Fluorescent labelling of histone H3: effect on histone-histone interaction and core particle assembly

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Substitution of Cys 110 of chicken histone H3 with *N*-iodoacetyl-*N*'-(5-sulpho-1-naphthyl)ethylenediamine or iodoacetamide prevents octamer formation in 2 M NaCl but does not prevent polyglutamic acid-mediated core particle assembly.

Histone H3 *Fluorescence* *Core particle* *Polyglutamic acid*

1. INTRODUCTION

Fluorescently labelled histones have been extensively used [1–7] to probe the internal organisation of the nucleosome and the changes in its structure brought about by the environment. The strategy has been either to reconstitute nucleosome core particles using histones that have been labelled as pure individual histones [2,3] or to dissociate nucleosomes with urea or guanidine hydrochloride at high NaCl concentrations, label with the desired probe and reassociate by removal of the denaturant by dialysis [1,4–7]. The most frequently used method of attachment of such a probe has been carboxymethylation with fluorescent derivatives of iodoacetamide assuming that such a probe specifically labels the single cysteine residue on chicken histone H3 [1,3–7]. The reassociated fluorescently labelled particles have been classified as nucleosome cores on the basis of their behaviour on sucrose density gradients and their DNase I cutting patterns. Moreover, pyrene-labelled *Physarum* H3 has been shown to be incorporated into nucleosomes in vivo [4] to demonstrate the fidelity in structure of the fluorescent product. Thermal denaturation studies [5], on reassociated nucleosome cores containing histone H3 fluorescently labelled at Cys 110, have however indicated that such a modification destabilises the reassociated particle. In addition, an investigation into the accessibility of such particles by small molecules [7] has shown increased accessibility relative to unlabelled reassociated nucleosome cores. It would thus appear that histone-DNA and histone-histone interactions are altered by the presence of a fluorescent probe on Cys 110 in histone H3. We have therefore investigated the effect of histone H3 sulphydryl modification on the reconstitution of histone-histone complexes.

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Abbreviations: IAEDANS, *N*-iodoacetyl-*N*-(5-sulpho-1-naphthyl)ethylenediamine; H3-(Cys-IAEDANS), histone H3 specifically labelled with IAEDANS at Cys 110; H3-(IAEDANS), histone H3 non-specifically labelled with IAEDANS; H3-H3-(IAEDANS), histone H3 Cys dimer non-specifically labelled with IAEDANS as the dimer

2. MATERIALS AND METHODS

Crude chromatin was prepared from digitonin-lysed chicken erythrocytes [8]. Core histones were extracted with 0.25 M HCl after washing the pellet with 5% (w/v) perchloric acid to remove histones

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H1 and H5 [9], and chromatographed on Biogel P60 in 50 mM NaCl (pH 1.7) [10] in the presence of pepstatin [11]. The H2A- and H4-containing fractions were pooled and stored at 4°C in the presence of 0.2% NaN_3.

The H3- and H2B-containing fraction eluted from the Biogel P60 column was pooled, concentrated by ultrafiltration and subjected to iodosobenzoate treatment as described [10] (25 μg/mg protein, 4 M urea, pH 7, 1 h at room temperature in the dark) before being chromatographed on a G-100 column in 50 mM acetate, 50 mM NaCl (pH 5.1) [10]. The fractions containing either pure H3 dimer or pure H2B were pooled separately, the former being reduced to H3 monomer by the addition of excess 2-mercaptoethanol. This was removed by dialysis against 0.25 M HCl.

Labelling by carboxymethylation of purified histone H3 with IAEDANS or iodoacetamide was carried out in 4 M urea, 50 mM phosphate (pH 8.3) in the dark for 20 min at room temperature with 1 mol label per mol cysteine. The reaction was stopped by the addition of excess (100 mol/mol) 2-mercaptoethanol. The labelled protein was recovered after dialysis against 0.25 M HCl, water and ultrafiltration. When the H3 dimer was labelled the addition of mercaptoethanol was only made after separation of the dimer via exclusion chromatography.

The reconstitution protocol of histone octamers incorporating modified histone H3 was attempted as in [8]. Histone H3-H4 tetramers were reconstituted from individual histones H3 and H4 [12]. Nucleosomal core particles were assembled with polyglutamic acid on long (1000 bp) DNA as described [13] SDS-polyacrylamide gel electrophoresis was carried out on 20% gels using a 5% stacking gel [14]. Fluorescence analysis of column eluates was by measuring the emission at 525 nm after excitation at 340 nm. Further experimental details are given in the figure legends.

