Volume 15 Number 22 1987 Nucleic Acids Research

Chicken erythrocyte β-globin chromatin: enhanced solubility is a direct consequence of induced histone hyperacetylation

William R.Alonso, Richard C.Ferris, Dong-Er Zhang and Daniel A.Nelson

Department of Biochemical and Biophysical Sciences, University of Houston, Houston, TX 77004, USA

Received July 26, 1987; Revised and Accepted October 19, 1987

ABSTRACT

Chicken immature red blood cells were incubated for 1 hour in Swim's medium containing ^{3}H-acetate and 10 mM *n*-butyrate. During the incubation period, the small percentage of dynamically acetylated and deacetylated histone is radiolabeled and hyperacetylated. A second effect of the *n*-butyrate incubation is to shift a small subset of nucleohistone into a soluble form. This chromatin is predominantly polynucleosome size (approximately dimer to pentamer) and can be separated from soluble mononucleosomes by 5-30% sucrose density gradient centrifugation. The soluble polynucleosomes are 25-30 fold enriched for adult β-globin (β^{A}) DNA and contain the hyperacetylated histones. We have tested whether histone hyperacetylation is responsible for the enhanced β-globin chromatin solubility by *in vitro* deacetylation of the soluble chromatin histones. This procedure converts the β-globin polynucleosomes to an insoluble form, demonstrating that histone hyperacetylation is in fact directly responsible for the increased solubility of the β^{A} chromatin.

INTRODUCTION

In chicken erythrocytes, only 2-4 percent of the total histone is actively acetylated and deacetylated (1,2), presumably a consequence of the reduced genomic activity in these cells. If dynamic acetylation is in some manner coupled to transcription, or involved in the maintenance of the active chromatin conformation, then this histone modification must be preferentially localized to active chromatin in the immature erythrocyte, including the adult β-globin region. Incubation of the erythrocytes with the histone deacetylase inhibitor *n*-butyrate (3) would result in the hyperacetylation of the histones associated with the β-globin DNA. The chromatin containing these highly acetylated histones would then be more soluble in $MgCl_2$ containing buffers compared to chromatin with low levels of histone-acetate (4,5), permitting isolation by differential centrifugation.

In earlier studies (6,7), we incubated chicken immature erythrocytes for 1 hour with *n*-butyrate, isolated the nuclei, mildly cleaved the DNA with an endonuclease that has no preference for chromatin containing hyperacetylated histones (4,8,9), and isolated the soluble chromatin by centrifugation. The soluble polynucleosomes were enriched for adult β-globin DNA (6) and highly acetylated histones (7). In this report, we provide direct evidence that the increased β-globin polynucleosome solubility is a result of histone hyperacetylation.

MATERIALS AND METHODS

Treatment of chickens and isolation and incubation of chicken immature erythrocytes

Anemia was induced in adult white leghorn chickens and the immature red blood cells were isolated as described previously (6). Typically, the immature erythrocytes prepared from two chickens were incubated for 60 minutes at 37°C in 100 ml of Swim's S-77 medium containing 10 mM *n*-butyrate and 20 mCi of ^{3}H-acetate [0.5 Ci/mmole, ICN].

Isolation, digestion and fractionation of nuclei

Nuclei were isolated as described earlier (7), resuspended at 70 $A_{260\ nm}$ units/ml in Nuclear Digestion Buffer [0.25 M sucrose, 60 mM KCl, 15 mM NaCl, 3 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM Na *n*-butyrate, 15 mM MES (2-N-morpholinosulfonic acid), 0.1 mM PMSF (phenylmethylsulfonylfluoride), pH 6.6 (NaOH)] and digested at 37°C for 10 minutes with micrococcal nuclease [Sigma] at 1 unit of enzyme per 50 µg of DNA. Reactions were terminated by the addition of 1/50 volume of 0.1 M EGTA [pH 7.4] and samples centrifuged at 9000 x g for 10 minutes to obtain soluble (S) and insoluble (P) chromatin fractions.

Sucrose density gradient centrifugation

3.5 ml of soluble (S) chromatin was overlayed directly onto 34 ml, 5-30% isokinetic sucrose gradients and centrifuged for 20 hours at 24,000 rpm in a SW-27 rotor [Beckman] at 4°C. The gradient buffer contained 60 mM KCl, 15 mM NaCl, 3 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM Na *n*-butyrate, 15 mM MES [pH 6.6], 0.1 mM PMSF, 2 mM EGTA. This buffer is essentially the same as the nuclear digestion buffer, except that EGTA is present.

