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Mode of Reconstitution of Chicken Erythrocyte and Reticulocyte Chromatin†

Robert A. Gadski and Chi-Bom Chae*

ABSTRACT: The mode of reconstitution of chicken erythrocyte and reticulocyte chromatin has been investigated. Chromatin was dissociated in 2 M NaCl, 5 M urea, and 0.01 M potassium phosphate (pH 7.2) and was dialyzed against various NaCl concentrations in 5 M urea and 0.01 M potassium phosphate (pH 7.2). Histone reassociation to DNA occurs with the binding of histone H5 at 0.5 M NaCl in 5 M urea, followed by histone H1 at 0.4 M NaCl in 5 M urea. All the classes of histones are reassociated with DNA at 0.2 M NaCl in 5 M urea and binding of all classes of histones is complete in 0.1 M NaCl and 5 M urea. Nonhistone proteins reassociate with DNA before and at the same time that histones reassociate with DNA. Binding of nonhistone proteins to DNA appears to be complete in 5 M urea and 0.01 M potassium phosphate (pH 7.2). There is also found in both erythrocyte and reticulocyte chromatin a nonhistone protein, present in relatively high concentrations, which remains associated with DNA in 2 M NaCl and 5 M urea. This tightly bound protein appears as one major band when chromatographed on sodium dodecyl sulfate-polyacrylamide gels, with a molecular weight of 95 000. This protein is soluble in phenol and sodium dodecyl sulfate but is insoluble in 5 M urea or 4 M guanidine hydrochloride. A fraction of reticulocyte nonhistone proteins was found to bind to DNA-cellulose in 5 M urea. The majority of these proteins elute at 0.15 M NaCl in 5 M urea but a significant fraction elutes at NaCl concentrations at which the bulk of the histones do not bind to DNA. The proteins that bind to free DNA have low molecular weights and do not show species specificity. Approximately 50% of the reticulocyte nonhistone protein does not bind to a DNA-cellulose column in 5 M urea and may require histones for complete reassociation.

Comparisons of native and reconstituted chromatin suggest that reconstituted chromatin has many of the same properties as native chromatin. RNA transcribed from reconstituted chromatin has been found to be similar to in vivo synthesized RNA (Gilmour and Paul, 1969), as well as RNA transcribed from native chromatin in vitro (Bekhor et al., 1969; Paul and Gilmour, 1968; Huang and Huang, 1969; Gilmour and Paul, 1970; Spelsberg et al., 1971). This is not only true for repetitive sequences of DNA but also for the transcription of a specific gene. The transcription of RNA from native and reconstituted avian reticulocyte chromatin (Barrett et al., 1974) and fetal liver chromatin (Paul et al., 1973) has shown similar (but not identical) fractional yields of globin-specific sequences. Also,

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reconstituted chromatin appears to have similar properties as native chromatin with respect to patterns of DNA digestion by nuclease (Axel et al., 1974), reactivity of thymine to γ rays (Roti et al., 1974), and formation of beaded structure (Oudet et al., 1975). These facts are interesting when considering the large numbers of histones and nonhistone-protein (NHP)[1] molecules that reassociate in some specific manner with DNA, which is large enough to contain several million genes, during the process of reconstitution. The above information suggests that chromatin has an intrinsic capacity for correct reassociation.

In this report we have studied the mode of reassociation of the histone and NHP from chicken erythrocyte chromatin and reticulocyte chromatin. These two chromatins offer an excellent system for studies on the mechanism of expression and repression of a specific gene, that is, the globin gene. We have determined the order of reassociation of the histones, which are believed to be nonspecific repressors of transcription (Paul and Gilmour, 1968), to DNA in various NaCl concentrations in 5 M urea. We have also studied the binding of the fractionated NHP to DNA under chromatin reconstitution conditions, since the NHP have been shown to be involved in specific RNA transcription (Gilmour and Paul, 1970; Paul et al., 1973; Spelsberg et al., 1971; Barrett et al., 1974). The order of binding of various protein fractions may give some insight into the structure and functional relationships of the various chromatin components. By attempting to elucidate which proteins determine the order of binding of the chromatin components necessary for specific RNA transcription we may be able to develop a fundamental understanding of how genes of eucaryotic organisms are expressed.

Experimental Section

Preparation of Chromatin. Chicken reticulocytes were prepared by daily 1.0-ml injections of phenylhydrazine (5 mg/ml) for a 5-day period (Attardi et al., 1966). On the 8th day the animals were decapitated and the blood was collected with the addition of $\frac{1}{10}$ volume of 10% sodium citrate. Following centrifugation at 12 000*g* for 10 min, the reticulocyte pellet was washed twice with 0.15 M NaCl. Packed erythrocytes were purchased from Pel Freez Biologicals Inc., Arkansas. The erythrocytes were washed twice with 0.15 M NaCl. Both reticulocyte and erythrocyte chromatin was prepared as previously described (Chae, 1975).

