Antibodies against the Folding Domain of Histone H5 Cross-react with H1* But Not with H1*

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James Allan§, Bryan J. Smith§§, Barbara Dunn***, and Michael Bustin**

From the §Department of Biophysics, Kings College, London, WC2B 5RL, England, the Institute of Cancer Research, London, SW3 England, and the ** Laboratory of Molecular Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

Antibodies to the folding domain (residues 22–100) of histone H5 were elicited in rabbits. Analysis of the specificity of these antibodies by enzyme-linked immunosassay and by diazobenzoyloxymethyl cellulose transfer techniques revealed that the antibody cross-reacts strongly with intact H5 and histones H1°a and H1°b purified from ox liver but not with the four core histones, with H1 purified from chick erythrocytes or calf thymus, or with high mobility group proteins. We conclude that the globular region of H5 is serologically homologous to that of H1* and suggest that possible functional similarities between the two proteins reside in this region.

In contrast to the highly conserved core histones, the lysine-rich histones H1 display considerable species and organ specificity in their primary structure (1, 2). One of the more specialized variants in this group is the chicken erythrocyte histone H5. During erythropoiesis histone H1 is replaced by H5 (3, 4). The switch from H1 to H5 in the nucleated erythrocytes is associated with chromatin condensation and gene inactivation (5). Recent studies demonstrated that one of the H1 subfractions, H1°, is similar to avian H5 with respect to sequence homology (6, 7), structural features (8), and immunological cross-reactions (9). The H1° histone seems to be homologous or identical with a differentiation-related, inducible protein called IP25 (10, 11). These proteins seem to accumulate in chromatin of cells which are either terminally differentiated or have a lower rate of cell division (12–15).

In addition to primary structure homology the lysine-rich histones share conformational similarities. Under appropriate conditions a part of their polypeptide chain adopts a folded conformation (16). In this folded conformation the lysine-rich histones are able to modulate the degree of compactness of the chromatin (17). The mechanism by which the H1 and H5 molecules bring about this compaction is not fully understood. Evidence has been presented, however, that the globular domain of these histones stabilizes the chromatosome at the exit points of the 165-base pair DNA strand (18).

Here we demonstrate that antiserum raised against highly purified folding domain of H5 cross-reacts strongly with H1° purified from ox liver but shows little cross-reactivity with H1 purified from calf thymus or chicken erythrocytes.

MATERIALS AND METHODS

Proteins—H1 and H5 were prepared from chicken erythrocyte chromatin by chromatography on hydroxylapatite and Amberlite CG-50 (19). Calf thymus H1, prepared by acid extraction, was further purified by hydroxylapatite chromatography. H1° from ox liver subfractions a and b were prepared as previously described (20). The folding domain of H5 (GH5) was prepared by limited trypsin digestion and was purified as described in a previous report (18). Protein concentrations were determined either by nitrogen analysis (21) or from their amino acid analyses.

Antiserum—Anti-GH5 antibodies were obtained from rabbits, each initially immunized by intradermal injection at multiple sites with 200 μg of GH5 in 2.0 ml of 66% complete Freund’s adjuvant. Identical booster injections were administered 14 and 21 days later. 28 days after initial immunization an intravenous injection with 25 μg of GH5 was administered. Starting 35 days after immunization, the rabbits were bled at weekly intervals. Antibodies were detected and characterized by an enzyme-linked immunosassay using alkaline phosphatase-conjugated goat anti-rabbit IgG obtained from Miles. Routinely, the antigen was added to microtiter plates for 18 h at 4 °C in 100 μl of sodium phosphate, pH 6.8. Unbound antigen was washed off with 0.05 M NaCl, 0.05 M sodium phosphate, pH 7.4, 0.05% Tween-20 (PBS-Tween). The wells were then treated with 1% horse serum in sodium phosphate. After removal of the horse serum, rabbit antibodies, diluted in 1% horse serum, were added for 2 h at 23 °C. Unbound sera were removed with PBS-Tween and alkaline phosphatase-labeled goat anti-rabbit diluted 1:500 was added. p-Nitrophenylphosphate was added in 1 M diethanolamine, pH 9.0.

Protein Electrophoresis and Transfer to DBM* Paper—Protein analyses were carried out by electrophoresis in 15% sodium dodecyl sulfate-polyacrylamide gels (22). The transfer of proteins from acrylamide gels to DBM paper was carried out essentially according to Renart et al. (23). After electrophoresis, the gel was washed with 0.025 M sodium phosphate, pH 6.5. The proteins were transferred electrophoretically in 0.025 M sodium phosphate, pH 6.5, 0.05% sodium dodecyl sulfate, at 2 A (15 V/cm) for 30 min.

RESULTS

The folding domain of histone H5 (GH5) purified to homogeneity (see inset Fig. 1A) is highly immunogenic. Each of the four rabbits immunized with this peptide, which consists of 78 amino acids, produced a relatively high antibody titer. The antiserum obtained from several bleedings of the rabbits were pooled and tested by an enzyme-linked immunosassay. The results presented in Fig. 1A indicate that the antiserum could be used to detect as little as 300 pg of GH5 with a serum dilution of 1:1,000. At higher antigen concentrations the serum could be effectively used at a dilution of 1:100,000. The antiserum cross-reacts strongly with intact H5 but not with intact H1 purified from chicken erythrocytes (Fig. 1B). This is not due to failure of histone H1 to bind to the microtiter wells since when tested with anti-H1 strong reaction was obtained. The fact that the antibodies did not distinguish between intact H5 molecules and the GH5 peptide derived from them suggest similarity in the conformation of the free peptide and the peptide region in intact H5.

