TWO FORMS OF RAT LIVER HISTONE H3

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1. Introduction

In higher eukaryotes, H3 is the only histone species which contains cysteine [1]. Earlier studies suggested a clear separation on the evolutionary scale for the number of thiols present in mammalian H3, since rodents were found to have only one cysteine, while the H3s of yet more advanced species were found to possess two such residues [2]. More recently, however, heterogeneity in calf H3 has been detected, with a minor fraction (20%) having serine in place of cysteine at position 96 [3,4]. This finding prompted us to develop a sensitive technique to examine possible heterogeneity in rodent H3. Using this method the present report demonstrates that a minor fraction of H3 of rat liver indeed contains at least two cysteines per molecule.

2. Materials and methods

2.1. *Isolation of oxidized H3 histone*

Liver histone was prepared from male Sprague-Dawley rats (Charles Rivers Labs, Wilmington, Mass.) as described earlier, except that chromatin was isolated with 1% Triton X-100 present in the saline-EDTA solution [5]. The histone powder was dissolved at 10 mg per ml in 10 mM Tris-40 mM H_2O_2 (pH 8.0), incubated 5 h at 25°C, and 2 volumes of 0.9 N acetic acid–8 M urea were added. Calf thymus chromatin was isolated as above but without sucrose centrifugation. Chromatin bound H3 was oxidized by vigorous homogenization, using both a Teflon homogenizer and a Virtus 60 homogenizer; both steps caused extensive foaming.

2.2. *Gel electrophoresis*

The 2.5 M urea system of Panyim and Chalkley [6] was used for both tube and slab gels, which were stained with Coomassie brilliant blue R-250 [7] and scanned at 600 nm. Slabs of 0.15 × 15 × 15 cm with wells 0.3 cm wide were run 12 h at 10 mA constant current in the first dimension, with a standard of 12 μg run in an outer well which was sliced from the gel. The sample load for two dimensional analysis was 48 μg. After the first dimensional run, slabs were soaked in the following solutions after being rolled in polypropylene mesh: 500 ml of 0.1 M Tris–2.5 M urea–10% mercaptoethanol (pH 8.0 at 55°C) for 45 min with shaking at 55°C; distilled water rinse; and 500 ml of 0.9 M acetic acid–2.5 M urea for 30 min with shaking at 55°C. Resulting gels were resealed after trimming excess material away (considerable expansion of gels occurs in the above regime) at 90° to the first dimension by use of 2% agarose, and run 10 h at 10 mA.

3. Results and discussion

As shown in fig.1A, oxidation of calf thymus histone leads to the formation of H3 homopolymers up to pentamers. The cross-links between H3 molecules are not homogeneous, since multiple peaks exist in each oligomer class. Five different dimer products are theoretically possible (cys 96–96, cys 110–110, cys 96–110, homologous and heterologous double disulfides); the precise molecular means by which dimers and higher oligomers are bridged, however, is not known. All H3 oligomers disappear upon reduction with the concomitant appearance of

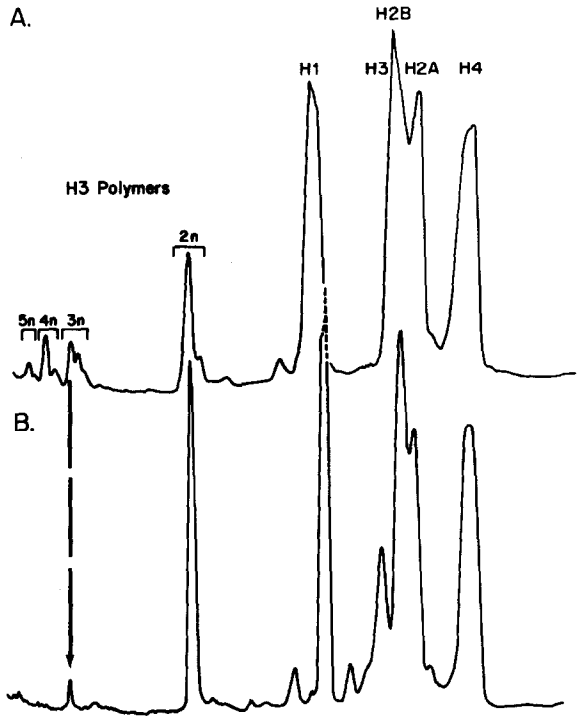

Fig.1. Electrophoretic profiles of oxidized histone. (A) Calf thymus. (B) Rat liver.