Chromatin Reconstitution. Erythrocyte chromatin was homogenized with a loose-fitting Dounce homogenizer in 2 M NaCl–5 M urea and 0.01 M potassium phosphate (pH 7.2) at a concentration of 500 μg of DNA/ml and stirred for 4 h at 4 °C. Portions of the dissociated chromatin were dialyzed against 0.6 M NaCl–5 M urea, 0.5 M NaCl–5 M urea, 0.4 M NaCl–5 M urea, 0.3 M NaCl–5 M urea, 0.2 M NaCl–5 M urea, 0.1 M NaCl–5 M urea, and 5 M urea, all in 0.01 M potassium phosphate (pH 7.2). After 4 h of dialysis at 4 °C, the fractions were centrifuged for 4 h at 200 000*g*. Following centrifugation, the supernatant was saved and the pellet was washed with the same buffer that it was dialyzed against. For reticulocyte chromatin it was necessary to include 1 mM phenylmethanesulfonyl fluoride and 1% dimethyl sulfoxide in 2 M NaCl–5 M urea solution to prevent proteolysis of proteins. However, erythrocyte chromatin shows no proteolytic activity in 2 M NaCl–5 M urea (Carter and Chae, 1976).

Gel Electrophoresis. Samples were either prepared for sodium dodecyl sulfate-gel electrophoresis or acid-urea-gel electrophoresis. Fractions prepared from sodium dodecyl sulfate-gel electrophoresis were made 1% sodium dodecyl sulfate–10 mM potassium phosphate (pH 7.0), and 0.1% β-mercaptoethanol. DNA pellets from reconstitution experiments were dissolved in 1.0 ml of 1% sodium dodecyl sulfate–10 mM potassium phosphate (pH 7.0), and 0.1% β-mercaptoethanol. DNA pellets from reconstitution experiments were dissolved in 1.0 ml of 1% sodium dodecyl sulfate–10 mM potassium phosphate (pH 7.0), and 0.1% β-mercaptoethanol with vigorous stirring for 16 h at room temperature. Samples prepared for sodium dodecyl sulfate-gel electrophoresis were electrophoresed as previously described (Chae, 1975). Histones were extracted from the fractions by suspending the pellet in, or dialyzing the supernates against, cold 0.2 M H_2SO_4 for 4 h. After centrifugation at 15 000*g* for 20 min the histone solution was dialyzed overnight against 7 M urea–0.9 N acetic acid and 0.1% β-mercaptoethanol at 4 °C. Histones were electrophoresed on 15% acrylamide gels in 2.5 M urea at a pH of 2.8 (Panyim and Chalkley 1969).

Preparation of [^3H] *NHP.* A chicken was made anemic by treatment with phenylhydrazine as described above. On the 6th day 5 mCi of [^{3}H]tryptophan (1.5 Ci/mmol) was administered intravenously over a 10-h period. On the following day blood was collected, and chromatin was prepared as described above. [^{3}H]NHP fraction was prepared by the procedure of Graziano and Huang (1971). Chromatin, treated for 30 min at 4 °C with 1 mM phenylmethanesufonyl fluoride and 1% dimethyl sulfoxide, was dissociated in 2 M NaCl–5 M urea and 0.01 M potassium phosphate (pH 7.2). All buffers in the following steps of preparation contained 1 mM phenylmethanesulfonyl fluoride and 1% dimethyl sulfoxide to inhibit protease activity (Carter and Chae, 1976). The chromatin was homogenized and stirred for 4 h at 4 °C and centrifuged for 15-h at 200 000*g*. The supernatant was dialyzed against 0.23 M NaCl–7 M urea and 0.01 M sodium acetate (pH 5.2). The fraction was then applied to a SP-C25 Sephadex column (2.3 × 20 cm). The column was washed with 0.23 M NaCl–7 M urea and 0.01 M sodium acetate (pH 5.2) until the [^{3}H]NHP were eluted. The column was next washed with 0.8 M NaCl–7 M urea and 0.01 M sodium acetate (pH 5.2) to elute the histone fraction. Proteins were determined by turbidity assay after precipitation with 20% trichloroacetic acid. Ehrlich ascites NHP were labeled by intraperitoneal injection of [^{3}H]tryptophan, 300 μCi per tumor-bearing mouse over a 16-h period. Cells were harvested and a NHP fraction was prepared as described above.

