Antibody against globular domain of H1° histone

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Antibodies against the globular domain of histones H1° and H5 were developed in rabbit. The antibody against the globular domain of H5 cross-reacted with H1° but not with H1; the antibody against the globular domain of H1° did not cross-react with H5, H1 or with HMG proteins. The globular domain of H1° therefore appears to have an immunological determinant(s) which does not exist in H1 and H5. By use of these antibodies, we show that nucleated erythrocytes of bullfrog contain an H1°-like protein (not an H5-like protein). This observation coincided with the report of Shimada, T. et al. [J. Biol. Chem. 256 (1981) 10577–10582]. These antibodies have application in detecting H1°-like proteins in eukaryotic cells.

Histone H1° *Histone H5* *Erythrocyte differentiation* *Immunoblotting*

1. INTRODUCTION

Very lysine rich histones have been implicated in the stabilization of higher order chromatin structures [2], in the processes of chromosome condensation, cell division and transcriptional activity [2–5]. In the current model of the nucleosome [6,7] the central globular domain of histone H1 is located at the DNA entry and exit points of the two turns of DNA [2,8]. One of the subfractions of the class of H1 histones called H1° [9] has been inversely correlated with mitotic activity [10]. It is of particular interest that the central globular domain of mammalian H1° has considerable sequence homology with the same domain of the very lysine rich histone H5. H5 is found in the nucleated erythrocyte of birds [11], reptiles [12], amphibians [13] and fish [13] and is thought to be involved in the terminal differentiation of erythrocytes. The sequence homology of these central domains of H1° and H5 also results in very similar conformations [10] and in immunological cross-reactivity [15,16]. The early report that bullfrog erythrocytes contain histone H5 [13] has been questioned by a recent report of the presence of an H1°-like protein rather than H5 [1]. Methods are therefore required to distinguish H1° from H5 to clarify this situation and also to identify H1° histone in cells and tissues.

Abbreviations: GH1, GH1°, GH5, globular H1, H1° and H5

2. MATERIALS AND METHODS

2.1. *Antigens and antisera*

Histones H1 and H1° were prepared from beef liver as in [17]. Histone H5 was prepared from chicken erythrocyte as in [18]. Globular parts of H1°, H1 and H5 were prepared by trypsin digestion as in [10,19,20] in the presence of 1.0 M sodium chloride and purified by Sephadex G-50. Antisera against globular H5 and globular H1° were obtained from rabbits immunized every two weeks with 100 μg protein-complete Freund's adjuvant (Difco) (1:1, v/v). After the second injection, the titer against each protein was observed. The sera used here were obtained after the fourth injection.

2.2. *Electrophoretic transfer of proteins and immunoblotting*

Proteins were electrophoresed by the use of 15% or 17.5% acrylamide gel containing SDS as in [21].

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The transfer of the proteins from SDS gels to nitrocellulose filter (Bio Rad) and immunoblotting were performed essentially as in [22].

2.3. *Isolation of histones from bullfrog*

The erythrocytes were obtained by heart puncture from bullfrog, *Rana catesbeiana*. The nuclei were isolated from erythrocytes of bullfrog or chicken by use of saponin as in [23]. After isolation of crude nuclei, acid soluble nuclear proteins were extracted by 0.4 N sulfuric acid, precipitated by 25% trichloroacetic acid and washed with acidified acetone and acetone.

3. RESULTS AND DISCUSSION

3.1. *Immunoassay of electrophoretically separated histones*

The globular part of H1° (GH1°) was highly immunogenic as also reported for the globular part of H5 (GH5) [16]. Lamb thymus core histones, globular domains of H1°, H5, H1, and total molecules of H1, H1° and H5 were transferred to nitrocellulose filter and checked for cross-reactivity against anti-GH1° or anti-GH5. Fig.1 shows that anti-GH5 serum cross-reacts with H5, H1° and GH1° but does not cross-react with H1, GH1 and core histones (fig.2). GH5 therefore has some immunological determinants in common with GH1°. This result agrees with [15,16]. In contrast, anti-GH1° serum cross-reacts with H1° but does not cross-react with GH5, H5, H1, GH1 and core histones. These data imply that there must be an immunological determinant(s) in GH1° which does not exist in GH1, GH5, H1 and H5. Although it is not clear which sequence or structure in GH1° is the immunological determinant which does not exist in GH5, this antiserum is very useful in determining the existence of H1° in organisms.

