dimers. Radioactivity was equally distributed between residual particles and released proteins, which is consistent with equivalent binding sites in the nucleosomal particle for H2A.H2B dimers, rather than with intrinsically different sites. The asymmetric release of H2A.H2B dimers would be caused by a change in the binding site of one dimer following the release of the other. This behavior might be related to the structural dynamics of nucleosomes.

The structural unit of chromatin, the nucleosome, includes the nucleosomal core, which is formed by 146 base pairs of DNA folded around the histone octamer $(H2A.H2B.H3.H4)_2$ (1–3). At high ionic strength (2 M NaCl), the histone octamer can be obtained in solution in the absence of DNA. Apparently, the structure of this isolated octamer is similar to that of the octamer in the nucleosomal particle, and both appear to be symmetrical (4, 5). Under different conditions, the isolated histone octamer dissociates to produce two H2A.H2B dimers and one $(H3.H4)_2$ tetramer (6–10). The two dimers dissociate asymmetrically, which is compatible with two alternative explanations: either the two binding sites for H2A.H2B in the tetramer are intrinsically different, or the sites are identical but the release of one dimer is accompanied by a change in the binding site of the undissociated dimer. Although the second explanation is the one generally accepted because it is consistent with a symmetrical particle, no direct evidence of its validity has been reported yet.

Modification with dimethylmaleic anhydride of both isolated histone octamers at high ionic strength (2 M NaCl)[1] and nucleosomal particles at low ionic strength (less than 0.1 M NaCl) (11) is accompanied by a biphasic release of H2A.H2B dimers. The reversibility of the modification caused by this reagent allows the reconstitution of typical nucleosomal particles from residual particles deficient in histones H2A and H2B (11, 12). This system is used in the present work to investigate the mechanism underlying the observed biphasic release of H2A.H2B dimers. The reported results are consistent with the presence of a symmetrical histone octamer in the nucleosomal cores. The biphasic release of histones H2A and H2B is probably due to a change in the binding site of one dimer upon release of the other.

* This work was supported in part by the Fondo de Investigaciones Sanitarias and the Comisión Asesora de Investigación Científica y Técnica (Spain). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] M. A. Nieto, J. Jordano, and E. Palacián, unpublished work.

MATERIALS AND METHODS

Preparation of Nucleosomal Particles and Radioactively Labeled H2A.H2B Dimers—Nucleosomal particles were prepared by digestion with micrococcal nuclease of chicken erythrocyte nuclei and isolation of the mononucleosomal fraction (11). Nucleosomal DNA had an average length of 160 ± 10 base pairs as compared with the digestion products of plasmid pBR322 treated with nuclease *Hae*III. Core histones were obtained from chicken erythrocyte nuclei by salt extraction (2 M NaCl), after removal of histones H1 and H5 (0.75 M NaCl) (6). From this preparation, histone H2A.H2B dimers were isolated by Sephadex G-100 chromatography, as described by Ruiz-Carrillo and Jorcano (6). The H2A.H2B dimers (20 μg) were iodinated in the solid phase with ^{125}I (400 μCi) (Amersham) and Iodogen (Pierce) as described by Fraker and Speck (13). The iodinated proteins were isolated by Sephadex G-25 chromatography.

Treatment of Nucleosomal Particles with Dimethylmaleic Anhydride and Separation of Residual Particles from Released Proteins—Nucleosomal particles (0.14 or 0.35 mg of DNA/ml), in 10 mM Hepes[2] (K^+) (pH 8.2), 5 mM EDTA, and 0.1 M phenylmethylsulfonyl fluoride, were treated with the desired amount of dimethylmaleic anhydride in dioxane. During reaction, the pH was maintained at 8.2 by addition of 1 M NaOH. The hydrolyzed reagent was eliminated by overnight dialysis at 2–4 °C against 10 mM Tris-HCl (pH 8.2), 5 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride. The modified preparation was centrifuged in linear 5–20% sucrose gradients, as previously described (11), the residual particles being separated from the released proteins and free DNA with an ISCO density fractionator.