DNA preparation, electrophoresis and filter hybridization

Southern transfer method. DNA was isolated, electrophoresed on 1% agarose gels, transferred to nitrocellulose, hybridized to nick-translated pHB1001 [a plasmid containing a chicken β^A cDNA; ref. 10], or to pOV230 [a plasmid containing an ovalbumin cDNA sequence; ref. 11] and autoradiograms prepared as described previously (6). Prior to electrophoresis, DNA samples were treated with 0.3 N NaOH at 37° C for 18 hours to remove RNA, thus all depicted DNA samples are single-stranded.

Dot blot analysis. DNA dot blotting was as described by Kafatos *et al.* (12). After incubation of the DNA in 0.3 N NaOH for 18 hours, samples were diluted with an equal volume of 2 M ammonium acetate and 5 µg dotted onto nitrocellulose filters using the Bio-Rad dot blot apparatus.

Histone preparation, electrophoresis and fluorography

Histone isolation, electrophoresis and the preparation of fluorograms were as described previously (7).

Preparation of the yeast histone deacetylase

A detailed report of the purification of the yeast histone deacetylase is in preparation. Briefly, nuclei were isolated from protoplasted yeast cells (13), extracted with 0.35 M NaCl, and

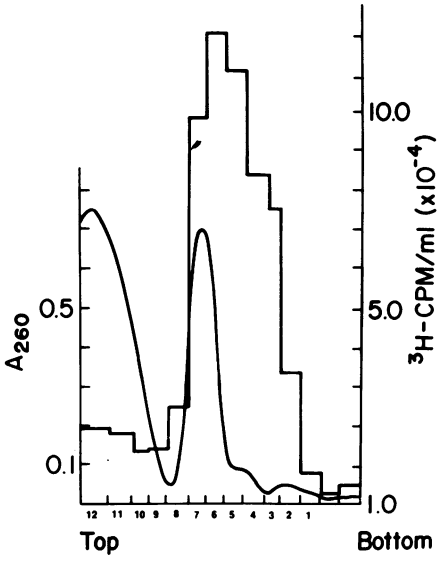

Figure 1. Sucrose density gradient centrifugation of the soluble chromatin. Soluble chromatin isolated from *n*-butyrate/^{3}H-acetate incubated immature erythrocytes was layered onto a 5-30% sucrose density gradient and centrifuged as described in the Materials and Methods. The smooth curve depicts the 260 nm absorbance profile, measured continuously during gradient fractionation. The histogram presents the ^{3}H counts per minute [cpm] in each fraction as determined by scintillation counting. Fraction numbers at the bottom of the figure [fractions 1-12] correspond to the fraction numbers used subsequently in figures 2 and 3.

the extract chromatographed on a 1.5 x 75 cm Sepharose 4B column equilibrated with 10 mM Tris, 2 mM EDTA, 1 mM β-mercaptoethanol, 10 mM Na *n*-butyrate, 100 mM NaCl, 0.1 mM PMSF, pH 7.4. Sepharose 4B fractions containing histone deacetylase activity were then applied to a DEAE Sephadex A-25 column [1.2 x 12 cm] pre-equilibrated with the above column buffer. The column was eluted with a 100 to 400 mM NaCl gradient and fractions containing the single peak of activity at 0.28-0.30 M NaCl were pooled. The yeast histone deacetylase was dialyzed into Nuclear Digestion Buffer containing 2 mM EGTA prior to use.

We estimate that after DEAE Sephadex chromatography the deacetylase is approximately 300-fold purified. The preparation of the nuclei and subsequent extraction results in about a 10-fold purification. Sepharose 4B chromatography results in another 10-fold, and DEAE Sephadex another 3-fold increase in purity. The preparation contains no detectable protease or DNase activities when incubated with histone and DNA substrates for 30 minutes at 37°C.

RESULTS

As we have described previously (6,7), a one hour incubation of chicken immature red blood cells in Swim's medium plus 10 mM *n*-butyrate increases the solubility of β^A chromatin.

