H1 and H5 HISTONE ARRANGEMENT IN CHROMATIN OF PIGEON ERYTHROCYTES

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1. Introduction

Erythrocyte-specific histone H5 may be regarded as one of the subfractions of the H1 histone family, which appears to be involved in supercompaction and transcription inactivation of erythrocyte chromatin [1–3]. H5 histone emerges very early in the course of erythrocyte maturation and then gradually increases its content [4]. However, the question as to how the replacement of H1 histone by H5 arises and the arrangement of these 2 types of histones in mature erythrocyte chromatin remains to be solved. Here we present the results of chemical crosslinking of H1 and H5 histones in erythrocyte nuclei.

2. Materials and methods

Nuclei from pigeon erythrocytes were isolated as in [5]. Nuclei were suspended in 0.01 M triethanolamine–HCl buffer (pH 7.4), 1 mM $MgCl_2$ at 1 mg/ml. Methyl-4-mercaptobutyrimidate (MMB, Pierce) was dissolved in the same buffer (1 mg/ml) and an equal volume was added to the nuclei at 4°C [6]. After 10–30 min incubation the excess of MMB was removed by centrifugation and repeated washing of the nuclei. SH-groups were reduced by adding 30% H_2O_2 up to 0.15% final conc. Free SH-groups were blocked with iodacetamide (Pierce) in the dark (1 mg/ml, 30 min). H1 and H5 histones and their oligomers were selectively extracted with 5% $HClO_4$. Crosslinked histones were chromatographed on an Amberlite CG-50 column (Bio Rad) [3]. Electrophoresis of crosslinked histones was carried out in a 7.5% polyacrylamide gel. Splitting of disulfide bridges was done by incubation of gel in the solution comprising 20% ethanol and 5% mercaptoethanol at 40–50°C.

3. Results and discussion

H1 and H5-containing mononucleosomes of chicken erythrocytes may be separated electrophoretically [7]. Pigeon erythrocytes contain 4 lysine-rich histone fractions: 3 of H1 and 1 of H5 (fig.1,2). This is the number of complete mononucleosomes one can expect to find after electrophoresis of chromatin digest in polyacrylamide gel. Although, only 2 such particles could be seen after electrophoresis in the first direction (fig.1), the second direction of electrophoresis reveals a diagonal distribution of 4 lysine-rich histones in a gel, indicating the presence of 4 lysine-rich histone-containing mononucleosomes. It means that complete mononucleosomes are heterogeneous with respect to the type of associated lysine-rich histone.

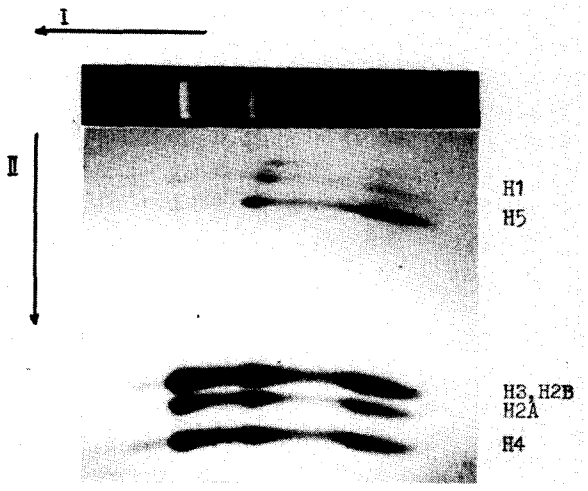


Fig.1. Two-dimensional electrophoresis of nucleosomes and nucleosome proteins: (I) direction – electrophoresis of nucleosomes in 0.01 M Tris–borate buffer (pH 8.3) (5); (II) direction – SDS electrophoresis of proteins of nucleosome fractions.

