Sci. U.S.A. 70, 2330.
Rosen, J. M., Woo, S. L. C., Holder, J. W., Means, A. R., and O'Malley, B. W. (1975), *Biochemistry 14,* 69.
Rosenfeld, G. C., Comstock, J. P., Means, A. R., and O'Malley, B. W. (1972), *Biochem. Biophys. Res. Commun. 46,* 1695.
Roy, A. K. (1973a) *Endocrinology 92,* 957.
Roy, A. K. (1973b), *J. Endocrinol. 56,* 295.
Roy, A. K. (1977), *Eur. J. Biochem. 73,* 537.
Roy, A. K., Dowbenko, D. J., and Schiop, M. J. (1977), *Biochem. J. 164,* 91.
Roy, A. K., McMinn, D. M., and Biswas, N. M. (1975), *Endocrinology 97,* 1501.
Roy, A. K., Milin, B. S., and McMinn, D. M. (1974), *Biochim. Biophys. Acta 354,* 213.
Roy, A. K., and Neuhaus, O. W. (1967), *Nature (London) 214,* 618.
Roy, A. K., Neuhaus, O. W., and Harmison, C. R. (1966), *Biochim. Biophys. Acta 127,* 78.
Roy, A. K., and Raber, D. L. (1972), *J. Histochem. Cytochem. 20,* 89.
Roy, A. K., Schiop, M. J., and Dowbenko, D. J. (1976a), *FEBS Lett. 64,* 396.
Roy, A. K., Schiop, M. J., and Dowbenko, D. J. (1976b), *FEBS Lett. 70,* 137.
Russel, D. H., Snyder, S. H., and Medina, V. J. (1970), *Endocrinology 36,* 1414.
Schutz, G., Beato, M., and Feigelson, P. (1972), *Biochem. Biophys. Res. Commun. 47,* 1426.
Sippel, A. E., Feigelson, P., and Roy, A. K. (1975), *Biochemistry 14,* 825.
Spelsberg, T. C., and Wilson, J. T. (1976), *Biochem. J. 154,* 439.
Talwar, G. P., Gupta, S. L., and Gros, F. (1964), *Biochem. J. 91,* 565.
Tata, J. R., and Widnell, C. C. (1966), *Biochem. J. 98,* 604.
Weintraub, H., and Groudine, M. (1976), *Science 193,* 848.
Widnell, C. C., and Tata, J. R. (1966), *Biochem. J. 98,* 21.

Studies on the Reactive Properties of Histone Amino Groups: Reactivities of Free Histones and Histones in Chromatin as a Function of Ionic Strength†

B. L. Malchy

ABSTRACT: The reactivity of the amino groups of the five histones towards acetic anhydride has been measured and with the exception of histone IIb2 the reactivities are very similar to those of exposed lysines with an average pK of 9.5. In addition the reactivities of these groups from 0.20 to 1.0 M NaCl and the reactivity of a peptide containing lysines 5, 8, 12 and 16 of histone IV have been measured in chromatin. It is concluded that at the lower ionic strengths the large proportion of the amino groups are buried for both the histones and the region of histone IV studied. Data obtained from the measurement of the reactivity of standard proline compounds and from a pH and ionic strength study indicate that the N-terminal proline of histone IIb2 is exposed.

The structure of the chromatin of a cell nucleus is a primary factor in determining the nature of the processes of transcription, replication and differentiation. These processes involve the interactions of DNA, RNA, histones, nonhistone proteins, and a number of enzymes. Recent developments have resulted in a widely accepted model for the structure of nucleohistone in which 4 of the 5 histones interact in pairs to form an octomeric complex with 200 base pairs of DNA (Kornberg, 1974). This structure is consistent with evidence from studies on histone–histone interactions, chemical cross-linking studies, nuclease digestion studies, observations by electron microscopy, and semiquantitative studies on the quantities of each of the histones (Kornberg, 1974; Noll, 1974; Olins and Olins, 1973). Although this model is consistent with many experimental observations, it has not explained many other aspects of nucleohistone structure. In particular it is not possible at present to account for the significance and timing of the variety of chemical modifications of histones that occur in chromatin or to account for the slow rate of evolution of histone primary sequence. It appears that further studies relevant to histone function are required to explain these phenomena.