Preparation of DNA and DNA–Cellulose. DNA was prepared by the procedure of Marmur (1961) as modified by Chae (1975). The pronase-digestion step was omitted, since final DNA preparation usually shows proteolytic activity in 5 M urea. DNA–cellulose was prepared from chicken erythrocyte and Ehrlich ascite DNA according to the procedure of Litman (1968). The amount of DNA bound to cellulose was 2.5 mg of erythrocyte DNA/gram of cellulose and 3 mg of Ehrlich ascites DNA/gram of cellulose. Two grams of DNA cellulose were used for each experiment. The DNA–cellulose column was equilibrated in a standard buffer at 0.01 M Tris-HCl (pH 8)–1 mM EDTA and β-mercaptoethanol (0.01 ml/500 ml). Also included was 5 M urea. Samples were dialyzed overnight at 4 °C against standard buffer including 5 M urea.

Iodination of NHP Fractions. Enzymatic iodination was carried out with a solid-state preparation of lactoperoxidase purchased from Worthington Biochemical (David and Reisfeld, 1974). NHP fractions were dialyzed against 0.1 M

[1] Abbreviations used are: NHP, nonhistone protein; EDTA, (ethylenedinitrilo)tetraacetic acid.

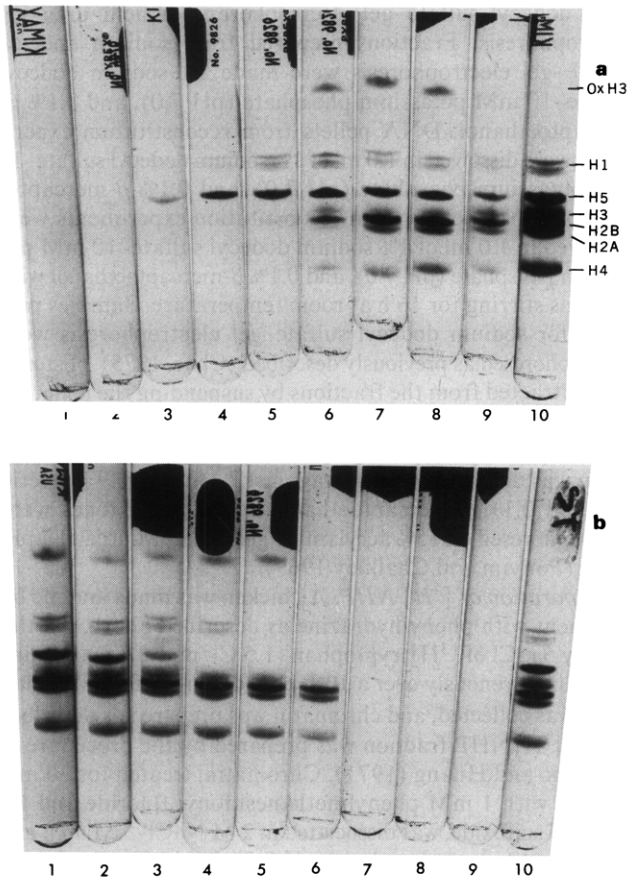

FIGURE 1: Electrophoretic pattern of histones of the erythrocyte chromatin dissociated in 2 M NaCl–5 M urea after dialysis into various concentrations of NaCl–5 M urea. (a) DNA pellet: (1) 2 M NaCl–5 M urea, (2) 0.6 M NaCl–5 M urea, (3) 0.5 M NaCl–5 M urea, (4) 0.4 M NaCl–5 M urea, (5) 0.3 M NaCl–5 M urea, (6) 0.2 M NaCl–5 M urea, (7) 0.1 M NaCl–5 M urea, (8) 0.0 M NaCl–5 M urea, (9) 0.0 M NaCl–0 M urea, (10) control histone fraction. (b) Supernate: (1) 2 M NaCl–5 M urea, (2) 0.6 M NaCl–5 M urea, (3) 0.5 M NaCl–5 M urea, (4) 0.4 M NaCl–5 M urea, (5) 0.3 M NaCl–5 M urea, (6) 0.2 M NaCl–5 M urea, (7) 0.1 M NaCl–5 M urea, (8) 0.0 M NaCl–5 M urea, (9) 0.0 M NaCl–0 M urea, (10) control histone fraction. The proteins soluble in 0.2 M H_2SO_4 were electrophoresed through the acid–urea–polyacrylamide gels as described under the Experimental Section. Proteins migrated from top (+) to bottom (−).

