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An approach to histone nearest neighbours in extended chromatin

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ABSTRACT

The primary sequence organization of histones upon the DNA molecule in chromatin has been analyzed by extension of the nucleoprotein at very low ionic strength and crosslinking with a reversible crosslinking reagent, methyl-4-mercaptobutyrimidate. Histones extracted after limited reaction were fractionated into different classes and the composition of the oligomers analyzed after reduction of the crosslinked material. We have found that the following dimers occur at a high frequency: (F3-F2b), (F3-F2a2), and (F2b-F2a2), whereas (F2b-F2a1), (F3-F2a1) and (F3-F3) occur with a lower frequency. F1 appears to polymerize rapidly to largely homogeneous polymers of high molecular weight. These results are analyzed in terms of several models proposed for chromatin structure.

INTRODUCTION

The periodic nature of the structure of chromatin observed by Pardon, Wilkins and Richards[1] has recently been re-interpreted in terms of repeating subunits of a histone-DNA complex organized somewhat like beads on a string[2]. Kornberg[3] postulated that an $(F2a1)_2(F3)_2$ tetramer and an $(F2b)_x(F2a2)_y$ oligomer form a protein core around which 200 base pairs of DNA are coiled. In a somewhat similar model, van Holde *et al.*[4] proposed that an octamer of non-F1 histones serves as the nucleation site for the coiling of 110 to 120 base pairs of DNA. Supportive evidence for these hypothetical subunits has been derived from electron microscopy[2,5,6,7] and from nuclease digestion of nuclei and chromatin[8,9,10,11]. Recently, Hyde and Walker[12] have proposed that linear co-polymers of F2a1-F3 and F2a2-F2b are plectonemically coiled and that the duplex DNA is supercoiled with a pitch of 53 Å around this protein core.

Such models have stimulated a widespread interest in histone-histone interactions in solution[13,14,15,16], in nucleoprotein[17] and in chromatin. These latter studies have revealed an F2a1-F2b dimer[18], oligomers of F2a1-F3 and F2b-F2a2[19], and a putative F3-F2a1 dimer[20]. Recent studies in this laboratory utilizing a battery of aldehydes, imidoesters and tetranitro-

methane as crosslinking reagents in chromatin have suggested a complex pattern of histone propinquity [21,22]. In this paper we probe the primary structure of chromatin with methyl-4-mercaptobutyrimidate, a readily reversible crosslinking agent.

MATERIALS AND METHODS

Calf thymus chromatin and nucleohistone were prepared as described previously [23], except the centrifugation of nuclei through sucrose was omitted. Methyl-4-mercaptobutyrimidate, dimethylsuberimidate, dimethyladipimidate and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide were obtained from Pierce Chemical Co.

Reaction With Cross-Linking Reagents

Nucleohistone, A_{260nm} = 10, was allowed to react with methyl-4-mercaptobutyrimidate (MMB), 1 mg/ml, at pH = 7.4, 4°. Aliquots were removed at appropriate times and dialyzed versus 0.5 mM triethanolamine-HCl (TEA), pH - 7.4, 4° for at least 4 hours to remove excess MMB. The nucleohistone was then oxidized with 4 µl 30% H_2O_2/10 A_{269nm} ml for 20 minutes at room temperature, and the oxidized nucleohistone then was reacted with 1.8 mg/ml iodoacetimide for 30 minutes at room temperature in the dark to block unoxidized sulfhydryls. The excess of these reagents was removed by dialysis versus 0.5 mM TEA for at least 8 hours, and the histones subsequently extracted by treatment with 0.2 M H_2SO_4 and precipitated with 95% ethanol.

Chromatin was treated with the other reagents under the following conditions: 1 mg/ml dimethylsuberimidate, 4°, pH = 7.4, 23 hours; 1 mg/ml dimethyladipimidate, 4°, pH = 7.4, 23 hours; and 3 mM carbodiimide, 22°, pH = 7.4, 1 hour. The diimidoester reaction was quenched by treatment with 0.2 M H_2SO_4 and the extracted histones were precipitated with ethanol. Chromatin treated with carbodiimide was dialyzed versus 0.5 mM TEA overnight prior to acid extraction of the histones.

Electrophoresis of Histones

The results of the various reactions were analyzed on acetic acid-urea [24] and SDS [25] polyacrylamide gels. Acid-urea gels were stained with Napthol Blue Black and SDS gels with Coomassie Blue R. Gels were scanned on a Beckman Acta CIII spectrophotometer; the resultant curves were resolved on a Dupont Curve Analyzer, and the area under the resolved peaks was measured.

The graph in Fig. 2 was obtained by correcting the amount of each histone on the gel (area under the curve) for the difference in volume of sample applied to the gel and expressing this value as a percentage of the largest amount present during the time course (either 0 or 0.5 hours). This

procedure was followed for 3 sets of gels of the same samples, and the average values for the amount of each histone present at each time point were plotted as a function of time of reaction with methyl-4-mercaptobutyrimidate (MMB).

Fractionation of Histones and Histone Polymers

Histones were fractionated into four groups, F1, F2b, F3, and (F2a1 + F2a2), by the modified procedure of Johns[26].

Extraction of Histone Polymers Associated with DNA

The acid insoluble pellet from extraction of MMB-treated nucleohistone was dissolved in 0.2 M H_2SO_4, 0.5 M 2-mercaptoethanol, kept at 4° overnight, and the supernatant precipitated with 95% ethanol.

RESULTS

Kinetics of Crosslinking by MMB

Essentially all histones associated with the DNA can be crosslinked together at low ionic strength at 4° during a 24 hr. time period utilizing methyl-4-mercaptobutyrimidate (MMB). However, since the addition of a butyryl side group to F2b changes its electrophoretic mobility slightly it is not possible to utilize a single electrophoretic system to analyze precisely the kinetics of crosslinking. If, however, the reaction products are analyzed on 2.5 M urea gels (where modified F2b co-migrates with F3) and 6.0 M urea gels (where F2b co-migrates with F2a2), then it is possible to assess the involvement of individual histones in the reaction. The results of such an analysis of a time course of fixation are shown in fig. 1. The gels were scanned and quantitated, and the rates of reaction of the various histones as assayed by disappearance of monomers are summarized in fig. 2. A least-squares analysis of the first eight time points reveals that the lysine-rich histone, F1, is polymerized most rapidly ($t_{\frac{1}{2}}$ = 1.4 hr.), consistent with several reports that this histone may exist in arrays. Histones F2b, F2a2, and F3 react to form crosslinks with $t_{\frac{1}{2}}$ = 2.5, 3.0, and 3.5 hrs. respectively; and the most slowly reacting histone is F2a1 ($t_{\frac{1}{2}}$ = 4.4 hr.).

