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Properties of Chromosomal Nonhistone Protein of Rat Liver*

Keiji Marushige,† Douglas Brutlag, and James Bonner

ABSTRACT: The nonhistone protein of interphase chromatin of rat liver has been isolated, using sodium dodecyl sulfate, and partially characterized. The chromosomal nonhistone protein is rich in acidic amino acids and contains tryptophan. The physical state of chromosomal nonhistone protein is markedly dependent upon detergent. In the presence of detergent (0.1%) the nonhistone protein gives $s_{20,w}$ of 2.7 S and an average molecular weight of 14,300. On removal or lowering of detergent concentrations the protein aggregates. The chromosomal nonhistone protein interacts with histones to form an insoluble complex under appropriate conditions. A chromosomal nonhistone protein–deoxyribonucleic acid complex can be made by a NaCl gradient dialysis of mixtures of deoxyribonucleic acid and the nonhistone protein. Such a complex shows the same activity in support of ribonucleic acid synthesis as does pure deoxyribonucleic acid.

Nonhistone proteins are present in isolated interphase chromatin as an integral constituent (Marushige and Bonner, 1966). Amounts of chromosomal nonhistone protein present in isolated chromatins vary markedly from one cell type to another. Analyses of chromatins from different tissues of pea (Bonner *et al.*, 1968a,b), those from different stages of the sea urchin embryo (Marushige and Ozaki, 1967), and those from different stages of trout testes (K. Marushige and G. H. Dixon, manuscript in preparation) indicate that template-active chromatins contain more nonhistone proteins than do template-less-active or -inactive chromatins. More nonhistone proteins are found in active euchromatin than in repressed heterochromatin of calf thymus (Frenster, 1965) and template-inactive nucleohistone fractions of pea embryo chromatin do not contain nonhistone proteins (Bonner and Huang, 1963). Despite the recent remarkable advance in chemistry and biology of histone (Bonner *et al.*, 1968a, b; Hnilica, 1967; Busch, 1965), little is known about nonhistone proteins of isolated chromatins. The present paper concerns the isolation and some of the physical, chemical, and biological characteristics of chromosomal nonhistone protein of rat liver.

Methods

Preparation of Chromatin. The rat liver chromatin was prepared as previously described (Marushige and Bonner, 1966). Frozen rat liver was ground (Waring Blendor) in saline–EDTA (0.075 M NaCl plus 0.024 M ethylendiaminetetraacetic acid, pH 8). The homogenate was filtered through four layers of Miracloth and centrifuged at 1500*g* for 10 min. The sediment was washed twice with saline–EDTA and then three times with 0.01 M Tris buffer (pH 8). The crude chromatin was purified by sedimentation through 1.7 M sucrose in 0.01 M Tris buffer (pH 8). The clear gelatinous pellet was resuspended in Tris buffer and again purified by sucrose centrifugation. The purified chromatin thus obtained was resuspended in Tris buffer (0.01 M, pH 8) and sheared in a Virtis homogenizer at 25 V for 90 sec followed by centrifugation at 17,000*g* for 20 min. The supernatant, in which more than 70% of the chromosomal DNA was recovered, was used for the preparation of chromosomal nonhistone protein.

Preparation of Chromosomal Nonhistone Protein. The chromatin (which contains 5–10 mg of nonhistone protein/10–20 mg of DNA) was first treated with 0.2 N HCl (0°, 30 min) in order to remove histones. The acid-insoluble material was collected by centrifugation at 14,000*g* for 20 min and dissolved in 15 ml of 0.05 M Tris buffer (pH 8) containing 1% SDS[1] following the procedure of Carusi and Sinsheimer (1963). After stirring overnight at 37°, the solution became clear. The solution was then dialyzed against 0.01 M Tris buffer (pH 8) containing 0.1% SDS at 37° for 24 hr and centrifuged at 36,000 rpm for 16–18 hr in a Spinco SW-39 head at approximately 25°. After the centrifugation, the upper two-thirds of the supernatant was pipetted and chilled in an ice bath. To the supernatant 1–2 drops of saturated KCl were added. The resulting dense precipitate of potassium dodecyl sulfate was sedimented at 12,000*g* for 20 min. The clear supernatant was decanted and saturated ammonium sulfate was added to a final 35% saturation. The protein precipitate was allowed to flocculate for 20 min in an ice bath and was collected by centrifugation at 12,000*g* for 20 min. The protein pellet was dissolved in 0.01 M Tris buffer (pH 8) and the precipitation with ammonium sulfate was repeated twice more. The recovery of chromosomal nonhistone protein was 50–60%. The final protein precipitate was dissolved