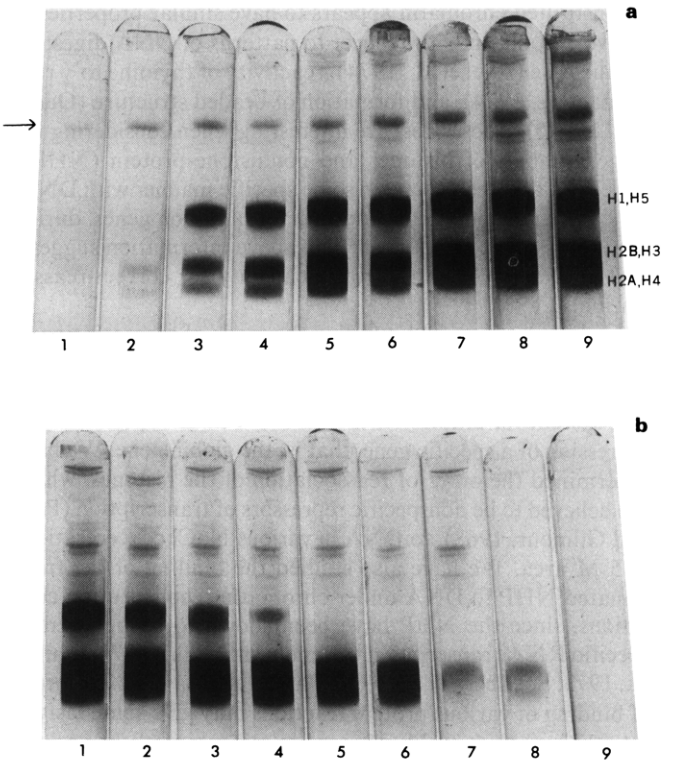

FIGURE 2: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of erythrocyte chromatin protein dissociated in 2 M NaCl–5 M urea after dialysis into various concentrations of NaCl–5 M urea. (a) DNA pellet: (1) 2 M NaCl–5 M urea, (2) 0.6 M NaCl–5 M urea, (3) 0.5 M NaCl–5 M urea, (4) 0.4 M NaCl–5 M urea, (5) 0.3 M NaCl–5 M urea, (6) 0.2 M NaCl–5 M urea, (7) 0.1 M NaCl–5 M urea, (8) 0.0 M NaCl–5 M urea, (9) 0.0 M NaCl–0 M urea. (b) Supernate: (1) 2 M NaCl–5 M urea, (2) 0.6 M NaCl–5 M urea, (3) 0.5 M NaCl–5 M urea, (4) 0.4 M NaCl–5 M urea, (5) 0.3 M NaCl–5 M urea, (6) 0.2 M NaCl–5 M urea, (7) 0.1 M NaCl–5 M urea, (8) 0.0 M NaCl–5 M urea, (9) 0.0 M NaCl–0 M urea. NHP that does not dissociate from DNA in 2 M NaCl–5 M urea is indicated by an arrow. Proteins migrated from top (−) to bottom (+).

NaCl–5 M urea–0.01 M potassium phosphate (pH 7.2)–1 mM phenylmethanesulfonyl fluoride, and 1% dimethyl sulfoxide. Following dialysis with 50 μCi of $Na^{125}I$, 2×10^{-5} M H_2O_2 and 10 μg of lactoperoxidase were added to a final volume of 1.0 ml. Incubation was carried out at 4 °C for 45 min. The solutions were centrifuged at 10 000g for 5 min and dialyzed against 1% sodium dodecyl sulfate–10 mM potassium phosphate (pH 7.0) and 0.1% β-mercaptoethanol overnight at room temperature. Phenylmethanesulfonyl fluoride was included in the reaction to reduce proteolytic degradation of NHP during iodination (Gadski and Chae, in preparation).

