HISTONES F2a1 AND F3 INTERACT REVERSIBLY AND COOPERATIVELY WITH DNA TO FORM AN EQUIMOLAR COMPLEX IN CHROMATIN

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1. Introduction

Recent studies on isolated histones in solution have highlighted the importance of specific interactions between f2a1 and f3 to give dimers, tetramers and higher oligomers [1–4]. Such studies take on a new significance with the demonstration that similar, though more extensive interactions also occur in chromatin where the histones are bound to DNA [4]. A model incorporating these observations has recently been proposed for chromatin structure [5]. Here we present data which demonstrate that f2a1 and f3 dissociate reversibly and cooperatively as an equimolar complex from calf thymus chromatin. This study complements previous results which showed that in native chromatin f2a1 and f3 can be covalently cross-linked into oligomeric products containing equimolar quantities of the two histones [4]. The cooperative reversible interaction of f2a1 and f3 with DNA provides a mechanism whereby the histones can be self-assembled into the highly ordered oligomers which may be an important feature of the structure of the chromatin supercoil [5].

2. Experimental

Chromatin was prepared from calf thymus [6] and characterised as described previously [7]. Histones were dissociated from DNA by dialysis to equilibrium for 3 hr. at 4°C against sodium chloride solutions containing sodium phosphate, 0.01 M, pH 6.3. Such conditions minimise the possibility of proteolysis which can, under other conditions, complicate the interpretation of the results [8].

Dissociated histones were separated from the DNA–histone complex by gel exclusion chromatography on Sepharose-4B [7]. Protein was measured using the Folin reaction [7] and DNA from A_{260} using an extinction coefficient of 7400 per mol phosphorus [9]. Circular dichroic spectra were recorded with a Jouan CD 185 Dichrograph [7]. Histone samples were analysed by electrophoresis either on polyacrylamide-urea [10,11], or on polyacrylamide-sodium dodecylsulphate gels at pH 10.0 [12]. Gels were stained with 0.1% Coomassie blue and scanned on a Joyce-Loebl double-beam microdensitometer. Traces were analysed with a Dupont curve analyser. The molar amounts of individual histone fractions were estimated from the areas of the stained electrophoretic bands. Pure histone fractions were kindly supplied by Dr E.W. Johns. The extinction coefficient of each stained fraction was estimated by using known amounts of histone, dried over P_2O_5, determined by weighing and from the A_{275} using the known tyrosine content [13] to estimate the extinction coefficient.

3. Results

A typical elution profile demonstrating the complete separation of depleted nucleohistone from dissociated histone (the two components in the dissociation reaction) by gel exclusion chromatography is shown in fig.1. The complete separation of the two components by the column shows that they are not in rapid, reversible equilibrium [14] and that the time scale for the re-establishment of the new equilibrium, caused by the perturbation of the original equilibrium

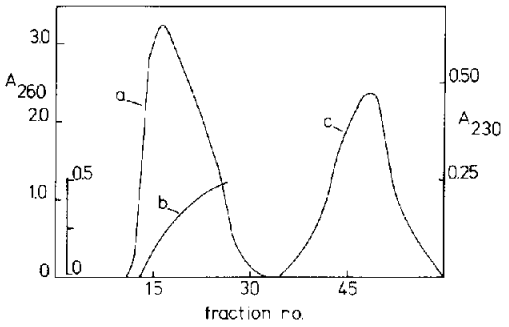

Fig.1. The fractionation of dissociated histone (c) from depleted nucleohistone (a) on Sepharose-4B equilibrated with 1.2 M NaCl. Curve (b) shows the variation of the protein: DNA ratio across the first peak (see inset ordinate)

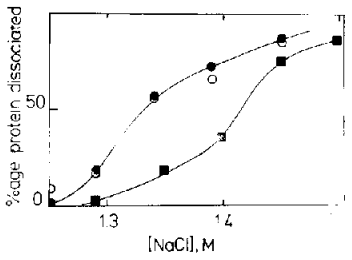

Fig.2. The percentage of histone dissociated from [illegible] a function of [NaCl], referred to zero dissociation [illegible] 1.20 M NaCl. (—●—), forward curve; (- -○- -), reverse curve, both at 4°C; (—■—), forward curve at 20°C. [DNH] = 427 μg/ml.