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† Present address: Department of Biochemistry, University of British Columbia, Vancouver, B. C., Canada

[1] Abbreviation used that is not listed in *Biochemistry 5*, 1445 (1966), is: SDS, sodium dodecyl sulfate.

in Tris buffer (0.01 M, pH 8) and dialyzed overnight against the same buffer at 37°.

Preparation of Histone. Histones were extracted from purified rat liver chromatin with 0.2 N H_2SO_4 and precipitated with ethanol as described by Bonner *et al.* (1968a).

Preparation of DNA. The purified rat liver chromatin (not sheared) was stirred in 1% SDS for 2–3 hr and NaCl was then added to a final concentration of 1 M. Deproteinization was carried out with the chloroform–isoamyl alcohol mixture according to Marmur (1961). The DNA was precipitated with ethanol and dissolved in dilute saline–citrate (0.015 M NaCl plus 0.0015 M sodium citrate). The DNA was then treated with Pronase (predigested at 37° for 2 hr) for 18 hr at 37° and further deproteinized with the chloroform–isoamyl alcohol mixture. The DNA was again precipitated with ethanol, dissolved in dilute saline citrate, and stored in an ice bath.

Amino Acid Composition. Chromosomal nonhistone protein was exhaustively dialyzed against H_2O, lyophilized, and hydrolyzed in 6 N HCl at 105° for 22 hr. The amino acid composition was determined using a Spinco amino acid analyzer. Tryptophan was estimated spectrophotometrically (Benze and Schmid, 1957).

Sedimentation Velocity Analysis. The sedimentation coefficient of chromosomal nonhistone protein was determined by band sedimentation in self-forming deuterium oxide (D_2O) gradients in the analytical ultracentrifuge (Vinograd *et al.*, 1963). The protein samples at a concentration of about 1.0 OD_{275} were dialyzed against 0.01 M Tris buffer (pH 8) containing various concentrations of SDS, and 20 μl of dialyzed protein was then layered on the same solvent containing D_2O (80–100%) using a band-forming centerpiece. The protein band was observed using absorption optics at a wavelength of 275 mμ with the Spinco photoelectric scanner and recorder. All velocity experiments were conducted at rotor velocities above 50,000 rpm for at least 90 min. The temperature was maintained at 20 or 25° and all sedimentation coefficients were corrected to the standard conditions (Svedberg and Pederson, 1940). Calculations were performed on the IBM 7094 and the IBM 360/50 computers.

Sedimentation Equilibrium. The protein solution (1.0 OD_{275} in 0.01 M Tris buffer, pH 8) was dialyzed against various concentrations of SDS in D_2O containing 0.1 M Tris buffer (pH 8) and 0.1 M NaCl. The protein solution was then diluted with the dialysate to 0.3 OD_{275}. A 3-mm column of protein solution was placed on the top of a 1-mm column of fluorocarbon (Spinco fluorocarbon, FC-43, density 1.8 g/cm³) in the analytical ultracentrifuge cell. The fluorocarbon provided a distinct bottom to the protein solution. The samples were brought to equilibrium at 12,590 rpm at 20°. Both the initial and the equilibrium conditions were recorded using absorption optics with the photoelectric scanner and recorder.

The data were treated as described by Van Holde and Baldwin (1958), plotting the natural log of the concentration of protein (expressed as optical density at 275 mμ) *vs.* the square of the distance from the center of rotation. The slope of such plots gives a weight-average molecular weight

$$\bar{M}_w = \frac{2RT}{(1 - \bar{v}\rho)\omega^2} \frac{d(\ln C)}{d(r^2)}$$

where R is the gas constant, T is the absolute temperature, $\bar{v}$ is the partial specific volume of the solute, ρ is the density of the solution, ω is the angular velocity of the rotor in radians per second, c is the solute concentration, and r is the distance from the center of rotation (Svedburg and Pederson, 1940). The value actually calculated in these experiments was

