PURIFICATION BY IMMUNOADSORPTION AND IMMUNOCHEMICAL PROPERTIES OF HISTONE H3

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1. Introduction

Antibodies to histones represent a powerful tool for investigating the structure of chromatin and chromosomes [1]. The distribution of histones in chromatin and in nucleosomes has been studied by complement fixation [2,3], immuno-electron microscopy [4], radioimmunoassay [5] and immunofluorescence [6]. It has been found, for instance, that histones H1 and H2B are more exposed in rat liver chromatin than histones H3 and H4 [3], and that more than 90% of the nucleosomes contain histone H2B [4]. The availability of histone antisera raises the possibility of locating the immunologically reactive regions of histones [7,8], and of preparing the corresponding antibody fractions. Such antibodies directed against well-defined portions of histone molecules could be used for determining the extent to which a particular histone is buried inside nucleosomes or is complexed with other chromatin components. In the present report we describe the location of three antigenic determinants in histone H3 from chicken erythrocytes, as well as the purification of this histone by immunoadsorption using antibodies to one of the determinants.

2. Experimental

Histone H3 from chicken erythrocytes was isolated and purified as described previously [9]. The twelve fragments of H3 illustrated in fig.1 and fig.4 were obtained by cleavage with cyanogen bromide, *N*-bromosuccinimide and dilute HCl [10].

Antibodies to H3 were obtained in rabbits and complement fixation tests were performed as described previously [8]. The antigenic activity of the fragments was tested by determining the ability of the peptides to inhibit binding between ^{14}C-labelled H3 and its specific antibodies. Reductive methylation with [^{14}C]formaldehyde was used to prepare ^{14}C-labelled H3 [11]. The reaction of labelled H3 with anti-H3 rabbit antibodies was followed by precipitation with goat antiserum to rabbit IgG. As illustrated in fig.2, maximum binding occurred when 0.5 mg/ml anti-H3 globulins were precipitated with 200 μl anti-rabbit IgG goat globulins. Globulin preparations were obtained from antisera by precipitation with 4 M ammonium sulphate. For the inhibition assays the various fragments were incubated with anti-H3 globulins for 30 min at 37°C, prior to the addition of labelled H3 under the conditions of maximum binding shown in fig.2.

Histone H3 was separated from a mixture of the various histones present in extracts of chromatin from chicken erythrocytes [9] and yeast [12] by means of a Sepharose 4B immunoadsorbent column prepared with antibodies specific for the CN1 fragment of H3. These antibodies were isolated from anti-H3 globulins on a column of activated CH-Sepharose 4B to which fragment CN1 had been attached covalently in the presence of 0.1 M bicarbonate buffer, pH 8.0. The CN1-specific antibodies were eluted with 0.1 M glycine–HCl buffer, pH 2.5, containing 0.5 M NaCl, concentrated by ultrafiltration, and attached to

Sepharose 4B activated with cyanogen bromide [13]. A total of 45 mg CN1 antibodies were coupled to 100 mg activated Sepharose.

3. Results and discussion

The ability of increasing quantities of the twelve fragments of H3 to inhibit the binding between ^{14}C-labelled H3 and its antibodies is illustrated in fig.3. The results are expressed as percentage inhibition produced by molar excesses of the fragments over 0.15 μg H3.

In a preliminary study of the antigenic activity of the three fragments CN1, CN2 and CN3 by the complement fixation technique [8], the CN3 peptide was found to possess the highest activity. In order to establish whether inhibition of complement

