Isolation of deuterated histones from yeast grown on media dissolved in ^2H_2O

Koei Hamana, Kazuei Mita*, Sachiko Ichimura*, Mitsuo Zama*, Keisuke Kaji[+] and Nobuo Niimura[†]

*College of Medical Care and Technology, Gunma University, Maebashi, Gunma 371, *Division of Chemistry, National Institute of Radiological Sciences, Chiba 260, [+]Institute for Chemical Research, Kyoto University, Uji, Kyoto-Fu 611 and [†]Laboratory of Nuclear Science, Tohoku University, Sendai 982, Japan*

We have succeeded in growing *Saccharomyces cerevisiae* (baker's yeast) on media containing ^2H_2O and isolating the core histones highly deuterated in the non-exchangeable positions. The deuterated histones obtained here are of great value for their possible widespread use for structural studies of chromatin.

Deuteration   *Histone*   *Chromatin*   Saccharomyces cerevisiae   *Yeast*   *NMR*

1. INTRODUCTION

The availability of deuterated biological macromolecules greatly increases the range of structural problems accessible to NMR or infrared spectroscopy as well as neutron scattering or neutron diffraction. For example, replacement of the protons in the macromolecule by deuterium leads to a much simpler ^1H-NMR spectrum by eliminating undesired resonances such as the signals of CH protons, or to allow ^2H-NMR studies to be made [1]. Since deuterated macromolecules have quite different neutron-scattering properties from their protonated equivalents, they can be studied as distinct unit in an otherwise protonated multicomponent system [2].

However, anyone familiar with biochemical procedures will realize that the difficulty of the biochemical aspects of such experiments to get deuterated biological materials is formidable. Thus the specific deuterated macromolecules which have been isolated to date are limited to ribosomal proteins from bacteria, *Escherichia coli* [3,4]. We here describe the first example of the isolation of the highly deuterated specific macromolecules, the histones, from an eukaryotic organism.

2. EXPERIMENTAL

For the purpose to extract histones, yeast cells were grown on the minimum media dissolved in fresh or recovered ^2H_2O (90–99.75%). Heavy water was recovered by distillation of the spent medium and then passing the distillate through a charcoal filter, followed by redistillation. The water recovered was assayed for ^2H_2O content by comparing its infrared absorption at 6600 A with that of dilutions of a freshly opened bottle of 99.75% ^2H_2O [5]. Measurements were made on a Cary 17D spectrometer. Stationary growing yeast cells were harvested and the cells were washed with cold buffer (25 mM Tris–HCl, pH 7.4, 10 mM $MgSO_4$, 0.5 mM EDTA, 40 mM $NaHSO_3$).

Isolation of yeast chromatin was done exactly as in [6] except that the cells were broken by a Braun homogenizer (4 min), instead of hand-shaking, with glass beads. The crude yeast histone fraction was extracted from chromatin by ice-cold 0.25 N HCl. The clear supernatant containing the acid-soluble proteins was supplemented with H_2SO_4 to

[†] To whom correspondence should be addressed

a final concentration of 0.2 N and the protein was precipitated overnight at 4°C with 10 vol. cold acetone and dried under vacuum. The crude histone fraction thus obtained was dissolved in 5 M urea in 0.05 M Tris acetate buffer (pH 5.1) and applied to CM Sephadex C-25 column chromatography and eluted with a linear 0–0.8 M NaCl gradient in 5 M urea in 0.05 M Tris acetate buffer (pH 5.1) to remove contaminants, RNA and non-histone proteins. Some other non-histone proteins of high molecular mass, still remaining as contaminants, were removed by preparative acid–urea gel electrophoresis. A Canalco preparative disc-electrophoresis apparatus (Canal Industrial) was used with an upper column of P/D-2/150. Polyacrylamide gels were prepared as in [7] except that they contained 7.5% acrylamide and 0.2% N,N'-bisacrylamide, to minimize the expansion of the gel volume during the electrophoresis. The crude histone mixture was dissolved in 8 M urea–1% mercaptoethanol and incubated at 37°C for 1 h prior to the preparative gel electrophoresis. The electrophoresis was performed at a constant voltage of 300 V (current 14 mA), with 0.9 M acetic acid as running buffer. The fastest moving fraction of the preparative gel electrophoresis was well separated from the other core histone fractions and was isolated as pure histone H4. The other eluted core histone fraction only poorly separated from one another were pooled together, followed by lyophilization. The lyophilized core histone mixture of H2A, H2B and H3 was dissolved in 8 M urea–1% mercaptoethanol and incubated at 37°C for 1 h, and then applied to Bio-Gel P-60 column chromatography. The core histones were eluted separately with 10 mM HCl, and were finally isolated as the pure histone species. The yield of H3 was lower than those of other histones, probably due to proteolysis during preparation. All the isolated pure histones were lyophilized.