$$M_c(1 - \bar{v}_c\rho) = \frac{2RT d(\ln C)}{\omega^2 \quad d(r^2)}$$

where M_c and $\bar{v}_c$ are the molecular weight and the partial specific volume of the protein–SDS complex, respectively. The molecular weight of the protein component alone, M_p, was calculated from the formula

$$M_p[(1 - \bar{v}_p\rho) + x(1 - \bar{v}_{SDS}\rho)] = M_c(1 - \bar{v}_c\rho) = \frac{2RT d(\ln C)}{\omega^2 \quad (dr^2)}$$

where $\bar{v}_{SDS} = 0.885$ cm³/g (Granath, 1953) and x is the weight of SDS bound per unit weight of protein (Hersch and Schachman, 1958). By performing equilibrium dialysis using ^{35}S-labeled SDS, the quantity x was determined for the various solvent conditions employed in the sedimentation experiments. These calculations were also performed using the IBM 7094 and the IBM 360/50 computers.

Binding of SDS. The amounts of SDS bound to chromosomal nonhistone protein at various concentrations of SDS were measured by the method of equilibrium dialysis (Carusi and Sinsheimer, 1963). The protein, ^{35}S-labeled SDS, carrier SDS, NaCl (0.1 M), and Tris buffer (0.01 M, pH 8) were appropriately mixed and 1.5 ml of this solution was dialyzed against 13.5 ml of Tris buffer containing 0.1 M NaCl and unlabeled SDS at the same concentration as that in the dialysis sack. Dialysis was carried out on a shaker at 37° for 48 hr. After the dialysis, aliquots of dialysate and dialyzed solution were dried in aluminum planchets and counted on a gas-flow counter. The concentration of SDS in dialysate and dialyzed solution was calculated from the measured radioactivity and the specific radioactivity of SDS. The amount of SDS bound per unit weight of protein was calculated from the difference in concentrations of SDS between dialysate and dialyzed solution.

RNA Synthesis. The standard incubation mixture (0.25 ml) contained: 10 μmoles of Tris buffer (pH 8), 1 μmole of $MgCl_2$, 0.25 μmole of $MnCl_2$, 3 μmoles of β-mercaptoethanol, 0.1 μmole of [^{14}C]ATP (specific activity 1 μCi/μmole), 0.1 mole each of GTP, CTP, and UTP, DNA template, and *Escherichia coli* RNA polymerase purified according to Chamberlin and Berg (1962) up to the step of their fraction 3. Incubation was carried out at 37° for 10 min. The reaction was stopped by the addi-

tion of 10% trichloroacetic acid. Acid-insoluble materials were collected on a membrane filter (Schleicher & Schuell C., B-6), washed with 10% trichloroacetic acid, glued to aluminum planchets, dried, and counted in a gas-flow counting system.

General Methods. Proteins were determined by the method of Lowry *et al.* (1951), calibrated with bovine serum albumin. DNA was determined by the diphenylamine reaction (Dische, 1955) after hydrolysis in 5% perchloric acid using calf thymus DNA as a standard. RNA was determined by the orcinol method (Dische, 1955) after hydrolysis in 0.3 M KOH using yeast RNA as a standard.

Results

Absorption Spectrum. The ultraviolet absorption spectrum of chromosomal nonhistone protein is shown in Figure 1. This spectrum indicates little contamination of the preparation by nucleic acids. Chemical analyses show that contents of DNA and RNA in the protein preparation are not more than 0.3 and 0.5% by weight, respectively.

Amino Acid Composition. The amino acid composition of chromosomal nonhistone protein is presented in Table I. Mole ratios of the amino acids determined by fitting these data with integers are also shown in Table I. The total number of residues per mole of protein is 145, and the minimum molecular weight calculated from this composition is 16,200. The partial specific volume of the protein was determined using the specific volume of the individual amino acids (Cohn and Edsall, 1943). The value obtained (0.730 cm^3/g) was used in all calculations in the sedimentation analysis involving the buoyancy term $1 - \bar{v}\rho$.