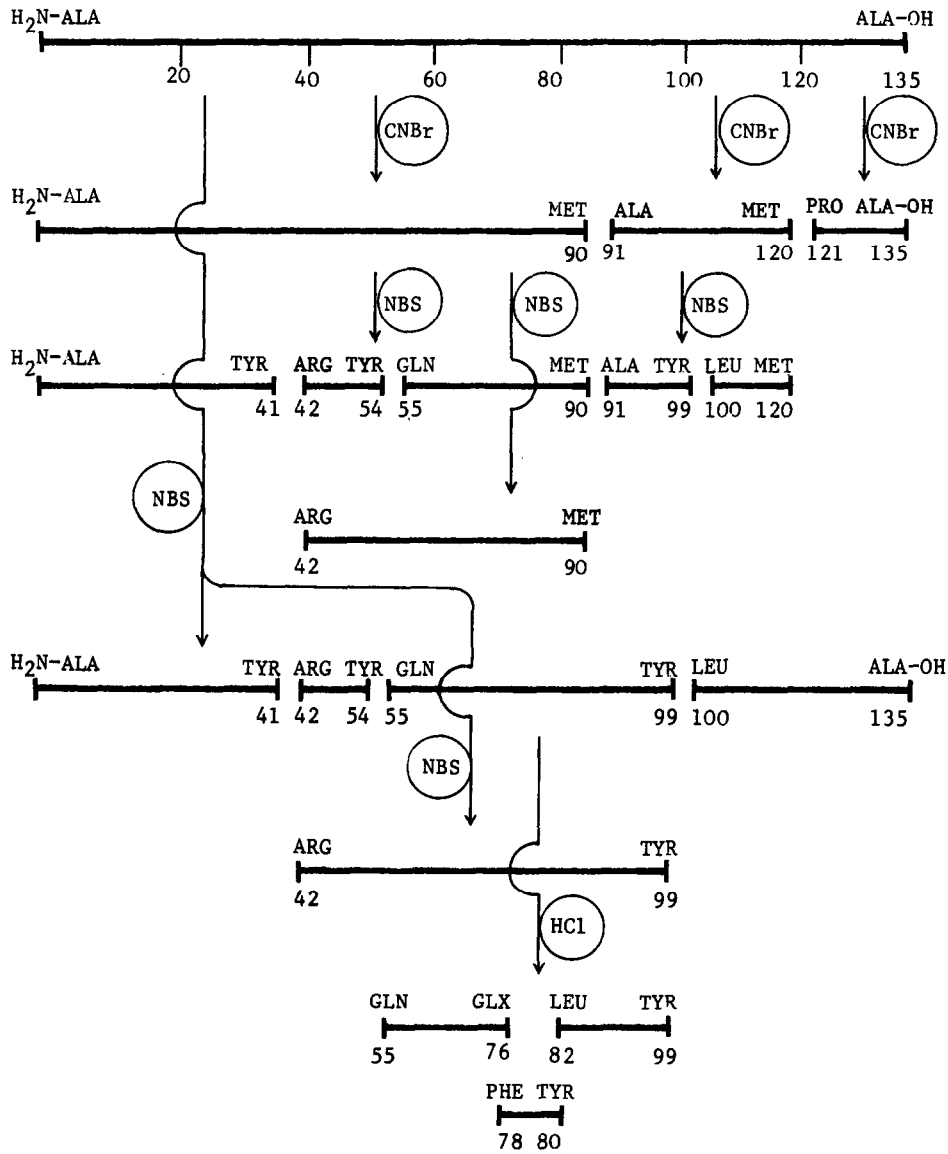

Fig.1. Chemical degradation steps used to obtain various H3 fragments.

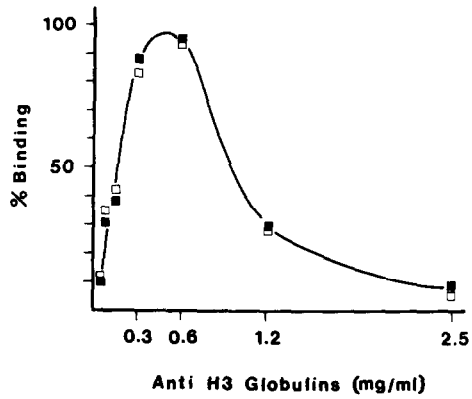

Fig.2. Binding curve of ^{14}C-labelled H3 ((■——■) 10 μg; (□——□) 20 μg) precipitated with anti-H3 rabbit globulins and goat anti-rabbit globulins (200 μl).

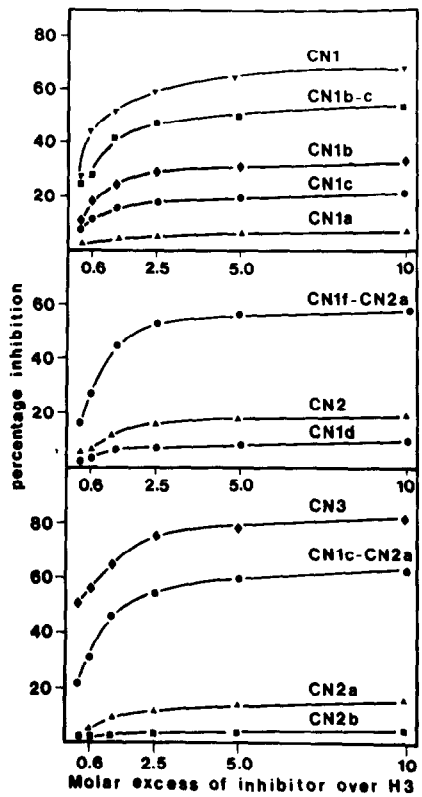

Fig.3. Inhibition of binding of H3 to anti-H3 globulins by various H3 peptide fragments. Prior to the addition of 0.15 μg H3, the globulins were incubated for 15 min., at 37°C with the inhibitor peptides.

fixation and inhibition of direct binding as measured by radioimmunoassays give comparable activity data, both techniques were compared with three peptides. The results showed that the relative immunological activities detected by the two tests were similar but that radioimmunoassays were slightly more sensitive.

