Specificity studies on anti-histone H1 antibodies obtained by different immunization methods

Ljuba Srebreva and Jordanka Zlatanova*

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

Received 3 March 1986

Antibodies were elicited against chromatographically purified histone H1 subfractions or against their complexes with RNA and their specificity studied by enzyme-linked immunosorbent assay. The results show that complexing of the pure protein with RNA (i) does not lead to any significant increase in the antibody titer and (ii) results in obtaining antibodies predominantly against the common antigenic determinants present in the H1 histone class. On the other hand, using pure histone fractions for immunization gives rise to antibody populations reacting mainly with the subfraction-specific determinants on the histone molecule. In view of these results the literature data should be interpreted with caution.

Histone H1 *Histone antibody* *Antibody specificity* *ELISA*

1. INTRODUCTION

The production of relevant antibodies with well defined specificity is a prerequisite for the application of immunological methods to chromatin studies. The reported poor immunogenicity of the histones [1] has been overcome by using histone-RNA complexes for immunization [2]. Bearing in mind that spatial folding of proteins plays an important role in determining their antigenic specificity [3,4], it is clear that complexing of the histones with nucleic acids might affect not only the titer but also the composition and specificity of the resulting antibody population. However, as pointed out by Stollar [5] no systematic comparison has been made between antibodies elicited against pure histone fractions and against histone-RNA complexes. The purpose of this study was to compare the two types of antibodies, obtained with and without RNA.

We have chosen to work with representatives of the lysine-rich (H1) histones as they represent a heterogeneous group of several closely related protein species [6] so (i) specificity studies can be performed on antibodies elicited against individual members of the group and (ii) the antigens compared can include both closely related (other members of the H1 group), more distantly related (nucleosomal histones) and totally unrelated proteins. In addition, we are specifically interested in one particular H1 subfraction, the so called H1o, which is shown to be involved in such vital cellular processes as proliferation [7], differentiation [8,9] and malignant transformation [10–12]. The availability of specific anti-H1o antibodies would be useful in approaching the issue of its role in chromatin structure and function as well as in evaluating the relatedness of this protein from different sources.

* To whom correspondence should be addressed at the Institute of Genetics

Abbreviations: PBS, phosphate-buffered saline; MCF, microcomplement fixation; ELISA, enzyme-linked immunosorbent assay; HMG, high mobility group (proteins)

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

In this work we show that injecting histone H1-RNA complexes (i) does not lead to any significant increase in the antibody titer and (ii) results in obtaining antibodies predominantly against the common antigenic determinants present in the H1 class. On the other hand using pure histone fractions for immunization gives rise to antibody populations reacting mainly with the specific determinants on the histone molecule. The conclusions reached in studies with anti-H1o antibodies were confirmed on antibodies raised against H1AB, a complex molecular mixture comprising several individual protein species.

2. MATERIALS AND METHODS

2.1. *Preparation and purity assessment of antigens*

Lysine-rich histones from mouse liver nuclei were extracted with 5% $HClO_4$ [13] and fractionated into H1AB and H1o by gel exclusion chromatography on a BioGel p100 column [14] as detailed in [15]. These fractions are considered sufficiently pure in view of the specificity of the $HClO_4$ extraction [13,16] and the ability of BioGel columns to separate the lysine-rich histones from co-extracted HMG proteins [16]. Our histone preparations revealed no sign of contamination even when heavily overloaded electrophoretic gels were inspected. On the other hand contamination with HMG proteins seemed unlikely as all antihistone antisera available in our laboratory gave only background cross-reactions with calf thymus HMGs, contrary to what was to be expected if these proteins were present in the histone preparations used for immunization.

Chicken erythrocyte histone H5 was prepared as in [17] and calf thymus HMG1 was chromatographically purified on CM-Sephadex according to [18]. Mouse liver nucleosomal histones were obtained from total 0.25 N H_2SO_4 nuclear extract depleted of H1 by 5% $HClO_4$ extraction. Human and bovine plasma albumins were purchased from Reanal, Hungary and Biomed, Poland.