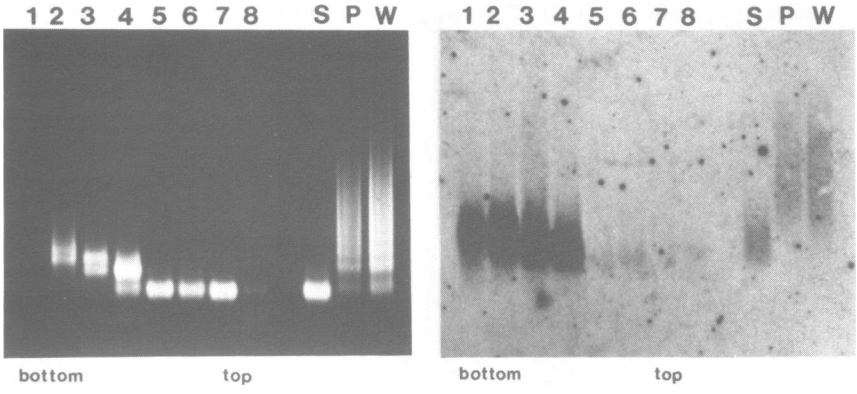

Figure 2. Analysis of DNA from the sucrose density gradient. Left Hand Panel: DNA from fractions 1-8 was electrophoresed on a 1% agarose gel, along with DNA from total soluble chromatin (S), insoluble (P) chromatin and from the whole (W) digest. Lanes 2 through 7 and lane S contain 5 μg of DNA, lanes P and W contain 20 μg of DNA. DNA in lanes 1 and 8 could not be quantitated. Right hand panel: DNA was transferred to nitrocellulose, hybridized to nick-translated pHB1001 [a plasmid containing the adult β-globin cDNA sequence] and an autoradiogram prepared as shown in the figure.

This enhanced solubility is detected after a mild micrococcal nuclease digestion and centrifugation of the isolated nuclei. The soluble chromatin from the *n*-butyrate incubated cells is up to 11-fold enriched in β^A DNA sequences compared to this chromatin from cells incubated without *n*-butyrate. The majority of the nucleohistone in the soluble chromatin fraction is nucleosome core size and is not enriched for the active sequences (6). It is the soluble polynucleosomes from the *n*-butyrate incubated cells that contain most of the β^A DNA.

The soluble polynucleosomes may be separated from the soluble mononucleosomes by exclusion chromatography (6), or sucrose density gradient centrifugation, as illustrated in figure 1. After sucrose gradient centrifugation, it is evident that the bulk of the soluble material absorbing at 260 nanometers consists of acid soluble oligonucleotides [fractions 9-12] and mononucleosomes [fractions 5-8]. Only a small portion of the soluble nucleohistone is polynucleosome size [fractions 1-4].

During the 1 hour *n*-butyrate incubation required to produce the soluble polynucleosomes, the immature red blood cells were also incubated with ^{3}H-acetate. The histogram in figure 1 shows the profile of ^{3}H in the sucrose density gradient. The amount of ^{3}H-acetate at the top of the gradient is low, indicating very little free ^{3}H-acetate or radiolabeled histone in the soluble chromatin fraction. The peak of the histogram is biased toward the higher molecular weight species [nucleosome dimers, trimers and tetramers] because the histone associated with the soluble polynucleosomes has become simultaneously hyperacetylated (7) and labeled with ^{3}H-acetate during the *n*-butyrate incubation. The soluble chromatin contains