H3 monomer (not shown). The key issue, however, is that a minor band present in oxidized rat liver histones migrates in the vicinity of calf H3 trimer (fig.1B). This band consists of approximately 3.4% of the total liver H3 protein as estimated by area analysis. Since it is shown below that this component consists of at least one molecule of H3 with at least two sulfhydryl groups, the minimum proportion of this new H3 is about 1%. An upper limit cannot be estimated since the relative specificities and efficiencies of such disulfide reactions are not known.

Strong evidence supporting the existence of the new H3 comes from two dimensional electrophoresis. As shown in figs. 2A, B, upon separation of oxidized rat liver histone in the first dimension followed by reduction in situ and electrophoresis at right angles to the first dimension, H3 molecules previously migrating as trimer and dimer are now converted to forms which co-migrate with H3 monomers. Furthermore, since reduction is not quantitative, a trace amount of H3 dimer originating from H3 trimer can be seen upon close inspection (figs.2A, B). The lack of a perfect diagonal line is the result of gel swelling which occurs during the in situ reduction step and affects the mobilities of certain proteins differentially in the second dimension.

Two controls were performed to rule out possible

Fig.2a

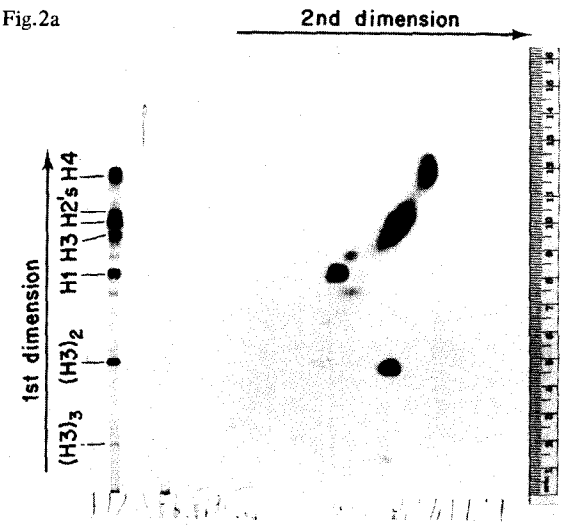

Fig.2b

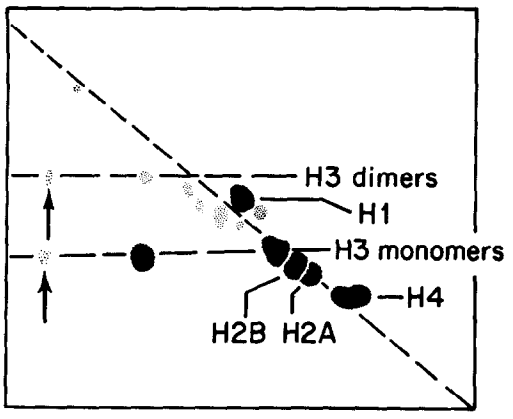

Fig.2. Two-dimensional electrophoresis of rat liver histone. Oxidized histone was separated in the first dimension, and after reduction in situ electrophoresis was repeated at a 90° angle relative to the first dimension. (A) Original gel including first dimension standard. (B) Diagram depicting faint spots present in fig.2A.

aggregates of H3 which might lead to a non-disulfide bridged trimer. The first was simply soaking the gel identically but in the absence of mercaptoethanol prior to the second dimension run. The second was soaking as above but with ethanol in place of mercaptoethanol. In both cases, more than 90% of H3 trimer remained intact after electrophoresis in the second dimension (not shown). The conclusion is that disulfide bridges maintain the trimer state.

Since heterogeneity in the primary structure of H3 has been found at position 96 in plant and animal systems [4,8], this locus is the expected site for the extra cysteine of the new rat species. It is not clear whether the different histone H3 genes are expressed randomly in different cell types, whether the concentrations of the two forms are proportionally reflected by the dosage of the various H3 genes present in histone gene repeats [9], whether heterogeneity is related to heterozygosity, and whether single nucleosomes [10] contain mixtures of the different H3 species.

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