Results

Mode of Reassociation of Chromosomal Proteins during Reconstitution. The mode of chromatin reconstitution was investigated by dissociating chromatin in 2 M NaCl–5 M urea and 0.01 M potassium phosphate, (pH 7.2). Portions of the dissociated erythrocyte chromatin were dialyzed directly against various NaCl concentrations in the presence of 5 M urea–0.01 M potassium phosphate (pH 7.2) and against 0.01 M potassium phosphate (pH 7.2). The DNA and associated proteins were removed by centrifugation as described above. Direct dialysis was chosen over sequential dialysis (against decreasing NaCl concentrations) because it has been shown (Chae, 1975) that the mode of reassociation of chromosomal proteins with DNA is about the same during sequential and direct dialysis of dissociated chromatin into the same salt and urea concentrations. In addition, in the majority of the reported reconstitution experiments, the bulk of the histones and NHP reassociate with DNA in the final dialysis step, that is, dialysis of 0.4 M NaCl–5 M urea solution against dilute buffer (Chae, 1975). Acid–urea–polyacrylamide gel electrophoresis of the unbound and reassociated erythrocyte histones is shown in Figure 1. At the concentration of protein applied to the gels, the histones appear to be completely dissociated from DNA in 2 M NaCl–5 M urea (Figure 1a). The first histone to bind to DNA is histone H5 in 0.5 M NaCl–5 M urea. This is followed by the binding of histone H1 in 0.4 M NaCl–5 M urea. The remaining classes of histones (H2A, H2B, H3, and H4) were found to reassociate with DNA at 0.2 M NaCl–5 M urea. The supernatant of these fractions (Figure 1b) shows that all of histone H5 is completely reassociated with DNA in 0.4 M NaCl–5 M urea. Histone H1 is completely reassociated in 0.3 M NaCl–5 M urea, histone H3 is completely reassociated in 0.2 M NaCl–5 M urea, and the remaining histone classes (H2A, H2B, and H4) are completely reassociated in 0.1 M NaCl–5 M urea. Similar results were obtained with reticulocyte chromatin.

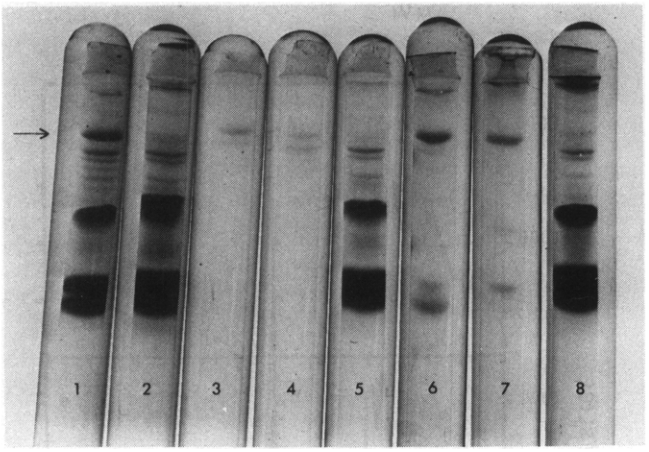

FIGURE 3: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of dissociated erythrocyte chromatin fractions. Chromatin dissociated in 2 M NaCl–5 M urea was fractionated on a Bio-Gel A50m column. The fractions obtained were electrophoresed on sodium dodecyl sulfate–polyacrylamide gels: (1) whole chromatin (control), (2) protein peak, (3) DNA (major peak), (4) DNA (trailing minor peak). Chromatin was dissociated in 2 M NaCl–5 M urea–1% β-mercaptoethanol–0.01 M Tris-HCl (pH 8.0) and centrifuged at 200 000g for 4 h: (5) supernate and (6) DNA pellet. Chromatin was dissociated in 4 M guanadine-HCl–1% β-mercaptoethanol–0.01 M Tris-HCl (pH 8.0) and centrifuged at 200 000g for 4 h: (7) DNA pellet and (8) supernate. The protein that does not dissociate from DNA in 2 M NaCl–5 M urea is indicated by an arrow.

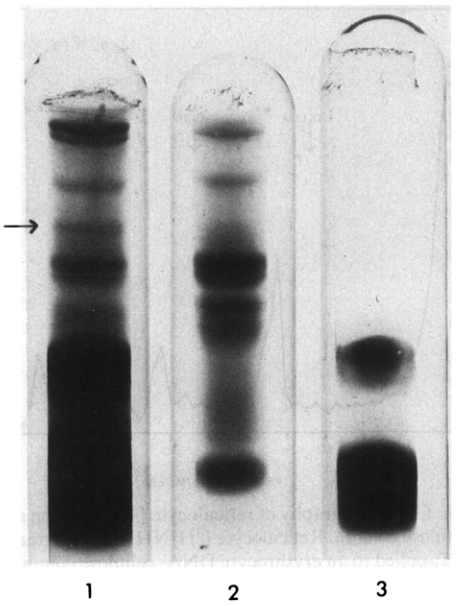

FIGURE 4: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of reticulocyte chromatin protein fractions obtained from SP Sephadex column. Approximately 100 μg of protein was applied to each gel: (1) whole chromatin, (2) NHP fraction, (3) histone fraction. The protein that does not dissociate from DNA in 2 M NaCl–5 M urea is indicated by an arrow.