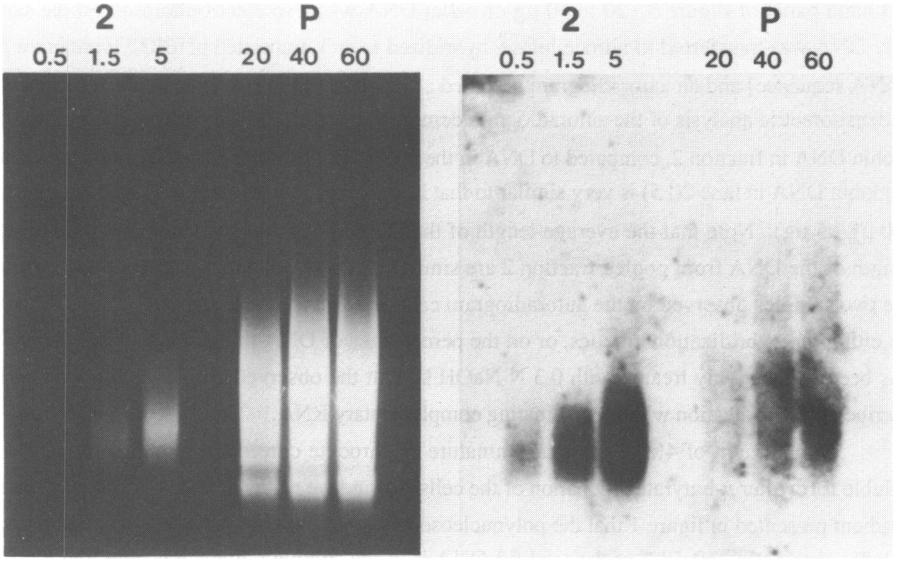

Figure 3. Quantitation of enrichment for adult β-globin DNA in the soluble polynucleosomes. The fraction 2 chromatin from a number of sucrose gradients was pooled, DNA isolated, incubated with alkali, precipitated and resuspended in agarose gel sample buffer. The absorbance at 260 nm was then accurately determined for fraction 2 DNA and for pellet (P) DNA. Left hand panel: Ethidium bromide stained agarose gel where (2) = fraction 2 DNA and (P) = DNA from insoluble, pelleted chromatin. 0.5, 1.5, 5, 20, 40 and 60 are the micrograms of DNA loaded in each slot. Right hand panel: Autoradiogram obtained after hybridization of the DNA to nick-translated pHB100l.

histones with a four-fold greater specific activity [cpm/μg histone] than those in the pellet. We also calculate that the soluble polynucleosome histones have a remarkable 30-fold greater specific activity than the pellet proteins. The soluble polynucleosomes are therefore highly enriched for the small percentage of dynamically acetylated immature red blood cell histone (2).

The left hand panel of Figure 2 illustrates the DNA sizes in fractions 1-8 of the sucrose density gradient. Fractions 1-4 contain the polynucleosome size, and fractions 5-8 the monomer size DNA species. The total soluble chromatin [lane S], from which the chromatin in fractions 1-8 is originally derived, is predominantly monomer size, as stated for the absorbance profile of the sucrose density gradient. The pellet (P) DNA banding pattern mimics that of the whole digest (W), since 95-97% of the total erythrocyte chromatin is in this insoluble form. The right hand panel of Figure 2 confirms that the soluble polynucleosomes are enriched for adult β-globin DNA.

To accurately quantitate the enrichment for active chromatin in the soluble polynucleosomes, fraction 2 chromatin from a number of sucrose gradients was pooled, the DNA isolated, and 0.5 to 5 μg of the sample electrophoresed on an agarose gel as shown in the

left hand panel of Figure 3. 20 to 60 μg of pellet DNA was also electrophoresed on the same gel. DNA was transferred to nitrocellulose, hybridized to nick-translated pHB1001 [contains a β^A cDNA sequence] and an autoradiogram prepared as depicted in the right hand panel of Figure 3. A densitometric analysis of the autoradiogram demonstrates a 25-30 fold enrichment for adult β-globin DNA in fraction 2, compared to DNA in the pellet. For example, the total amount of adult β-globin DNA in lane 2(1.5) is very similar to that in lane P(40), suggesting a 27 fold enrichment (40 μg/1.5 μg). Note that the average length of the DNA in the pellet fraction and the average length of the DNA from pooled fraction 2 are similar. Thus the difference in globin content of the two samples observed on the autoradiogram cannot be attributed to the effects of DNA size on either the hybridization kinetics, or on the percentages of DNA transferred. Also, the DNA has been exhaustively treated with 0.3 N NaOH so that the observed globin content cannot be ascribed to hybridization with contaminating complementary RNA.

A maximum of 4% of the total immature erythrocyte chromatin may be prepared in a soluble form after *n*-butyrate incubation of the cells (6), and we estimate from the sucrose density gradient presented in figure 1 that the polynucleosomes represent approximately 10% of the total soluble chromatin. 40-50% of the total β^A DNA is in the soluble polynucleosome fraction (6), thus we estimate overall, that the soluble polynucleosomes are 12-15 fold enriched for β^A sequences compared to the whole digested DNA.

These results, and those presented previously (7) are consistent with the hypothesis that histone hyperacetylation enhances the solubility of the β^A chromatin. A final conclusion that this preferential solubilization is due exclusively to histone hyperacetylation has not, however, been possible due to the pleiotropic effects of *n*-butyrate during cell incubations. *n*-Butyrate incubation alters histone phosphorylation patterns (14) and H1 composition (15), both of which could contribute significantly toward increased solubility.

We have devised a method to test whether histone hyperacetylation is the major factor responsible for the increased globin chromatin solubility, and our scheme is illustrated in figure 4. We have asked whether deacetylation of the histones in the soluble chromatin fraction [now designated S1 in figure 4] reduces the solubility of the β-globin polynucleosomes. S1 chromatin is subjected to the action of a purified yeast histone deacetylase [see Materials and Methods] and the deacetylated S1 chromatin fractionated by centrifugation into soluble (S2) and insoluble (P2) components. As illustrated in figure 4, we predict the P2 chromatin fraction to be enriched for the β^A containing polynucleosomes. The basis for this prediction is that in the absence of *n*-butyrate incubation of the cells, the β^A polynucleosomes are not sequestered into the soluble chromatin fraction (6), suggesting to us that these polynucleosomes are insoluble when deacetylated.

