Chemical crosslinking of histone H1° to histone neighbours in nuclei and chromatin

Viktor K. Dashkevich, Lev G. Nikolaev, Jordanka S. Zlatanova*, Boris O. Glotov and Evgenii S. Severin

Institute of Molecular Biology, USSR Academy of Sciences, Moscow 117984, USSR and **Institute of Molecular Biology, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria*

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Crosslinking of histones in mouse liver nuclei and extended chromatin with a bifunctional reagent leads to the formation of H1–H1° heterodimers as well as H1°–H1° homodimers. H1° can be also crosslinked to the core histones. Thus, the location of histone H1° within the basic repeating chromatin structure seems to be analogous to that of H1 histone.

Chromatin structure *Histone H1° location* *Protein crosslinking*

1. INTRODUCTION

Mammalian tissues with restricted proliferative activities are characterized by the presence of a specific lysine-rich histone subfraction H1° [1]. Initially this histone was considered as a factor involved in the induction or maintenance of a quiescent cellular state [2]. Later it was shown to be related also to the expression of differentiated function in a number of cellular systems both in vitro [3,4] and in vivo [5]. In addition, it is probably the only histone fraction that considerably changes during malignant transformation. Usually tumors are characterized by a decreased level of H1° [6,7], although reports to the contrary are also available [8–10].

Primary structure and immunological studies performed with the central part of the H1° molecule revealed a high degree of homology between H1° and H5 histones [11–13]. The homology with histone H1 is less pronounced, in agreement with a peptide map analysis [3,14,15]. Histone H1° has been shown to bind to the linker DNA segments in chromatin [14,16] protecting them from nuclease attack [17]. Like H1 and H5 histones, the H1° molecule consists of 3 distinct structural domains: a cationic N-terminal part or 'nose', a central globular part or 'head', containing about 80 amino acid residues, and a basic structureless C-terminal part or 'tail' [18]. These data suggested that H1° histones bound to the nucleosomes in a manner similar to the binding of other H1 subfractions.

In view of the possible involvement of H1° in fundamental cellular functions such as gene expression, differentiation and carcinogenesis, studies on the location of H1° in chromatin are of particular interest.

This report presents an analysis of the spatial proximity of H1° histones to neighbouring histones in intact nuclei and extended chromatin.

Abbreviations: PMSF, phenylmethylsulphonyl fluoride; TEA, triethanolamine; Tris, tris-(hydroxymethyl)-aminomethane; EGTA, ethylene glycol bis(2-aminoethyl ether)-*N,N'*-tetraacetic acid

2. METHODS

Mouse liver nuclei were isolated as in [19] with modifications. Mouse liver tissue was homogenized in 0.25 M sucrose, 4 mM $MgCl_2$, 25 mM KCl,

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5 mM Tris–HCl (pH 7.5), 1 mM EGTA, 0.5 mM PMSF, and the homogenate was filtered through 4 layers of cheesecloth. Nuclei were pelleted by centrifugation at 1000 × *g* for 5 min, and resuspended in 1.5 M sucrose, 4 mM $MgCl_2$, 25 mM KCl, 10 mM Tris–HCl (pH 7.5), 0.5 mM EGTA and 0.5 mM PMSF. The resulting suspension was layered onto a cushion of the latter buffer but with 2.2 M sucrose and centrifuged in a SW 25.2 rotor (Beckman) at 22000 rev./min for 60 min. The nuclei were then washed twice with 10 mM TEA–HCl (pH 7.9), 0.25 M sucrose, 5 mM $MgCl_2$, 0.5 mM PMSF, and finally resuspended in this buffer at 5 mg DNA/ml.

To obtain chromatin, the nuclear pellet after high-speed centrifugation was washed twice in 5 mM Na-cacodylate (pH 6.0), 0.25 M sucrose, 4 mM $MgCl_2$ and 1 mM PMSF. The nuclei at 5 mg DNA/ml were subjected to endogenous nucleolysis for 40 min at 37°C in the above buffer additionally supplied with 1 mM $CaCl_2$. The reaction was terminated by the addition of EGTA to a final concentration of 2 mM and chilling on ice. The degree of chromatin digestion under these conditions was moderate, the chromatin fragments being on average 10–20 nucleosomes long.

Further steps were done at 4°C. The autodigested nuclei were lysed during overnight dialysis against 3 mM TEA–HCl (pH 7.2), 0.5 mM ethylenediamine tetraacetic acid and disodium salt. After centrifugation of the lysate (10000 × *g*, 20 min) chromatin in the supernatant was obtained and used for crosslinking after adjustment of pH to 7.9 with 1 M TEA. Chromatin concentration during crosslinking was about 3 mg/ml in DNA.