One approach that has been used in the analysis of chromatin structure is that of measuring the chemical reactivities of potential histone reactive groups by the method of competitive labeling (Malchy and Kaplan, 1974). In this procedure an internal standard is included directly in the reaction mixture and chemical reactivities relative to this standard are determined by measuring the extent of reaction of the groups under study (Kaplan et al., 1971). This procedure also allows estimation of the degree of exposure of any group by the determination of its "reactivity index" (Visentin et al., 1973). Even with a system as complex as chromatin, it is possible to obtain precise information on the properties of individual reactive amino acids. Thus the object of this approach is to provide information about the chemical properties of specific areas within the nucleohistone structure and to assess these properties in terms of nucleohistone function.

† From the Department of Biochemistry, Queen's University, Kingston, Ontario, Canada K7L 3N6. *Received February 22, 1977.* This work was supported by the Medical Research Council of Canada.

Experimental Section

Materials

Acetic anhydride (^{3}H- and ^{14}C-labeled) was purchased from Amersham/Searle Corp., Oakville, Ontario, Canada. Prolylleucine and prolylglycine were purchased from Nutritional Biochemicals Corp., Montreal, Quebec, Canada. Trypsin (2× crystallized) was obtained from Worthington Biochemical Corp. Other chemicals used were of reagent grade quality.

Methods

Histone Reactivities. Histones were prepared according to a standard procedure (Malchy and Kaplan, 1974) and then were dissolved in 5 mM KH_2PO_4–5 mM sodium Veronal to a concentration of 0.83 mg/mL. Subsequently, phenylalanine internal standard was added and a 25-mL aliquot of the solution was titrated to the desired pH. Histones prepared in this manner have been shown to form tetramers in the case of the arginine-rich histones and dimers in the case of the moderately arginine-rich histones (D'Anna and Isenberg, 1974). Then to each of the vigorously stirred histone solutions, 50 μL of acetonitrile containing 0.28 μmol of [^{3}H]acetic anhydride (sp act. 4 Ci/mol) was added. The temperature was maintained at 10 °C. After completion of the reactions, the samples were freeze-dried, fully acetylated with [^{14}C]acetic anhydride, and the histones and internal standard were purified and counted as previously described (Malchy and Kaplan, 1976).

Reactivities of Proline Standards. Pro-Gly (0.9 mg) and Pro-Leu (1.3 mg) were each dissolved in 5.0 mL of 0.1 M KCl containing 1.6 mg of phenylalanine and the pH was adjusted to 12.0. The sample was equilibrated at 10 °C and then 50 nmol of [^{14}C]acetic anhydride (120 mCi/mmol) was added. After 30 min, the samples were fully acetylated at pH 8 with 100 μL of unlabeled acetic anhydride (Kaplan et al., 1971).

Ionic Strength Study. Chromatin was prepared and sheared according to a standard procedure (Marushige and Bonner, 1966). For the experiments at 0.20 M and 0.25 M NaCl, the sample of chromatin was diluted in 5 mM NaH_2PO_4–5 mM sodium Veronal until its A_{260} value was 0.33 and then the pH was adjusted. To 250 mL of this solution, 5 μmol of phenylalanine was added and the solution equilibrated to 10 °C. Then 0.75 μmol of [^{3}H]acetic anhydride (sp act. 5 Ci/mol) in 150 μL of acetonitrile was slowly added to the vigorously stirred solution. For the samples at 0.34, 0.47, and 0.91 M NaCl, the reaction solutions contained 310 mL of chromatin with an A_{260} of 0.61, 130 mL of chromatin with an A_{260} of 2.0 and 55 mL of chromatin with an A_{260} of 4.5. The 0.35 M NaCl sample contained 5 μmol of phenylalanine, while the other two solutions contained 2 μmol. These solutions were reacted with 0.5 μmol of the above [^{3}H]acetic anhydride solution. After the trace labeling, the samples were diluted to give an NaCl concentration of 0.20 to 0.25 M, and $CaCl_2$ and $MgCl_2$ were added to give final concentrations of 0.01 M. Then the samples were recentrifuged at 13 000g for 4 h. From the centrifuged chromatin, histones were isolated using two extractions with 20 and 10 mL of 0.4 M H_2SO_4. An aliquot of the supernatant solution (approximately 0.5 μmol of phenylalanine) was freeze-dried and then remixed with the histones from the corresponding reaction. The histones and internal standard were then dissolved in 20 mL of 8 M urea, fully acetylated with [^{14}C]acetic anhydride, purified, and counted as described above. Before electrophoretic separation, the fully acetylated histones were treated with 0.1 M mercaptoethanol at pH 8.0 for 20 min.