The amount of yeast histone deacetylase to be added to the S1 chromatin fraction to obtain an adequate amount of deacetylation was determined empirically. A major advantage in using the yeast deacetylase is that it is not inhibited by *n*-butyrate (13), thus this short chain fatty

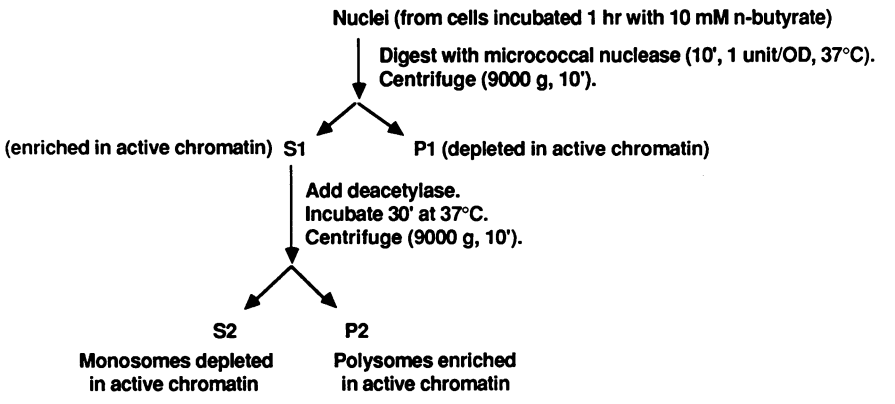

Figure 4: Scheme to test whether histone hyperacetylation is responsible for the *n*-butyrate induced adult β-globin polynucleosome solubility. Isolated chicken immature erythrocyte nuclei were digested with micrococcal nuclease and centrifuged to yield soluble [now designated S1] and insoluble [designated P1] nucleohistone. The soluble fraction (S1) is mixed with the yeast histone deacetylase, incubated at 37°C for 30 minutes and centrifuged to yield the soluble material designated S2 and pelleted nucleohistone designated P2.

acid can be included in the preparation of chicken nuclei and the S1 fraction, and maintained in the S1 fraction to inhibit the endogenous chicken deacetylase. The deacetylation reaction is controlled solely by the quantitiy of added yeast deacetylase. Using aliquots of the S1 fraction and of the yeast deacetylase [both in Nuclear Digestion Buffer containing 2 mM EGTA], conditions were established in which 30-50% of the labeled acetyl groups were removed from the histone during a 30 minute incubation period.

We first demonstrated that the yeast histone deacetylase not only removed acetyl groups from the S1 histone, but that its action was sufficient to produce a P2 chromatin fraction. Cells were simultaneously incubated in *n*-butyrate and ^{3}H-acetate for one hour, nuclei isolated and digested with micrococcal nuclease. Lane W of figure 5 depicts the levels of acetylation of the labeled histone in the whole digested sample. The small percentage of histone dynamically acetylated in the red blood cells is hyperacetylated [note the considerable amount of radiolabel in H4Ac4] and preferentially fractionates into the S1 fraction [see lane S1], as described previously (7). The majority of the histone [96-98%] is unlabeled, is not hyperacetylated and fractionates into the P1 fraction. This information regarding the characteristics of the unlabeled histone cannot be conveyed by the fluorogram in figure 5, lane P1, but has been described before (7). When the S1 chromatin is deacetylated and then fractionated by centrifugation into S2 and P2, it is evident that the radiolabeled histones in S2 and P2 are not as highly acetylated compared to those in the original S1 sample. For example, radiolabeled H4 in the S2 and P2 fractions is predominantly mono- and di-acetylated, whereas in the original S1 sample, much of the radiolabel is in the tetra-acetylated form. Thus, visual comparison of lane S1 with lanes S2 and