Crosslinking involves an initial reaction of an ε-amino group of lysine with the imidoester followed by an oxidation of the thiol groups. The rate of reaction with the imidoester itself can be followed by an attendant small decrease in electrophoretic mobility of the histone. Thus modified (but not crosslinked) F2a1 migrates in the same position as the acetylated form of the molecule, as demonstrated by the increase in intensity of this band as the reaction proceeds (fig. 3b). Likewise modified, acetylated F2a1 migrates even more slowly in an empty region of the gel and can be measured by

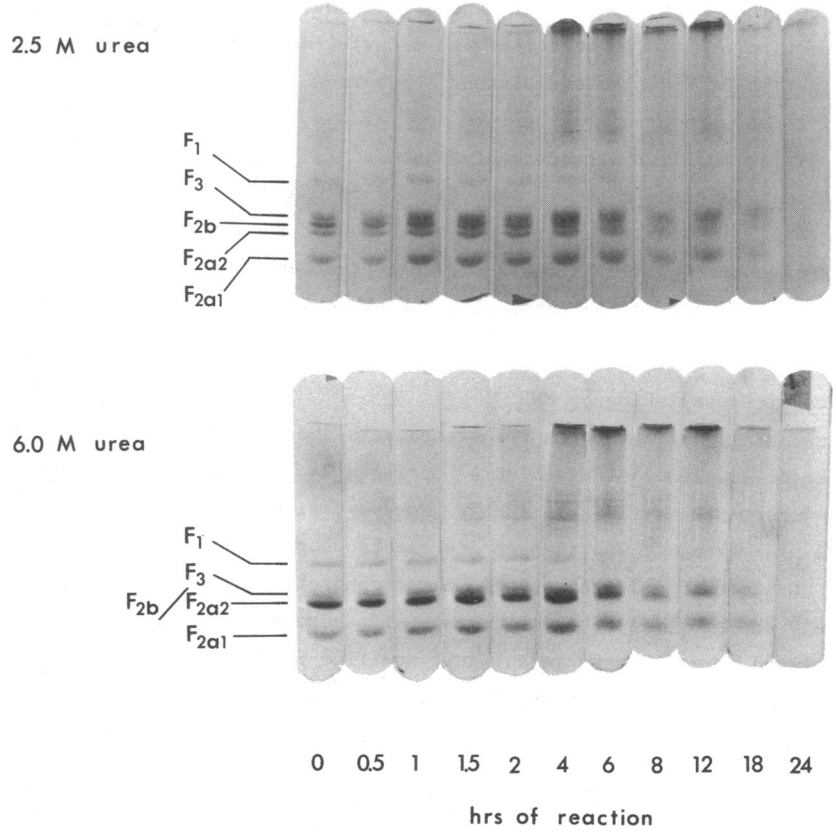

Fig. 1: 0.9 M Acetic acid-urea gels of histones extracted at the indicated times during reaction of nucleohistone with MMB. 2.5 M and 6.0 M refer to the concentration of urea in the gels.

microdensitometry and as such necessarily reflects the rate of modification (fig. 4a). The amount of histone in this band then remains constant for 8 hrs (fig. 4a), demonstrating an equal rate of modification and crosslinking to other histones. A similar plateau in the time course of reaction of diacetylated F3 (fig. 4b), shows that F3 is also modified and crosslinked at equal rates. Although similar data are more difficult to obtain for the other histone fractions, we have no reason to expect deviation from this pattern.

The Products of Crosslinking with MMB

In fig. 3 (b,c) we see the nature of the crosslinked products during a

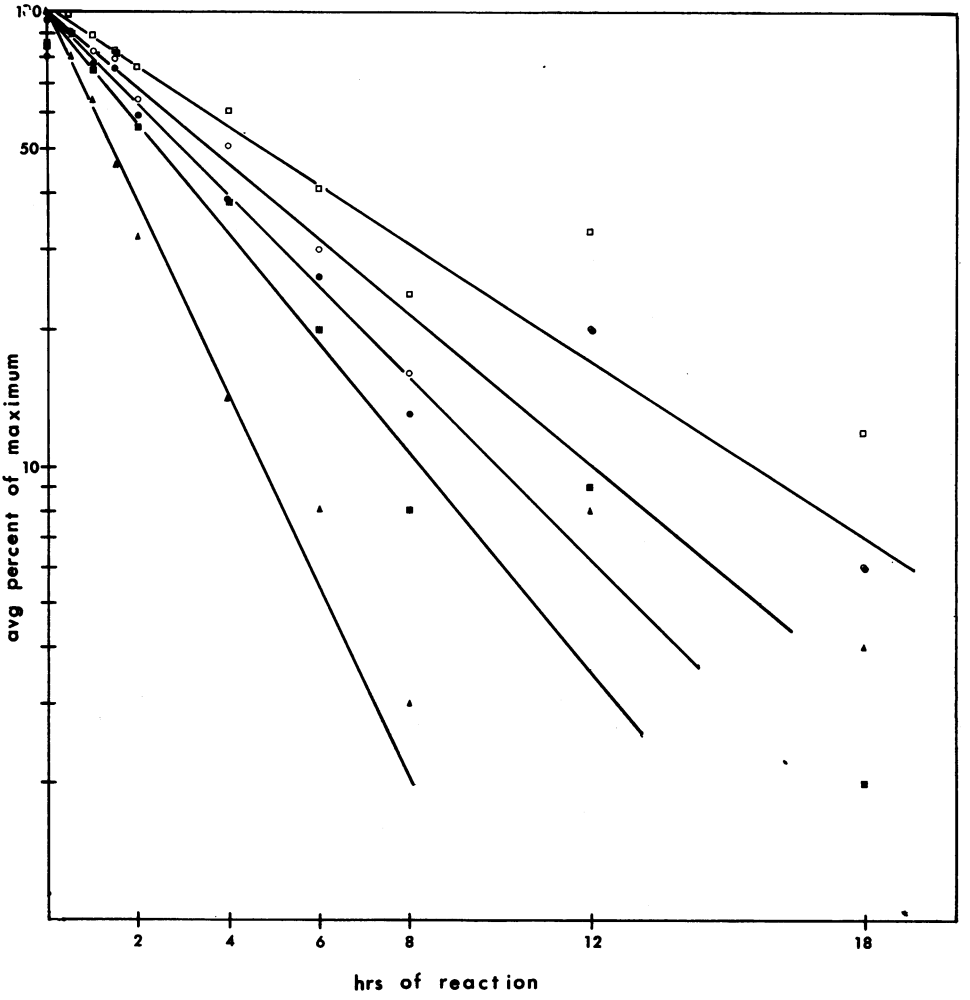

Fig. 2: Amount of each histone remaining as monomer after reaction of nucleohistone with MMB for the indicated times. ▲, F1: ■, F2b; ●, F2a2; o, F3; □, F2a1.