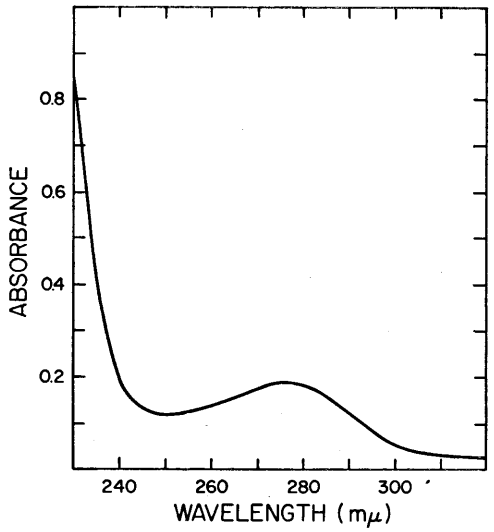

FIGURE 1: Absorption spectrum of chromosomal nonhistone protein. Solvent, 0.01 M Tris buffer (pH 8) containing 0.01% SDS. Concentration, 162 μg/ml.

Sedimentation Velocity. The sedimentation coefficient of chromosomal nonhistone protein varies depending upon the concentration of SDS. The ability of SDS to disperse the protein varies from one protein preparation to another. The sedimentation coefficients of several experiments at various SDS concentrations are presented in Table II. The sedimentation coefficient of the protein without SDS is not significantly different from that with 0.001% SDS. Increasing concentrations of SDS causes a corresponding decrease of the sedimentation coefficient. Different protein preparations re-

TABLE I: Amino Acid Composition of Chromosomal Nonhistone Protein.

Amino Acid	Mole %	Integral No.[a]
Lysine	6.25	9
Histidine	1.51	2
Arginine	5.60	8
Aspartic acid	9.48	14
Threonine	5.50	8
Serine	7.54	11
Proline	4.96	7
Glutamic acid	12.39	18
Glycine	7.54	11
Alanine	7.65	11
Valine	5.60	8
Cystine (half)	1.29	2
Methionine	1.94	3
Isoleucine	4.31	6
Leucine	9.91	14
Tyrosine	2.59	4
Phenylalanine	3.88	6
Tryptophan	2.05	3

[a] The integral numbers of moles of each amino acid per mole of protein were calculated by a program which reduced the sum of the squares of the deviations from integers to a minimum.

TABLE II: Sedimentation Coefficient of Chromosomal Nonhistone Protein.

Concn of SDS (%)	$s_{20,w}$ (S)[a]
None	6.16
	5.92
	6.26
0.001	5.88
0.01	3.69
	3.41
0.01–0.1	2.58
	2.60
	2.76
	2.78
0.1	2.07

[a] All sedimentation coefficients have been corrected to the standard conditions of solvent density and viscosity but have not been corrected for the effects of SDS binding.