Isolation of Histone IV Peptide. Fully acetylated histone IV was purified by gel electrophoresis (Malchy and Kaplan, 1976) and isolated by the procedure of Bray and Brownlee (1973). Histone IV was isolated from 2 mg of total histone and then digested with 20 μg of trypsin in 0.25 mL of 0.5% ammonium bicarbonate at pH 8.0 for 6 h. In order to identify the histone IV peptides, ^{14}C-labeled fully acetylated histone IV was prepared as a marker. The histone was prepared by the Johns procedure (1967) and after acetylation was digested with 1/20 w/w trypsin in 0.5% ammonium bicarbonate for 6 h at 37 °C. The histone IV sample and marker were separated by high voltage electrophoresis at pH 6.5. The position of the histone IV peptide containing lysines 5 to 16 was identified after chromatography with butanol–acetic acid–water–pyridine (15:3:12:10 by volume). After chromatography, the sample peptide was then purified by electrophoresis at pH 2.1, eluted, and counted.

Results

The structure of free histones appears to be quite relevant to their structure in chromatin. Individual histones are known to aggregate in solution at neutral pH. This is especially true for histones III and IV (Hnilica, 1972). However, it has been shown that histones interact with each other to form complexes and these complexes do not appear to aggregate further in solution (Kornberg and Thomas, 1974). The nature of histone complex formation in vitro may or may not reflect in vivo complexes. At present it has been observed that the complexes are a 1:1 proportion for histones III and IV and IIb1 and IIb2. In high salt at pH 9.0, IIb1, IIb2, III, and IV have been cross-linked with the reagent dimethyl suberimidate to form a species corresponding to a histone octomer with an approximate molecular weight of 120 000 (Thomas and Kornberg, 1975). In addition in 2 M NaCl at pH 7.0 Weintraub has observed a complex containing histones IIb1, IIb2, III, and IV with a molecular weight corresponding to a histone tetramer (Weintraub et al., 1975).

The reactivities of each of the histones as a function of pH are shown in Table I. It can be seen (Figure 1) that, with the exception of histone IIb2, the data fit titration curves in which the lysines have normal reactivities and averaged ionization constants of around 9.5. Thus it is possible to conclude that the histone lysines are exposed to solvent under the above conditions. In addition it appears that the average pK for these proteins is lower than that which might be expected for an exposed lysine. Titration studies reveal that lysines have pK's in the range of 9.9 to 10.7 (Tanford, 1962). In addition competitive labeling analysis of the lysines of elastase gave a pK of 10.3 for lysine 87 and lysine 224 (Kaplan et al., 1971). The low average pK for histone lysines appears to result from the very large percentage of basic residues in these proteins. Thus, the highly positively charged environment of these proteins would likely tend to suppress protonation of a given lysine and this result is observed.

It has been observed previously that at pH 8.4 the N-terminal proline of histone IIb2 was 11 times as reactive as a standard exposed lysine (Malchy and Kaplan, 1974). In order to quantitate the degree of exposure of any group, one must obtain its ionization constant and in addition determine its reactivity relative to a standard having the same ionization constant. As a result the reactivities and ionization constants of two proline peptides have been determined. The reactivities obtained were 8.7 for Pro-Leu and 9.2 for Pro-Gly relative to the reactivity of phenylalanine. Figure 2 shows these reactivities, along with that of proline, plotted in comparison with a standard Brönsted plot for primary amines. The data show that in these three cases the reactivities of the secondary amino

TABLE I: Reactivity Data for the Reaction of Free Histones with Acetic Anhydride.

	^3H dpm/^{14}C dpm						$10^2 \times \alpha r$				
pH	Histone I	Histone IIb2	Histone IIb1	Histone III	Histone IV	Acetyl-Phe	Histone I	Histone IIb2	Histone IIb1	Histone III	Histone IV
7.51	74.2	248	63.3	116	81.0	51.7	1.43	4.80	1.22	2.25	1.56
7.99	96.4	316	96.9	205	83.0	70.4	4.03	13.2	4.05	8.59	3.47
8.51	168	393	117	186	111	136	11.2	26.2	7.80	12.4	7.37
9.08	210	353	145	163	146	147	38.4	64.6	26.6	29.9	26.7
9.51	197	281	160	143	124	141	70.3	100	57.2	36.7	44.4
10.14		189	141	91.9	115	91.3		169	126	82.0	103