partial reaction of MMB with chromatin. Both SDS and acid-urea gels indicate that a range of products of varying sizes is produced. As judged by acid-urea gels (fig. 3b) the major products seem to be a rather complex group of dimers and at later times a substantial amount of highly polymerized material (see also fig. 1). Material which migrates at about half the rate of the histone monomers on acid-urea gels was identified as dimer material by (1) its identity of migration relative to that of known F3-F3[30] and F2a1-F2b[18] dimers and by (2) SDS gel molecular weight determination. In addition to a dimer doublet, the SDS gel reveals a trimer doublet, distinct

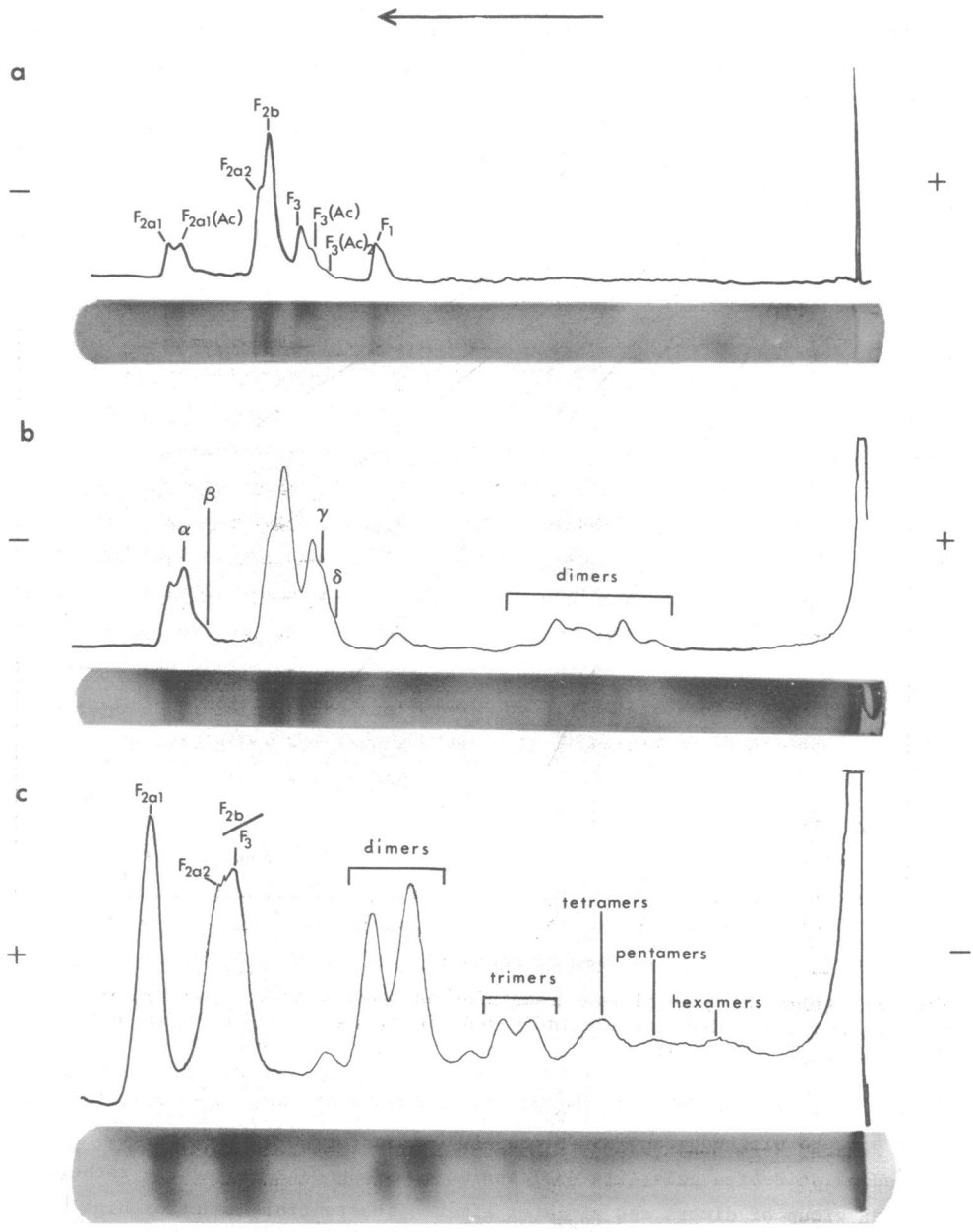

Fig. 3: a) 16 cm, 0.9 M acetic acid, 3.0 M urea gel of unreacted histones.
b) Gel as in a) of histones extracted from nucleohistone reacted for 4.0 hours with MMB. α is F2a1(Ac) + F2a1 (modified), β is F2a1 (Ac) (modified), γ is F3(Ac) + F3 (modified), δ is $F3(Ac)_2$ + F3(Ac) (modified).
c) SDS gel of histones from nucleohistone reacted for 6.0 hrs with MMB.