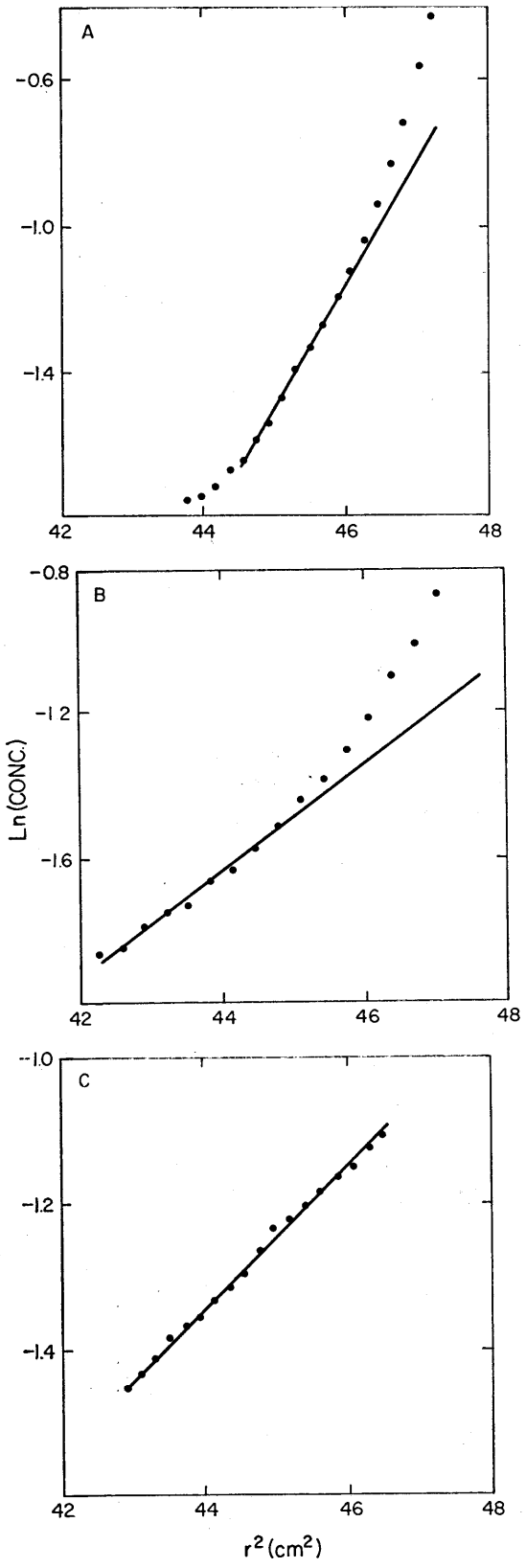

FIGURE 2: Equilibrium concentration distribution of chromosomal nonhistone protein. (A) The protein under conditions which cause extreme aggregation. The solvent contained 0.01% SDS. (B) The protein under conditions which cause moderate polydispersity. The solvent contained 0.1% SDS. (C) The protein under conditions which cause little polydispersity. The solvent contained 0.1% SDS.

quire different concentrations of SDS to reduce the $s_{20,w}$ to the 2.6S region. For instance, two preparations of protein required 0.01% SDS to reduce the sedimentation coefficient to 2.58 and 2.76 S, whereas two others required 0.1% SDS to give protein bands with sedimentation coefficients of 2.60 and 2.78 S. A single experiment at 0.1% SDS yielded a band having a sedimentation coefficient of 2.07 S. All bands appeared as single broad peaks.

Sedimentation Equilibrium. Sedimentation equilibrium experiments were employed to further characterize the chromosomal nonhistone protein and to investigate the effects of SDS on the protein. Equilibrium dialysis studies using [^{35}S]SDS show that 0.196 and 1.01 mg of SDS are bound per mg of protein in the presence of 0.01 and 0.1% SDS, respectively. Corrections for SDS binding can be made since conformational changes do not influence the equilibrium conditions. Table III summarizes the results of sedimentation equilibrium experiments. As with the sedimentation coefficients, the concentrations of SDS required to disperse the protein are variable from one preparation to another. In general, at concentrations of 0.01% or less, the protein aggregates to give a corrected weight-average molecular weight of 60,000 over the measurable range of protein concentrations, with 30–40% of the material pelleting and hence not measurable. The plot of ln (c) *vs.* r^2 for such experiments (Figure 2A) shows that the material is extremely polydisperse. The protein dissolved in the 0.01–0.1% SDS range shows a much lower degree of polydispersity (Figure 2B) with no material pelleting. The initial linear slope near the meniscus of the plot of

TABLE III: Molecular Weight of Chromosomal Nonhistone Protein.

Concn of SDS (%)	Wt-Av Mol Wt[a]	Range of Mol Wt in a Single Expt[b]
0–0.01	66,900	24,500–112,000[c]
	60,400	
0.01–0.1	24,000	12,000–45,000
	24,100	
	29,200	
0.1	14,300	12,000–20,000

[a] Obtained by fitting all of the measured data points with a straight line by the method of least squares. All molecular weights were corrected for SDS binding and represent only the molecular weight of the protein component. [b] These values were determined by fitting data from various regions throughout the cell with a straight line and determining the molecular weight in that region from the slope of the fitted line. [c] In experiments with this degree of aggregation, usually a large percentage of the material pellets and is not measureable.

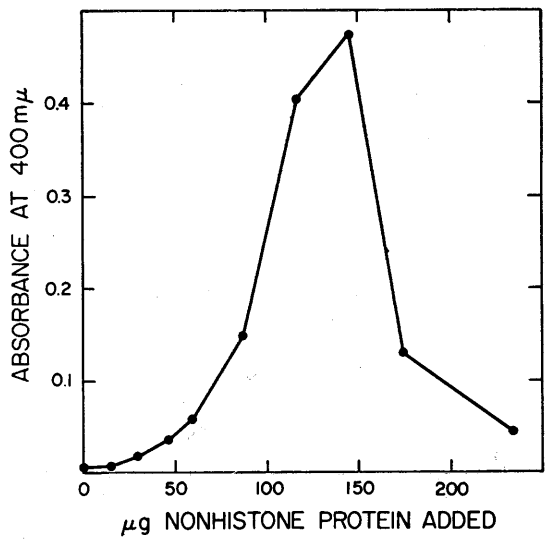

FIGURE 3: Interaction of chromosomal nonhistone protein with histone. Histone (115 μg) was mixed with various amounts of chromosomal nonhistone protein in a total volume of 1.1 ml (0.01 M Tris buffer, pH 8). Turbidity formed was measured at 400 mμ 15 min after mixing.