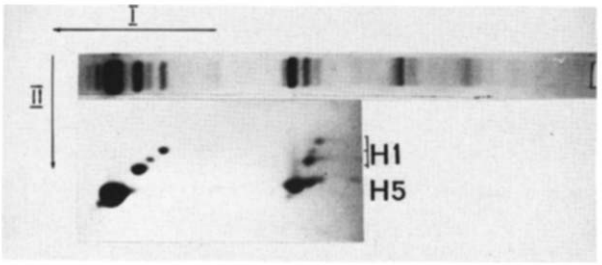


Fig.2. Two-dimensional electrophoresis of H1 and H5 histones and their oligomers: (I) direction – H1 and H5 oligomers in 7.5% gel; (II) direction – dimer composition after cleavage of crosslinks with 5% mercaptoethanol (10% gel).

Chemical crosslinking of lysine histones enables us to study the arrangement of H1 and H5 histones in chromatin belonging to neighbouring mononucleosomes.

Reversible chemical crosslinking of histones with MMB was performed in nuclei to avoid a possible rearrangement of lysine-rich histones in the process of chromatin isolation. H1 and H5 histones and their oligomers were selectively extracted with 5% $HClO_4$ and separated electrophoretically in a 7.5% gel (fig.2). As seen from fig.2 crosslinked lysine-rich histones give an oligomeric series up to 5–6 monomer histones in these conditions of electrophoresis. The dimer zone shows 4 dimer bands, the composition of which was analyzed by two-dimensional electrophoresis after cleavage of crosslinks with β-mercaptoethanol (fig.2). A rapidly migrating and the most intensive dimer band is exclusively represented by H5 histone, i.e., this band is a H5 homodimer. All 3 of the H1 histone subfractions were recovered in dimers, which always contain as a counterpart H5 histone, thus indicating that they are heterodimers. These results of crosslinking reflect the linear arrangement of H1 and H5 histones in chromatin since the same data were obtained when isolated di- and trinucleosomes were treated with MMB (not shown).

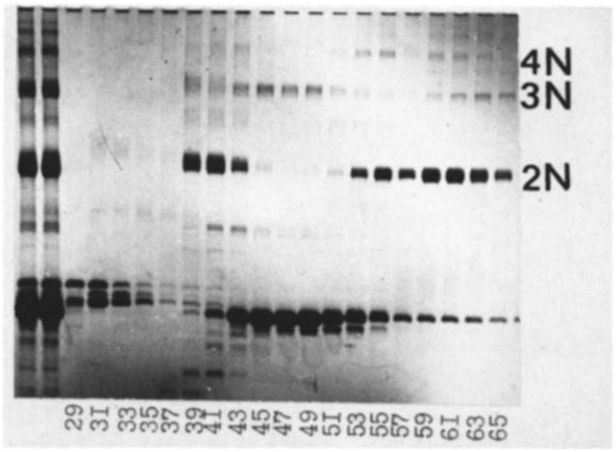
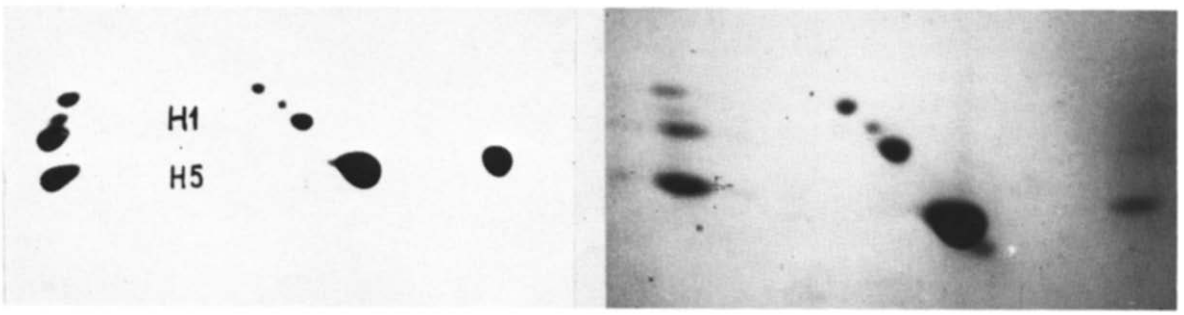


Fig.3. SDS electrophoresis of histone fractions after chromatography of crosslinked histones on an Amberlite CG-50 column. Each second chromatographic fraction is analyzed. Left two runs – total crosslinked lysine-rich histones from erythrocyte nuclei.