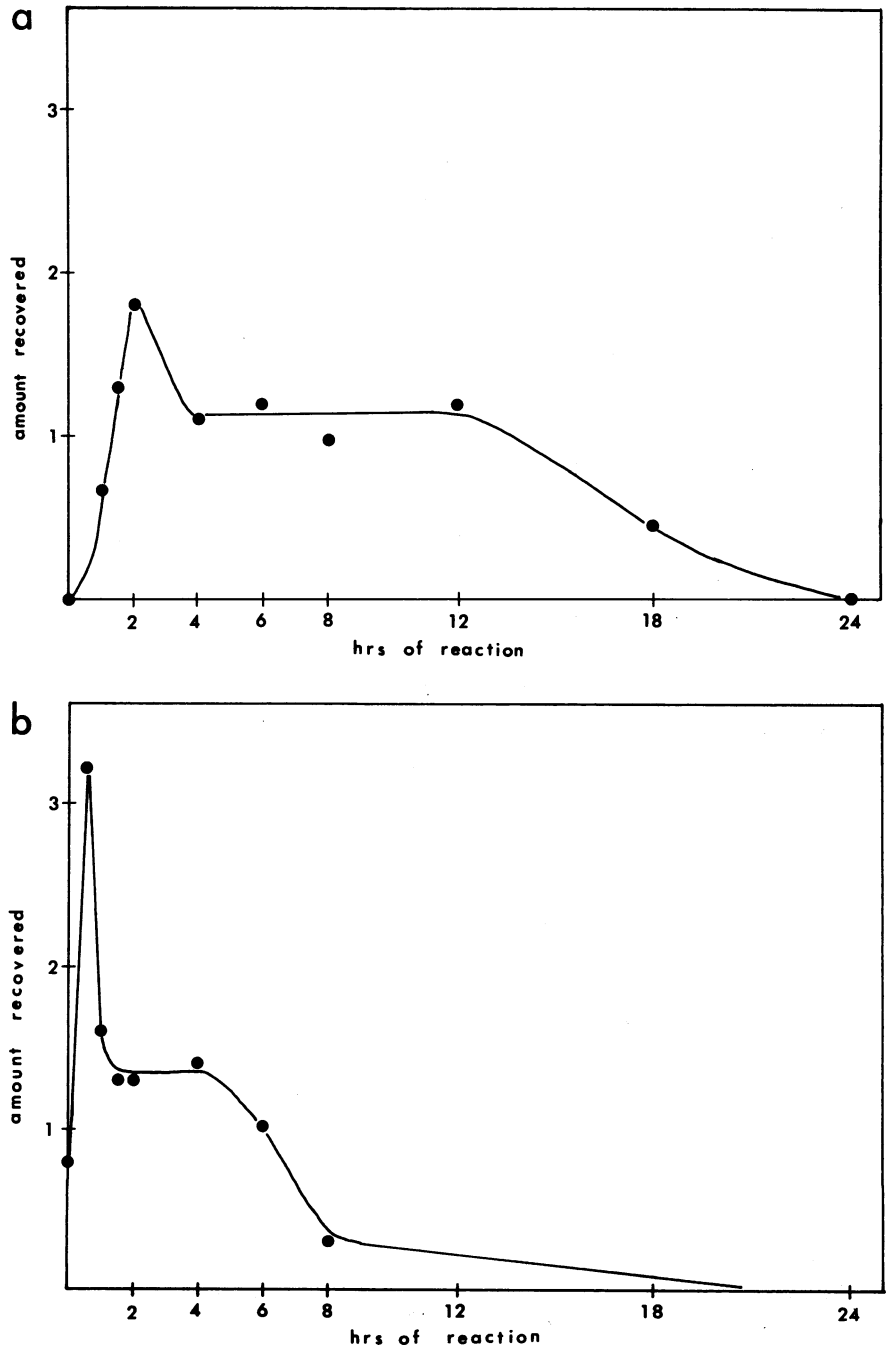

Fig. 4: Amount of modified, acetylated F2a1 (a) and F3 (b) recovered as a function of time of reaction with MMB.

oligomers up to a hexamer and large polymers not able to enter the gel (fig. 3c).

The crosslinked material is not produced as an artifact after the histones have been dissociated from the DNA; this was shown by a failure to produce any changes in the gel patterns by prolonged (45 min) treatment of free, modified histone with H_2O_2 under the conditions which had generated the initial disulfide bonds. Evidently the blockage of unoxidized thiol groups by iodoacetamide before histone extraction was successful.

The amount of material present as dimer can be quantitated after gel scanning, and the fraction present as dimer relative to that left as monomer is shown in fig. 5. It appears that the amount of dimer develops to a constant fraction of uncrosslinked material and that it disappears as the last monomer material reacts (see also fig. 1), indicating that it is probably an intermediate on the way to more complex, high molecular weight products. This would explain the low yields of small oligomer relative to initial amounts of monomer (fig. 1), since most of the material lost as monomer should be present as high polymers, not entering the gel. To test this, a sample of histones extracted after extensive crosslinking of nucleohistone with MMB was treated with 2-mercaptoethanol to convert all polymers to monomers. The amount of histone monomer was then equal to about 85% of that extractable from untreated nucleohistone (data not shown). The remaining 15% probably reflects either histone

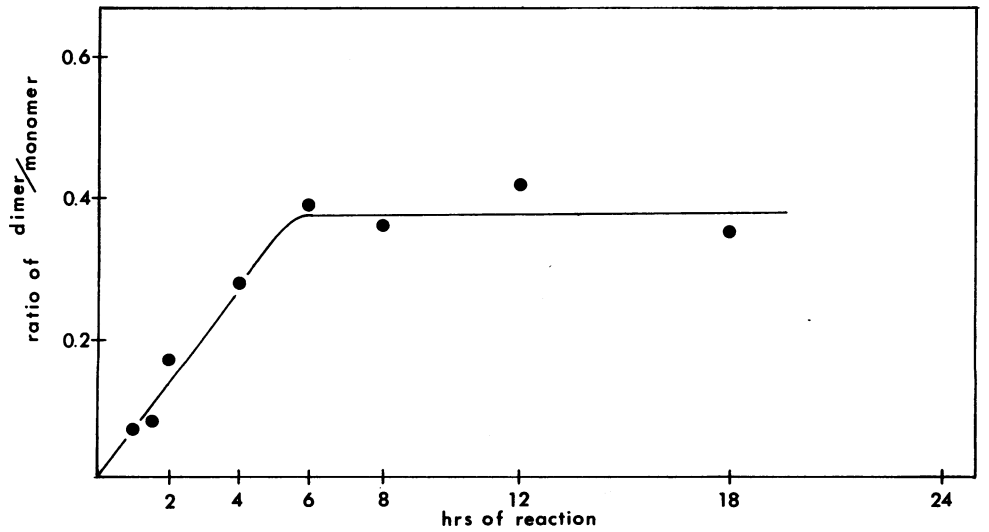

Fig. 5: Ratio of amount of dimer to amount of total monomer as a function of time of reaction with MMB.

linked to the DNA or acid-insoluble very high polymers (vida infra).

Crosslinking can also be achieved using bifunctional imidoesters and carbodiimides, and we have compared the products of the reaction of MMB with those of dimethylsuberimidate, dimethyladipimidate and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide as shown in fig. 6. It is clear that discrete, heterogeneous dimers are produced in all cases, though there are small variations im mobility. Since it is known that major contributions to the dimers from suberimidate-fixed chromatin come from histones F2b and F2a2 [22], it is intriguing to note the multiplicity of dimers in the gels of fig. 6b indicating a possibility of several dimer isomers. The position of the dimer bands relative to F2a1 provides a convenient and precise means of identification, and the different products from fixation by these various imidoesters are summarized in this form in Table I.

Histone Polymers Associated with DNA

At late stages in the crosslinking reaction we noted that the yield of polymer material at the top of the gels decreased appreciably (see fig. 1). Evidently some crosslinked material was no longer extracted into acid during the standard isolation procedures. Accordingly the acid-insoluble pellet (containing DNA) was treated with 2-mercaptoethanol, re-extracted with acid, and analyzed on polyacrylamide gels as shown in fig. 7. Essentially no crosslinked histone is found in the acid-insoluble pellet until the eighth hour of reaction. At this time only F1 is found in this fraction. Since it is clear from figure 1 that there is no unpolymerized F1 extractable