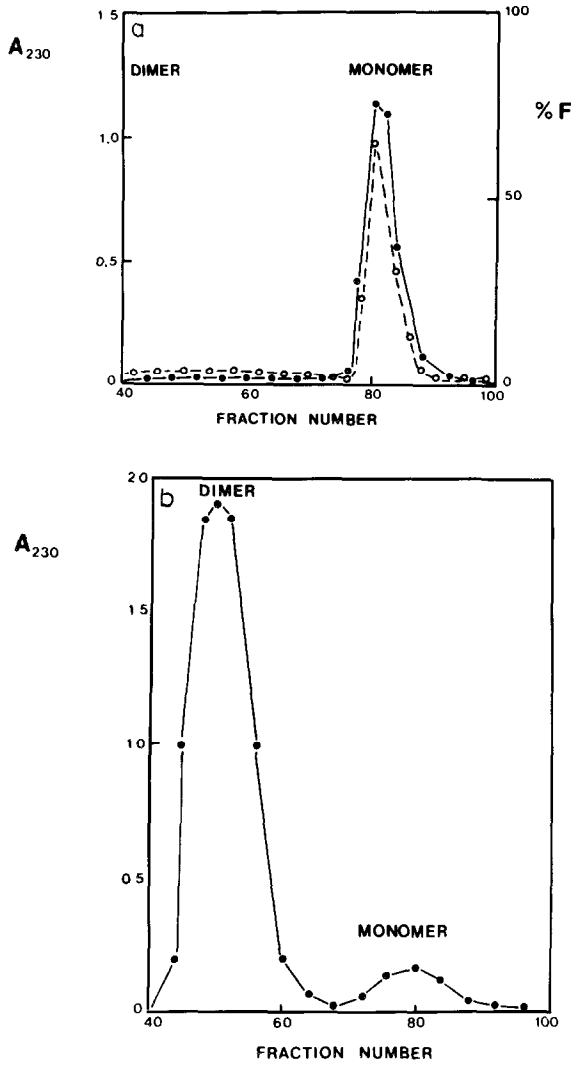

Fig.1.(a) Sephadex G-100 chromatography of iodosobenzoate-treated IAEDANS-labelled histone H3, (H3-(Cys-IAEDANS)). Eluant: 50 mM NaCl, 50 mM acetate (pH 5.1). (●—●), A_{230} (○—○) fluorescence. (b) Sephadex G-100 chromatography of unlabelled histone H3 after treatment with iodosobenzoate. Conditions as for a.

3. RESULTS

Isolated histone H3 can be stoichiometrically and specifically labelled at the cysteine residue with IAEDANS. This shown by the inability of the labelled histone H3 to dimerise (fig.1a) whereas unlabelled histone H3 dimerises to 95% on oxidation with iodozobenzoate (fig.1b). This IAEDANS-labelled histone H3 was used to attempt the reconstitution of histone octamers. However, IAEDANS substitution of the Cys 110 residue in histone H3 rendered this histone unsuitable for octamer formation (fig.2); the fluorescent histone H3 eluted from the Sepharose 6B col-

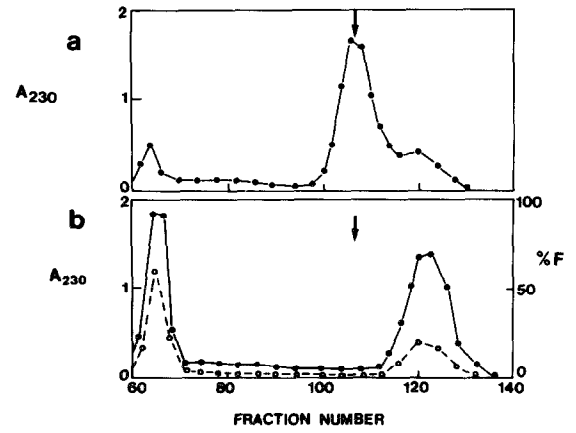

Fig.2. Reconstitution of histone octamers from: (a) chicken core histones [8], shown for comparison; (b) H3-(Cys-IAEDANS) and H2A, H2B and H4. Solid urea was added to 8 M to the ultrafiltration-concentrated histone solution which was then dialysed against 2 M NaCl, 10 mM Tris (pH 7.4). Any precipitate was removed by centrifugation (12000×g, 5 min) before application to the Sepharose 6B column equilibrated in the same buffer. The peaks eluted represent H3-H4 aggregate, histone octamers and dimers respectively by SDS electrophoretic criteria. (↓) Denotes octamer elution volume. (●—●) A_{230}, (○—○) fluorescence.

