

The effect of Ca^{2+} on the stability of chicken erythrocyte histone octamers

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Histones can be extracted from chicken erythrocyte chromatin with 2 M CaCl_2 10 mM Tris (pH 7.4). The core histones so extracted exist as H3–H4 tetramers and H2A–H2B dimers since calcium at >0.5 M result in dissociation of the histone octamer. Reconstitution of octamers occurs on removal of the Ca^{2+} by dialysis. Although <0.5 M calcium do not result in octamer dissociation, perturbations in the structure can be detected by CD and tyrosine fluorescence spectroscopy.

Histones Octamer Reconstitution Calcium Erythrocyte chromatin

1. INTRODUCTION

The core histones H2A, H2B, H3 and H4 can be extracted from chicken erythrocyte chromatin in equimolar amounts with 2 M NaCl at pH 7 [1]. High ionic strength solution presumably interrupts the electrostatic interactions between the histones and the DNA and simultaneously induces sufficient higher structure such that the core histones so isolated exist as an octamer [1]. Decreasing the salt concentration to around 0.2 M [2] or the pH to around 5 [3] results in dissociation of the octamer to an H3–H4 tetramer and two H2A–H2B dimers. The pH-dependent dissociation starts at around pH 5.4 [4] and suggests the requirements of ionised groups with pK -values in this region for octamer integrity. Such groups could be either the carboxylic acid group of aspartate or glutamate or the phosphate group from phosphorylated histones. Divalent metal cations readily form complexes with both phosphates and carboxylates thereby neutralising them. Therefore, we have investigated the ef-

fects of the divalent cation calcium on the extractability of the histone complexes from chicken erythrocyte chromatin and their stability.

2. METHODS

Nuclei were isolated from washed chicken erythrocytes by digitonin lysis and crude chromatin subsequently prepared by washing with 10 mM citrate 150 mM NaCl (pH 7.4) until the supernatant was free of proteins ($A_{230} < 0.1$) [5]. Histone octamer was isolated by the high (2 M) NaCl extraction method [2] preceded by an initial 0.8 M NaCl extraction to remove H1 and H5. Sephadex 6B chromatography was done on 2.5 cm $\times$ 1 m columns under the conditions given in the corresponding figures. SDS–polyacrylamide gel electrophoresis was done on 20% slab gels with a 5% stacking gel [6]. CD spectroscopy, fluorescence spectroscopy, crosslinking and sedimentation velocity determinations were as in [5]. Calcium binding studies were carried out with $^{45}\text{CaCl}_2$ using the flow dialysis method [7]. All experiments done at pH < 5.5 used buffers containing 10 μg pepstatin/ml to inhibit contaminant lysosomal proteases [8]. All Tris-

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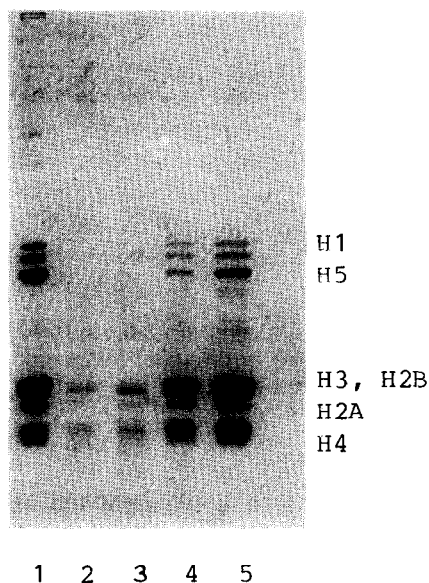


Fig. 1. SDS-PAGE of histones extracted with 10 mM Tris (pH 7.4) containing CaCl_2 : (1) standard; (2) 0.5 M CaCl_2 ; (3) 1 M; (4) 2 M; (5) 4.5 M.

buffers were adjusted with HCl to the desired pH-values. Further experimental details are in the figure legends.

3. RESULTS AND DISCUSSION

SDS-PAGE of the products of extraction of chicken erythrocyte chromatin with 10 mM Tris (pH 7.4) containing increasing concentrations of CaCl_2 is shown in fig. 1. Little histone was extracted at 0.5 M CaCl_2 with a slight increase in yield at 1 M CaCl_2 . Increasing the CaCl_2 concentration to 2 M resulted in an increase in core histone extraction together with H1 and some H5. A further increase in CaCl_2 to 4.5 M yielded more H5. This is in sharp contrast to extraction of chicken erythrocyte chromatin with increasing [NaCl] at around pH 7 where the order of release from DNA is H1 and H5 around 0.5 M, H2A and H2B at around 1.2 M and H3 and H4 by 2 M [9].