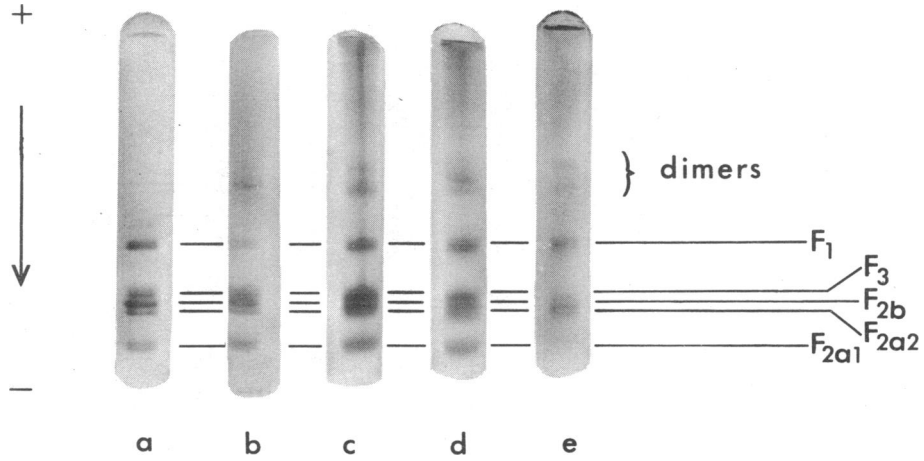

Fig. 6: 0.9 M acetic acid, 3.25 M urea gels of the histone products of reaction of chromatin with MMB (b), dimethylsuberimidate (c), dimethyladipimidate (d), carbodiimide (e). a) is unreacted control.

Table I: Catalogue of Dimer Mobilities

Reagent	Dimer mobility*, F2a1 = 1.0				
	0.53	0.48	0.46	0.44	0.41
Methyl-4-mercaptobutyrimidate	+**	+++		+	+
Dimethylsuberimidate		+++	+		++
Dimethyladipimidate		++		+++	+
Carbodiimide		+			+++

* Although these mobilities vary slightly with the batch of gels and conditions of the run, they are completely comparable within a single set run simultaneously, as these gels were.

** The +'s give an estimate of the relative amount of that particular dimer band present.

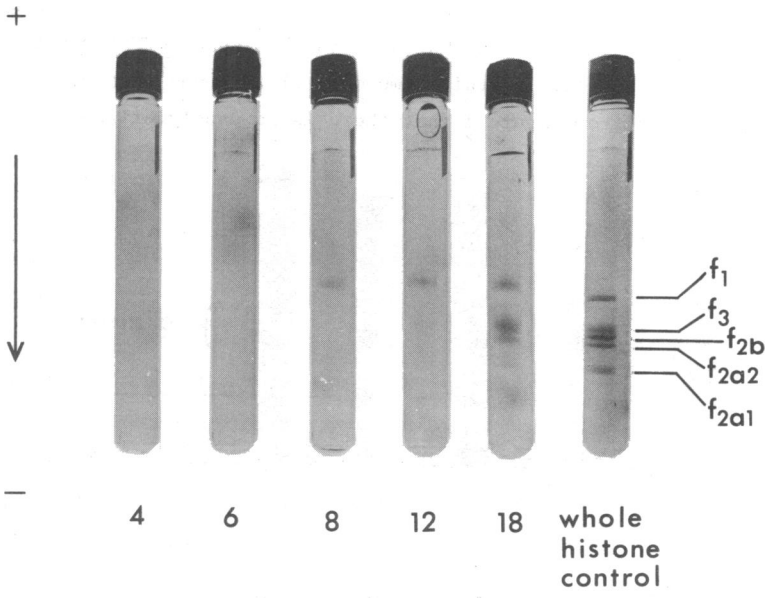

Fig. 7: Histones recovered after reduction from the acid-insoluble pellets of nucleohistone reacted with MMB for the indicated times.

at times immediately before the eighth hour, we conclude that the F1 isolated in this manner during the eighth through its twelfth hours is in a polymerized form and does not consist of individual F1 molecules bound to DNA. Also the F1 polymers do not contain significant quantities of other histones at these times.

At later stages in the reaction, all histone fractions were found in the acid-insoluble material. We do not know whether this represents polymeric material which had become bound to DNA, or whether the increase in molecular complexity had led to it becoming less soluble in acid.

Identification of Components of Dimer Bands

In principle the MMB-dimer bands could contain components from any of the five histones, though from the rate of disappearance of the various histones and the fairly rapid conversion of F1 to polymers (figs. 2 and 7), we expect that they would be rich in F2a2, F3, and F2b. Initial reversal experiments indicated that a rather complex situation prevailed and directed us to subfractionate the histones to try to simplify the system. Chemical separation schemes for histones separate them into F1, F2b, F3, and (F2a1 + F2a2). We anticipated that homodimers would fractionate into the parental histone fraction. It was difficult to predict how heterodimers would fractionate, but as will be described below, the separation is usually quite efficient and certainly provides a considerable simplification in the analysis of crosslinked products. In general, as documented below, we find that dimers containing an F2b component tend to fractionate into the F2b fraction irrespective of the other dimers component and that with the exception of (F3 - F2b), dimer containing an F3 compound fractionate into the F3 group.

The results of a chemical fractionation of crosslinked histones is shown in fig. 8. It is apparent that relatively little polymeric material is found in the F1 fraction. On the other hand both F2b and F3 fractions exhibit substantial amounts of dimer, falling in similar but somewhat different regions of the gel. The F2a1 + F2a2 fraction (F2A) possesses a relatively small complement of dimer or more complex material.

In order to define the nature of the histones in the various crosslinked products we have separated the bands by electrophoresis on 18 cm gels and then cut the gel in a series of 3 mm slices. The gel slices were incubated for 2 hrs. at room temperature in a medium containing both 4 M urea and 1.0 M 2-mercaptoethanol. The slices were then applied to conventional short gels and analyzed electrophoretically. In order to satisfactorily analyze for

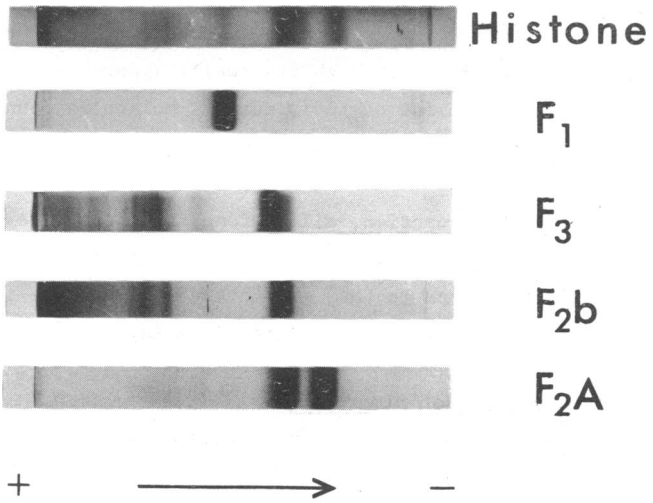

Fig. 8: Results of chemical fractionation of histones and polymers.

F2a2, F2b, and F3 it is necessary to slice two parallel gels and analyze each in turn on 1 M and 6 M urea gels. Furthermore, judicious selection of the time of reduction gives a product which still contains some original polymer so that proper identification of the source of the crosslinked material is ensured.

