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ERYTHROID SPECIFIC NUCLEAR ANTIGEN AND GLOBIN GENE BINDING PROTEIN

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SUMMARY: Using the procedure of Bekhor and Mirell (Biochem, 18, 609, 1979), we isolated a nonhistone protein fraction tightly bound to DNA which putatively has a role in globin gene regulation in chicken reticulocytes. This fraction was tested by gel electrophoresis and microcomplement fixation and appears by these criteria very similar to the chicken nuclear antigen previously identified in reticulocyte chromatin and structurally altered erythrocyte chromatin. This antigen is tissue and species specific (Pumo et al, Biochem. 19, 2362, 1980).

We and other laboratories have previously reported the existence of a nuclear antigen found in chicken erythroid nuclei (1-4). The antigen is detected by using antisera from rabbits injected with dehistonized chicken reticulocyte chromatin. This antigen, actually a nonhistone protein-DNA complex (1,3), is identified by microcomplement fixation and horseradish peroxidase localization in chicken reticulocytes (1-4). When intact erythrocyte chromatin is examined for the presence of nuclear antigen, only a small amount is detectable. However, upon mechanical or chemical disruption of the chromatin, large amounts of nuclear antigen are detectable, equal to or greater than the amount found in reticulocyte chromatin (4). These and other observations pointed to the conclusion that a structural phenomenon was involved. Altering the structure of the chromatin coincidently altered the apparent antigenicity of the nonhistone-DNA complex.

Other laboratories have reported a fraction of chicken nonhistone proteins which bind tightly and specifically to fractions of chicken DNA (5-8). In two cases the work has been done with chicken reticulocyte nuclei and evidence indicates that these nonhistone proteins which bind

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tightly to DNA are important regulatory molecules for the globin gene (7,8).

In this paper, we present data indicating that the nonhistone protein-DNA complex against which we have prepared antibody is similar to the putative regulatory proteins described by Bekhor and Mirell and Ross, et al. (7,8).

METHODS

The isolation of erythroid nuclei and chromatin has been described previously (1,4). Briefly, blood cells were collected and washed in saline-sodium citrate and heparin. Cells were lysed in 1 mM $MgCl_2$, then sucrose was added to a concentration of 0.25 M and the lysate was centrifuged. Membrane ghosts were removed from the crude nuclear pellet by washing in 0.25 M sucrose, 0.5% Triton X-100-TKMC [50 mM Tris-HCl (pH 7.4)-24 mM KCl-5 mM $MgCl_2$-0.2 mM $CaCl_2$]. The nuclear pellet was further purified by centrifugation through 1.6 M sucrose-TKMC at 17,000 g for 30 min. To make chromatin, the nuclear pellets were gently homogenized with a loose pestle in a glass homogenizer in 10 mM Tris-HCl (pH 7.9)-1 mM ethylene diaminetetraacetic acid. The chromatin was collected by centrifugation at 2000 g for 10 min and then washed in dilute saline-sodium citrate buffer.

The antibody sera was prepared according to the method of Chytil and Spelsberg (9). The microcomplement fixation test is that of Wasserman and Levine (10). The fraction of nonhistone proteins tightly bound to DNA was obtained by the method of Bekhor and Mirell (8). Briefly, the chromatin was dissolved in 2 M NaCl-TPD [10 mM Tris-HCl (pH 8.0)-0.1 mM phenylmethane sulfonyl fluoride-0.2 mM dithiothreitol] and centrifuged through 1 M sucrose- 2 M NaCl-TPD at 350,000 g for 20 hrs. The pellet was redissolved in NaCl-TPD and the centrifugation repeated. The second pellet was dialysed against TPD for 20 hrs. The dialysate was centrifuged at 15 K for 15 min. The resulting pellet was resuspended in TPD and centrifuged 3 times. The final pellet was used for the complement fixation test.

The sodium dodecylsulfate polyacrylamide gel electrophoresis was done according to the discontinuous buffer system of Laemmli (11). The separating gel is 10% acrylamide, the stacking gel is 4.5% acrylamide. The gel was stained with Coomassie Blue.

RESULTS

Figure 1 illustrates the results of microcomplement fixation tests comparing the reactivity of whole chicken reticulocyte chromatin with the nonhistone protein tightly bound to DNA isolated by the procedure of Bekhor and Mirell (8). The level of activity per μg of chromatin (measur-