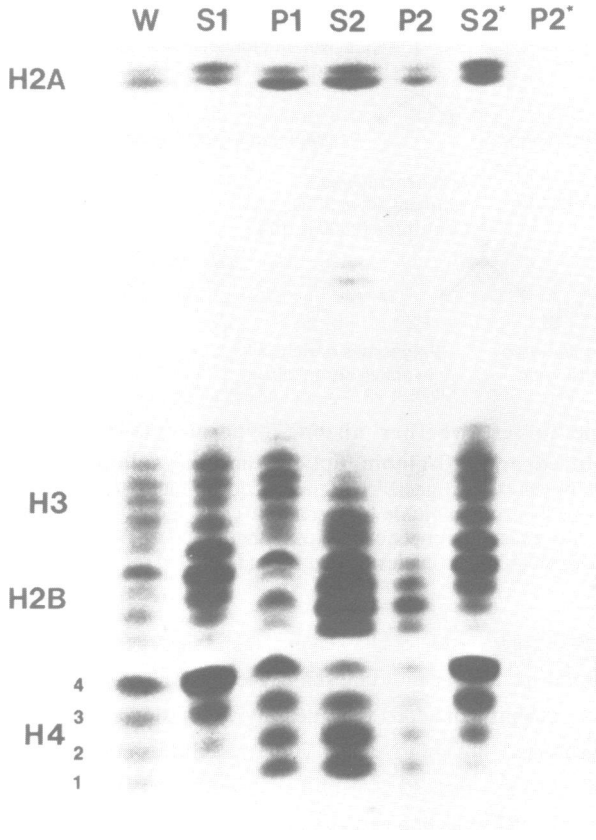

Figure 5: Fluorogram of a Triton acid-urea gel after electrophoresis of histone isolated from W, S1, P1, S2, P2, S2* and P2* chromatin fractions. The preparation of S1, P1, S2 and P2 chromatin fractions is as outlined in figure 4. S2* and P2* are samples from a control experiment where the yeast histone deacetylase was mixed with the chicken S1 chromatin and placed on ice for 30 minutes. Histones were extracted from each sample, electrophoresed on a Triton acid-urea gel and a fluorogram prepared as shown in the figure. Lane W contains histones from the unfractionated nuclei after digestion with micrococcal nuclease. The numbers 1, 2, 3 and 4 for H4 mark the respective positions of the mono-, di-, tri- and tetra-acetylated forms.

P2 demonstrates that deacetylation has occurred as a result of the addition of the deacetylase. The radiolabeled histone in P2 indicates that some chromatin is now insoluble and sequestered into this chromatin fraction.

Three control experiments were performed to verify the causal relationship between the action of the deacetylase and the isolation of a P2 chromatin fraction. First, a sample identical to that described above, containing S1 chromatin and the deacetylase, was incubated on ice for 30 minutes, rather than at the 37°C. The result is shown in figure 5, lanes S2* and P2*. The

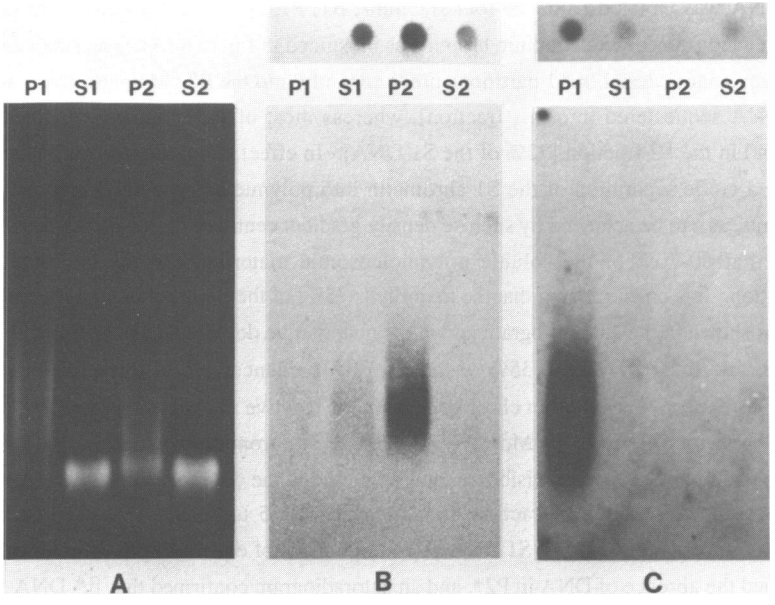

Figure 6: Analysis of DNA from the P1, S1, P2 and S2 chromatin fractions. DNA was prepared from the P1, S1, P2 and S2 chromatin fractions as described in figure 4 and 20 μg of each was electrophoresed on a 1% agarose gel [panel A: ethidium bromide stained gel]. The chicken DNA was then transferred to nitrocellulose, hybridized to nick-translated pHB1001 [panel B], or pOV230 [panel C] and autoradiograms prepared. pHB1001 contains a chicken β^A cDNA sequence and pOV230 a chicken ovalbumin cDNA sequence. Above panels B and C is a dot blot analysis of the same DNA samples. Each dot contains 5 μg of DNA.