The approach is illustrated by a typical result for one of the dimer bands in the F3 fraction. The dimer band (R_f = 0.44) was reduced and analyzed on 1 M and 6 M urea gels (fig. 9). In the 1 M system F2b and F3 migrate together so that one can compute the relative amount of F2a2 (28 units relative to 43 for F3 + F2b). Subsequently on the 6 M urea system F2a2 and F2b (24 units) migrate together and F3 can be determined directly (45 units). Then we conclude that in this particular dimer from the F3 class we have essentially no F2b and that the other histone fractions are present to the extent of F3 = 45 units, F2a2 = 28 units and F2a1 = 15 units. From these numbers we would conclude that two dimer species are present in the original dimer band, namely (F3-F2a2) approximately 28 units and (F3-F2a1) approximately 15 units. We do not expect that we have dimers such as F2a1-F2a2 as these should not have been extracted into the F3 fraction which possibly extracts only those dimers with a composition F3-X.

We have analyzed the various histone fractions in this way, at different times of fixation, utilizing two gel systems in all cases for the final analysis. The data so obtained are compendious in the extreme and we present only a typical analysis of a reversal of polymers from F3 analyzed on a 6 M

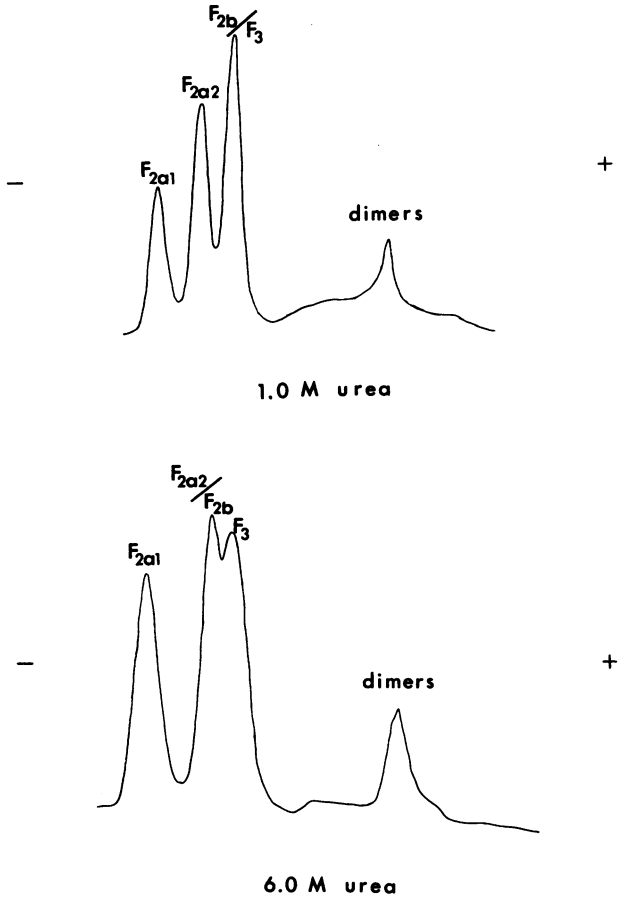

Fig. 9: Histones recovered after reduction of the R_f = 0.44 dimers run on 1.0 and 6.0 M urea gels.

gel. The results for this particular instance are shown in fig. 10. As the polymer bands of slower mobility are analyzed, different compositions for the various crosslinked products appear. Thus for F3-containing dimer products moving with a mobility 0.49-0.50 relative to F2a1, we see primarily F3-F2a1 dimers, at 0.47 and 0.43 we see a substantial accumulation of F3-F2a2 dimer; finally, dimers at 0.41 are enriched in F3-F3, though some F3-F2a1 and F3-F2a2 are also present. It is also clear that there is more than one dimer of a given type. Thus we find F3-F2a2 dimers at 0.47, 0.43 and 0.39, probably reflecting different sites of crosslinking between the two histone molecules. This type of behavior was noted for all the dimers to be described.

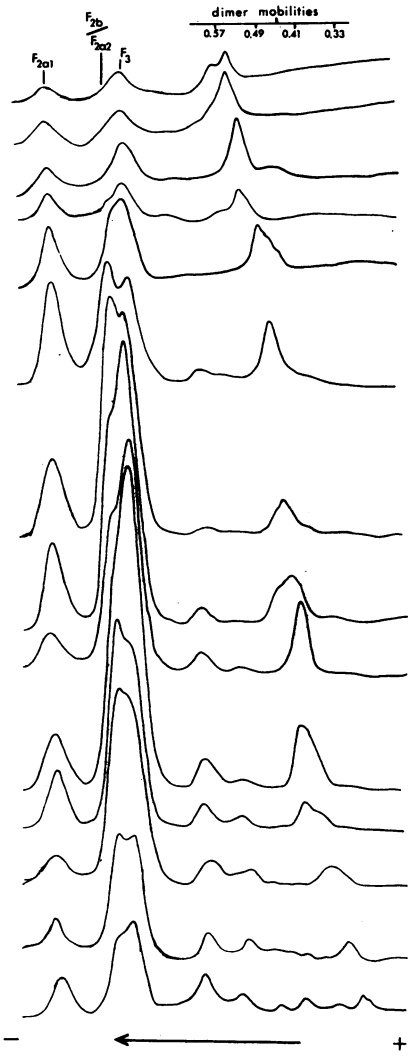

Fig. 10: Histones recovered after reduction of gel slices containing oligomers of the indicated relative mobilities, from the F3 fraction. Microdensitometric traces of 6.0 M urea, 0.9 N HOAc gels.

As one moves beyond the dimer region one finds an increasing complexity and all histones are now present. Reduction of trimers also produces dimers with mobilities similar to those analyzed on previous slices; this supports the idea of the dimers being a steady state intermediate in the formation of high polymers. The amount of F1 in the crosslinked products increases until the top of the gel is reached where F1 now contributes 20% to the total product.

Table II

F3 fraction			F2b fraction		
Rf*	dimer	amount	Rf*	dimer	amount
>0.50	F3-F2a1	trace	0.48-0.50	F2b-F2a2	
0.50	F3-F2a1	+		F2b-F3	trace
				F2b-F2a1	
0.49	F3-F2a1	+			
	F2a2-F3	+	0.46	F2b-F2a2	+
				F2b-F3	+
0.47	F3-F2a1	+			
	F2a2-F3	++++			
0.43	F2a1-F3	+	0.44	F2b-F2a2	++
	F2a2-F3	+++		F2b-F3	++
0.41	F3-F1	trace	0.42	F2b-F2a2	++
	F2a1-F3	trace		F2b-F3	++
	F2a2-F3	+		F2b-F1	+
	F3-F3	+++			
0.39	F2a2-F3	+++	0.40	F2b-F2a2	++
	F2a1-F3	trace		F2b-F3	++
	F1-F3	trace	0.38	F2b-F2a2	+
				F2b-F3	+
				F2b-F1	+

* relative mobility, F2a1 = 1.0

The results from this study are summarized in Table II, in which we report the relative frequencies of occurrence of a given dimer as a function of the mobility of the dimer.