Reconstitution of Nucleosomal Particles—The modified residual particles were mixed with a complementary amount of radioactively labeled H2A.H2B dimers and dialyzed at 2–4 °C for 6 h against 10 mM maleate (pH 6.0), 1 M NaCl, 5 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride. This treatment causes the regeneration of the modified amino groups of the residual particles (14). The reconstitution mixture was dialyzed a second time for 18 h at 2–4 °C against 10 mM Tris-HCl (pH 8.2), 5 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride, and centrifuged in a linear 5–20% sucrose gradient, from which the reconstituted nucleosomal particles were isolated with an ISCO density fractionator (11).

Analytical Procedures—Prior to electrophoresis of histones, samples were precipitated with 20% trichloroacetic acid, washed with mixtures of ethanol ether, and dried under vacuum. Electrophoresis was conducted on polyacrylamide gels prepared from 15% acrylamide and containing 0.1% sodium dodecyl sulfate (15). Proteins were stained with a solution containing 0.05% Coomasie Blue, 7.5% (v/v) acetic acid, and 10% (v/v) ethanol. DNA was isolated as described by

[2] The abbreviation used is: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Marmur (16) and subjected to electrophoresis on mixed gels of 0.5% agarose and 3% polyacrylamide (17).

Protein concentration was determined by the methods of Lowry *et al.* (18) and Bradford (19). The concentration of nucleosomal particles was determined spectrophotometrically, taking $A_{260\,nm} = 20.0$ for a solution containing 1.0 mg of DNA/ml.

RESULTS AND DISCUSSION

The experimental design consists of the following steps: 1) preparation, by treatment of nucleosomal particles with dimethylmaleic anhydride, of residual particles lacking one H2A.H2B dimer; 2) reconstitution, from these particles and ^{125}I-labeled H2A.H2B dimers, of nucleosomal particles containing one radioactive H2A.H2B dimer; 3) treatment of these reconstituted particles with the amount of reagent required to release 50% of all H2A plus H2B histones; and 4) evaluation of the radioactivity present in the residual particles and in the released proteins. Fig. 1 shows a scheme of the events expected from each of the two hypotheses being considered. According to the asymmetrical model, at the end of the experiment, all the radioactivity would be present in the fraction of released proteins, while the symmetrical model predicts an equal distribution of radioactivity between the released proteins and the residual particles.

The validity of this experimental design requires that the biphasicity of the H2A.H2B release allow the preparation of residual particles containing only one H2A.H2B dimer. Previous work shows that the release of H2A plus H2B is biphasic (11). In addition, the structural properties of preparations of residual particles devoid of at least 50% of H2A plus H2B, after regeneration of the modified amino groups, indicate the practical absence of particles containing two H2A.H2B dimers (12). If particles containing the full complement of histones H2A and H2B were present, they would not require the addition of extra H2A and H2B to show the properties of the original nucleosomal particles, which is not supported by the data (12). Moreover, according to a previous quantitative study (14), the acid treatment used in the reconstitution procedure is more than adequate to obtain complete regeneration of the modified amino groups in the residual particles.

The treatment of the original nucleosomal particles with dimethylmaleic anhydride was accompanied by release of 50–60% of histones H2A plus H2B, and the liberation of a small amount of free DNA. The sedimentation pattern of the modified preparation, and the electrophoresis of the histones present in the residual particles and in the released fraction, are shown in Figs. 2*A* and 3, *B* and *C*. This preparation of residual particles was used to reconstitute nucleosomal particles containing one ^{125}I-labeled H2A.H2B dimer. Electrophoresis of the labeled H2A.H2B preparation is shown in Fig. 3, *D* and *E*. Of the two histones, only H2B is modified, in agreement with previous results (20). The reconstituted nucleosomal particles have the core histone composition (Fig. 3, *F* and *G*), sedimentation coefficient (Fig. 2*B*), CD spectrum (not shown), thermal denaturation profile (not shown), and DNase I digestion pattern (not shown) of the original particles. Autoradiography of the electrophoretic gel shows the incorporation of the labeled dimer to the nucleosomal particle, while no degradation products are observed. Treatment of the reconstituted particles with dimethylmaleic anhydride caused the release of 60% of histones H2A plus H2B and 20% of DNA. The same percentages of these histones and DNA were released from control nucleosomal particles treated with the reagent under identical conditions. Approximately 50% of the total radioactivity incorporated into the reconstituted particles was associated with each of the two fractions: 12,652 cpm were found in the residual particles and 11,274 in the released fraction. This result is in agreement with the symmetrical model (Fig. 1). The small amount of free DNA released on modification should not affect the interpretation of the results, even if, contrary to the conclusion of a previous work (11), its production were not independent of the liberation of H2A and H2B. Therefore, our results seem to exclude the existence in nucleosomal particles of two intrinsically different binding sites for the two H2A.H2B dimers as the cause of the biphasic dissociation obtained upon treatment with dimethylmaleic anhydride. In spite of the equivalence of the

