ISOLATION AND PROPERTIES OF NONHISTONE CHROMOSOMAL PROTEINS FROM PEA CHROMATIN*

Paul PO-CHAO LIN*, Ralph F. WILSON and James BONNER

Division of Biology, California Institute of Technology, Pasadena, California 91109 (U.S.A.)

Summary

We have separated the nonhistone chromosomal proteins of pea chromatin from other chromosomal constituents and have studied some of the biological functions of these proteins. After dissociation of pea chromatin in 3 M NaCl, 5 M urea (pH 8) and subsequent removal of urea, the chromosomal proteins are separated from DNA on Bio-Gel A50. Histones are then separated from nonhistones by chromatography on Bio-Rex 70 in the presence of 0.35 M NaCl and 5 M urea. The resulting nonhistone proteins are concentrated on a DEAE-cellulose column.

No detectable histone remains in the nonhistone protein fraction. All of the nonhistone proteins are qualitatively but not quantitatively recovered. The major nonhistone proteins are acidic in amino acid composition, heterogeneous in molecular weight (10,000 to 68,000) and freely soluble at low ionic strength. The nonhistone proteins co-precipitate with histones at low ionic strength to form complexes. These can be redissolved in solutions of higher ionic strength. At physiological ionic strengths most nonhistone chromosomal proteins do not bind to histones. In reconstitution experiments, the mass ratio of nonhistone chromosomal protein to DNA is 0.17 at saturation binding. An input ratio, protein to DNA, of about 20 is required to give half-maximal binding. In vitro transcription by E. coli RNA polymerase is not significantly affected by the presence of nonhistone proteins, either with pea DNA or pea chromatin as template.

Introduction

There have been several studies of methods for the preparation of nonhistone chromosomal proteins (NHC proteins). Earlier work on this matter involved clearly denaturing conditions in order to avoid problems of interaction of NHC proteins with histones. More recently several attempts to avoid clearly denaturing conditions have been reported. We have tried to apply all findings, new and old, to the development of methods for the separation, under the gentlest possible conditions, of NHC proteins. The method which we here propose is the result of this study, as applied to pea plant chromatin. We have in addition studied some properties of the separated NHC proteins.

Experimental procedures

Materials

Materials and reagents were obtained from: Asgrow Seed Company (Alaska peas [Pisum sativum. Var.]); Schwarz/Mann Res. (sucrose [ribonuclease free], 3H-GTP); Bio-Rad Laboratories (electrophoresis grade acrylamide and bisacrylamide, Bio-Res 70 (10.2 meq/dry g.), Bio-Gel A50, and N,N,N',N'-tetramethylenediamine (TEMED)); Gallard-Schlesinger (DEAE-cellulose powder (0.7 meq/g.)); Sigma (ribonuclease A (bovine pancreas, 5X crystalline));
NONHISTONE PROTEINS FROM PEA CHROMATIN

CalBiochem (pronase [A grade], dithiothreitol and nucleotide derivatives). Sodium dodecyl sulfate (SDS) was 2X recrystallized technical grade from Matheson, Coleman and Bell. Urea solutions were freshly deionized before use. All standard chemicals were reagent grade.

Purification of Chromatin
Pea embryo chromatin was prepared from the embryonic axes of 3-day old germinating seedlings, pea bud chromatin from the apical bud (approximately 1.5 cm of stem plus bud) of 6-day old dark-germinated seedlings, and developing pea cotyledon chromatin (harvested in the field, shelled, quick-frozen and stored at \(-20^\circ\)) by the methods of Bonner et al. with the following modifications. 1 mM NaHSO₃ was included in all buffers to prevent proteolytic activity. The 1.7 M sucrose-pelleted chromatin was resuspended and sheared at 60 V for 1 min in the Virtis homogenizer. After centrifugation at 12,000 g for 10 min, the supernatant (purified chromatin) constituted the starting material for the preparation of NHC proteins. All preparations of NHC proteins were made from freshly purified chromatin. Procedures were carried out at 0-4^\circ unless otherwise stated.

Separation of Chromosomal Proteins from DNA on Bio-Gel A50
The sheared chromatin was dialyzed against 20 volumes of dissociating agent (3 M NaCl, 5 M urea, 1 mM NaHSO₃, 10 mM Tris buffer, pH 8) for 24 hr. After removal of urea by dialysis, 70 ml (600 A₂₆₀ units) of dissociated chromatin was applied to a Bio-Gel A50 column, 3.5 x 95 cm, equilibrated with and eluted by 3 M NaCl, 1 mM NaHSO₃ and 10 mM Tris buffer, pH 8. The flow rate was 30 ml/hr and 6-ml fractions were collected. The appropriate fractions were combined into DNA and protein pools.

Separation of NHC Proteins from Histones on Bio-Rex 70
Bio-Rex 70 resin was washed with deionized distilled water, and equilibrated with 0.35 M NaCl, 5 M urea, and 10 mM Tris-acetate buffer, pH 7.2. The protein pool from Bio-Gel A50 column chromatography was dialyzed against 5 M urea, 10 mM Tris-acetate, pH 7.2, to give a final NaCl concentration of 0.35 M, and then applied to a Bio-Rex 70 column (2.5 x 8.5 cm) at a flow rate of 100 ml/hr. The column was then washed with 100 ml of the buffer solution. The run-off, referred to as the NHC protein fraction, was dialyzed to remove salt, applied to a DEAE-cellulose column (2 x 10 cm, previously equilibrated with 10 mM Tris buffer, pH 8, and 5 M urea), and eluted with 0.4 M NaCl in the above buffer solution. The proteins which adsorbed to the Bio-Rex 70 column were eluted with 3 M NaCl in the above buffer solution, and are referred to as the histone fraction.

Polyacrylamide Disc Gel Electrophoresis
SDS-gel electrophoresis was carried out according to the method of Shapiro et al., with the following alterations: final gel composition was 7% polyacrylamide, 0.18% bisacrylamide, 0.1% SDS, 0.1 M Na-phosphate buffer (pH 7.1), 0.05% TEMED, and 0.05% ammonium persulfate. Generally, 50–100 μg of protein were applied to 0.6 x 6 cm gels and run at constant voltage (47 V) for 2 hr. Gels were stained with 0.25% Coomassie Blue and destained electrophoretically according to the method of Elgin & Bonner.

Urea-acrylamide gel electrophoresis of histones at pH 4.3 was performed as described by Bonner et al. The pH 8.5 urea (7 M)-7% acrylamide gel electrophoresis of NHC proteins was performed by the methods of Davis and of Lin & Varner. All NHC protein samples were dialyzed against 7 M urea, 10 mM Tris buffer (pH 8). The gels were run at 2 mA per tube at 4^\circ for 45 min with bromophenol blue as a tracking dye.

Preparation of DNA
The DNA from Bio-Gel A50 column chromatography was precipitated with 2 volumes of ethanol, recovered by centrifugation, redissolved in and dialyzed against 10 mM Tris buffer (pH 8). The DNA solution (0.5 mg/ml) was then treated with bovine pancreatic ribonuclease A (2.5 μg/mg of DNA) at 37^\circ for 30 min, further deproteinized with pronase A (2.5 μg/mg of DNA) at 37^\circ for 30 min, extracted twice with phenol, and precipitated in 0.1 M K-acetate and 2 volumes of ethanol. After centrifugation, DNA was dissolved in H₂O, dialyzed, and stored in liquid nitrogen. After dialysis against 0.25 mM Na-EDTA (pH 8), with Na⁺ of 0.75 mM, the purified DNA shows a monophasic melting curve with a T m of 43^\circ and has a hyperchromicity of 38%.