by passage of the components down the column, is slow compared to the separation time on the column. This means that the chemical composition of the separated dissociated species may be used as an accurate measure of the original equilibrium position. However, it is also clear that the protein/DNA ratio of the leading peak varies across the peak, implying an incipient tendency for a new equilibrium to be established across this peak as it moves down the column. This phenomenon has been noted previously by us [7] and by others [15]. Despite this, an accurate measure of the amount of protein bound to the DNA in the original equilibrium may be obtained by pooling all the fractions from the first peak and measuring the overall protein–DNA ratio. In this way the curve for histone dissociation between 1.0 and 2.0 M NaCl at 4°C was constructed (fig.2). A comparison of the gel electrophoresis patterns of the protein dissociated in 1.2 M NaCl and the protein remaining bound to the DNA (fig.3) shows that all of the fractions f1, f2a2 and f2b have dissociated completely whereas f2a1 and f3 remain completely bound. Thus the curve shown in fig.2 represents the dissociation of f2a1 and f3 only.

The reassociation curve obtained by completely dissociating all histones from the DNA by adding solid NaCl to make the solution 2 M in NaCl and then dialysing the solution to lower salt before separating the components on the column coincides with the forward dissociation curve and demonstrates that the system is in thermodynamic equilibrium with respect to dissociation by NaCl. At a temperature of 20°C the degree of dissociation is decreased at all salt concentrations (fig.2), indicating that the dissociation reaction is endothermic. However, the two curves were not the same shape, implying that there may be salt-induced conformational changes contributing to the overall change.

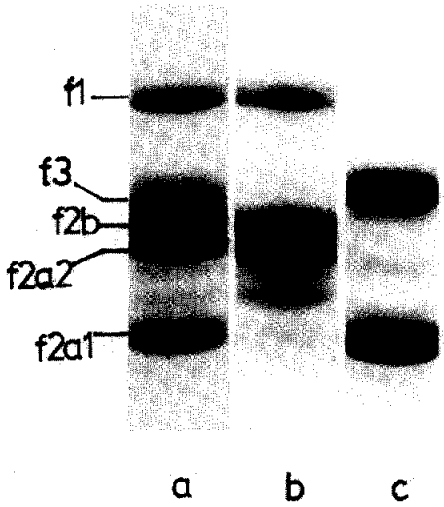

Fig.3. Acrylamide-urea gel electrophoresis patterns of (a) whole histone, (b) histone dissociated from DNA in 1.20 M NaCl, (c) histone bound to DNA in 1.20 M NaCl.

The effect of protein concentration on the degree of dissociation was investigated at two different salt concentrations by decreasing the initial nucleoprotein concentration. There was a marked increase in the degree of dissociation with dilution at both 1.25 and 1.37 M NaCl as might be expected from a consideration of mass action effects on a disproportionation reaction. For technical reasons it was not possible to investigate the reversibility of the process by the

methods employed here. However, if we assume that dilution at constant [NaCl] produces a new thermodynamic equilibrium, it is possible to construct binding curves at each [NaCl]. As shown in fig.4, the sigmoidal nature of the binding curves indicates that the binding of f2a1 and f3 to DNA is highly cooperative. No quantitative analysis of these curves will be attempted at this stage.

To determine whether f2a1 and f3 were differentially dissociated by NaCl, the proteins remaining bound to the DNA at various points across the dissociation curve were analysed by gel electrophoresis. The gel patterns show that f2a1 and f3 dissociate in equimolar proportions both when the degree of dissociation is increased by raising the [NaCl] (table 1) and when it is increased by dilution of nucleohistone at constant [NaCl]. Furthermore, an analysis of the reassociation shows that the two histones also reassociate as an equimolar complex.