Material from the top of the F2b fraction has the composition $(F1)_{10}(F2b)_5(F2a2)_2(F3)_2(F2a1)_1$ and from the top of the F_3 gels we find material of composition $(F1)_3(F3)_5(F2a1)_1(F2b)_1(F2a2)_2$. It seems most likely that these reflect a mixture of different polymers. However it also would appear that if large homogeneous polymers of F1 are produced, they also possess the ability to crosslink to at least some other histone molecules with a sufficient frequency to cause much of the F1 to be co-extracted with other

histone fractions.

DISCUSSION

Analysis of the kinetics and nature of product formation during the reaction of nucleohistone with methylmercaptobutyrimidate (MMB) at low ionic strength leads to the following conclusions. The rate of crosslinking is, with a single exception, determined by the lysine content of the histones, since the half-life for crosslinking of all histones except F2a1 is linearly and inversely related to their lysine content (fig. 11). Histone F2a1 is crosslinked at a much lower rate than expected (fig. 11). This effect could be caused by the involvement of a greater percentage of F2a1 lysines in salt bridges to the DNA or in as yet undefined interactions with other histones. The rate of histone crosslinking is apparently determined by the rate of amidination (see fig. 4); this crosslinking leads to the production of a heterogeneous set of dimers which subsequently polymerize further to eventually form very high molecular weight species. Similar complex oligomeric products are found with other reagents differing in length of the crosslinking bridge and in type of bond formed (fig. 6 and Table I).

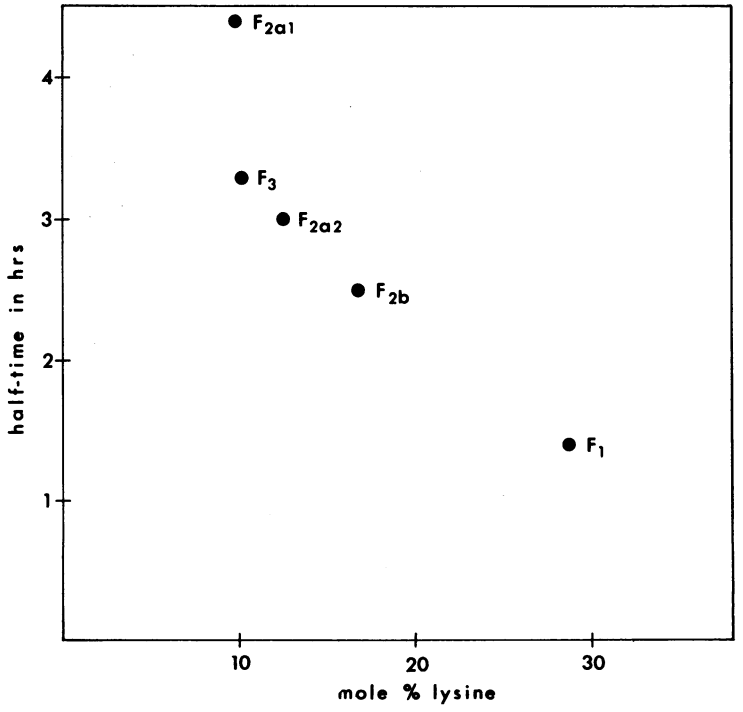

Fig. 11: Relationship between the half-time of crosslinking of histones by MMB and their lysine content.

The most frequently occurring dimers are (F3-F2b), (F2b-F2a2) and (F3-F2a2), and we find a significant but smaller quantity of (F3-F2a1), (F2b-F2a1) and (F3-F3). Very little F1 was found in the form of heterodimers, and very low yields were obtained of (F2b-F2b), (F2a2-F2a2), (F2a1-F2a1) or (F2a1-F2a2). F1 is polymerized very rapidly to form high molecular weight material which is relatively pure F1, though at later stages in the cross-linking reaction the poly-F1 becomes associated with other histones and as such appears in the F2b and F3 fractions. One would expect F2a1 to appear more frequently in higher polymers than in dimers or trimers since its slow rate of reaction with the imidoester demands that on the average the neighboring histones would already be incorporated into oligomeric units by the time F2a1 reacts.

Our data corroborates much previous data on histone association in chromatin obtained with a variety of probes. In particular, the finding of long arrays of F1 confirms the earlier results of Olins and Wright[27] and Hunter and Chalkley[21]. A dimer of F2a1-F2b has also been produced by Martinson and McCarthy[18] using tetranitromethane, and by Van Lente, et al.[31] utilizing formaldehyde as a histone crosslinking reagent. Several workers have found F2a1-F3 interactions in chromatin, Hyde and Walker[19] by using formaldehyde, Rubin and Moudrianakis[17] by measuring effects of F3 on F2a1 binding to DNA, and Bonner and Pollard[20] by utilizing a water soluble carbodiimide. It should be noted, however, that our results with this reagent as a crosslinker (fig. 6e) demonstrate a much more complex spectrum of products than those found by Bonner and Pollard[20]. Dimers and higher oligomers of F2a2-F2b were also found by Hyde and Walker[19] and by Van Lente, et al.[31] using formaldehyde and by ourselves[22] using dimethylsuberimidate.

In order to interpret these results it is important to distinguish between primary and secondary structure of chromatin. Primary structure refers to the order in which histones are bound directly to DNA along its long axis; secondary structure reflects those additional interactions between histones in the compact form of the chromatin fiber. We assume that the transition from secondary to primary structure can be accomplished without major reorganization and breaking of electrostatic bonds by exposing the chromatin to urea solutions. Similarly exposure of chromatin to very low ionic strength ($<10^{-4}$) causes massive increases in viscosity[32] and we expect that much of the basic structure is primary in nature, though some secondary interactions may give rise to the typical chromatin gels. These studies were performed on chromatin which was crosslinked at low ionic strength and we presume we are studying

essentially primary structure. This notion is supported by the observation that similar results are obtained when crosslinking occurs in 6 M urea (unpublished observation).

The dimer frequencies described above will then in all probability reflect the primary structure to a substantial degree. Nonetheless the primary structure must also be consistent with its logical formation from the extension of a secondary structure while preserving most of the histone-DNA electrostatic bonds. The model of Kornberg[3] would lead to expectations of (F3-F2a1) and (F2b-F2a2) dimers, otherwise the interactions between the various histones have not been defined in detail and this approach does not really provide a test of the model. The model of Van Holde et al.[4] is insufficiently precise with respect to details of internal organization of histones, and it is not possible to test the model by histone crosslinking studies. Furthermore it is based on limit nuclease digests and the possibility of histone migration has not been adequately excluded.