The specificity of the serum was further tested by reaction...
Similarity of the Folding Domain of Histones H5 and H1

with various peptides derived from calf thymus H1. Enzyme-linked immunoassay revealed that the antibody did not react with the following peptides: GH1 (residues 36–121), for a detailed description of the peptides see Ref. 18, NH1 (residues 1–98), NGH1 (residues 1–121), and CH1 (residues 122–231). These results were supported in tests in which the protein fragments were transferred from polyacrylamide gels to DBM paper and the stained protein gel was compared with an autoradiograph of the DBM paper treated with the anti-GH5 serum and 125I-protein A (Fig. 2). In this sensitive assay, a very weak signal with GH1 and NGH1 was observed. It is possible that the antiserum may contain a small fraction of antibodies which recognize the folding domain of H1. The enzyme-linked immunoassays, however, indicate that the reaction of the serum with GH5 was at least 500-fold higher than with GH1.

The anti-GH5 serum was tested for possible cross-reaction with the major proteins known to be associated with the chromatin fiber. Proteins separated on polyacrylamide gels were transferred to DBM paper, and the paper was treated with anti-GH5 and 125I-protein A. The results presented in Fig. 3 reveal that the antiserum does not cross-react with calf

Fig. 1. Enzyme-linked immunoassay for sensitivity and specificity of anti-GH5. A, enzyme-linked immunoassay for the specificity of anti-GH5. The microtiter plates were coated with 100 ml of antigen at the indicated concentration. The bound antigen was reacted with the antiserum at dilutions of 1:1,000 (a), 1:5,000 (b), 1:20,000 (c), 1:50,000 (d), and 1:100,000 (e). The curves were obtained 30 min after addition of substrate. The absorbance values were read with a Multiscan (Flow Labs). The maximum absorbance readable is 2.0. Inset, purity of proteins: a, histone H5; b, fragment GH5; c, chicken histones H1. B, enzyme-linked immunoassay for the sensitivity of the anti-GH5 serum. Varying amounts of GH5 (A and B), H5 (O), or chicken erythrocytes H1 (Δ) were tested either with anti-GH5 at 1:5,000 (O, A, Δ) or with preimmune sera at 1:2,500 (A).

Fig. 2. Lack of reaction of anti-GH5 with peptides derived from H1. Peptides were transferred by Southern blotting from 15% polyacrylamide gels to DBM paper. The paper was treated with anti-GH5 diluted 1:400 and with 125I-protein A at 5 x 10^6 cpm/ml. A, Coomassie blue stain of the peptides remaining in the gel; B, corresponding autoradiogram. I, mixture of H5 and GH5; 2, from top: HMG-1, HMG-2, HMG-17; 3, calf thymus histones; 4, ox liver H1a; 5, ox liver H1b.

Fig. 3. Anti-GH5 reacts with H1a but not with other chromosomal proteins. A, Coomassie blue; B, corresponding autoradiogram. 1, mixture of H5 and GH5; 2, from top: HMG-1, HMG-2, HMG-17; 3, calf thymus histones; 4, ox liver H1a; 5, ox liver H1b.

Fig. 4. Enzyme-linked immunoassay demonstrating cross-reaction of anti-GH5 with H5 and H1a and H1b but not with H1. The antiserum to GH5 was diluted 1:5000. H1a and H1b were purified from ox liver H1 from calf thymus.
thymus HMG-1, HMG-2, and HMG-17 or with the histones purified from calf thymus. Since ox liver contains two electrophoretically distinguishable H1 fractions (20), one of which may cross-react, both were tested for cross-reactivity. The antisera reacted very strongly with both subfraction H1\(^a\) and H1\(^b\) purified from ox liver. Analysis by enzyme-linked immunoassay indicated that both H1\(^a\) and H1\(^b\) reacted with the serum to the same degree (see Fig. 4). The globular region of H1\(^a\) was obtained by trypsin digestion. Analysis by the DBM transfer technique indicated that this peptide reacts strongly with the anti-GH5 serum (Fig. 5).

It is concluded that the folding domain of H5 is immunologically similar to that of H1\(^a\) and distinct from that of H1 or other major proteins associated with the chromatin fiber.

**DISCUSSION**

Recent evidence suggested that histone H5 which is specific for nucleated erythrocytes from various sources is homologous to histone H1\(^a\). Although the overall amino acid composition of these two lysine-rich histones suggests that the proteins are not identical (20), sequence analysis has revealed that H1\(^a\) is more similar to H5 from chicken erythrocytes than to H1 obtained from the same source as H1\(^a\). Mura and Stollar (9) reported that antibodies to H5 react with H1\(^a\) but not with H1. In the present study we demonstrate that this immunological cross-reaction may be localized to the folding domains (residues 22-100) of the histones. These results are in agreement with sequence analysis (6, 7) and with NMR studies which revealed a number of strong downfield spectral shifts associated with the folding domain of H1\(^a\) and H5 but not with H1 (8).

It has been proposed that the occurrence of H5 in nucleated erythrocytes is associated with repression of both replication and transcription. The accumulation of H1\(^a\) and protein IP-25 (which apparently is identical with H1\(^o\)) has been correlated with lack of DNA synthesis and commitment to differentiation. If indeed H1\(^o\) and H5 share the capacity to restrict transcription and replication of DNA and are involved in cell differentiation, then the close homology between these two proteins in the folding domain would tend to implicate this region in that repressive activity. The availability of a potent antiserum which is specific for this domain will facilitate a variety of studies on the role of these proteins in cellular processes.

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J Allan, B J Smith, B Dunn and M Bustin