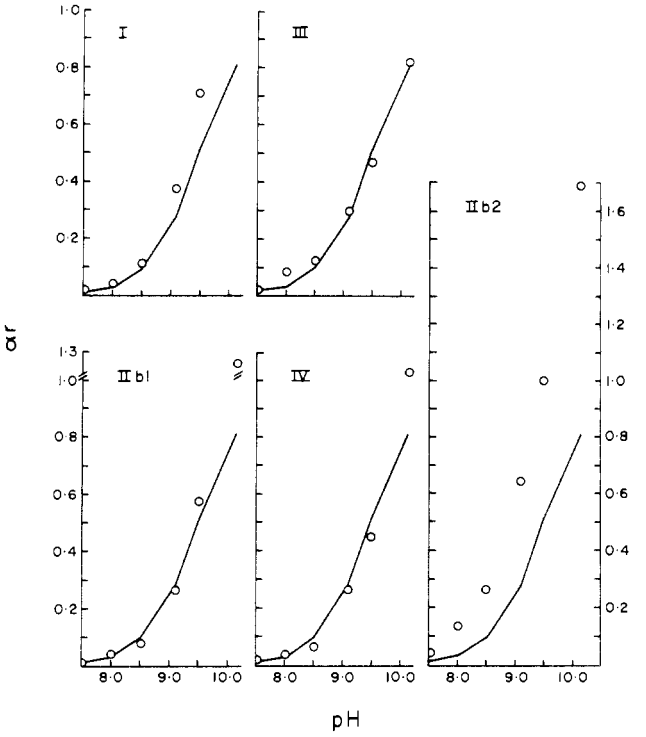

FIGURE 1: Graphs of αr vs. pH for isolated histones. The solid line is a theoretical curve for a group with a pK of 9.5 and an r value of 1.0.

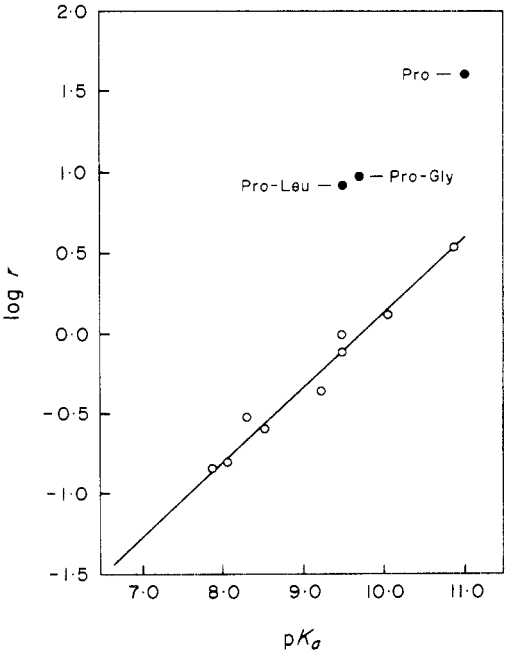

FIGURE 2: Reactivities of three secondary amino compounds plotted relative to a standard Brönsted plot for primary amines. The open circles are measured reactivities for primary amines and the closed circles represent the secondary amines.

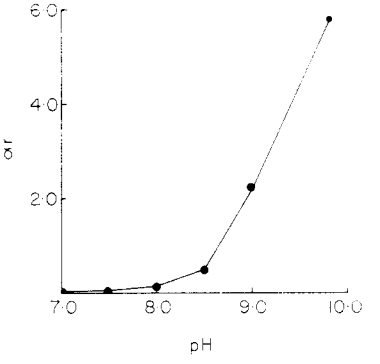

FIGURE 3: Estimation of values of αr vs. pH for the N-terminal proline of histone IIb2 based on the difference in reactivity of histones IIb2 and IIb1. Calculated values used in this plot are pH 7.00, αr = 0.0090; pH 7.50, αr = 0.030; pH 8.01, αr = 0.132; pH 8.50, αr = 0.52; pH 9.10, αr = 2.2; pH 9.8, αr = 5.8. The solid line is a theoretical curve for a group with a pK of 9.7 and a relative reactivity of 10.1.

groups are an order of magnitude greater than the primary amino compound with a corresponding pK.