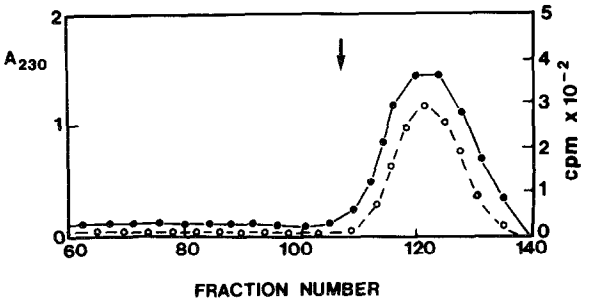

Fig.3. Attempted reconstitution of histone octamers from H3 labelled with iodo[^{14}C]acetamide and subsequently treated with iodosobenzoate and chromatographed on a Sephadex G-100 column to remove dimerised unsubstituted material. The absence of an aggregate peak is explained by the precipitation of 35% of the radioactivity during octamer reconstitution. Conditions as for fig.2. (●—●) A_{230}, (○—○) cpm.

umn with the aggregate and dimer fractions. To investigate whether it is the size of the substituting group on the cysteine that prevents octamer reconstitution, reconstitution was attempted with histone H3 labelled with the smaller radioactive iodoacetamide moiety. It was found that no octamers were reconstituted after such treatment (fig.3). We conclude that the substitution of cysteine in histone H3 with a bulky as well as a small residue prevents normal histone-histone interactions necessary for octamer formation in 2 M NaCl. However, if the fluorescent label on histone H3 is attached to other residues, octamer formation is not disturbed. This is shown by the ability of histone H3, labelled with IAEDANS under conditions preventing substitution of the sulphydryl group (fig.4), to form octamers (fig.5).

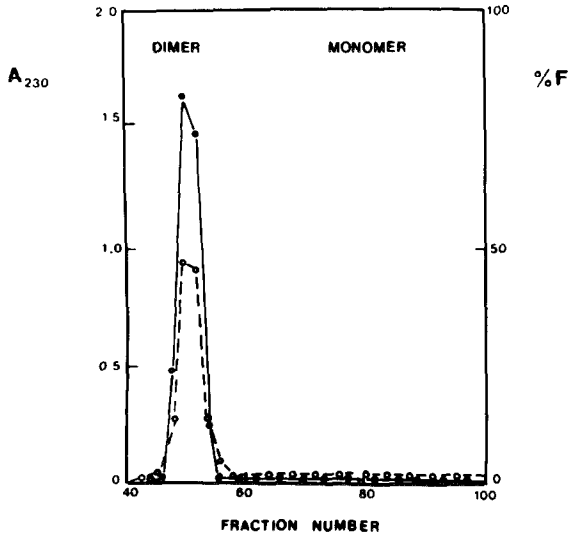

Fig.4. Sephadex G-100 chromatography of IAEDANS-labelled H3 dimer, (H3-H3-(IAEDANS)). (●—●) A_{230}, (○—○) fluorescence.

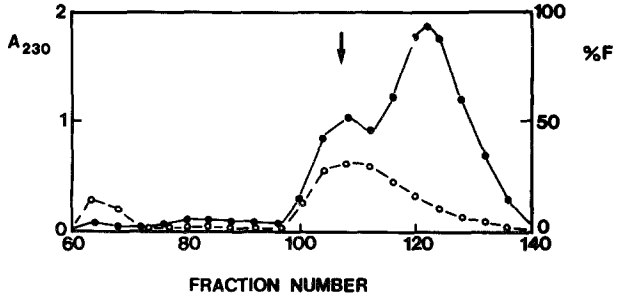

Fig.5. Reconstitution of histone octamers from H3-(IAEDANS) H2A, H2B and H4. The H3-(IAEDANS) was produced from the labelled dimer H3-H3-(IAEDANS) (fig.4) via reduction with 2-mercaptoethanol. Conditions and symbols as for fig.2.

Fluorescent nucleosome formation has been reported [4] in *Physarum* after uptake of histones fluorescently labelled with a 10-fold molar excess of dye. These labelled core particles may have arisen either because the in vivo pathway of nucleosome formation is less stringent and will accept a histone H3 substituted at Cys 110 or through labelling at sites other than Cys 110 especially at dye:SH ratios greater than unity. To test the former hypothesis, polyglutamic acid-mediated assembly of core particles on long DNA was attempted using an equimolar mixture of H2A, H2B, H4 and H3-(Cys-IAEDANS). Micrococcal nuclease digestion of the assembly mixture indeed revealed that approx. 145 bp DNA were protected from digestion after assembly (fig.6). We have not yet established the nature of the histone complex associated with this DNA.