labeled histone in lane S2* yields a pattern on the gel that is identical to that in lane S1. There is no histone deacetylation, and no nucleohistone could be isolated in the P2* fraction. If the deacetylase binds to the S1 chromatin, then this result demonstrates that binding alone is not sufficient for precipitation. In a second experiment, no deacetylase was added and the sample incubated at 37°C for 30 minutes. The result was the same as depicted in lanes S2* and P2*. Thirdly, the deacetylase was first inactivated by incubation at 60°C for 10 minutes. The heat inactivated enzyme was then equilibrated to 37°C, the S1 chromatin added, and the sample further incubated at 37°C for 30 minutes. The result of this experiment was also identical to that illustrated in lanes S2* and P2*. There was no histone deacetylation and no P2 chromatin. These last two experiments demonstrate that an active histone deacetylase is required for nucleohistone precipitation. It is interesting to note that when an appropriate amount of active deacetylase is added to the S1 sample and incubated at 37°C, within 15 minutes, the samples become turbid, whereas control samples remain clear. We conjecture that the increase in turbidity is a direct result of deacetylase action and nucleohistone aggregation.

DNA was isolated from the four fractions, S1, P1, S2 and P2, electrophoresed on an agarose gel and stained with ethidium bromide as illustrated in figure 6 (A). The small amount of polynucleosomal material in S1 partitions predominantly into the P2 chromatin fraction [8% of the S1 DNA sequestered into this fraction], whereas most of the mononucleosomes remain soluble and in the S2 fraction [92% of the S1 DNA]. In effect, then, the histone deacetylation results in a crude separation of the S1 chromatin into polynucleosome and mononucleosome components, as can be achieved by sucrose density gradient centrifugation [figures 1 and 2]. We estimate that 60-70% of the soluble polynucleosomal material is in the P2 fraction after deacetylation. It is not surprising that the majority [~75%] of the β^A chromatin in S1 fractionates into P2, as shown in the autoradiogram and in the quantitative dot blot analysis of the β^A DNA in figure 6B, and thus overall, 30-35% of the total β^A sequences are in this minor chromatin fraction. As expected, ovalbumin chromatin, which is inactive in chicken erythrocytes, has the opposite behavior [figure 6C]. Most of the ovalbumin chromatin fractionates into P1, with almost no ovalbumin sequences visible in the P2 fraction in the dot blot analysis. DNA was also purified from the S2* and P2* fractions [data not shown]. 5 μg of S2* DNA had an identical adult β-globin content as 5 μg of S1 DNA. Electrophoresis of equal sample volumes of P2 and P2* verified the absence of DNA in P2*, and an autoradiogram confirmed that β^A DNA was not present in the P2* fraction. We conclude that the *n*-butyrate incubation preferentially induces hyperacetylation of the β^A associated histones, and this hyperacetylation is directly responsible for the enhanced β^A polynucleosome solubility. When these polynucleosomes are deacetylated, they aggregate and can be pelleted by centrifugation.

DISCUSSION

A very small class of polynucleosomes may be prepared in a soluble state from micrococcal nuclease digested nuclei, if immature erythrocytes have been incubated for 1 hour with *n*-butyrate. The soluble polynucleosomes are 25-30 fold enriched for adult β-globin DNA sequences compared to the pellet DNA. The inclusion of ^{3}H-acetate during the *n*-butyrate incubation demonstrates that these polynucleosomes contain histones with specific activities [cpm ^{3}H/μg histone] some 30-fold greater than pelleted chromatin histones. This high specific activity is a diagnostic tag, not only for the small percentage of histone undergoing the active modification, but for those regions of the erythrocyte genome associated with these histones. When the soluble β-globin polynucleosomes are deacetylated, they aggregate and can be pelleted by centrifugation, providing evidence that histone hyperacetylation alone, is responsible for their previous soluble state. The results are fully consistent with experiments demonstrating that histone hyperacetylation enhances the solubility of chromatin in buffers containing a divalent cation such as Mg^{2+} (4,5). Our own fractionation buffer contains 3 mM $MgCl_2$, and is used throughout the fractionation scheme depicted in figure 4. The main conclusion from these experiments is that dynamic histone acetylation and deacetylation are preferentially occurring on

the β^A chromatin in the chicken immature red blood cell. The inactive chromatin, representing the vast majority of nucleohistone in this cell type, is not participating in active histone modification.