The same reconstitution experiments were conducted, but sodium dodecyl sulfate–polyacrylamide gel electrophoresis was carried out on the various fractions to determine the mode of reassociation of the erythrocyte NHP (Figure 2). At least one major high-molecular-weight and two minor low-molecular-weight erythrocyte NHP remain associated with DNA in 2 M NaCl–5 M urea. This tightly bound NHP will be discussed in greater detail later. Various dissociated NHP appear to reassociate with DNA before and at the same time as the bulk of the histones. At 0.3 M NaCl–5 M urea it is evident that a significant fraction of the NHP have become reassociated with the DNA. At 0.3 M NaCl–5 M urea only histones H1 and H5 are reassociated with the DNA. It is interesting to consider what effect, if any, these NHP have on the binding of the remaining histones H2A, H2B, H3, and H4, and if the binding of certain sequence specific NHP is necessary for the correct reconstitution of chromatin. These sodium dodecyl sulfate gels were intentionally overloaded with respect to the histone proteins in order to show the binding pattern of the NHP, which are present in relatively low quantities in chicken erythrocyte and reticulocyte chromatin. All of the NHP appears to reassociate with DNA in the presence of 5 M urea, as judged by sodium dodecyl sulfate–gel electrophoresis of the supernate fraction (Figure 2b). The results obtained with reticulocyte chromatin are similar to those of erythrocyte chromatin (not shown here). The binding of some NHP to DNA in 5 M urea is not completely unexpected, since some enzymes, bovine lactoperoxidase (David and Reisfeld, 1974), chromatin protease (Carter and Chae, 1976), subtilisin (Markland and Smith, 1971), etc., have been found to retain their activity in 5 M urea. In the reconstitution techniques used the NaCl is first removed by dialysis followed by removal of urea. Since histones are known to bind to DNA in urea it would be necessary for the NHP, that protect a specific region of DNA from becoming repressed, to specifically bind to the DNA sequences in urea.

Proteins Tightly Bound to DNA. The presence of proteins tightly bound to DNA in 2–3 M NaCl–5 M urea has been observed in HeLa cell chromatin (Pederson and Bhorjee, 1975), in rat liver chromatin (Chiu et al., 1975), and in Pea cotyledon chromatin (Dahmus and Bonner, 1970). Erythrocyte chromatin dissociated in 2 M NaCl–5 M urea is distinct from other tissues because of the presence of the major tightly bound high-molecular-weight NHP. Chromatography of dissociated chromatin on a Bio-Gel A-50 m column equilibrated in 2 M NaCl–5 M urea (not shown here) shows separation of DNA from dissociated proteins. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the various peaks (Figure 3) shows that the major DNA peak has one major high-molecular-weight protein associated with it. A smaller trailing DNA peak shows the presence of this same protein band, as well as another protein of lower molecular weight. The major tightly bound protein is soluble in sodium dodecyl sulfate and phenol, but it is not soluble in 2–3 M NaCl–5 M urea (in the presence or absence of β-mercaptoethanol), or 4 M guanidine chloride. Reticulocyte chromatin also contains this protein (see Figure 4). In erythrocyte chromatin the presence of a small number of the tightly bound proteins in relatively large quantities suggests that these proteins are not involved in specific transcription. Pederson and Bhorjee (1975) find tightly bound NHP present in high concentrations associated with inactive chromatin that is in agreement with our conclusions. It is interesting that the investigators who extracted NHP by phenol from dehistonized chromatin reported that 99 000-dalton NHP is one of the major NHP in avian red blood cells (Vidali et al., 1973). However, the 99 000-dalton protein appears to be absent in the NHP prepared from 3 M NaCl-dissociated chromatin (Sanders, 1974).

Binding of Reticulocyte [^{3}H]NHP to DNA–Cellulose. DNA–cellulose chromatography was undertaken to determine at what concentrations of NaCl in 5 M urea would NHP bind to DNA. This method of separating DNA-bound NHP from unbound NHP was chosen over centrifugation method because a significant amount of NHP in 5 M urea pellets during