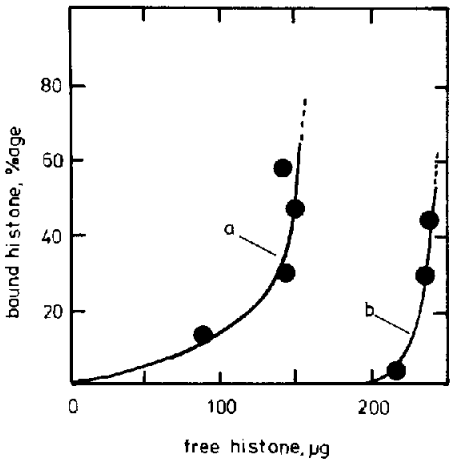

Fig.4. Histone binding isotherms at 4°C constructed as described in the text, by decreasing the initial DNH concentration: (a) 1.25 M NaCl, (b) 1.34 M NaCl. [DNH] was varied from 560 to 98 µg/ml.

The circular dichroic spectra measured over the range from 320 to 210 nm of depleted nucleohistone and the corresponding reassociated nucleohistone were identical at a given [NaCl], indicating that the association–dissociation was reversible with respect to the secondary structure of the DNA and histone components.

In contrast to the slow kinetic processes responsible for the reestablishment of the association–dissociation equilibrium brought about by perturbations at constant [NaCl], altering the equilibrium by changing the [NaCl] was relatively much faster. This could be demonstrated by applying a nucleohistone sample which had been dialysed to equilibrium against 1.25 M NaCl to a Sepharose-4B column previously equilibrated with 1.37 M NaCl. The degree of dissociation was identical to that of a sample which had been dialysed to equilibrium directly against 1.37 M NaCl. Thus, the new salt-induced equilibrium is reestablished on a time scale which is small compared to the time required to separate the products by gel exclusion chromatography.

4. Discussion

Although most previous studies on the salt-induced dissociation of histones have suggested that, with the exception of f1, they dissociate non-specifically [16, 17], detailed analysis shows that the dissociation curve has three distinct stages [18]. As shown here the final stage, which occurs between 1.1 and 2.0 M NaCl involves the discrete dissociation of f2a1 and f3.

Table 1
Molar percentages of f2a1 and f3 bound to DNA at various degrees of dissociation induced by varying [NaCl]

[NaCl], M	% Histone dissociated from DNA*	Molar % of each histone bound	
		f2a1	f3
1.25	0	48	52
1.29	4	59	41
1.35	19	56	44
1.39	46	49	51
1.45	75	49	51

* The degree of dissociation at 1.20 M NaCl is referred to as zero.

These two histones dissociate and reassociate reversibly with DNA in the form of a stoichiometric equimolar complex. Solution studies on mixtures of f2a1 and f3 have shown that they associate in the absence of DNA to form dimers, tetramers and higher oligomers [1–4]. Thus it appears that interaction with DNA also takes place through this equimolar complex. Although the molecular weight of the interacting species is not known, it must be at least the dimer.

The binding curves obtained at constant [NaCl] (fig.4) indicate that histones interact co-operatively between themselves when binding to DNA. Furthermore, the sigmoidal nature of the salt-induced dissociation curve at constant [DNH] suggests, but does not prove, that NaCl also interacts cooperatively with DNH. The cooperative nature of this interaction provides a kinetic mechanism for the generation of a self-assembling, highly ordered, regular arrangement of the histone complex along the DNA backbone in the native chromatin. This is an important feature of a recent proposal for the structure of chromatin which is based on an 'open' polymerisation of f2a1–f3 dimers [5].

The establishment of a reversible thermodynamic equilibrium has an important bearing on the question of salt-induced histone rearrangements recently studied in several laboratories [19,20]. It has been argued on the basis of these results that the structural properties of depleted nucleohistones prepared by salt-dissociation bear little relation to the properties of the native chromatin on account of the extensive rearrangements of histones which take place under the effect of the salt. From a structural point of view such rearrangements become unimportant if it can be shown that the histones, DNA and salt are in rapid, reversible equilibrium. This study shows that this is the case for f2a1 and f3.

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