The nature of the histone complexes extracted by 2 M CaCl_2 were next investigated. Histone octamer prepared by 2 M NaCl 10 mM Tris (pH 7.4) extraction of chicken erythrocyte chromatin and Sepharose 6B chromatography in the same buffer was used as a comparison (fig. 2a). Extraction of

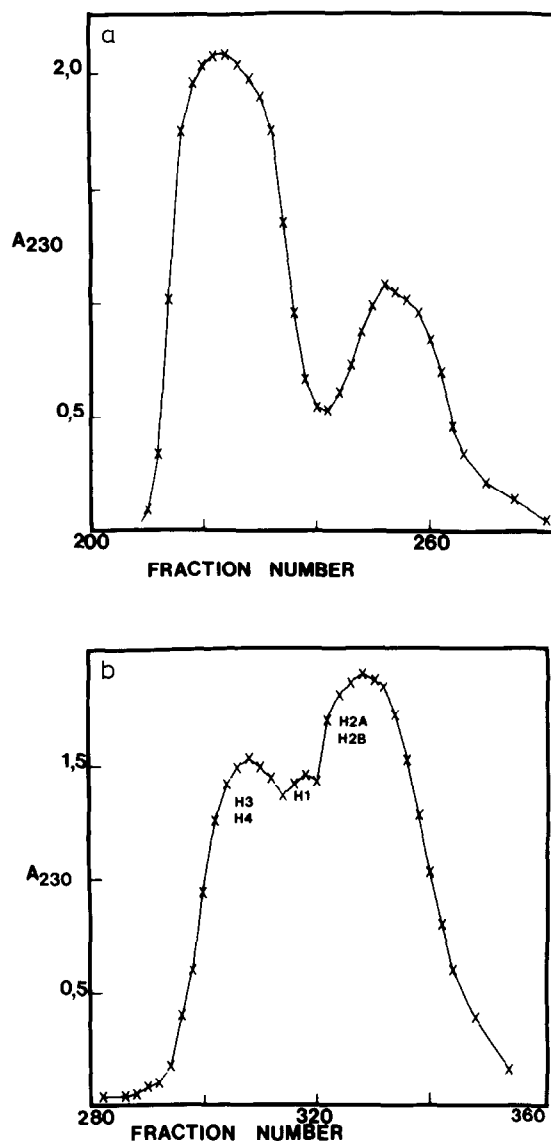


Fig. 2. Sepharose 6B chromatography of extracted histones. The column was run in either 2 M NaCl, 10 mM Tris (pH 7.4) (a) giving rise to a large octamer peak and a smaller peak of H2A-H2B dimer of 2 M CaCl_2 , 10 mM Tris (pH 7.4) (b).

chicken erythrocyte chromatin with 2 M CaCl_2 10 mM Tris (pH 7.4) and subsequent Sepharose 6B chromatography in the same buffer did not yield an octamer but rather H3-H4 tetramer and H2A-H2B dimer together with H1 and H5 (fig. 2b). Dialysis of the histone octamer against 2 M

CaCl_2 10 mM Tris (pH 7.4) and subsequent Sepharose 6B chromatography in this buffer resulted in an elution profile identical to fig. 2b but lacking the H1 and H5 peak. This CaCl_2 dissociation of the octamer was found to be reversible. Dialysis of the total eluate from this Sepharose 6B column against 2 M NaCl 10 mM Tris (pH 7.4) and re-chromatography in this buffer resulted in octamer reformation with an identical elution profile to fig. 2a.

The tetrameric nature of the H3–H4 complex produced by octamer dissociation in 2 M CaCl_2 was confirmed by determination of the sedimentation velocity and by crosslinking. The sedimentation velocity in 2 M CaCl_2 10 mM Tris (pH 7.4) was found to be 0.68 S corresponding to a $s_{20,w}$ of 2.2 S. This value is slightly lower than those of 2.5 or 2.8 S reported in [6,10] but may represent a different conformation of the tetramer (see later). Crosslinking of the H3–H4 complex in 2 M CaCl_2

10 mM triethanolamine (pH 7.4) proceeded to an *n*-mer via a tetrameric intermediate (fig. 3) which is characteristic of the H3–H4 tetramer [5].

The effect of varying $[\text{CaCl}_2]$ on octamer stability at pH 7.4 was investigated. Histone octamers were chromatographed on a Sepharose 6B column in 2 M NaCl 10 mM Tris (pH 7.4) containing varying $[\text{CaCl}_2]$. Octamer dissociation occurred yielding elution profiles similar to fig. 2b if the chromatography was carried out with ≥ 0.5 M CaCl_2 . Using 0.1 M CaCl_2 resulted in no apparent dissociation and an elution profile similar to fig. 2a was obtained.

Although octamer dissociation could not be detected by column chromatography at 0.1 M CaCl_2 slight perturbations in the octamer structure could be observed using CD spectroscopy at this concentration (fig. 4). Comparing the spectra of the octamer in Tris–EDTA and Tris–100 mM calcium, Ca^{2+} appear to increase the β -sheet (217 nm) and random coil (200 nm) content at the expense of the α -helical content (222 nm). The effect of calcium concentration on the structure of the octamer is more pronounced when the tryosine emission is followed by fluorescence spectroscopy (fig. 5). Increasing calcium concentrations resulted in increasing fluorescence without a change in the maxi-