In the case of the N-terminal proline of histone IIb2, its pK can only be determined by measuring its reactivity as a function of pH. In the absence of these data, its pK can be inferred from the measurement of the reactivities of histone IIb2 and IIb1 as a function of pH. It has been observed previously that the reactivity of histone IIb2 is primarily because of this N-terminal proline. Also it has been observed that as a function of pH the amino group reactivities fall into three classes depending on the lysine to arginine ratio of the histones (Malchy and Kaplan, 1976). Thus the difference in reactivities between IIb2 and IIb1 at those pHs where the lysines are buried can be used as an estimate of the reactivity of the N-terminal proline. When these reactivities are plotted as a function of pH, they are observed to fit a titration curve with an ionization constant of 9.7 and a relative rate constant of 10 (Figure 3). By comparison with the proline standards, it can be seen that the estimated properties of this proline are consistent with those of a fully exposed group.

The structure of chromatin undergoes substantial changes with ionic strength. For example, the intrinsic viscosity of chromatin has been observed to be lower by a factor of 10 in chromatin at 0.15 mol/L ionic strength than in chromatin at 0.01 mol/L ionic strength (Lewis et al., 1976). The low viscosity appears to be dependent on those proteins which are washed off by low concentrations of salt as it can be altered dramatically by washing chromatin with 0.5 M NaCl. In addition reconstitution complexes of histone I and DNA show

TABLE II: Reactivity Data for the Reaction of Histones in Chromatin at Various Ionic Strengths.

NaCl (M)	pH	^3H dpm/^{14}C dpm: Histone I	Histone IIb2	Histone IIb1	Histone III	Histone IV	Acetyl-Phe	Reactivity index: Histone I	Histone IIb2	Histone IIb1	Histone III	Histone IV
0.20	7.98	6.25	41.1	8.43	8.50	4.85	49.7	0.31	2.0	0.42	0.42	0.24
0.25	7.97	12.7	75.2	13.8	14.7	8.08	91.4	0.34	2.0	0.37	0.40	0.22
0.34	7.92	23.3	71.9	16.6	18.9	9.82	120	0.48	1.5	0.34	0.39	0.20
0.47	7.76	18.5	54.4	18.7	23.7	9.06	74.4	0.62	1.8	0.63	0.79	0.30
0.91	8.02	33.0	81.0	43.6	47.0	19.9	79.3	1.0	2.5	1.4	1.5	0.62
0.25	9.78	46.6	164	44.3	36.9	28.4	76.8	0.69	2.4	0.65	0.54	0.42

a contraction behavior similar to that observed for whole chromatin (Bradbury, 1975).

In order to perform experiments at "physiological" ionic strengths, it was necessary to work with dilute solutions as described. Other laboratories have also performed chromatin studies at these ionic strengths by working with dilute solutions (Thomas and Kornberg, 1975). The results obtained at several NaCl concentrations are shown in Table II. It should be noted that the variation in rate constant for the reaction of standard amines with acetic anhydride as a function of ionic strength has not been measured. However, in these experiments the rates of reaction of the amino groups are measured relative to the phenylalanine standard and a substantial variation in relative reactivities of standard amines over the range of ionic strengths used would not be expected.[1]

The results show that histone reactivity indices are higher at "physiological" ionic strengths than at the lower ionic strengths used previously. In addition as the NaCl concentration is raised up to 1 M, these values approach those obtained for exposed lysines. The histone reactivity indices observed in the 0.2 to 0.5 M NaCl range are again those which would be expected for proteins whose lysines are substantially buried. The values obtained can be accounted for by three possible explanations. The first would be that at these ionic strengths a specific structure exists in which a fraction of the lysine residues is exposed in chromatin. This does not appear likely since one of the primary histone functions is presumably to neutralize DNA phosphate residues. The second would be that, in the range 0.2 to 0.35 M NaCl, the lysine residues still are interacting in the chromatin but not as strongly as they are at lower ionic strengths. The third possibility is that a change in the overall environment of the chromatin has occurred and this has caused a change in lysine reactivity indices. Discrimination of these possibilities would require a detailed study involving peptide isolations and pK determinations. Comparison of the histone I reactivity index with those of IIb1, III, and IV shows that it is not substantially different than the others. This agrees with the earlier observations at lower ionic strength. Over the range 0.2 to 0.34 M NaCl, it can be seen that IIb1, IIb2, III, and IV reactivity indices remain essentially constant. However, the value for histone I increases significantly from 0.3 to 0.48. This difference may possibly reflect a different type of interaction of histone I in chromatin. Thus those histones which interact to form an octomeric complex in chromatin form a stable structure unaffected by small changes in ionic strength while histone I chromatin interactions are weakened in proportion to the salt concentration. In addition, histone I is clearly different from the others on the basis of its extractability by salt (Ohlenbush et al., 1967; Bartley and Chalkley, 1971) and on the basis of the degree of exposure of its ϵ-amino groups in chromatin at high pH (Malchy and Kaplan, 1976); whereas it appears similar in reactivity to the others as the ionic strength of the chromatin is raised. Although it may be possible to explain the data on the basis of selective loss of histone I from chromatin, a more plausible explanation is based on the fact that even when the ionic strength of chromatin is raised the histone I molecules would all be in a lower free energy state if they were interacting with negative ions such as phosphates than if they became exposed to solvent. On the other hand, since the other histones have substantially more arginine residues which may still be interacting strongly with DNA, the removal of histone I from the chromatin would be significantly easier. Also, since at high pH, 11 to 12.0, the lysines are essentially unprotonated, there is no free energy decrease due to charge interaction and the lysines thus become exposed to solvent.

