Mutual arrangement of histone H1 molecules in extended chromatin

Chymotryptic digestion of cross-linked H1 histone dimers

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Mutual arrangement of histone H1 molecules in chromatin extended in low salt–EDTA buffer and additionally in the presence of urea was studied by means of reversible cross-linking combined with chymotryptic digestion. In the chromatins tested, the chymotryptic halves of H1 were cross-linked in all possible combinations; i.e., C–C, C–N and N–N. The results imply that the mutual arrangement of H1 histones is determined by the structure of extended nucleosomal chain, rather than chromatin superstructure.

Chromatin structure *Histone H1 arrangement* *Protein cross-linking* *Chymotryptic digestion*

1. INTRODUCTION

Histone H1 molecules are considered now as direct participants in chromatin condensation (review [1]). From this point of view studies of the H1 histsone arrangement with respect to the structural components of chromatin are of particular interest.

One approach to elucidate the arrangement is cross-linking of the H1 molecules with bifunctional reagents [2–8]. Using chemical or enzymatic splitting of cross-linked histone molecules, it has been shown [4] that the N-terminal parts of the H1 molecules were in close proximity to the core histones. The globular domains of neighbouring H1 molecules could also be cross-linked to each other, and their proximity was conserved in extended chromatin and destroyed only in the presence of 8 M urea [8].

Earlier we studied the proximity between the N- and C-terminal parts of H1 molecules in compact chromatin of intact nuclei using chymotryptic digestion of cross-linked H1 histone dimers [7]. Here we present an analysis of the proximity in chromatin extended in a low salt–EDTA buffer, and in chromatin unravelled in the presence of 8 M urea.

Abbreviations: EDTA, ethylenediamine tetraacetic acid, disodium salt; SDS, sodium dodecyl sulphate; MMB, methyl-4-mercaptobutyrimidate–HCl; TEA, triethanolamine

2. METHODS

Calf thymus nuclei were isolated at pH 6.0 to inhibit proteolysis of histones and purified by sedimenting them through 1.4 M sucrose. The nuclei obtained were subjected to endogenous nucleolysis and lysed by dialysis against 3 mM TEA–HCl buffer (pH 7.2), 0.5 mM EDTA or, alternatively, against the same buffer, but additionally containing 8 M deionized urea. Nuclear debris was removed from chromatin solutions by

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centrifugation for 30 min at 10000 × *g*. A detailed procedure will be published [8].

To cross-link the histones, MMB was added to the chromatin solution up to a concentration of 0.5 mg/ml, and the pH was adjusted to 7.9 with 1 M TEA. After incubation for 1 h at 4°C, H_2O_2 was added to a final concentration of 0.3%. The mixture was kept for 30 min, and then free SH-groups of MMB were blocked with iodoacetamide (2 mg/ml) for 30 min in the dark. Unreacted imidoester groups of MMB molecules were blocked by treatment with 50 mM glycine for 15 min.

Histone H1 mono- and oligomers were extracted with 5% $HClO_4$ and precipitated with 15% trichloroacetic acid. After cross-linking in urea chromatin was first dialyzed overnight against distilled water and then extracted as pointed out.

Histone H1 dimers were isolated by gel filtration on a 1.5 × 100 cm column of Sephadex G-200 [7], equilibrated with 10 mM HCl.

Chymotryptic digestion of H1 dimers and diagonal SDS gel electrophoresis were done as in [7,8].

3. RESULTS AND DISCUSSION

Limited chymotryptic digestion of H1 histone leads to the splitting of the molecules into two fragments – the N- and C-terminal halves (residues 1–106 and 107–213, respectively) [9]. The N-terminal half of H1 histone includes the so called 'nose' and 'head' of the molecule, while practically only the 'tail' of the H1 is present in the C-terminal half [9,10].

Using the chymotryptic digestion of cross-linked H1 histone dimers, it is possible to reveal closely-spaced H1 chymotryptic halves belonging to different molecules both in nuclei [7] and in extended chromatin. For such an analysis, digested H1 dimers were resolved in SDS-containing polyacrylamide gel in the first dimension and, after splitting of the cross-links with 2-mercaptoethanol, were further electrophoresed in the second dimension on the same gel. On the diagonal gels obtained, initially cross-linked H1 molecules and their chymotryptic fragments migrated in the second dimension as the H1 and its fragments, respectively, thus finally occupying positions outside the diagonal.