Homogeneous trimers and tetramers of lysine-rich histones were obtained at preparative scale by means of chromatography on an Amberlite CG-50. Fig.3 shows an electrophoretic analysis of each second fraction after chromatography. Fractions of interest were analyzed by means of two-dimensional electrophoresis (fig.4). Cleavage of crosslinks and electrophoresis confirmed the above conclusion that H5 forms homogeneous dimers and oligomers. However, trimers and tetramers comprising H1 histone subfractions always contain H5 histone (fig.4).

Fig.4. Protein composition of chromatographic histone oligomers. (a) Dimers: middle, uncrosslinked lysine-rich histones; left, dimer composition of fraction 41 (fig.3); right, dimer composition of fraction 61 (fig.3). (b) Trimers and tetramers: left, trimers from fraction 47 (fig.3); right, tetramers from fraction 53 (fig.3).

a b



It may be concluded that H1 and H5 histones alternate along chromatin in erythrocyte nuclei. H5 forms a marked amount of homopolymers since it has a great excess over H1 (H5/H1 ratio is ~3). A little amount of suggested H1-homodimers is found in the region of dimer fractions 31–33 (see fig.3). Because of the small quantity of the material it was not analyzed by two-dimensional electrophoresis, but it would be reasonable to point out that the marker H1 homodimers exhibit similar electrophoretic mobility (not shown). These suggested homodimers contain <5% of the total H1.

It is known that during erythropoiesis H1 is replaced by erythrocyte-specific H5 histone, but this replacement is not complete [1–3]. In the mature pigeon erythrocytes the H5/H1 ratio is ~3. Alternation of H1 and H5 histones permits the discrimination between different possible ways of histone replacement. Long chromatin stretches such as transcriptional units or axial loops [8] could be occupied by only one type of lysine-rich histones. The order of histone replacement might reflect the sequence of genes switching off during erythrocyte maturation. The process of H1 histone replacement, however, appears to be more random. Data obtained indicate that in most cases H1 histone molecules are neighbouring H5.

The arrangement of H1 subfractions with respect to each other is of general interest in connection with study of the mechanism of lysine-rich histone replacement. The neighbouring of H1 subfractions to each other is obscured in erythrocyte chromatin by the presence of H5 histone. We have analyzed the H1 subfractions neighbouring in the rat thymus nuclei. The results are given in fig.5, from which it is seen that both of the two H1 subfractions form homodimers. However, ⩾50% of the dimers is the heterodimers, i.e., H1 subfractions alternate along chromatin fiber like H1 and H5 histones in erythrocyte nuclei. Thus, the partial alternation of different types of the lysine-rich histones is characteristic not only for mature erythrocytes but is a more typical phenomenon.

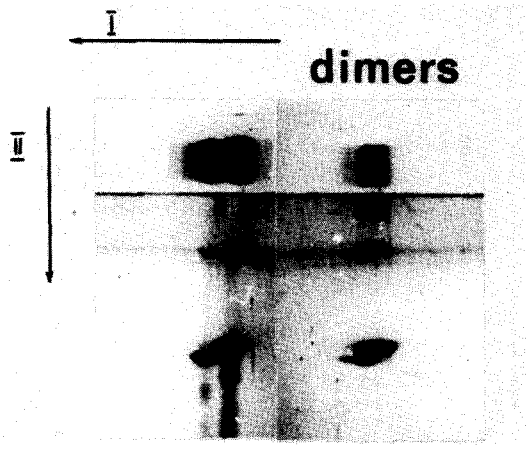


Fig.5. Neighbouring of H1 histone subfractions in rat thymus nuclei as revealed by MMB crosslinking: (I) direction – monomers and dimers of H1 histone in 7.5% gel; (II) direction – dimer composition after cleavage of crosslinks with β-mercaptoethanol (10% gel).

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