In addition Table II shows reactivity indices measured in 0.25 M NaCl at pH 9.8. The values obtained are higher than those obtained at lower pH and a substantial change in histone I reactivity has occurred. These changes may reflect the fact that the unmasking phenomenon observed for histone lysines may be occurring at lower pHs at the higher ionic strength. Also, the substantial reactivities obtained may help to explain why it is possible to generate exclusively a histone octomer in chromatin by cross-linking in 0.2 M NaCl, pH 9 (Kornberg and Thomas, 1974), whereas this has not been possible at neutral pH.

In the case of histone IV, a reactivity index of 0.20 to 0.24 in the NaCl range of 0.2 to 0.34 M NaCl raised the possibility that a small number of lysines might be exposed. Histone IV is known to have an irregular distribution of positive charges and the amino-terminal half of the molecule, where the high concentration occurs, has been postulated to be a major DNA binding site (DeLange et al., 1969). However, a part of this region, that from lysine 5 to lysine 16, contains the only known sites for histone acetylation and in fish testis the primary sites were found to be lysines 8, 12, and 16 (Candido and Dixon, 1971). Therefore the question arose as to whether or not these lysines were largely exposed to solvent, thereby facilitating acetylation, or remained buried in ionic linkage.

Figure 4 shows an autoradiogram obtained after electrophoresis and chromatography of trypsin-digested fully acetylated histone IV. The autoradiogram shows a particularly dark spot which is positively charged at pH 6.5. This peptide appeared to be identical with a peptide which contains lysine 5, 8, 12, and 16 isolated by Candido and Dixon (1971) and referred to as peptide TA1. Preparative purification of the peptide corresponding to this spot and subsequent amino acid analysis confirmed this identification (unpublished results). When this peptide was isolated from a sample reacted in 0.25 M NaCl, a $^3H/^{14}C$ ratio of 12.6 was obtained corresponding to a reactivity index of 0.34. Thus it appears that in 0.25 M NaCl the region of histone IV containing lysines 5, 8, 12, and

[1] The pK of phenylalanine was found to vary by no more than 0.10 over the range studied.

FIGURE 4: Autoradiogram of neutral and basic peptides obtained from ^{14}C-labeled fully acetylated histone IV after tryptic digestion. Peptides were separated by electrophoresis at pH 6.5 and chromatography using butanol–acetic acid–water–pyridine. The position of the peptide containing lysines 5, 8, 12, and 16 is indicated by the small arrow.

16 remains largely unavailable to solvent, although it remains possible that one of these groups may be exposed.

Discussion

Studies on nucleohistone or chromatin structure provide information which then may be useful in understanding the function of the nucleus. Although it can be argued that properties measured in this isolated structure to not reflect the situation inside the nucleus, a clear example of the maintenance of similar properties in chromatin and in the nucleus is the existence of the same repeat pattern obtained upon nuclease digestion (Kornberg, 1975). Another important question with regard to this problem is the use of ionic conditions in the study of chromatin structure. Although the content of ions in the nucleus has been measured (Langendorf et al., 1961), the ionic conditions under which chromatin behaves most like the nucleus is still unknown (Hancock, 1975). The present study represents an analysis of the properties of histone amino groups from the range of low to high ionic strength and thus should encompass ionic strengths representative of the in vivo situation.

In the analysis of chromatin structure, it is also important to establish the presence of exposed and buried groups in order to evaluate the structure. Thus, in the case of much simpler systems such as monomeric proteins it has been shown many times that specific structures exist with functional groups in well-defined environments (Blow and Steitz, 1970). However, it is possible to envision a structure for isolated chromatin which is a nonspecific aggregate and thus a poor representative of the in vivo situation.

