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Accessibility of histone H1° and its structural domains to antibody binding in mononucleosomes

Todor Banchev, Ljuba Srebreva and Jordanka Zlatanova*

Institute of Molecular Biology and **Institute of Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

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This work is devoted to the study of the immunoreactivity of histone H1° and its major structural domains in mononucleosomes. Three types of antibody populations were used: (i) anti-H1° which reacted with antigenic determinants situated along the whole polypeptide chain; (ii) anti-GH5 which recognized epitopes located in the globular region; and (iii) anti-C-tail antibodies reacting specifically with fragment 99–193 of the protein molecule. The anti-GH5 antibodies gave a weak reaction, the C-tail-specific antibodies reacted relatively strongly and the antiserum to the intact molecule showed an intermediate level of reactivity. The relative intensities of the immunoreaction could be interpreted as reflecting the exposure of the antigenic determinants of the individual protein domains in the monosome particle.

Histone H1°; Structural domain; Antibody binding; Mononucleosome; ELISA

1. INTRODUCTION

All lysine-rich histones studied thus far possess a characteristic three-domain structure consisting of a short basic random-coiled N-terminal region ('nose'), an apolar globular central region ('head') and a flexible highly basic C-terminal half [1–4]. The globular part of H1 occupies a position close to the entry and exit points of the DNA in the nucleosome [5,6], while the C-terminus extends over the linker DNA [7]. It is the C-tail of the molecules that is responsible for the folding of the fibre into higher-order structures.

Histone H1 and its differentiation-specific counterparts (histones H5 and H1°) perform distinct functions in the cell. H1 is present in all cell types, irrespective of their proliferation and differentiation status. The accumulation of the erythrocyte-specific histone H5 is connected to the irreversible inactivation of the genome during the final stages of maturation of the red blood cell [8,9]. H1° is characteristically present in tissues with little cell division both in vertebrates and invertebrates [10–17]. This protein species is also involved in terminal differentiation [18,19] but its action can be easily reversed under conditions inappropriate for the execution of the differentiated function [20]. The functional differences among the distinct subtypes of histone H1 suggest that the interaction of the respective homologous domains with chromatin might be different.

In an attempt to approach the issue of how the different structural domains of histone H1° are situated in monosomes we studied the interaction of antibodies to defined regions of the protein molecule with the monosome-contained antigen. The antibody binding was assayed by solid-phase ELISA.

Correspondence address: T. Banchev, Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

Abbreviations: ELISA, enzyme-linked immunosorbent assay; GH5, globular domain of histone H5; PMSF, phenylmethylsulphonyl fluoride

2. MATERIALS AND METHODS

2.1. *Isolation of nuclei and mononucleosomes*

Mouse liver nuclei were obtained by detergent treatment [15]. Chromatin, highly enriched with monosomes, was isolated essentially as in [21]: the autodigestion being performed in 5

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mM Tris-HCl, pH 8.0, 0.25 M sucrose, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM PMSF, and the dialysis in 5 mM Tris-HCl, pH 8.0, 0.5 mM EDTA. Alternatively, monosomes were purified by sucrose density centrifugation of chromatin released from nuclei by mild micrococcal nuclease treatment.

2.2. *Purification of histone H1° and its individual domains*

H1° was isolated from mouse li· r by 5% $HClO_4$ and gel filtration [15]. Its purity was checked by electrophoresis in 20% polyacrylamide gels in the presence of 0.1% SDS [22]. The central globular domain was obtained by trypsin treatment [23]. Pure C-terminal tail containing no part of the globular region (amino acids 99–193) was obtained by acetic acid hydrolysis followed by SDS-electrophoresis [23].

2.3. *Immunochemical procedures*

Antibodies were elicited in rabbits against purified H1° [24]. Anti-GH5 was kindly provided by Russanova et al. [25]. Anti-C-tail antibodies were obtained out of the total anti-H1° antiserum by adsorbance to nitrocellulose filters containing the C-fragment and elution of the bound antibodies [26,27]. ELISA and immunoblotting were performed as in [28].

2.4. *DNA electrophoresis*

The DNA content of the monosome preparations was analysed by electrophoresis in 1.2% agarose gels after standard phenol extraction of the DNA.

3. RESULTS AND DISCUSSION

3.1. *Specificity of the various antibody populations used*

The polyclonal antiserum obtained against purified intact H1° is specific to this protein [24,29] and recognizes epitopes along the whole polypeptide chain (with the possible exception of the short N-nose) (Banchev et al., submitted).

The anti-GH5 antibody reacted with those fragments of H5 which contained the globular region or a part of it [25]. On the basis of the existing homology between the structured domains of H1° and H5 [30] it was expected that this antibody population would react preferentially with the globular region of H1°, which turned out to be the case (not shown).

The anti-C-tail antibodies were thoroughly characterized in [27]: they reacted mainly with the fragment used for adsorption and showed only background reaction with peptides derived from other parts of the molecule.

3.2. *Accessibility of H1° and its structural domains in mononucleosomes*

The availability of H1° antigenic determinants to antibody binding in monosomes immobilized to microtitre wells is an important prerequisite to the further studies of the localization of the individual protein domains in chromatin particles by ELISA. Monosomes were prepared following micrococcal nuclease treatment of isolated nuclei (fig.1). Alternatively, chromatin preparations highly enriched with monosomes were obtained following endogenous nucleolysis and dialysis against low ionic-strength buffer (fig.1). The monosome preparations obtained by either method contained some of the H1° antigenic determinants in a form accessible to antibodies (fig.2).