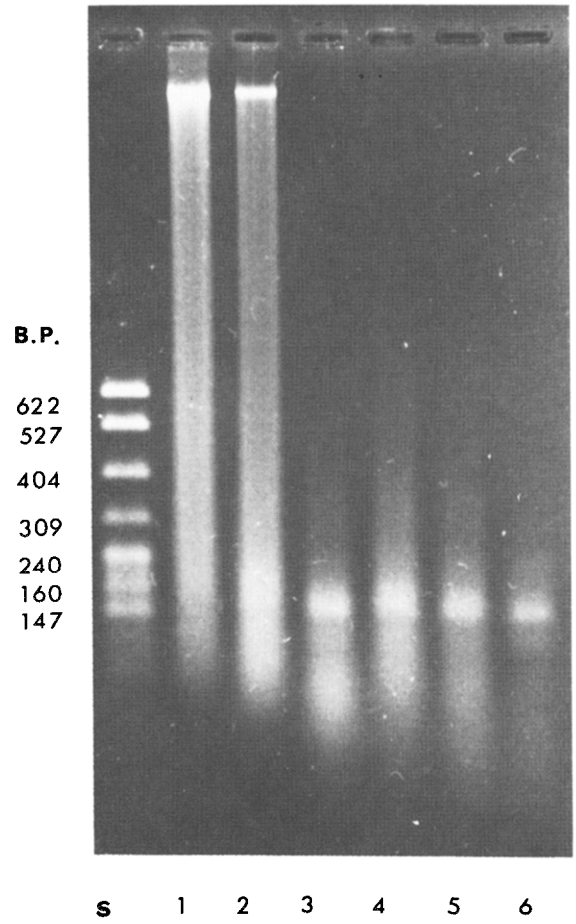

Fig.6. Agarose electrophoresis of micrococcal nuclease digestion of the product of polyglutamic acid-mediated assembly of equimolar amounts of H2A, H2B, H4 and H3-(Cys-IAEDANS) on excess long DNA in 0.2 M NaCl. The standard shown (S) is an *HpA*II digest of pBR322. Aliquots were taken immediately after digestion commenced and thereafter at 30, 60, 90, 120 and 300 (lanes 1–6).

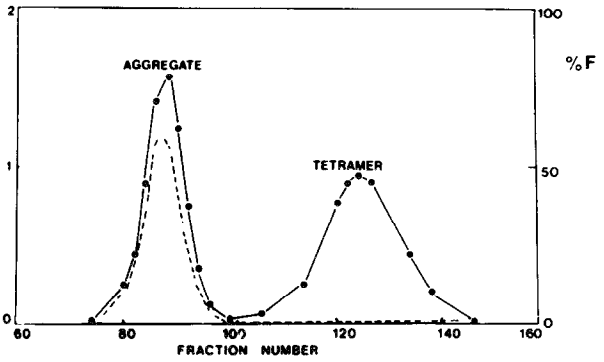

Fig.7. Sephadex G-100 chromatography of the products of reconstitution after urea treatment of H4 and H3 partially labelled with IAEDANS. Label:SH ratio = 0.5:1. Eluant: 2 M NaCl, 0.1 M phosphate (pH 7). (●—●) A_{230}, (○—○) fluorescence.

The prevention of octamer formation in 2 M NaCl after Cys 110 substitution is probably due to inability of the substituted histone to form the correct H3-H4 tetramer. This is confirmed by the lack of fluorescent tetramer formation when labelled H3 and unlabelled H4 were subjected to the reconstitution procedure described by Beaudette et al. [12] after having been unfolded at high urea concentration (fig.7).

4. DISCUSSION

The specific attachment of fluorescent probes has been used to investigate the structure of the nucleosome, the effect of the ionic environment on the latter and the pathways of its assembly [1–7, 15,16]. The DNase I susceptibility and sedimentation velocities ($s = 10.3$) on ultracentrifugation of such labelled nucleosomes have been shown to be similar to that of undissociated unlabelled material [2]. The size criterion, however, is not very discriminating in view of the finding that histones H3 and H4 alone can also yield particles of 9.1 S [17] and 9.8 S [18,19] associated with about 140 bp of DNA.

The finding reported here, that modification of the chicken H3 Cys 110 with either the dansyl or carboxymethyl group results in an inability of H3 to undergo complex formation in 2 M NaCl to either the natural histone octamer or histone H3-H4 tetramer, agrees well with the suggestion [5,7] that modification of Cys 110 in H3 destabilises the reassociated core. It appears that it is the inability to form a faithful H3-H4 tetramer once Cys 110 of H3 has been modified which prevents octamer formation (fig.7). This emphasises the role of the Cys 110 environment during this process. Non-specific labelling of histone H3 at sites other than Cys 110 allows the reconstitution of octamers carrying a probe and demonstrates the importance of using dye:SH ratios of unity or less in order to achieve specific attachment, since carboxymethylation is not a reaction specific to sulphydryl groups [20].

Polyglutamic acid mediates in vitro assembly of core particles at physiological ionic strength possibly by mimicking the action of assembly factors [21]. Since assembly of core-like particles occurred using Cys 110-labelled histone H3, DNA binding must overcome the unfavourable histone-histone interactions. Our results further indicate that the pathways of octamer formation in 2 M NaCl and core particle assembly in the presence of polyglutamic acid may be different with respect to the interference of substituted Cys 110 in histone H3.

ACKNOWLEDGEMENTS

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