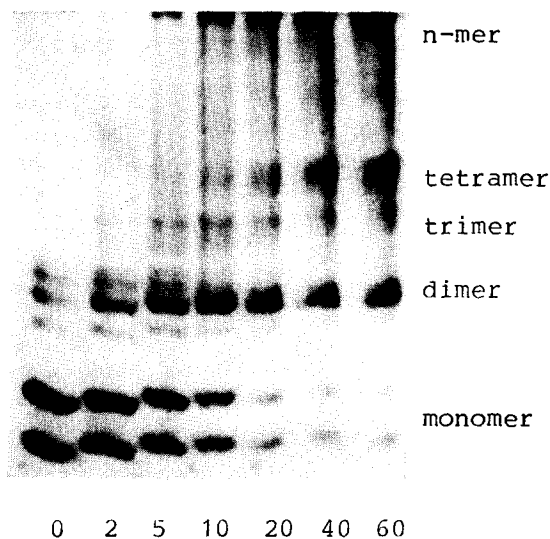


Fig. 3. SDS–PAGE of histone H3–H4 complexes cross-linked with dimethyl suberimide in 2 M CaCl_2 , 10 mM triethanolamine (pH 7.4). The reaction was quenched with 50 mM glycine at the stated times. Since quenching is not completely efficient the samples were applied directly to the gel. The poor gel quality is due to calcium dodecylsulphate precipitation. The size of the crosslinked components was checked by comparison with H3–H4 tetramers crosslinked in 2 M NaCl, 10 mM Tris (pH 7.4) [5].

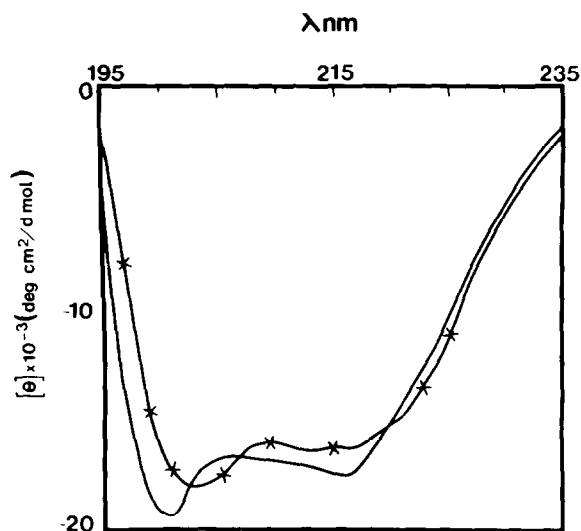


Fig. 4. CD spectroscopy of histone octamer in 2 M NaCl, 10 mM Tris, 1 mM EDTA (pH 7.4) (x—x) or 2 M NaCl, 10 mM Tris, 0.1 M CaCl_2 (—).

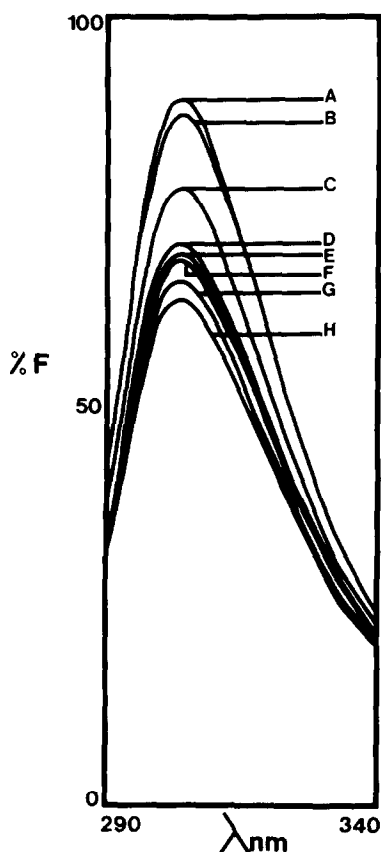


Fig. 5. Fluorescence spectra of histone octamers in 2 M NaCl₂, 10 mM Tris (pH 7.4) containing decreasing concentrations of CaCl₂: (a) 2 M; (b) 1 M; (c) 0.5 M; (d) 0.1 M; (e) 0.01 M; (f) 0.001 M; (g) no addition; (h) 1 mM EDTA. Excitation was at 269 nm.

imum wavelength of the emission spectrum. The greatest change in intensity occurred in the region of octamer dissociation between 0.1 and 1 M CaCl₂. Although octamer dissociation is complete at 0.5 M CaCl₂ the fluorescence emission intensity increased at higher CaCl₂ concentrations thus suggesting that Ca²⁺ alter the conformation of the products of dissociation. This may account for the decreased *s*_{20,w}-value obtained for the H3-H4 tetramer. The calcium-dependent dissociation is

not brought about by the binding of Ca²⁺ to high affinity sites on the octamer since we were unable to detect any such sites using flow dialysis with ⁴⁵CaCl₂ [7].

We hypothesise that electrostatic interactions contribute to maintaining the integrity of the octamer in 2 M NaCl, the negative charge for such an interaction arising from carboxylic acid and phosphate groups, though the abundance of the latter will be low. These groups have p*K*-values in the region pH 5–6 and the dissociation constants for calcium binding of the order of 10⁻¹ M [11]. Decreasing the pH to 5 or increasing the Ca²⁺ to >0.1 M would neutralise these negative charges and thereby destabilise the octamer.

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