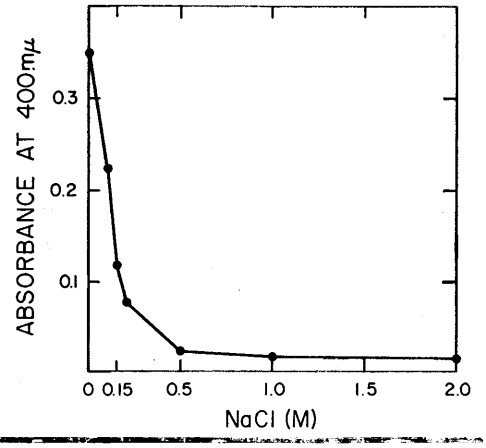

FIGURE 4: Interaction of chromosomal nonhistone protein with histone at various concentrations of NaCl. Chromosomal nonhistone protein (100 μg) was mixed with histone (100 μg) at various concentrations of NaCl (abscissa) in a total volume of 1.0 ml (0.01 M Tris buffer, pH 8). Turbidity was measured at 400 mμ 15 min after mixing.

ln (c) *vs.* r^2 of three such experiments gave the corrected molecular weights of 17,800, 16,700, and 12,000. All of these experiments, however, give average molecular weight about 26,000 due to polydispersity. A single experiment at 0.1% SDS gave a linear plot of ln (c) *vs.* r^2 over most of the liquid column (Figure 2C) indicating a homogeneous material, the molecular weight (corrected for SDS binding) being 14,300.

Interaction with Histone. When clear solutions of chromosomal nonhistone protein and histones are mixed (both in 0.01 M Tris buffer, pH 8), precipitation occurs instantaneously. Figure 3 presents data on turbidimetric determinations of chromosomal nonhistone protein–histone interaction. Precipitation increases with increasing ratio of nonhistone protein to histone, reaches a maximum at a ratio between 1 and 1.5, and then decreases sharply with further increase of the ratio of nonhistone protein to histone. The precipitate formed at a nonhistone protein/histone ratio of 1.1 was collected by centrifugation (17,000g, 20 min) and analyzed for acid-soluble and acid-insoluble proteins. Results show that essentially all the nonhistone protein precipitates under these conditions and that the ratio of nonhistone protein to histone in the insoluble complex is approximately 2. Figure 4 shows turbidimetric measurements of the precipitation at different salt concentrations. The precipitation is very sensitive to ionic strength and little or no precipitation occurs at NaCl concentration of 0.5 M or higher. The complex once formed is not, however, soluble in 2 M NaCl.

Interaction with DNA. Chromosomal nonhistone protein does not form a complex with DNA when the two are mixed at lower ionic strength (0.01 M Tris buffer or 0.15 M NaCl). A complex is, however, formed when DNA and nonhistone protein are mixed at higher salt concentrations and then subjected to a NaCl gradient dialysis similar to that described for the reconstitution of DNA and histone (Huang *et al.*, 1964). Rat liver DNA was mixed with various amounts of chromosomal nonhistone protein in 1 M NaCl–0.01 M Tris buffer (pH 8), dialyzed against first 0.4 M NaCl and then 0.2 M NaCl in Tris buffer (0.01 M, pH 8) for 4 hr each, and finally against 0.01 M Tris buffer (pH 8) for 18 hr. Aliquots (2 ml) of the reconstitution mixture were then layered on top of 2.5 ml of 0.6 M sucrose in D_2O containing 0.01 M Tris buffer (pH 8) and centrifuged at 36,000 rpm for 17 hr in a Spinco SW-39 rotor. After centrifugation, 12-drop fractions were collected from the bottom of the tubes and ultraviolet absorbance was examined. DNA was run as a control (Figure 5A). An additional control consisted of nonhistone protein alone (dotted line in Figure 5B–D). That chromosomal nonhistone protein complexes with DNA follows from the facts that A_{230}/A_{260} of DNA fractions increases with an increase of input chromosomal nonhistone protein in the reconstitution mixture, and from the reduction in size of the free nonhistone protein peak in the presence of DNA (Figure 5). The peak fractions of chromosomal nonhistone–DNA complex shown in Figure 5 were collected and further analyzed for their chemical composition and template activity in support of RNA synthesis. As shown in Table IV, the ratios of nonhistone protein to DNA increase linearly by increasing concentrations of input nonhistone protein in the reconstitution mixture. The DNA complexed with nonhistone protein supports RNA synthesis catalyzed by exogenously added RNA polymerase as effectively as does DNA not so complexed. It is also observed that chromosomal nonhistone protein when directly added to the incubation mixture does not inhibit RNA synthesis directed by DNA (Table V).