3.2. *Detection of an H1°-like protein in bullfrog erythrocyte*

Acid extractable nuclear proteins from bullfrog were transferred to nitrocellulose filter and reacted with anti-GH1° serum or anti-GH5 serum. The proteins of the H1 and H5 region of the gel are less

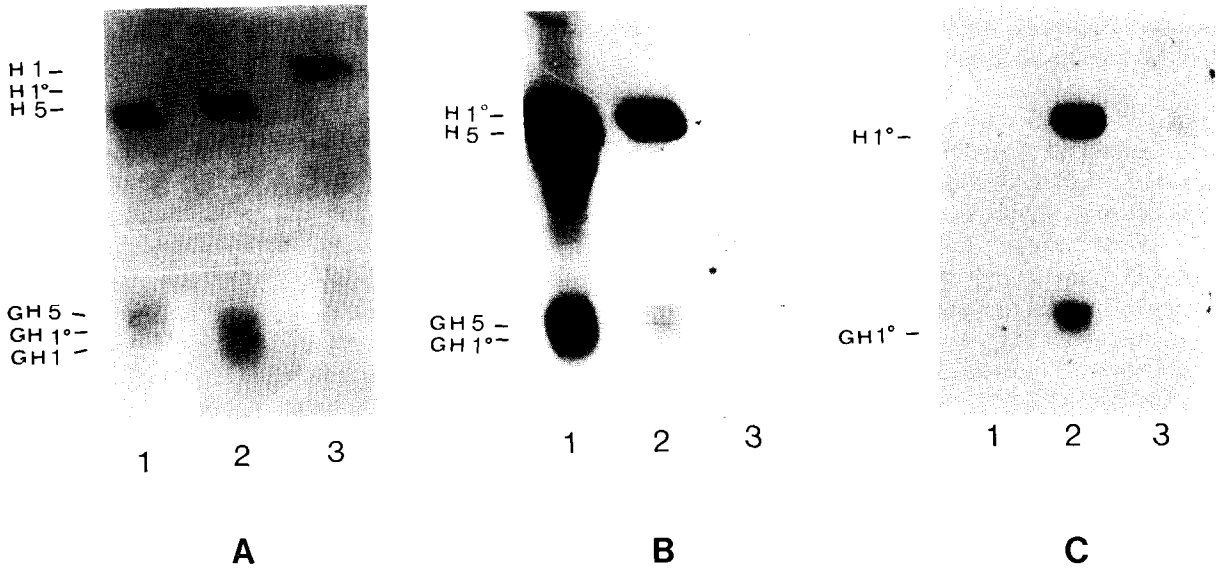

Fig.1. Reaction of anti-GH1° and anti-GH5 serum with H1, H5, H1°, GH1, GH1° and GH5. Purified H1, H1°, H5, GH1, GH1° and GH5 were electrophoresed in SDS gels and transferred to nitrocellulose filter as described in section 2. After staining with Amido black, the transferred proteins were incubated with indicated antisera (1 : 40 dilution), with ^{125}I-labelled protein A and autoradiographed by use of Kodak XAR-5 or XS-5 film. (1) H5 and GH5 (chicken erythrocyte), (2) H1° and GH1° (beef liver), (3) H1 and GH1 (beef liver). (A) Protein transferred to nitrocellulose filter from SDS gel and stained with Amido black. Autoradiography: (B) Reaction with anti-GH5 serum (serum also reacted with degraded H5 molecule, lane 1). (C) Reaction with anti-H1° serum.