Fig.1 presents the results of diagonal gel electrophoresis of chymotryptically digested H1 dimers from different chromatins.

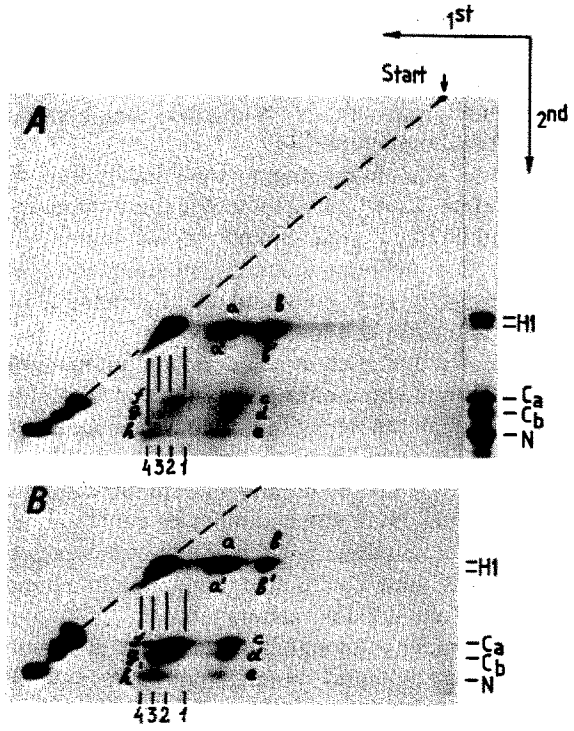

Fig.1. Diagonal SDS gel electrophoresis (16% in both dimensions) of chymotryptic digest of H1 histone dimers, obtained from extended at low ionic strength in the presence of EDTA chromatin (A) and chromatin in the same conditions but with 8 M urea added (B). Standard, chymotryptic digest of histone H1. For details see text.

Extra-diagonal spots in each case were identified as in [7]. In particular, spots b and b′ originated from cross-linked undigested H1 dimers. The spots a and a′ represent intact H1 histone, and the spots c, d and e apparently corresponded to fragments of partially digested complexes H1–C_a, H1–C_b and H1–N, respectively. Note that the C-terminal halves of H1 are resolved into two bands, C_a and C_b [7].

In as much as the C_b- and N-fragments were not found beneath the spot f on the vertical line 1 and the electrophoretic mobility of this part of the spot f precursor in the first dimension was somewhat less than that of H1, the right part of the spot f represents the C-fragments from C_a–C_a cross-linked dimers. These dimers have the least mobility

of all possible pairs of the H1 chymotryptic fragments. The left part of the spot h (vertical line 4) can be similarly concluded to contain the N-fragments from split N–N dimers, which should have the highest mobility.

Both C- and N-fragments were located on the vertical lines 2 and 3 and therefore these fragments originated from cross-linked C_a–N and C_b–N species. It is difficult to locate precisely the spots corresponding to C_a–C_b and C_b–C_b species, but these cross-links are also very likely.

The patterns depicted in fig.1A, are qualitatively very similar to those obtained earlier for nuclei (see fig.6 in [7]) where chromatin was condensed. Following the same line of argument as in [7], one can conclude that the N- and C-terminal halves of neighbouring H1 histone molecules can be cross-linked to each other in all possible combinations (N–N, N–C and C–C), also in chromatin extended in low salt–EDTA buffer.

This similarity strongly suggests that proximity between the C- and N-terminal halves of H1 molecules is determined essentially by the arrangement of histones along the nucleosomal chain itself rather than by chromatin superstructure.

The H1 molecule halves can be cross-linked in all possible combinations, also in chromatin, isolated and kept in 8 M urea (fig.1B). Thus, even unfolding of the nucleosomes in 8 M urea [11] and denaturation of the H1 globular domains did not lead to the loss of the proximity between the H1 chymotryptic halves, although, as we demonstrated in [8], the yield of H1 histone dimers was decreased 3-fold and the proximity between H1 globular domains disappeared.

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