Discussion

Nonhistone proteins are present in the rat liver chromatin as a real constituent, dissociation of which from DNA requires higher concentrations of salt than do histones (Marushige and Bonner, 1966; Fambrough and

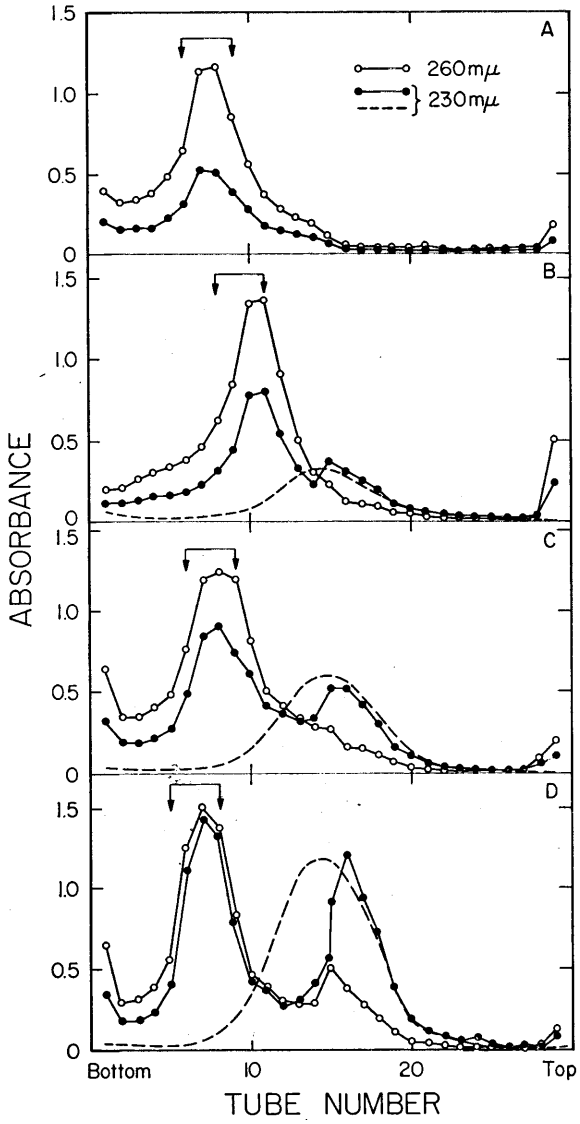

FIGURE 5: Reconstitution of chromosomal nonhistone protein with DNA. DNA (410 μg) was mixed with 0 (A), 410 (B), 820 (C), and 1640 μg (D) of chromosomal nonhistone protein in a total volume of 2.5 ml of 1 M NaCl containing 0.01 M Tris buffer (pH 8) and subjected to NaCl gradient dialysis. Aliquots (2 ml) were then layered on top of 2.5 ml of 0.6 M sucrose in D_2O containing 0.01 M Tris buffer (pH 8) and centrifuged at 36,000 rpm for 17 hr. After the run, 29 fractions were collected from each tube, each was diluted with 0.5 ml of H_2O, and absorbance was determined at 230 and 260 mμ. Nonhistone protein in the absence of DNA was also subjected to salt gradient dialysis and processed in the same manner (dotted line). Fractions indicated by arrows were combined and used for further analyses.