3. RESULTS AND DISCUSSION

To prepare deuterated histones, eukaryotic organisms must be grown on a minimum medium dissolved in ^2H_2O or in a medium in which all the precursors for macromolecular synthesis are supplied in deuterated form. We chose the former way.

We have searched for various eukaryotic organisms which can grow in ^2H_2O and from which a sufficient amount of histones is extractable to perform our structural studies of chromatin. The protozoa (*Tetrahymena pyriformis* GL) hardly grew in 99.75% ^2H_2O. All the algae we examined (*Chlamydomonas mowusii, Chlorella vulgaris, Chlorella ellipsoidea, Scenedesmus acutus* and *Euglena gracilis*) were able to grow in ^2H_2O but they have some experimental difficulties in preparing histones. Finally we found *S. cerevisiae* (ATCC 1600) as the most suitable organism for our present purpose, among the fungi we examined (*Aspergillus niger, Penicillium chrysogenum* and *S. cerevisiae*).

Fig.1 shows the growth curves of yeast in the complete or the minimum medium dissolved in ^1H_2O or ^2H_2O. In ^1H_2O, the growth rate in the
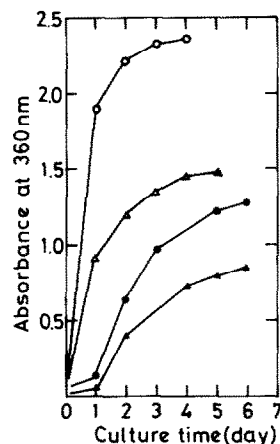

Fig.1. Growth curves of yeast. *Saccharomyces cerevisiae* (ATCC 1600) was grown on the complete or the minimum media dissolved in ^1H_2O or 99.75% ^2H_2O (Norskhydro or Merck): (—○—) complete medium in ^1H_2O; (—●—) complete medium in ^2H_2O; (—△—) minimum medium in ^1H_2O; (—▲—) minimum medium in ^2H_2O. The complete medium contains glucose (2%), polypeptone (1%) and yeast extract (1%). The minimum medium contains glucose (2%) as the carbon, and $(NH_4)_2SO_4$ (0.1%) as the nitrogen sources, respectively, and the trace elements and vitamins [8]. Growth was done at 28°C with continuous agitation to ensure aeration, and was followed by measuring the increase in turbidity at 360 nm. In the minimum medium dissolved in ^2H_2O, sucrose was a good carbon source comparable to glucose in the ability to grow yeast cells, but glycerol was a poor carbon source to support the growth of the cells.

minimum medium is about a half of that in the complete medium. By transferring the cells in ^2H_2O the growth rate decreases to ~⅓ of that in ^1H_2O, with the time lag in the growth rate up to 24 h, either in the complete or the minimum medium.

Yeast chromatin was prepared from deuterated cells grown on the ^2H_2O-minimum medium and the crude histone fraction was obtained from the chromatin by acid-extraction, as in [6]. The crude histones, still considerably contaminated with non-histone proteins and RNA, were usually isolated in amounts of about 100 mg/100 g pressed yeast. The SDS–polyacrylamide gel electrophoretic pattern of the crude histone fraction was indistinguishable from that of the hydrogenated histone fraction extracted from yeast cells grown on the complete medium dissolved in ^1H_2O (not shown). Fig.2 shows the SDS and acid–urea polyacrylamide gel electrophoresis of purified deuterated yeast core histones H2A, H2B and H4. The gel electrophoresis of hydrogenated core histones from chicken erythrocytes is also shown for comparison.

The extent of deuteration in H4 was determined from the loss of resonances in its ^1H-NMR spectrum, as in [10]. It is clear from the NMR spectra of H4 shown in fig.3 that there is almost a total loss of the non-exchangeable proton resonances from the deuterated H4 sample. To determine the percentage of deuteration the area under the proton peaks in the two spectra of the range 0–3.5 ppm was integrated. The extent of deuterium replacement of purified histone H4 thus determined was 86%. The deuteration estimate of the crude histone fraction was 78%.