2.2. *Antiserum production*

Antibodies were elicited in rabbits against either pure histone fractions or their complexes with yeast RNA [2]. The immunogens were dissolved in PBS and 150 μg protein was administered at each injection. For the first three injections the samples were emulsified with an equal volume of complete Freund's adjuvant and applied intradermally to multiple sites on the back of the animals at weekly intervals. Intravenous boosters were done roughly once a month.

2.3. *Immunological tests*

ELISA was performed on tissue culture multiwell plates (Linbro, Flow, USA). The histone (2–5 μg/ml in PBS) was adsorbed to the surface by overnight incubation at 0°C. The remaining unadsorbed material was washed away by PBS-Tween 20 (0.5 ml Tween/l) and the plates were incubated with 1–3% albumin in PBS for 1–2 h at 37°C. Following another wash with PBS-Tween the plates were treated with the specific antihistone antiserum appropriately diluted with 1% albumin in PBS-Tween (3 h at 37°C). The plates were further washed and the attached specific antibodies detected by means of peroxidase-conjugated anti-rabbit IgG serum (Sigma, MO, USA) diluted 100-fold with 1% albumin in PBS-Tween. Incubation was for 1 h at 37°C. At the last step the plates were washed and incubated for 30 min at 37°C with 200 μl *o*'-diphenylamine (20 mg in 60 ml citrate buffer, pH 5.6, 10 μl H_2O_2). The reaction was terminated by addition of 50 μl 4 N H_2SO_4. The colour intensity was determined on a Multiscan (Labsystems, Finland).

3. RESULTS AND DISCUSSION

MCF is the most widely used specificity assay in histone immunology [1]. However, a few years ago Moorman et al. [19] suggested that this method was not applicable to test antihistone serum specificity. Our own comparison of MCF and ELISA data (Zlatanova et al., submitted) confirmed the conclusions of these authors; in view of the additional advantages of ELISA (ease of performance, sensitivity, etc.) it was chosen for further work.

To permit a proper comparison between the antisera the proteins used as immunogens and antigens in ELISA were from one and the same batch. The data for the anti-H1o antisera are presented in fig.1. As can be seen the specificity of the antiserum elicited against H1o alone is much

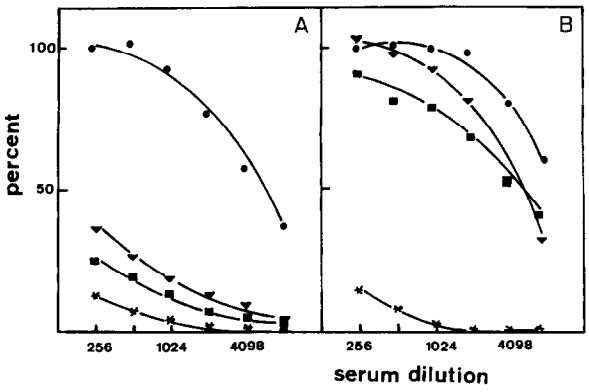

Fig.1. Cross-reactivity data for anti-histone H1o antisera, raised against either the pure histone fraction (A) or its complex with RNA (B). The intensity of the ELISA reaction is expressed as percentage of the reaction of the homologous antigen (mouse liver H1o) at the lowest antiserum dilution tested (256). The points represent averages from 7 independently performed assays – individual points differed from each other by no more than 10%, usually by much less. (●) Mouse liver H1o, (▼) mouse liver H1AB, (■) chicken erythrocyte H5, (★) nucleosomal histones from mouse liver, HMG1 from calf thymus and bovine plasma albumin.

higher than that of the respective antiserum obtained against H1o-RNA. This concerns the cross-reactivity with the other members of the H1 class. It is worth noting that the two sera show only a background reaction with the nucleosomal histones which are indistinguishable from the totally unrelated proteins used as controls: calf thymus HMG1 and human (or bovine) plasma albumin. Thus we are dealing with antibodies highly specific to the H1 class. The differences between the two antibody populations lie in their specificity concerning the closely related proteins of the H1 family: the H1o-RNA antiserum does not possess high specificity for the homologous antigen and seems to contain antibody molecules predominantly against the antigenic determinants common to all members of the H1 family. On the other hand the anti-H1o antiserum is highly specific for H1o, i.e. it contains antibodies mainly against the antigenic determinants specific to this protein.