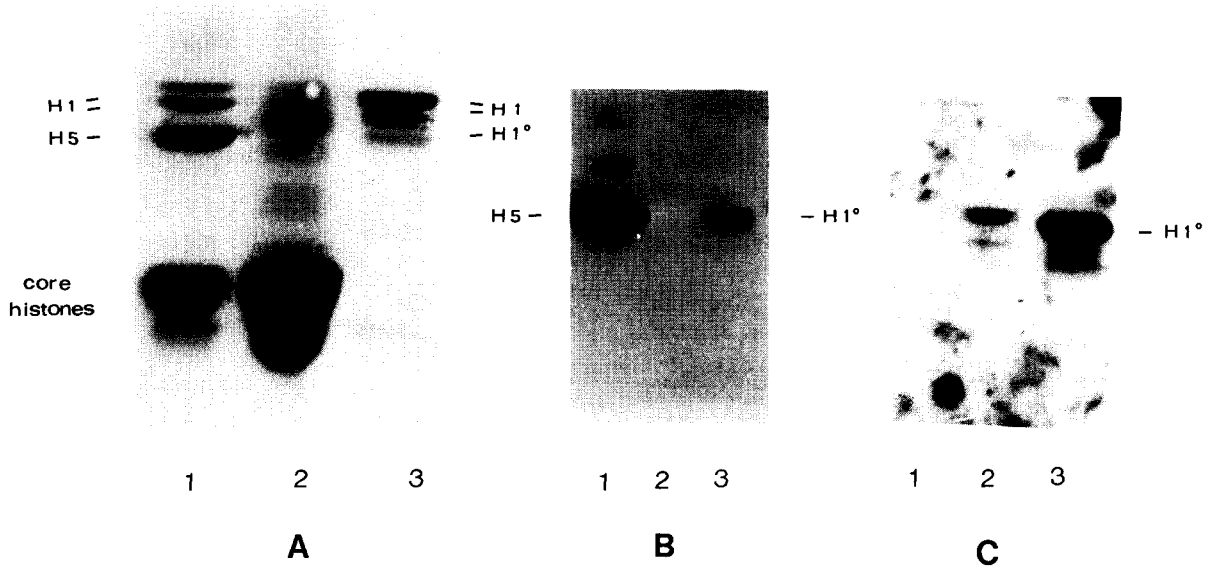

Fig.2. The existence of the H1°-like protein in bullfrog erythrocyte. The histones from chicken erythrocytes (1), bullfrog erythrocytes (2) and H1/H1° from beef liver (3) were electrophoresed and processed for immunoblotting with anti-H1° and anti-H5 serum as described for fig.1. (A) Proteins transferred to nitrocellulose filter, stained with Amido-black. Autoradiography: (B) Reacted with anti-GH5 serum. The band above H5 shown in (1) was a slower migrating protein than H1. (C) Reacted with anti-GH1° serum.

clear with bullfrog than with chicken erythrocyte, probably due to contaminating proteins. However, anti-GH5 serum did not react with any proteins from bullfrog while anti-GH1° serum reacted with one of these proteins (fig.2). The M_r-value of this protein is a little higher than H1° of mammalian cells as determined by SDS-gel electrophoresis. This result shows that this protein has an immunogenic determinant(s) in common with GH1° but does not have any determinants of GH5. This protein is not identical with mammalian H1° and can be called an H1°-like protein as reported earlier.

When the injection was continued after the anti-GH1° serum reported here was obtained, this rabbit developed anti-GH1° serum which reacted both with GH1° and GH5. The separation of anti-GH1° antibody from anti-GH5 antibody is proceeding now in our laboratory. Recently, by use of this serum, an H1°-like protein was detected in chromosomal proteins of the slime mold *Physarum polycephalum* (in preparation). This anti-GH1° serum seems to be very useful to distinguish H1°-like proteins (or H1°) from H5-like proteins (or H5).

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