Bonner, 1968). Such nonhistone proteins are not dissociated from chromatin in 2 M NaCl, a concentration which dissociates all histones (Fambrough and Bonner, 1968). It has been shown also that treatment of the chromatin with dilute acid at low temperature selectively removes histones without denaturing DNA and that such acid-treated chromatin retains the majority of nonhistone proteins complexed to DNA (Marushige and Bonner, 1966). Chromosomal nonhistone proteins can be separated from DNA by centrifugation in 4 M CsCl (100,000*g*, 18–24 hr), under which conditions DNA sediments, whereas the protein floats and forms a skin (Bonner *et al.*, 1968a). This protein skin is, however, difficult to resolubilize at neutral pH although Benjamin and Gellhorn (1968) preserved it in soluble form by conducting the centrifugation at pH 11.6. In the present experiments, the chromosomal nonhistone protein is dissociated and solubilized by SDS. The DNA is then removed by centrifugation and the protein is further purified by ammonium sulfate precipitation. The chromosomal nonhistone protein thus isolated is soluble in Tris buffer. However, sedimentation analyses indicate that the protein is aggregated under this solvent condition. SDS binds to the protein and disperses it, from an originally very high molecular weight aggregate (average 6.1 S) to a fairly homogeneous SDS–protein complex with the molecular weight of protein component being 14,300. Difficulty in obtaining a precise agreement with the minimum molecular weight given by its amino acid com-

TABLE IV: RNA Synthesis with Chromosomal Nonhistone Protein–DNA Complex as Template.[a]

Nonhistone/DNA in the Reconstitution Mixture		RNA Synthesis[b] (μμmoles of AMP incorp/0.25 ml of incubn mixture)
Input	Product	
0	0.02	3130
1	0.40	3200
2	0.87	2920
4	1.60	2950

[a] Incubation mixture and other conditions given in Materials and Methods. The mixture contained 3.6 μg of DNA as the complex and 33 μg of RNA polymerase. Incubation for 10 min at 37°. [b] Incorporation by enzyme alone (20 μμmoles) substracted.

TABLE V: Effect of Chromosomal Nonhistone Protein on RNA Synthesis with DNA as Template.

μg of Nonhistone Protein Added/0.25 ml of Incubation Mixture[a]	RNA Synthesis[b] (μμmoles of AMP incorp/0.25 ml of incubn mixture)
2	2700
2.5	2730
5	2850
10	2740

[a] Incubation mixture and other conditions given in Materials and Methods. The mixture contained 5 μg of DNA, 33 μg of RNA polymerase, and chromosomal nonhistone protein as indicated. Incubation for 10 min at 37°. [b] Incorporation by enzyme alone (18 μμmoles) subtracted.

position is probably due to errors involved in dealing with a highly interacting system.

The chromosomal nonhistone protein complexes with histones (Figure 3). Similar interactions have also been observed with nuclear phosphoprotein (Langan and Smith, 1966), nuclear residual protein (Wang, 1966), and "chromatin acidic protein" (Wang, 1967). Langan (1967) has in addition shown that the ability of histones to decrease template activity of DNA is diminished if histone is complexed to nuclear phosphoprotein. The interaction of chromosomal nonhistone protein with histone appears to be ionic and NaCl as low as 0.15 M considerably reduced the interaction (Figure 4). Wang and Johns (1967) have also found that the formation of the histone–nonhistone protein complex is diminished at high ionic strength. This suggests that nonhistone protein–histone complexes may not occur at a physiological ionic strength in the cell. The chromosomal nonhistone protein froms a complex with DNA (Figure 5). It is, however, difficult to make an assessment from this experiment as to how much nonhistone protein can bind to DNA because of the highly self-interacting nature of this protein.

The isolated chromosomal nonhistone protein does not inhibit DNA-dependent RNA synthesis *in vitro* when added to the incubation mixture. The reconstituted chromosomal nonhistone protein–DNA complex (Table IV) as well as acid-treated chromatin (Marushige and Bonner, 1966) possess essentially the same template activity in support of RNA synthesis as does pure DNA. The facts that template-active chromatins generally contain more nonhistone protein (Bonner *et al.*, 1968b), that the active euchromatin fraction is rich in nonhistone protein in calf thymus (Frenster, 1965), and that nonhistone protein is nearly absent from the template-inactive nucleohistone fractions of pea embryo chromatin (Bonner and Huang, 1963), may suggest that one portion of the chromosomal nonhistone protein is associated with the active portion of the genome.

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