Such a picture is surprising only at first sight. It is analogous to the situation in chromatin: the interaction of histones with DNA leads among other things to a burial of the specific antigenic determinants for each H1 molecular subtype inside the complex macromolecular structure, leaving predominantly the common determinants exposed to the solvent and hence to interaction with the bulky antibody molecules [20]. The interaction of the purified histone fractions with RNA may have a similar effect: burial of the specific determinants inside the insoluble protein-RNA complex formed and exposure of the common H1 class determinants.

It is known that the specificity of a given antiserum broadens with prolonged immunization until a plateau specificity is reached (e.g. [21]). Although the two sera studied are of comparable 'age' (blood withdrawn about 4 months after the beginning of immunization) there exists the possibility that the establishment of the plateau specificity occurs faster in the case of the H1o-RNA antiserum (because of either the presence of RNA or the individual rabbit reaction) and hence its lower specificity. In such a case it would be relevant to compare the cross-reactivity data for sera obtained after immunization programmes of different duration. The results of such a comparison are presented in fig.2. As expected, antisera of different ages show different behaviour with a well expressed broadening of specificity with time. This, however, concerns only the reaction of the closely related H1 proteins. As far as the cross-reactivities with the other proteins are concerned, the degree of reaction with these antigens lessens with time.

As evident from these results even very 'young' sera obtained against H1o-RNA are considerably less specific than sera against the pure histone frac-

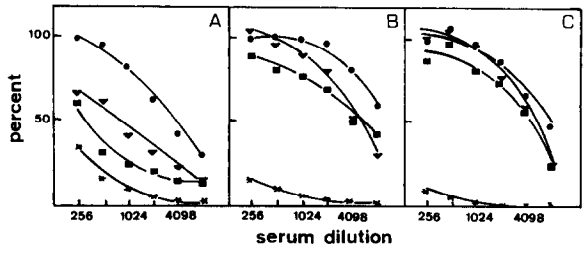

Fig.2. Cross-reactivity data for anti-H1o-RNA antisera obtained after immunization programmes of different duration: (A) 2, (B) 4 and (C) 6.5 months, respectively. See legend to fig.1.

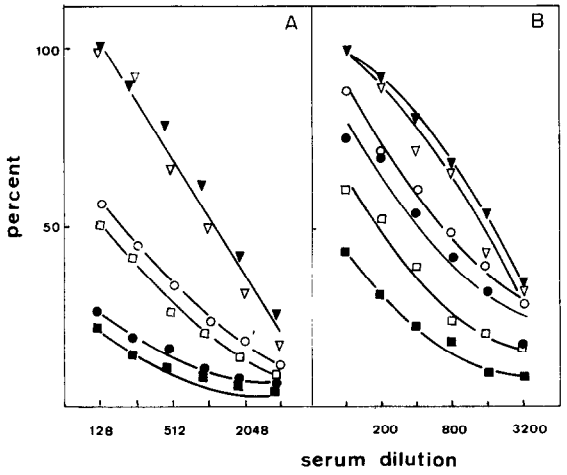

Fig.3. Comparison of cross-reactivities for two pairs (A and B) of anti-mouse liver H1AB antisera elicited against pure histone fractions (closed symbols) and histone-RNA complex (open symbols). See legend to fig.1.

tion. Thus, the degree of cross-reactivity among members of the H1 class seems to depend on whether the immunization has been performed with pure histone fraction or with its complex with RNA.