Two lines of evidence both indicate that the N-terminal proline of histone IIb2 is an exposed group. The first is its very high reactivity index which was found to be 11.2 at pH 8.4 (Malchy and Kaplan, 1974). Determination of the reactivities of standard proline compounds revealed that an exposed proline would have a reactivity in this range. In addition, estimation of the pK and reactivity of the N-terminal proline has yielded values which would be obtained for a fully exposed group. The second line of evidence results from studies of the variations in reactivities of histones in chromatin with changes in pH and salt concentration. If the N-terminal proline in histone IIb2 was buried, it would be expected that high pH or high salt would disrupt the structure and expose the group. This would result in a substantial change in reactivity. However, it has been observed that at high pHs (Malchy and Kaplan, 1976) the reactivity index for histone IIb2 decreases while that of the other histones is increasing. Similarly, examination of Table II shows that between 0.2 and 1.0 M NaCl histones I, IIb1, III, and IV undergo increases in reactivity of from three to four times. Over this same range histone IIb2, whose lysines are presumably also becoming exposed, only increases in reactivity by 25%. This evidence again indicates that the proline is exposed and therefore does not increase in reactivity in high salt.

It should be noted that changes in the reactivity index of histone IIb2 are observed in the neutral pH range and in the low ionic strength range (Table II, Malchy and Kaplan, 1976). These may reflect changes in the properties of the N-terminal proline. Also, it has been observed that in sheared chromatin histone IIb2 is relatively more susceptible to degradation by trypsin than it is in nuclease-treated chromatin (Kornberg, 1975). Thus a more complete understanding of the properties of the N-terminal proline would require further studies.

The chemical reactivity of the histone IV peptide containing lysines 5, 8, 12, and 16 was measured because of the interesting biological properties of this region of histone IV. This region of histone IV is considered to be its only site of in vivo acetylation (Candido and Dixon, 1971). The function of in vivo acetylation is not clear but is believed to be an important process occurring in the nucleus. In particular, models which attempt to explain chromatin function can be divided into those in which the histones move along the DNA before or during the processes of transcription and replication and those in which the histones remain fixed (Cantor, 1976; Weintraub et al., 1976). A model in which nucleosomes do not move along the DNA could explain histone acetylation in terms of weakening ionic interactions between histones and DNA and thus contributing to one or both of the processes of transcription and replication. A rolling nucleosome model might require that the DNA enter the nucleosome at many different entry points (Cantor, 1976). This may appear to be inconsistent with the fact that in vivo acetylation only occurs at a small number of specific places. However, since it is known that the sites of acetylation are in the most basic parts of the histones (Dixon et al., 1975), it could be postulated that acetylation functions to weaken interactions between basic amino acids and phosphates in those areas where ionic interactions may be rate limiting to movement. Recent experimental evidence has indicated that in that part of the DNA which is being actively transcribed the DNA has a different susceptibility to DNAse I digestion (Weintraub and Groudine, 1976; Garel and Axel, 1976). This indicates that histone location in relation to DNA must vary. Also recent evidence has shown that in vitro acetylation of histones alters the rate of in vitro DNA transcription (Marushige, 1976). It appears clear that at present the available experimental evidence does not provide a satisfactory explanation for the function(s) of histone acetylation.

In itself the unreactive nature of the N-terminal part of

histone IV toward acetic anhydride would indicate that histone acetylation does not occur on chromatin or that it proceeds slowly. In fact, recent evidence demonstrates that histone IV acetylation occurs in the cytoplasm (Carillo et al., 1975). This would be a facile process since there histone lysines should be exposed. However, the evidence for nuclear histone acetylation appears to be very strong. It has been shown to occur in both testis and thymus nuclei and to be not inhibited by puromycin (Candido, 1975; Vidali et al., 1968). In a recent article (Jackson et al., 1975), calculations have estimated that 25 to 30% of all histone IV molecules are acetylated in a 10-min period. This would probably imply a very fast turnover rate for the acetylating enzyme in addition to the lysines under consideration being freely accessible to solvent and this appears to be inconsistent with data presented in this paper. However, the estimation of the extent of acetylation depends on the assumption that the specific radioactivity of the acetate pools inside the hepatoma cell are the same for α-NH_2 acetylation and ϵ-NH_2 acetylation. Since the two types of acetylation occur in different parts of the cell and since the ϵ-NH_2 acetylation shows a very fast turnover rate while the α-NH_2 acetylation is permanent, this does not appear to be likely. Thus it appears that histone acetylation does occur on histone IV lysines which are likely to be primarily interacting with DNA phosphates. In view of the apparent specificity of histone acetylases(s) in its ability to distinguish among buried lysines, it would thus be of interest to know if the in vitro acetylation of chromatin by nuclear acetyltransferases produces the same specific acetylated products as observed in vivo.