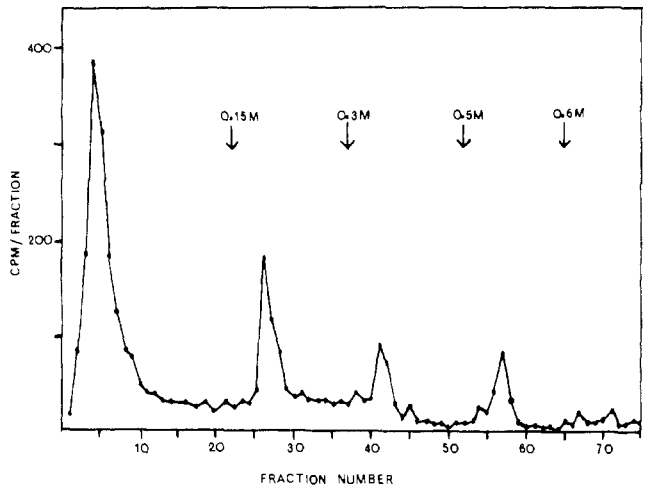

FIGURE 5: Chromatography of reticulocyte [^{3}H]NHP on an erythrocyte DNA-cellulose column. Reticulocyte [^{3}H]NHP in 5 M urea and standard buffer was applied to an erythrocyte DNA-cellulose column equilibrated in 5 M urea and standard buffer. The column was eluted with various concentrations of NaCl-5 M urea and standard buffer. Fractions of 1.3 ml were collected.

high-speed centrifugation. Chromatography on a Bio-Gel A-50 m column was not used because it was found that some NHP have an affinity for the agarose in the presence of 5 M urea.

A reticulocyte [^{3}H]NHP fraction was obtained by [^{3}H]-tryptophan labeling of the chromatin proteins and fractionation of the NHP from the histones on SP Sephadex by the procedure of Graziano and Huang (1971) as described under the Experimental Section. The histone fraction appears to contain approximately 15% of the [^{3}H] protein applied to the SP Sephadex column. Graziano and Huang (1971) report that chromatography of chicken brain chromatin proteins on SP Sephadex resulted in 10% or so contamination of the histone fraction with NHP. The NHP and histone protein fractions prepared by this method were electrophoresed on sodium dodecyl sulfate-polyacrylamide gels together with whole chromatin (Figure 4). The NHP fractions show a protein migrating in the position of the histones H2B and H3. Ruiz-Carrillo et al. (1974) also report a similar small-molecular-weight NHP that they attribute to the presence of globin in the nucleus. There does not appear to be histone contamination of the NHP fraction as judged by acid-urea-gel electrophoresis (not shown here). A fraction of the NHP was dialyzed against 5 M urea and standard buffer, as described under the Experimental Section, and applied to a DNA-cellulose column prepared with erythrocyte DNA. Approximately 50% of the NHP applied to the DNA-cellulose column did not bind to the column (Figure 5). Since earlier reconstitution experiments with erythrocyte (Figure 2) and reticulocyte chromatin showed that the binding of NHP to DNA was complete in 5 M urea, it is probable that many of the NHP bind to a histone-DNA complex. Of the NHP that bind to DNA in 5 M urea 21% of the NHP elutes with 0.15 M NaCl-5 M urea, 6.3% of the NHP elutes with 0.3 M NaCl-5 M urea, and 5% of the NHP elutes with 0.5 M NaCl-5 M urea, all in standard buffer. At 0.15 and 0.3 M NaCl-5 M urea the bulk of the histones would not bind, this includes histones H2A, H2B, H3, and H4. At 0.5 M NaCl-5 M urea only histone H5 reassociates with DNA. In the control experiment there is very little binding of reticulocyte NHP to cellulose alone. Similar DNA binding patterns were seen when [^{3}H]NHP from Ehrlich ascites chromatin was chromatographed on Ehrlich ascites DNA-cellulose (not shown here). It is of interest that in at least two tissues there