The specificity of binding was shown by the inability of fragment CN3 to inhibit the reaction between ^{14}C-labelled CN1 fragment and CN1 antibodies purified by immunoadsorption.

The extent of inhibition produced by the twelve fragments in radioimmunoassays is summarized in fig.4. Results are expressed as percentage inhibition caused by a ten-fold molar excess of peptide over intact H3. By comparing the activities of fragments CN1b, CN1c, CN1d and CN1b–c one may conclude that one antigenic determinant lies in the region corresponding to residues 40–60. From a comparison of the activities of fragments CN1d, CN2, CN1c–CN2a and CN1f–CN2a it appears that a second determinant is located in the vicinity of residues 80–100, while a third one is obviously located in the CN3 region. Thus the present results confirm earlier indications [8] that the H3 molecule possesses three antigenically active regions.

Attempts to prepare an immunoadsorbent with complete H3 failed, but good results were obtained

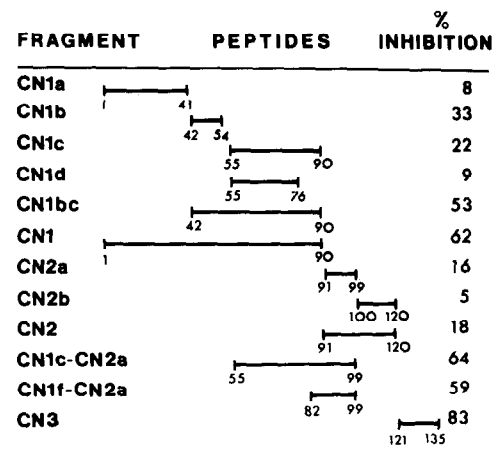

Fig.4. Percentage inhibition of the H3-anti H3 reaction observed at a ten-fold molar excess of the various fragments over ^{14}C-labelled H3.

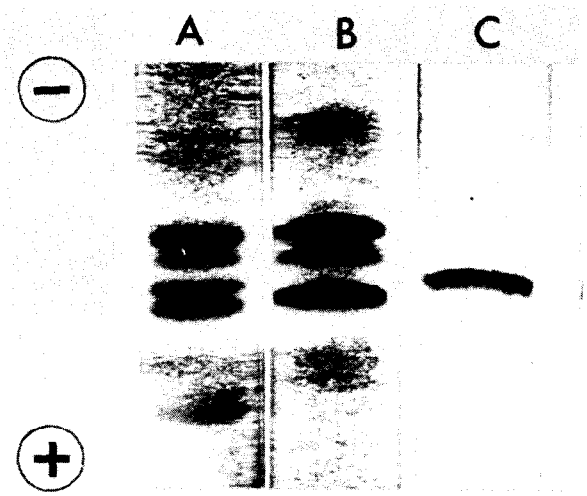

Fig.5. Polyacrylamide gel electrophoresis patterns of chicken histones. (A) Total histones (30 μg) applied onto Sepharose immunoadsorbent column. From top to bottom, bands correspond to H4, H2A, H2B, H3, H5 and H1. (B) Eluate of histones (40 μg) not retained by column: H4, H2A, H2B, H5 and H1. (C) Histones H3 (10 μg) released from column with glycine buffer, after specific adsorption to CN1-antibodies. Gels were made according to [15] and stained with amido black.

when fragment CN1 was coupled to Sepharose. This allowed the separation of CN1-specific antibodies from the total IgG fraction of a rabbit anti-H3 serum. The CN1 antibodies were coupled to Sepharose and were used to separate H3 from mixtures of total histones from chicken erythrocytes and yeast cells. When a mixture of 8 mg chicken histones was applied to the immunoadsorbent column, 750 μg pure H3 were recovered (fig.5). The identity and purity of H3 was confirmed by amino acid analysis.

Since histone H3 is an evolutionary extremely stable protein [14] one could have assumed that the same H3 purification procedure [9] would be applicable to materials from various sources. However, all H3 histones do not have a single cysteine residue [16], a property which is needed for dimerization and subsequent separation by gel filtration [9]. The purification of H3 by immunoadsorption illustrated in the present report is an attractive alternative purification method, and may also resolve the difficulties presented by possible conformational changes in histone structure that occur during certain extraction procedures [17].

Acknowledgements

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