We describe the β^A chromatin from *n*-butyrate incubated cells as polynucleosomal in character because of the location of the DNA on filters after Southern transfer, and because we previously observed no enrichment for β^A DNA in the soluble mononucleosomes (6). This does not preclude the possibility, however, that the mononucleosomes contain active, or potentially active DNA sequences. We have some evidence that different regions of active chromatin are digested at different rates by the micrococcal nuclease. To cite an example, we previously showed a region 5' of the ρ (embryonic) β-globin gene to be soluble after *n*-butyrate incubation (7). This region was cleaved more rapidly to mononucleosome size compared to the adult β-globin chromatin, and thus the DNA appeared on the filter as mono- and dinucleosome size, while β^A globin was observed to be polynucleosomal. This latter information could explain why some soluble mononucleosomes become insoluble after deacetylation of the histones (see figure 6A, lane P2).

We have repeated many of our solubility experiments using mature red blood cells, and find that with these cells as starting material, *n*-butyrate enhances the solubility of β^A chromatin, as reported for the immature erythrocytes (Zhang and Nelson, manuscript submitted). This information is significant since Ridsdale and Davie (16,17) have recently reported a powerful method for the purification of a rare class of soluble polynucleosomes from chicken mature erythrocytes. These soluble polynucleosomes are nearly 50-fold enriched for β^A DNA and contain histones somewhat more acetylated than the bulk of these proteins. Their purification scheme differs from ours in two respects: 1) *n*-butyate incubation and histone hyperacetylation are not required, and 2) the fractionation buffer does not contain divalent cations. The differential solubility of the polynucleosomes reported by Ridsdale and Davie depends on the concentration of the monovalent salts KCl, or NaCl. Because both we, and Ridsdale and Davie report a substantial enrichment for β^A chromatin, it is likely that the same subset of chicken erythrocyte polynucleosomes are being isolated. As partial evidence for this, the slightly increased levels of acetylation of the histones in the polynucleosomes purified by Ridsdale and Davie suggest that these are the regions of chromatin participating in active histone acetylation. Thus two independent fractionation methods demonstrate that β^A chromatin can be prepared in a polynucleosomal form after mild micrococcal nuclease digestion.

We incorporate these recent findings in the context of chicken erythrocyte β-globin chromatin structure as follows: The β^A chromatin in the chicken erythrocyte is DNase I sensitive and is the paradigm for potentially active chromatin (18). After a mild micrococcal nuclease digestion, the polynucleosomes containing the β^A DNA are preferentially soluble in 0.15-0.20 M NaCl (16,17), this being due to the specific structural and compositional features of active chromatin. We surmise that *in vivo*, the acetyltransferase and deacetylase enzymes are actively

engaged in acetylating and deacetylating the histones in these polynucleosomes. The histones associated with the β^A DNA are not maintained in a fully hyperacetylated state. Thus the β^A polynucleosomes are insoluble in $MgCl_2$ containing buffers in the absence of *n*-butyrate incubation of the cells (6). Inhibition of the chicken histone deacetylase with *n*-butyrate forces histone hyperacetylation, and enhances the solubility of this chromatin in the presence of $MgCl_2$.

We suggest that the evidence is quite compelling that histone acetylation and deacetylation are actively occurring on the globin chromatin in the chicken red blood cell. More refined experiments will be required, however, to ascertain the reasons for dynamic histone acetylation on active and potentially active β^A chromatin. There are at least two interpretations as to the function of this dynamic modification. One is that an open chromatin conformation in active regions dictates the sites of dynamic histone modification. Histone acetylation and deacetylation occur fortuitously on open chromatin because the N-terminal portions of the histones are more exposed and the lysine residues thereby more available to the modifying enzymes. An alternative argument asserts that active histone acetylation and deacetylation are part of the dynamics of maintaining the active chromatin conformation. Ridsdale and Davie (17) demonstrate increased levels of histone acetylation and ubiquitination, and slightly decreased amounts of linker histone associated with their active polynucleosome preparation. The latter result is in agreement with recent studies suggesting that, with the exception of the DNase I hypersensitive regions, linker histone content in β^A chromatin is similar to that in inactive chromatin regions (19). Since the rates and extent of histone modification are presumably regulated by the cell, and the H1 and H5 content of active chromatin is in dynamic equilibrium with other chromatin regions, Ridsdale and Davie promote the notion that the maintenance of active chromatin is a dynamic process. The histone acetyltransferases and deacetylases, in conjunction with other proteins or activities that regulate chromatin composition and DNA topology, would therefore be responsible for the maintenance of the active chromatin conformation.

ACKNOWLEDGEMENTS

This research was supported by a NIH Biomedical Research Support Grant.

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