Acknowledgments

I would like to thank Mrs. R. Croxford for valuable technical assistance and Dr. H. Kaplan for discussions and assistance with the amino acid analyses.

References

Bartley, J. A., and Chalkley, R. (1971), *J. Biol. Chem. 247,* 2647.

Blow, D. A., and Steitz, T. A. (1970), *Annu. Rev. Biochem. 39,* 63.

Bradbury, E. M. (1975), *Ciba Found. Symp. 28,* 141.

Bray, D., and Brownlee, S. M. (1973), *Anal. Biochem. 55,* 213.

Candido, E. P. M. (1975), *Can. J. Biochem. 53,* 796.

Candido, E. P. M., and Dixon, G. H. (1971), *J. Biol. Chem. 246,* 3182.

Cantor, C. R. (1976), *Proc. Natl. Acad. Sci. U.S.A. 173,* 3391.

Carillo, A. J., Wangh, L. J., and Allfrey, V. G. (1975), *Science 190,* 418.

D'Anna, J. A., and Isenberg, I. (1974), *Biochemistry 13,* 4992.

DeLange, R. J., Fambrough, D. M., Smith, F. L., and Borner, J. (1969), *J. Biol. Chem. 244,* 317.

Dixon, G. H., Candido, E. P. M., Honda, B. M., Louie, A. V., Macleod, A. P., and Sung, M. T. (1975), *Ciba Found. Symp. 29,* 229.

Garel, A., and Axel, R. (1976), *Proc. Natl. Acad. Sci. U.S.A. 173,* 3966.

Hancock, R. (1975), *Ciba Found. Symp. 28,* 26.

Hnilica, L. S. (1972), Structure and Biological Function of Histones, Cleveland, Ohio, C.R.C. Press, p 47.

Jackson, U., Shires, A., Chalkley, R., and Granner, D. K. (1975), *J. Biol. Chem. 250,* 4856.

Johns, E. W. (1967), *Biochem. J. 105,* 611.

Kaplan, H., Stevenson, K. J., and Hartley, B. S. (1971), *Biochem. J. 124,* 289.

Kornberg, R. D. (1974), *Science 184,* 868.

Kornberg, R. D. (1975), *Science 187,* 1203.

Kornberg, R. D., and Thomas, J. O. (1974), *Science 184,* 865.

Langendorf, H., Siebert, G., Lorenz, I., Hanover, R., and Beyer, R. (1961), *Biochem. Z. 335,* 273.

Lewis, E. A., DeBuysene, M. S., and Rees, A. W. (1976), *Biochemistry 15,* 186.

Malchy, B. L., and Kaplan, H. (1974), *J. Mol. Biol. 82,* 537.

Malchy, B. L., and Kaplan, H. (1976), *Biochem. J. 159,* 173.

Marushige, K. (1976), *Proc. Natl. Acad. Sci. U.S.A. 173,* 3973.

Marushige, K., and Bonner, J. (1966), *J. Mol. Biol. 15,* 160.

Noll, M. (1974), *Nature (London) 251,* 249.

Ohlenbush, H. H., Olivera, B. M., Tuan, D., and Davidson, N, (1967), *J. Mol. Biol. 25,* 299.

Olins, A. C., and Olins, D. E. (1973), *J. Cell Biol. 59,* 252.

Tanford, C. (1962), *Adv. Protein Chem. 17,* 69.

Thomas, J. O., and Kornberg, R. D. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 2626.

Vidali, G., Gorshey, E. L., and Allfrey, V. G. (1968), *J. Biol. Chem. 243,* 6361.

Visentin, L. P., Yaguchi, M., and Kaplan, H. (1973), *Can. J. Biochem. 51,* 1487.

Weintraub, H., and Groudine, M. (1976), *Science 793,* 848.

Weintraub, H., Palter, K., and VanLente, F. (1975), *Cell 6,* 85.

Weintraub, H., Worcel, A., and Alberts, B. (1976), *Cell 9,* 409.