Fig.2. SDS– and acid–urea polyacrylamide gel electrophoresis of the purified histones from yeast grown on the minimum media dissolved in ^2H_2O. (A) SDS–polyacrylamide gel electrophoresis: (b) H2A; (c) H2B; (f) H4; (a,d,e) hydrogenated core histones from chicken erythrocytes. (B) Acid–urea polyacrylamide gel electrophoresis: (b) H2A; (c) H2B; (e) H4; (a,d,f) hydrogenated core histones from chicken erythrocytes. Acid–urea polyacrylamide gels were made as in [7]. The gels were stained in 0.25% Coomassie blue and de-stained in 20% ethanol/7% acetic acid. SDS–15% polyacrylamide gels as described in [9] were stained in 0.25% Coomassie blue and de-stained in 10% acetic acid.
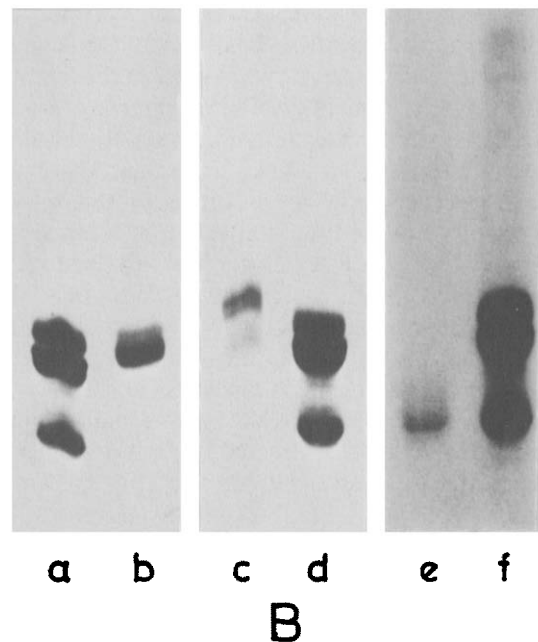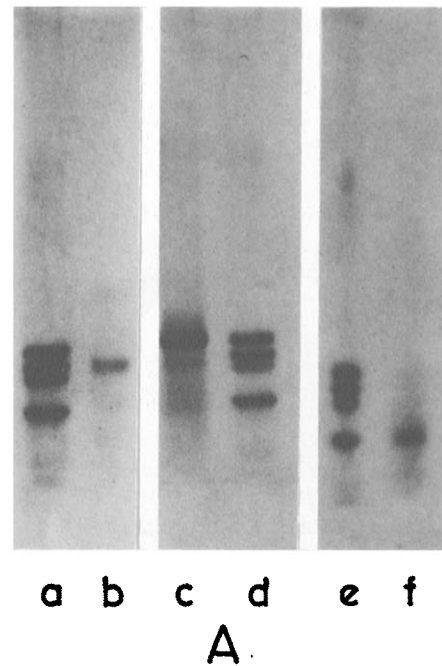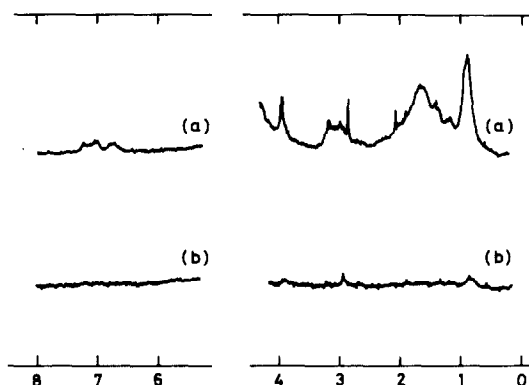
Fig.3. 100-MHz ^{1}H-NMR spectra of equal concentrations (5 mg/ml) of samples of (a) normal protonated, (b) deuterated yeast H4 histone. The concentration was determined by weight. The two spectra are plotted on the same scale. Dried H4 histone was dissolved in 99.75% ^{2}H$_2$O and then lyophilized. After this freeze-dried process was repeated 3-times, the finally lyophilized sample was dissolved in 99.75% ^{2}H$_2$O. ^{1}H-NMR measurements were carried out on a JEOL JNM-FX 100 spectrometer.

In the proposed model of the nucleosome core particle, consisting of 146 basepairs of DNA wrapped around an octamer of two each of the histones H2A, H2B, H3 and H4, the arrangement of the individual histones was determined on the basis of various chemical cross-linking data and the image reconstruction to 22 Å resolution of the histone octamer, and the boundaries of the individual histone molecules were not yet defined [11]. Authors in [3] have extensively developed the neutron scattering triangulation method for the determination of the 3-dimensional organization of ribosomal protein assemblies. They [4] reconstituted prokaryotic 30 S ribosomal subunits containing specific deuterated subunit proteins obtained from bacteria, *Escherichia coli*, grown on media containing ^{2}H$_2$O. Since it is well-known that the nucleosome core is reconstituted from 146 basepair DNA and the equimolar mixture of the 4 core histones [12], the triangulation method could also be applied to clarify the 3-dimensional organization of the histone octamer of the nucleosome core particle. That is, the neutron scattering analysis of the nucleosome core, in which specific histone species are deuterated in non-exchangeable positions, will directly give the information of the 3-dimensional organization of the histone octamer within the core particle.

Fortunately the core histones obtained here are highly deuterated and therefore they should be good substituents of hydrogenated histones to reconstitute nucleosome samples for neutron scattering analysis. Signals from NMR or infrared spectra of deuterated histones will also give us new structural information on histones and chromatin. Here we have shown the way to get deuterated histones that are of great value for their possible widespread use for structural studies of chromatin.

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