Recently Hyde and Walker[12] suggested an interesting model for chromatin secondary structure which accounts for most of the existing relevant data. They envisage a histone core consisting of dimers of (F3-F2a1) and (F2a2-F2b) interacting in an isologous manner with identical dimer groups such that both pairs together interact with 96 base pairs of continuous DNA supercoil. However, if (F3-F2a1) complexes are arranged heterologously and (F2b-F2a2) isologously, then the predicted primary sequence has essentially all of the dimers produced by reactions of nucleohistone with MMB. We may imagine that F1 is organized on the outmost aspect of the structure so that it has ample opportunity to crosslink to other F1 molecules, but so that crosslinks to other histones are infrequent as observed in this study. F1 is sufficiently long and has so little second degree structure[28] that it is possible to envisage it stretching over a period approaching 200 base pairs. This would be consistent with the recent calculations of Wright and Olins[29] of the data of Panyim et al.[23], who showed that there is approximately 1 mole of F1 for every 10 moles of the other histones. However, we note that the molar ratios calculated by Wright and Olins[29] were F1 * 1, F2a2 * F2a1 * F3 * 2, and F2b * 4. Essentially all previous models have chosen to ignore the extra amount of F2b, assuming that F2b and F2a2 are present in equimolar proportions. However, the model of Hyde and Walker[12] could easily be modified without affecting the overall structure by inserting $(F2b)_2$-F2a2 instead of (F2b-F2a2), thus accounting for the large amount of F2b. Such a change can also be accommodated within the dimer frequencies we have observed. Certainly a trimer as a basic unit is not excluded by any data of which we are aware.

The ability of F3 and F2b to interact with all other histones (excluding F1) and the restricted ability of F2a1 and F2a2 to form certain dimers argue against a simple linear repeating sequence in the primary structure (it also argues against a random sequence). Because of the hypothesized compact histone core, Hyde and Walker[12] suggest that adjacent histones may be interacting in part within both grooves of the DNA. This provides an opportunity for primary structure interactions of the type shown in fig. 12.

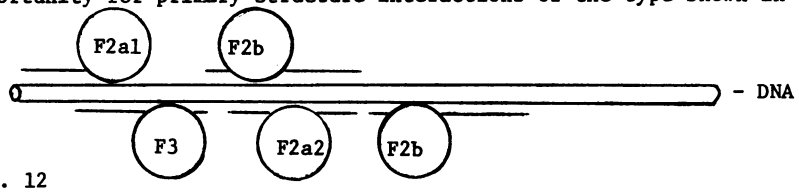

Fig. 12

A repeating structure analogous to this in the extended form of chromatin (either as a pentamer or a decamer) would clearly generate all the dimers observed, and at the same time prohibit the formation of apparently forbidden dimers such as F2a2-F2a1. As such this would appear to be consistent with the model of Kornberg or of Hyde and Walker, but would appear to exclude that of Van Holde in which interaction of the histone specifically with the major groove was emphasized.

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REFERENCES

1. Pardon, J. F., Wilkins, M. H. F. and Richards, B. M. (1967) Nature 215, 508-509.
2. Olins, A. L. and Olins, D. E. (1974) Science 183, 330-332.
3. Kornberg, R. O. (1974) Science 184, 868-871.
4. van Holde, K. E., Sahasrabuddhe, C. G. and Shaw, B. R. (1974) Nucleic Acids Res. 1, 1579-1586.
5. Senior, M. B., Olins, A. L. and Olins, D. E. (1975) Science 187, 173-175.
6. van Holde, K. E., Sahasrabuddhe, C. G., Shaw, B. R., van Bruggen, E. F. J. and Arnberg, A. C. (1974) Biochem. Biophys. Res. Comm. 60, 1365-1370.
7. Griffith, J. D. (1975) Science 187, 1202-1203.
8. Axel, R., Melchoir, W., Jr., Sollner-Webb, B. and Felsenfeld, G. (1974) Proc. Nat. Acad. Sci. 71, 4101-4105.
9. Noll, M. (1974) Nucleic Acids Res. 1, 1573-1578.
10. Sahasrabuddhe, C. G., and van Holde, K. E. (1974) J. Biol. Chem. 249, 152-156.
11. Burgoyne, L. A., Hewish, D. R. and Mobbs, J. (1974) Biochem. J. 143, 67-72.

12. Hyde, J. E. and Walker, I. O. (1975) Nucleic Acids Res. 2, 405-421.
13. D'Anna, J. A., Jr. and Isenberg, I. (1974) Biochemistry 13, 4992-4997.
14. Roark, D. E., Geoghegan, T. E. and Keller, G. H. (1974) Biochem. Biophys. Res. Com. 59, 542-547.
15. Kornberg, R. D. and Thomas, J. O. (1974) Science 184, 865-868.
16. Kelley, R. I. (1973) Biochem. Biophys. Res. Com. 54, 1588-1594.
17. Rubin, R. L. and Moudrianakis, E. N. (1975) Biochemistry 14, 1718-1726.
18. Martinson, H. G. and McCarthy, B. J. (1975) Biochemistry 14, 1073-1078.
19. Hyde, J. E. and Walker, I. O. (1975) FEBS Letters, 50, 150-154.
20. Bonner, W. M. and Pollard, H. B. (1975) Biochem. Biophys. Res. Com. 64, 282-288.
21. Chalkley, R. and Hunter, C. (1975) Proc. Nat. Acad. Sci. 72, 1304-1308.
22. Chalkley, R. (1975) Biochem. Biophys. Res. Com. 64, 587-594.
23. Panyim, S., Bilek, D. and Chalkley, R. (1971) J. Biol. Chem. 246, 4206-4215.
24. Panyim, S. and Chalkley, R. (1969), Arch. Biochem. Biophys. 130, 337-345.
25. Panyim, S. and Chalkley, R. (1971) J. Biol. Chem. 246, 7557-7560.
26. Oliver, D., Sommer, K. R., Panyim, S., Spiker, S. and Chalkley, R. (1972) Biochem. J. 129, 349-353.
27. Olins, D. E. and Wright, E. B. (1973) J. Cell Biol. 59, 304-317.
28. Bradbury, E. M., Cary, P. D., Chapman, G. E., Crane-Robinson, C., Danby, S. E., Rattle, H. W. E., Boublik, M., Palau, J. and Aviles, F. J. (1975) Eur. J. Biochem. 52, 605-613.
29. Wright, E. B. and Olins, D. E. (1975) Biochem. Biophys. Res. Com. 63, 642-650.
30. Panyim, S., Sommer, K. R. and Chalkley, R. (1971) Biochemistry 10, 3911-3917.
31. Van Lente, F., Jackson, J. F. and Weintraub, H. (1975) Cell 5, 45-50.
32. Bartley, J. and Chalkley, R. (1973) Biochemistry 12, 468-474.