To check to what extent the observed difference between the two types of antisera was a general phenomenon, we repeated the specificity studies on comparable sera elicited against mouse liver H1AB; a mixture of several different H1 molecular types. Once again the specificity of the antisera elicited against the pure protein was greater than that of the antisera against the protein-RNA complex (fig.3A and B).

4. CONCLUDING REMARKS

The systematic comparison of antisera elicited against one and the same histone immunized either alone or as a complex with RNA has led to the following conclusions: (i) the antibody titer is not dependent on whether the protein is injected alone or with RNA at least for the histones of the H1 class and (ii) the pure histone used as immunogen evokes the formation of more specific antibody populations as compared to the histone-RNA complex.

In the light of our results, data in the literature obtained predominantly with antibodies elicited against histone-RNA complexes should be viewed with some caution. This is especially true for studies using immunological cross-reactions as a measure of sequence homologies between chromosomal proteins from different sources or between individual members of one and the same histone class from the same source. It might well be that the calculated homologies represent overestimations as antibodies elicited against histone-RNA complexes give artificially stronger cross-reactions as compared to antibodies against pure proteins.

REFERENCES

[1] Bustin, M. (1978) in: The Cell Nucleus (Busch, H. ed.) vol.IV, pp.195–238, Academic Press, New York.
[2] Stollar, B.D. and Ward, M. (1970) J. Biol. Chem. 245, 1261–1266.
[3] Sela, M. (1969) Science 166, 1365–1374.
[4] Sela, M. (1971) Ann. NY Acad. Sci. 190, 181–202.
[5] Stollar, B.D. (1978) Methods Cell Biol. 18, 105–122.
[6] Elgin, S.C.R. and Weintraub, H. (1975) Annu. Rev. Biochem. 44, 725–774.
[7] Panyim, S. and Chalkley, R. (1969) Biochem. Biophys. Res. Commun. 37, 1042–1049.
[8] Zlatanova, J., Oberhummer, K. and Swetly, P. (1980) in: In Vivo and In Vitro Erythropoiesis: the Friend System (Rossi, G.B. ed.) pp.297–307, Elsevier/North-Holland, Amsterdam, New York.
[9] Gjerset, R., Gorka, C., Hasthorpe, S., Lawrence, J.J. and Eisen, H. (1982) Proc. Natl. Acad. Sci. USA 79, 2333–2337.
[10] Lea, M.A., Youngworth, L.A. and Morris, H.P. (1974) Biochem. Biophys. Res. Commun. 58, 862–867.
[11] Maraldi, N.M., Cocco, L., Papa, S., Capitani, S., Mazzotti, G. and Manzoli, F.A. (1978) IRCS Med. Sci. 6, 78.
[12] Tsanev, R. and Hadjiolov, D. (1978) Z. Naturforsch. 91, 237–247.
[13] Johns, E.W. (1964) Biochem. J. 92, 55–59.
[14] Böhm, E.L., Strickland, W.N., Strickland, M., Thwaits, B.H., Van der Westhuizen, D.R. and Von Holt, C. (1973) FEBS Lett. 34, 217–221.
[15] Srebreva, L. and Zlatanova, J. (1983) Biochim. Biophys. Acta 740, 163–168.

[16] Goodwin, G.H., Walker, J.M. and Johns, E.W. (1978) in: The Cell Nucleus (Busch, H. ed.) vol.VI, pp.181–219, Academic Press, New York.
[17] Srebreva, L. and Zlatanova, J. (1983) J. Biochem. Biophys. Methods 8, 85–86.
[18] Goodwin, G.H., Nicolas, R.H. and Johns, E.W. (1975) Biochim. Biophys. Acta 405, 280–291.
[19] Moorman, A.F.M., Cossé, A.A., De Graaf, A. and Charles, R. (1980) FEBS Lett. 114, 25–29.
[20] Zick, Y., Goldblatt, D. and Bustin, M. (1975) Biochem. Biophys. Res. Commun. 65, 637–643.
[21] Prager, E.M. and Wilson, A.C. (1971) J. Biol. Chem. 19, 5978–5989.