TISSUE-SPECIFIC NONHISTONE CHROMATIN PROTEINS WITH AFFINITY FOR DNA

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Received 8 March 1973

1. Introduction

As was shown by several investigators, the nonhistone proteins of chromatin are necessary for the tissue-specific restriction of DNA in eukaryotes [1–5]. It was possible to elicit antibodies against mixtures of certain nonhistone proteins with DNA which were shown by the microcomplement fixation technique to be highly tissue and species specific [6, 7]. We report here the isolation, purification and partial characterization of the immunospecific, DNA-binding proteins of rat liver chromatin.

2. Methods

The isolation of rat liver chromatin was described previously [8]. The tissue-specific nonhistone proteins were obtained by selective removal of phosphoproteins and histones with buffered 5.0 M urea containing increasing amounts of NaCl [9]. The DNA–protein complex was recovered after each extraction by ultracentrifugation at 110,000 *g* for a time period necessary to sediment most of the protein-bound DNA (36–48 hr). The final nonhistone–DNA pellet was homogenized in 0.15 M NaCl, 0.015 M sodium citrate solution (SSC) and used for the immunization of rabbits [6]. The immunospecificity of the nonhistone protein–DNA complexes was determined by the microcomplement fixation method of Wasserman and Levine [10]. The antisera were purified on DEAE-cellulose column and the antigen–antiserum incubation was at 37° for 90 min.

The affinity for DNA of the isolated nonhistone proteins of chromatin was tested by chromatography on DNA-containing columns. Rat spleen DNA–agarose–polyacrylamide gel was prepared according to the method of Cavalieri and Carroll [11], equilibrated with 5 M urea in 50 mM Tris-HCl buffer, pH 8.0 in a 1.2 × 30 cm column and loaded with 1 mg of ^{125}I-labeled nonhistone protein [12]. A stepwise gradient of 0–0.6 M KCl in 5.0 M urea in 50 mM Tris-HCl buffer, pH 8 was used to elute the DNA-bound proteins. Polyacrylamide electrophoresis of the eluted proteins was performed in the presence of 0.1% sodium dodecyl sulfate [13, 14].

3. Results

As was shown previously, a fraction of chromatin nonhistone proteins is immunospecific and the tissue-specificity depends on the presence of both, the DNA and nonhistone proteins in the complex. Shown in fig. 1 is the microcomplement fixation of antibodies against rat liver nonhistone protein–DNA complex. The immunospecificity of chromatin depends on the presence of DNA complexed with a fraction of nonhistone proteins. Neither the proteins nor the DNA alone are reactive. However, the immunospecificity can be partially restored by reconstitution of chromatin proteins with homologous DNA.

The nonhistone proteins isolated from their complexes with DNA by ultracentrifugation in 5.0 M urea, 2.5 M NaCl and 50 mM Tris-HCl buffer pH 8.0 were

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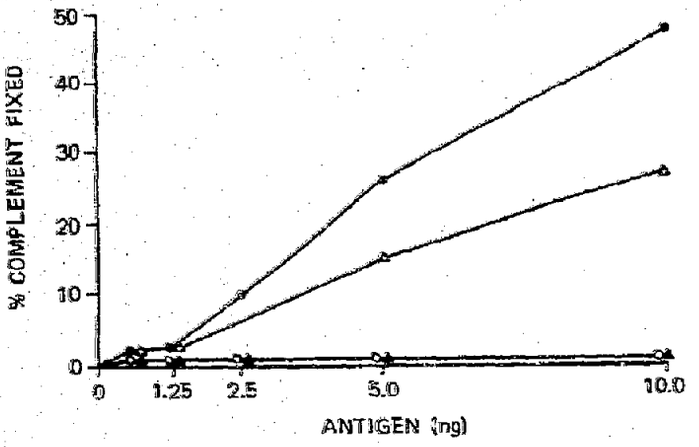

Fig. 1. Complement fixation in the presence of antiserum against rat liver nonhistone protein–DNA complex resulting from the removal of phosphoprotein and histone fractions in 5 M urea containing phosphate buffer pH 7.6 or 2.5 M NaCl and 0.1 M succinate buffer pH 5.0, respectively. (●—●—●) Rat liver chromatin dissociated in 2.5 M NaCl in 5 M urea and 50 mM Tris-HCl buffer pH 8.0 and reconstituted by gradual decrease of NaCl concentration in the presence of 5 M urea. Native, not dissociated chromatin was immunochemically identical. (△—△—△) Chromatin obtained by reconstitution of the total rat liver chromatin protein to isolated, pure rat spleen DNA. (○—○—○) Total chromatin protein of rat liver chromatin obtained by dissociation in 5 M urea, 2.5 M NaCl and 50 mM Tris-HCl buffer, pH 8.0 followed by ultracentrifugation (supernatant). (▲—▲—▲) Rat spleen DNA.

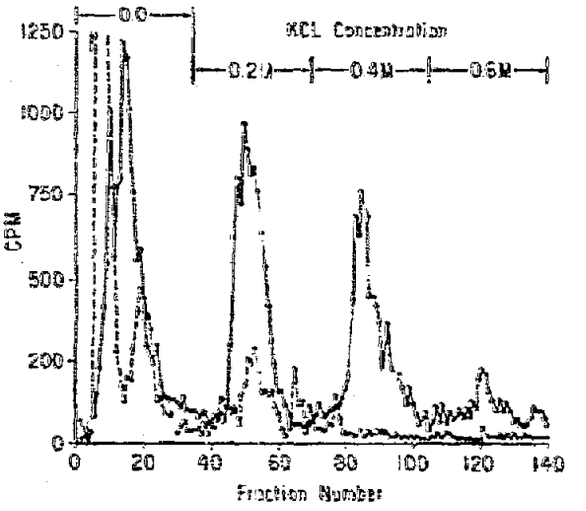

Fig. 2. Affinity chromatography of rat liver nonhistone protein (preparation used for immunization in fig. 1) and histone. Both protein mixtures were labeled *in vitro* with ^{125}I. Rat spleen DNA bound to agarose–polyacrylamide matrix was used for chromatography. (●—●—●) Radioactivity of rat liver nonhistone proteins. (○—○—○) Radioactivity of histones.