Nuclei and chromatin were crosslinked with dimethyl-3,3′-dithiobispropionimidate·2 HCl (Pierce) at 0.5 and 2.5 mg/ml, respectively, for 1 h. After this, 1 M glycine was added to a final concentration of 50 mM to block unreacted imidoester groups, and the incubation was continued for 15 min. Histones and their crosslinked products were extracted with 5% $HClO_4$ overnight and then precipitated after addition of trichloroacetic acid to 15%. Histone pellet was washed with acetone, dried and dissolved for electrophoresis in the sample buffer without 2-mercaptoethanol [20].

Extracted with 5% $HClO_4$, nuclei and chromatin were resuspended once more in 0.25 HCl to extract other crosslinked species, in particular, heterodimers of histones H1 and H1° with the core histones. The HCl extracts were prepared for electrophoresis as above.

Diagonal gel electrophoresis of the extracted histones and their crosslinked oligomers was done in 0.1 × 15 × 15 cm polyacrylamide slab gels in the presence of Na-dodecylsulphate [20] as in [21]. Prior to the separation in the second dimension the S–S bridges of the crosslinks were split with 2-mercaptoethanol.

3. RESULTS AND DISCUSSION

3.1. *Identification of histones crosslinked in compact nuclear chromatin*

Chemical crosslinking of histones in nuclei results in H1 dimers and higher oligomers [21–29]. These can be selectively extracted with 5% perchloric acid and analysed by diagonal gel electrophoresis. After fractionation of the crosslinked products in the first dimension, the identities of the histone partners in the crosslinked products can be established by electrophoresis in the same gel in the second dimension after cleavage of the crosslinks. All the protein spots that lie in a resulting gel on the diagonal represent protein species with electrophoretic mobilities not influenced by the cleavage of the crosslinks; e.g., histone monomers. The spots that fall off the diagonal correspond to the oligomer species initially kept together due to crosslinks. Individual histone constituents from separated histone heterooligomers fall on the corresponding vertical lines.

Fig.1A shows a diagonal gel obtained for perchloric acid-soluble protein from crosslinked nuclei. The proteins of the extra-diagonal spots along the vertical lines a, b and c moving in the second dimension like markers H1°, H1B or H1A, behaved in the first dimension as H1 dimers as judged from their app. M_r-value [21].

Since the extra-diagonal spots on the verticals a and c are unique, they can be interpreted in terms of formation of homodimers H1A–H1A (line c) and H1°–H1° (line a). The presence of H1° spot on the vertical line b points to the existence of H1°–H1A and/or H1°–H1B crosslinked heterodimers. The crosslinked dimers H1A–H1B and H1B–H1B were demonstrated independently [30].

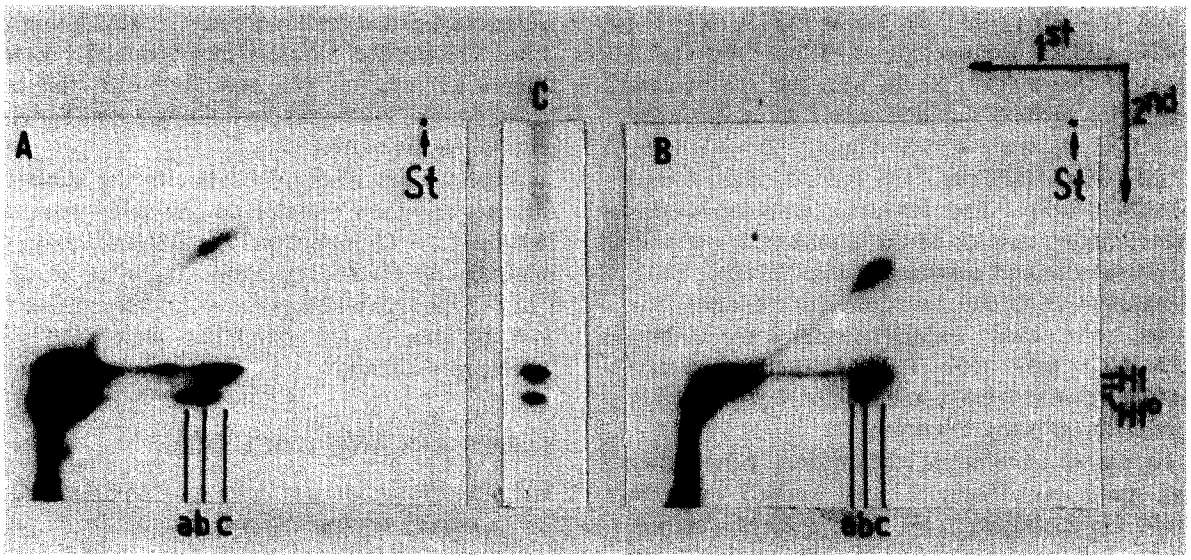

Fig.1. Diagonal gel electrophoresis of 5% perchloric acid-soluble protein from crosslinked nuclei (A) and extended chromatin (B); C, histone standard from mouse liver nuclei; 10 and 12% polyacrylamide gels in the first and the second dimensions, respectively. St, start position.