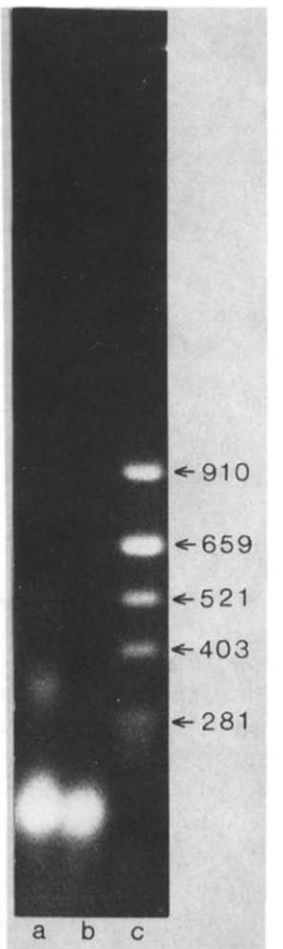

Fig.1. Agarose gel electrophoretic analysis of the DNA content of: (a) chromatin preparations highly enriched with monosomes; (b) monosomes obtained following micrococcal nuclease digestion of isolated nuclei and sucrose density gradient centrifugation; (c) molecular mass marker: *Alu*I-digested pBR322.

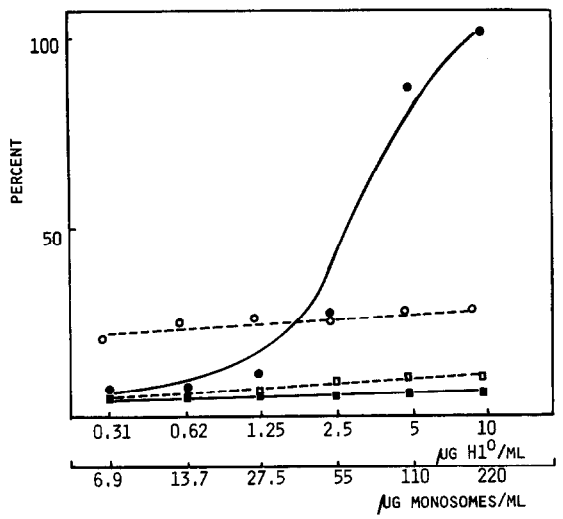

Fig.2. Comparison of the reactivity of the pure antigen to that of the antigen in monosomes by ELISA. The intensity of the reaction with H1° loaded at 10 μg/ml was taken as 100%. (●) Purified mouse liver H1° and (○) monosomes reacted with anti-H1° antiserum, (■) purified mouse liver H1° and (□) monosomes reacted with nonimmune serum.

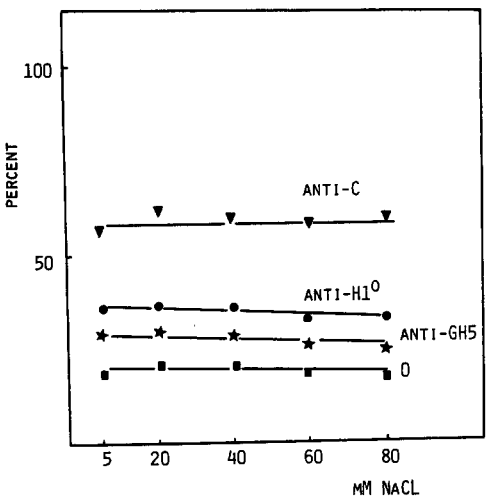

Fig.3. Immunoreactivity of monosomes as a function of the ionic strength with antibodies to intact H1° (●), to the trypsin-resistant core (★), to the C-tail (▼); nonimmune serum (■). The intensity of the reaction with pure H1° at the lowest anti-H1° antiserum dilution is taken as 100%.

The immunoreactivity of monosomes with domain-specific antibody populations was studied as a function of the ionic strength following the procedure of Russanova et al. [25]. Monosomes were incubated in solutions of different NaCl concentrations, the induced conformational changes, if any, were fixed with glutaraldehyde and the material was used as an antigen in ELISA performed under standard ionic conditions.

The data in fig.3 show the following things: (i) as expected the immunoreactivity was independent of the ionic strength (the ion concentration in the studied range does not significantly affect the conformation of the monosomes [i.e. 31,32]; (ii) the anti-GH5 antibodies gave a relatively weak reaction which was nevertheless reproducibly higher than that of the nonimmune serum. Literature data concerning monosomes are lacking but studies with chromatin have yielded controversial results. Russanova et al. [25] found that the globular domain was inaccessible to antibodies at all salt concentrations studied while Mendelson et al. [33] obtained an intense immunoreaction even in 80 mM NaCl. Our results seem to be closer to those of Russanova et al. [25] as even in monosomes the globular domain seems to be relatively inaccessible.

The C-tail-specific antibodies reacted relatively strongly and the antiserum to the intact molecule showed an intermediate level of reactivity, somewhat closer to the reaction with the anti-GH5. It should be noted that the dilutions of the respective antibodies were carefully chosen so as to give comparable response with the intact H1° molecule. In such a case, the relative intensities of the immunoreaction could be interpreted as reflecting the relative exposure of the antigenic determinants of the individual protein domains in the monosome particle.

4. CONCLUDING REMARKS

The results of this study show that the antigenic determinants of the globular part of histone H1° are somehow hindered from interaction with antibodies, most probably due to participation in binding to other components of the monosomes (DNA or proteins). The C-terminal tail seems to be much more accessible to antibodies, meaning that it is relatively exposed in the particles.

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