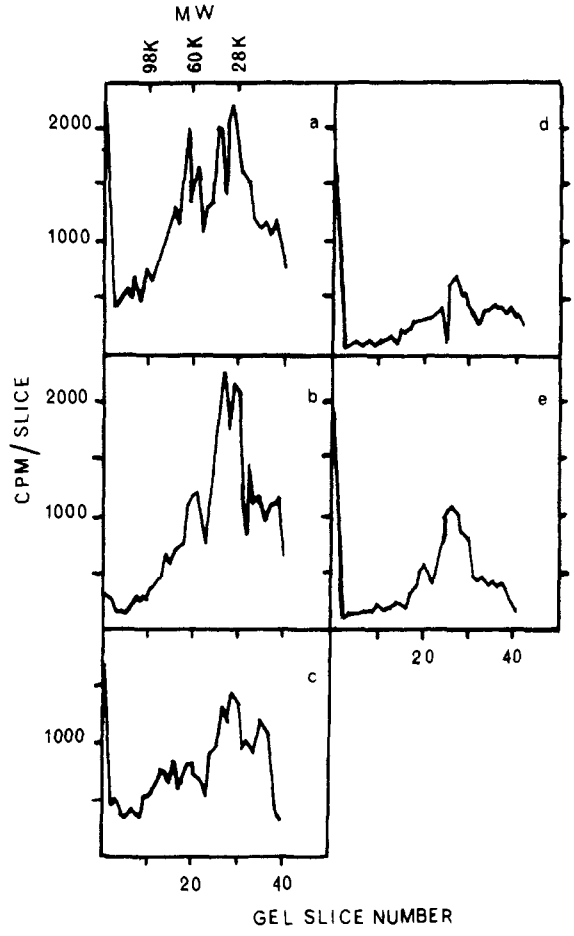

FIGURE 6: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of reticulocyte NHP fractionated on an erythrocyte DNA-cellulose column (Figure 5). The various NHP fractions obtained from chromatography on DNA-cellulose (Figure 5) were labeled with ^{125}I and electrophoresed: (a) control NHP fraction, (b) NHP that did not bind to DNA-cellulose, (c) 0.15 M NaCl-5 M urea eluate, (d) 0.3 M NaCl-5 M urea eluate, and (e) 0.5 M NaCl-5 M urea eluate. Proteins migrated from left (−) to right (+).

are NHP that bind to DNA in 0.3 M NaCl-5 M urea, which are eluted by 0.5 M NaCl-5 M urea. Only histones H5 and H1 bind to DNA under these conditions. Each fraction of reticulocyte [^{3}H]NHP eluted from the erythrocyte DNA-cellulose columns was iodinated as described under the Experimental Section and electrophoresed on sodium dodecyl sulfate-polyacrylamide gels. The pattern (Figure 6) of the NHP shows that most of the protein in the reticulocyte NHP fraction are below 60 000 daltons in molecular weight. This is in agreement with data obtained from gels stained with Coomassie blue. Both the reticulocyte NHP fraction that elutes from DNA-cellulose in 5 M urea and the NHP that elute at 0.15 M NaCl-5 M urea show electrophoretic patterns very similar to that of the whole NHP fraction applied to the DNA-cellulose column. However, the proteins eluting at 0.3 M NaCl-5 M urea and 0.5 M NaCl-5 M urea show a slightly higher average molecular weight than the original NHP fraction, and the protein patterns are complex. The reticulocyte [^{3}H]NHP that bind to erythrocyte DNA-cellulose also bind to Ehrlich ascites DNA-cellulose (not shown here) and therefore are not species specific.

Discussion

It has been the purpose of this study to obtain some under-

standing of the mode of chromatin protein reassociation with DNA. Since reconstituted chromatin has many of the properties of native chromatin, an appreciation of the intrinsic self-assembly properties of chromatin may lead to an understanding of the function of the various components in chromatin. In our method of reassociation, dissociated chromatin is dialyzed directly against various NaCl concentrations in 5 M urea. This technique is used so that the time period for reassociation is shorter-limiting the chance of proteolytic degradation of chromatin proteins (Carter and Chae, 1976) and also because in the reconstitution procedure of Bekhor et al (1969) and Gilmour and Paul (1969) the majority of the histones and NHP appear to reassociate with DNA in the last dialysis step (Chae, 1975).

Chromatin NHP can be divided into several groups based on the affinity these proteins have for DNA. There is in chromatin a group of proteins that remain tightly bound to DNA. Pederson and Bhorjee (1975) report a special class of NHP in HeLa cells that remain associated with DNA in high NaCl and 5 M urea buffers. The majority (70%) of these proteins have molecular weights less than 30 000. Chiu et al.'s (1975) data shows that in rat liver chromatin dissociated in 2.5 M NaCl–5 M urea and 0.05 M Tris-HCl (pH 8.0) 3–5% of the total chromatin protein remains associated with DNA. In chicken erythrocyte and reticulocyte chromatin it is interesting that the tightly bound proteins appear to consist of one major high-molecular-weight protein found in relatively high concentrations. NHP dissociated from DNA in high NaCl concentrations (2–3 M) and 5 M urea are responsible for transcriptional specificity of chromatin (Paul et al., 1973). Since the proteins remaining tightly bound to DNA are then not directly involved in transcription specificity they may have a structural function or may be implicated in some way as general repressors of transcription. The presence in relatively large amounts of one major protein would support this hypothesis. Proteins involved in specific gene expression are probably a heterogeneous mixture of proteins present in relatively low concentrations. Concerning the binding to DNA of the group of NHP that dissociate from DNA in 2 M NaCl and 5 M urea our data is in agreement with an earlier report (Chae, 1975). The dissociated NHP reassociate with DNA before, at the same time, and after the bulk of the histones reassociate with DNA. At the present time further experiments are in progress that are designed to determine what significance the binding of a small amount of NHP to DNA at NaCl concentrations where the bulk of the histones do not bind to DNA has on the reassociation process.

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