These data show that in nuclei all H1 subfractions including H1° are interspersed; i.e., they can be found in close proximity to each other. Although no exact quantitation of the different dimers can be done, the finding that all 3 H1 subfractions are presented in the crosslinked dimers in roughly the same relative amounts (line b) as in intact nuclei, speaks in favour of effectively random location of these subfractions in nuclei.

Fig.2A presents the diagonal gel electrophoresis of crosslinked nuclear proteins insoluble in 5% $HClO_4$ but extractable with 0.25 M HCl. Extradiagonal spots on the vertical line $(CH)_3$ (the zone of trimers of the core histones) correspond to histone H1° (spot X) and the core histones (lower spots), H4 being present in barely detectable amounts. Lower spots might result from cleavage of H1°-core histones heterodimers as well as from cleavage of core histone trimers which comigrate with the heterodimers in the first dimension. The presence of an H1° spot X and the core histone spots beneath on the same vertical line suggests that the H1° histones can form dimers with some of the core histones upon crosslinking. Unfortunately, it is not possible to determine precisely to which of the core histones H1° is actually crosslinked.

3.2. *Identification of crosslinked histones in extended chromatin*

In view of the fact that in intact nuclei additional contacts and proximities among the histones might exist due to higher order structure, we studied the location of H1 subfractions with respect to each other and to the core histones in so called extended chromatin, where lateral contacts between chromatin fibres are believed to be greatly diminished. In this case the observed crosslinked products would reflect mainly mutual disposition of histone fractions along the chromatin fibre. The results are presented in fig.1B,2B.

Fig.1B demonstrates the H1–H1 proximities in extended chromatin. The characteristic positions of spots on the vertical line b points to close location of H1° to other H1 subfractions. Similarly, it can be concluded (line a) that a small fraction of the H1° molecules are close enough to be crosslinked in homodimers. The pattern of crosslinking is generally analogous to that obtained with nuclei. The only visible but essential difference is a markedly lower yield of H1° homodimers in extended chromatin than in nuclei (compare the intensities of the H1° spots on the vertical lines a in fig.1A and 1B).

The similarity of the patterns obtained with

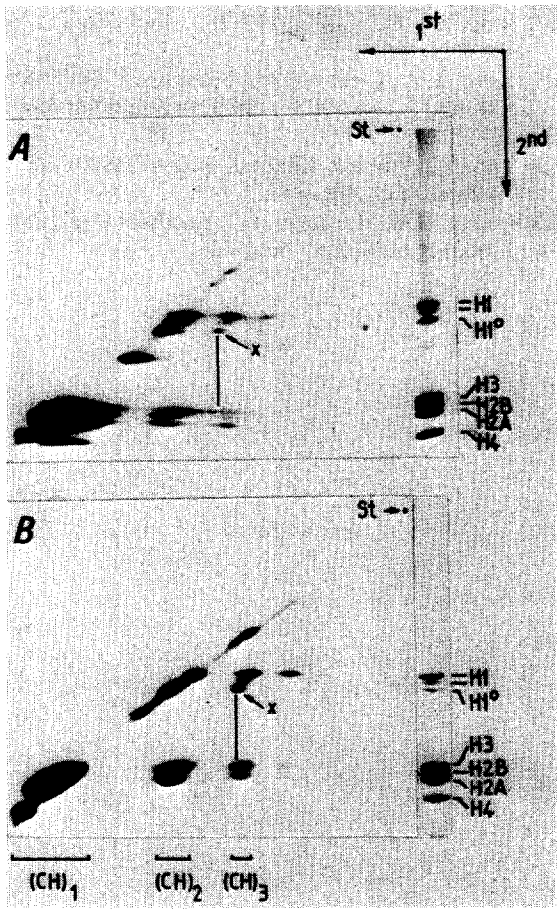

Fig.2. Diagonal gel electrophoresis of 5% perchloric acid-insoluble protein extracted with 0.25 M HCl from crosslinked nuclei (A) and extended chromatin (B); 16% polyacrylamide gel; $(CH)_i$ designates positions of core histone *i*-mers in the first dimension; St, start position.

nuclei and extended chromatin also gives evidence of crosslinking of histones H1 to the core histones (fig.2A and 2B). We note, that the mutual arrangement of H1 subfractions (including H1°) and the core histones in nuclei and chromatin seems to be alike.

It follows that in nuclei and in extended chromatin histone H1° is in close proximity to other H1 subtypes as well as to the core histones. This in turn implies that H1° is located within the repeating chromatin structure in a way similar to that of H1 histone [27], thus supporting a suggestion originally put forward on the basis of similarities in the secondary structure of histones H1°, H5 and H1. In particular, the occurrence of H1° homodimers indicates that H1° molecules can be found as close to each other as 12 Å, possibly on neighbouring nucleosomes.

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