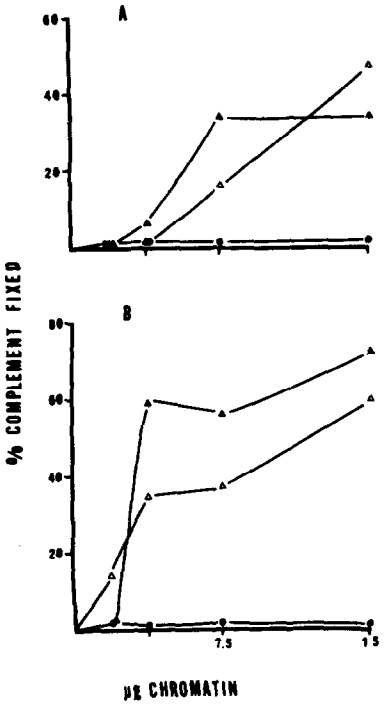

Figure 1: Complement fixation tests to determine the amount of chicken reticulocyte nuclear antigen present in nonhistone proteins tightly bound to DNA isolated by the procedure of Bekhor and Mirell (8). (A) Complement fixation test with antiserum prepared according to Chytil and Spelsberg (9) used at a 1:150 dilution. (B) Complement fixation test with antisera from rabbits injected with nonhistone protein-DNA complex after treatment with 50 mM Tris (pH 8.0)-2.5 M NaCl, 5 M Urea. The dilution of antiserum is 1:200. Reticulocyte chromatin (▲—▲); tightly binding nonhistone chromatin proteins (△—△); erythrocyte chromatin (●—●).

ed as DNA in 5 M urea) is similar in both the standard reticulocyte chromatin and the Bekhor and Mirell fraction. For plots A and B, antisera from two different rabbits was used. The same antibody was used in experiment 1A as in our previous papers (1,4). The antisera for experiment 1A were raised against dehistonized chicken reticulocyte chromatin. The antisera for experiment 1B were raised against tightly bound nuclear protein-DNA complexes. The tightly bound nuclear protein-DNA complexes were prepared by extracting chromatin with 50 mM Tris-HCl (pH 8.0)-2.5 M NaCl-5 M urea to remove most histone and nonhistone proteins (12). As

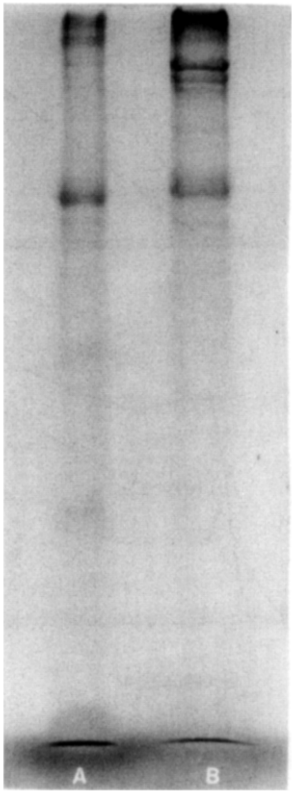

Figure 2: Polyacrylamide gel electrophretic profiles of chicken reticulocyte nonhistone proteins which remained tightly bound to DNA during two different isolation procedures. All major bands are present in both samples. (A) Proteins removed from DNA by extraction with 2% sodium dodecyl sulfate (12). (B) Proteins removed from DNA by DNase I (3.1.4.5) digestion of the nonhistone protein-DNA complex described by Bekhor and Mirell (8).

shown in Fig 1A and 1B, both fractions of whole chromatin and tightly bound nuclear protein-DNA complexes have similar antigenic characteristics.

Figure 2 shows the gel electrophoresis profile comparing the tissue specific immunogenic nuclear proteins and the protein fractions obtained following the procedure of Bekhor and Mirell (8). The immunogenic nuclear proteins were isolated from chromatin previously treated with 2.5 M NaCl-5 M urea-50 mM Tris (pH 8.0) to remove most of the nuclear proteins

which are not immunogenic. The chromatin residue was extracted with 2% sodium dodecyl sulfate to isolate nuclear antigen by following the procedure of Elgin and Bonner (13). As shown in Figure 2, there are some differences in relative quantities of some of the protein bands but all major species are present in each sample.

DISCUSSION

Our data from microcomplement fixation and polyacrylamide gel electrophoresis indicate that the major immunogenic proteins in reticulocyte chromatin are similar to the globin gene binding fraction of nonhistone chromosomal proteins described by others (7,8).

In our previous work on the chicken erythroid nuclear antigen we showed that antibody to these antigens could distinguish between intact reticulocytes and erythrocytes and between chromatin from the two cell types. Upon chemical or mechanical disruption of the erythrocyte cells or chromatin which causes a structural change, the antibody is able to detect the presence of the nuclear antigen (1,4). The unmasking of nuclear antigen of the chicken erythroid cell is a structural phenomenon which correlates with globin gene expression and erythrocyte maturation.

Chae and associates (7,14) have identified an apparent regulatory protein fraction in reticulocytes. These are nonhistone proteins which bind very tightly to chicken DNA; during reconstitution these proteins must bind to the DNA before the histones in order to assure globin gene transcription. This fraction can specifically locate globin gene sequences.

Bekhor and associates have also identified fractions of nonhistone proteins tightly bound to DNA in both chicken liver cells (5,6) and chicken reticulocytes (8). Both of these fractions are associated with specific active DNA sequences in their respective cell type. Given the close electrophoretic and immunological similarities between our chicken erythroid cell nuclear antigen and these nonhistone protein fractions, we

would like to suggest that an intimate relation exists in reticulocytes (and probably other cells as well) between chromatin structure and regulation. Possibly in this case the two cannot be separated. This communucation indicates that antibodies to reticulocyte nuclear antigens might be very useful in elucidating the exact nature of the chromatin structure-regulation relationship especially with respect to globin gene expression.

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