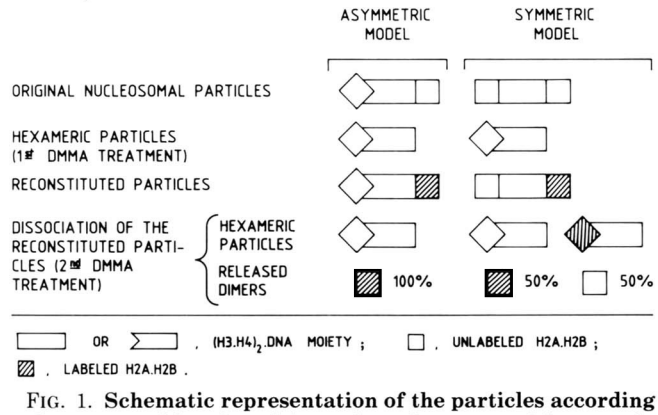

FIG. 1. **Schematic representation of the particles according to the two considered models, and results expected from each.** *DMMA*, dimethylmaleic anhydride.

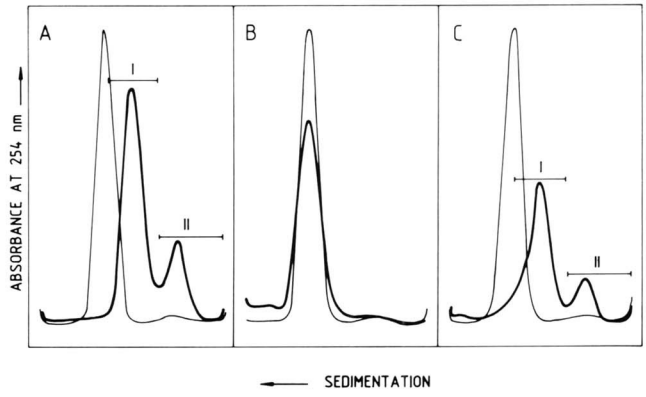

FIG. 2. **Sedimentation patterns of the different nucleosomal preparations.** The superimposed *thin tracing* refers to unmodified control nucleosomes centrifuged under the conditions of the samples. The *bars* (*I* or *II*) correspond to the isolated fractions. *A*, original nucleosomal particles (0.35 mg of DNA/ml, 6 ml) treated with 0.7 mg of dimethylmaleic anhydride/ml, as indicated under "Materials and Methods." *B*, reconstituted nucleosomal particles containing ^{125}I-labeled H2A.H2B. *C*, reconstituted particles (0.14 mg of DNA/ml, 1 ml) treated with 0.5 mg of dimethylmaleic anhydride/ml.

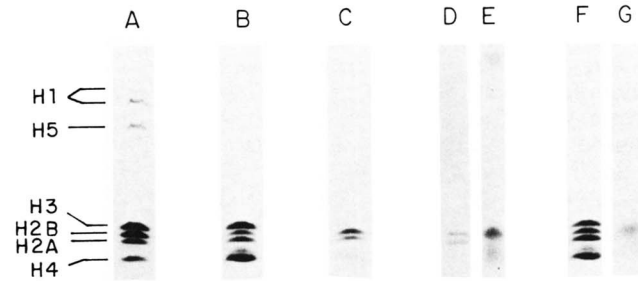

FIG. 3. **Histone electrophoresis of different preparations.** *A*, original nucleosomal particles. *B*, residual particles (Fraction I in Fig. 2*A*). *C*, released fraction (Fraction II in Fig. 2*A*). *D*, preparation of ^{125}I-labeled H2A.H2B dimers. *E*, autoradiography of *D*. *F*, reconstituted nucleosomal particles. *G*, autoradiography of *F*.

two binding sites for H2A.H2B dimers shown in this work, a functional asymmetry has been observed in the assembly and disassembly of both histone octamers (6–10) and nucleosomal particles (21, 22). The properties responsible for this behavior might be important for correct assembly and disassembly of the nucleosomal particles, and for the production of structural changes of functional significance.

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