iodinated *in vitro* with ^{125}I and fractionated by affinity chromatography (fig. 2). A large portion of the iodinated nonhistone protein is strongly attached to the DNA and can be eluted only with 0.4 M KCl in 5.0 M urea, 50 mM Tris-HCl buffer, pH 8.0. As seen in fig. 2, this protein fraction is eluted at a higher KCl concentration than is necessary to elute ^{125}I iodinated unfractionated histone mixture.

The ^{125}I-labeled nonhistone protein eluted from the column was concentrated and analyzed by polyacrylamide electrophoresis in the presence of 0.1% sodium dodecyl sulfate. The radioactivity pattern of the sliced gel is compared in fig. 3 with the electrophoretic pattern (scan) of nonhistone protein fraction used, in complex with the DNA, for immunization of rabbits to demonstrate its immunospecificity. The main protein species represented by the stained bands appear to be identical with the ^{125}I-labeled DNA binding species recovered from the column.

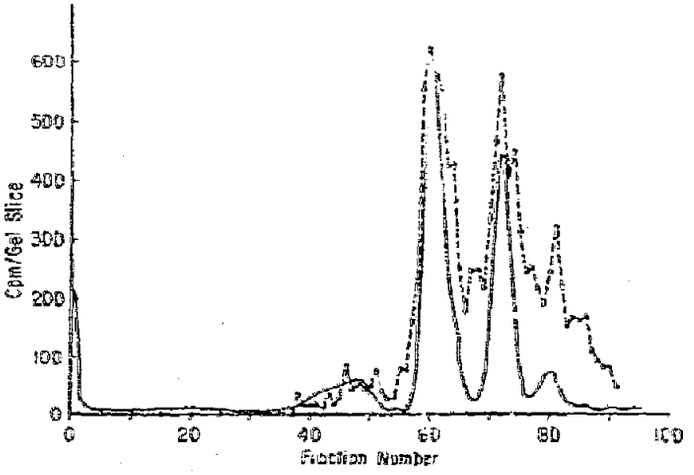

Fig. 3. Polyacrylamide gel electrophoresis of ^{125}I-labeled protein eluted from rat spleen DNA column with 5 M urea and 0.4 M HCl in 50 mM Tris-HCl buffer, pH 8.0. (○—○—○) Radioactivity. (——) Optical scan at 555 nm. The electrophoresis was carried out in 10% polyacrylamide gel containing 0.1% sodium dodecyl sulfate, 4 M urea and 0.1 M phosphate buffer pH 7.0. The gels were scanned by Gilford spectrophotometer gel scanner.

4. Discussion

Our earlier observation [7] that the immunospecificity of certain nonhistone proteins in chromatin depends on their selective associations with homologous DNA leads to a suggestion that it may be possible to obtain and purify these proteins by affinity chromatography. Indeed, as was shown in figs. 2 and 3, a considerable protein portion of the immunospecific DNA–protein complex pelleted by ultracentrifugation in 5.0 M urea, 2.5 M NaCl and succinate buffer pH 5.0 binds to rat spleen DNA. Although low molecular weight in 0.1% sodium dodecyl sulfate, these DNA-binding proteins differ from the histones in electrophoretic mobility, amino acid composition, and in their stronger binding to the DNA. As can be seen in fig. 2, ^{125}I-iodinated histones are completely eluted from rat spleen DNA columns with 0.2 M KCl. A higher concentration of KCl (0.4 M) is necessary to elute the nonhistone protein fraction. Because the evolutionary conservative histones are extremely poor antigens, the immunochemical tissue-specificity of the DNA-binding proteins described here further supports our conclusion that these proteins are not histones. According to our preliminary studies, the DNA-binding proteins shown in fig. 3 are extensively phosphorylated.

The DNA-binding protein fraction shown in fig. 3 associates only with homologous (rat) DNA. When applied to calf thymus DNA columns, these proteins were not retained. This feature of the DNA-binding nonhistone proteins is in a good agreement with our earlier finding that the tissue-specific nonhistone proteins formed immunospecific complexes only with homologous (rat) DNA but not with heterologous (sea urchin) DNA [7]. It is of interest, that competitive hybridization studies with RNA species templated *in vitro* from various reconstituted chromatin preparations showed that the selective transcription of tissue specific RNA species is directed by a fraction of nonhistone proteins similar to that described in this paper [3, 4]. The histones were shown to be necessary only for relatively nonspecific, quantitative DNA restriction in chromatin [4].

A mixture of low molecular nonhistone proteins with affinity for DNA was recently shown to be present in the chromatin of sea urchin embryos [15, 16] and other tissues [17, 18]. The electrophoretic behavior of these proteins is quite similar to that shown for the tissue specific nonhistone protein fraction (fig. 3), suggesting that the DNA-binding proteins are relatively simple and may be present in all cells. Their simplicity, however, may not compete with the specific biological functions of these proteins since a similar situation exists in immunoglobulins which are known for their ability to distinguish between minor structural differences of various antigens.

Acknowledgements

The authors are indebted to Ms. Susan Getz for her assistance in the isolation of chromatin and DNA. This investigation was supported by the U.S. Public Health Service Grant CA-07746 and by the NIH-NCI Contract E-72-3269.

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