PURIFICATION OF THE HISTONE H1 FROM THE FRUIT FLY *CERATITIS CAPITATA*

Isolation of a high mobility group (HMG) non-histone protein and aggregation of H1 through a disulphide bridge

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Received 14 April 1977

1. Introduction

Histone H1 has been isolated from several Diptera species either by methods involving perchloric acid extraction [1–3] or by preparative electrophoresis [4]. Dipteran H1 differs to some extent from the homologous mammalian histone in both electrophoretic mobility and amino acid composition [1–4]. Rodríguez-Alfageme et al. [4] first reported the occurrence of cysteine in H1 preparations from *Drosophila* embryos, and we also found low amounts of this amino acid in H1 obtained from *C. capitata* pharate adults by perchloric acid extraction [3]. Our preparation was, however, contaminated by another protein (see fig.1a) and it was not possible to decide by that time whether or not cysteine was actually present in H1. Present studies mainly show two facts:

(i) The contaminant protein, which has been isolated, has proved to be a non-histone chromosomal protein related to high mobility group (HMG) proteins, first studied in mammals by the Johns group [5–9].

(ii) Cysteine is actually present in H1 and it confers to the histone the ability to aggregate under oxidizing conditions.

2. Materials and methods

2.1. *Purification of H1 histone from* Ceratitis capitata

Crude H1 was obtained from pharate adults by the method of Butler and Johns [10], as previously described [3]. Crude H1, 100 mg loaded onto a carboxymethyl-cellulose column (18 × 1.5 cm) and processed according to Johns [11].

2.2. *Oxidation of H1 histone*

Oxidation of pure H1 histone was carried out essentially as described by Marzluff et al. [12]. Briefly, histone samples were dissolved (4–10 mg/ml) in 6.0 M guanidinium chloride, 0.3 M Tris, pH 8.3, and aerated at 37°C. Guanidinium chloride was removed by dialyzing against distilled water and the histone was recovered by adding 100% trichloroacetic acid to a final concentration of 18%. Separation of oxidation products was achieved by Bio-Gel P-100 chromatography, following the procedure of Sommer and Chalkley [13].

2.3. *Analytical methods*

Electrophoresis was performed in 20% polyacrylamide gels according to Johns [14]. In some instances, samples were also run in 15% polyacrylamide–SDS gels by the procedure of Laemmli [15] as modified by Chapman (personal communication). Amino acid analyses were carried out after acid hydrolysis in a Durrum D-500 autoanalyzer. Cysteine was determined in some samples as the carboxymethyl derivative, after treating the protein with iodoacetate [16].

3. Results

3.1. *Carboxymethyl-cellulose chromatography of crude H1*

Crude H1 was resolved into two main components,

CMC-1 and CMC-2, by carboxymethyl-cellulose chromatography. CMC-1 eluted before starting the NaCl gradient and it was identified as the minor component of crude H1 (fig.1b), which was previously referred to as 'fraction 7' [3]. The other main fraction, CMC-2, eluted later and it corresponded to the major component of the starting material (fig.1c). Its amino acid composition is given in table 1, and it will be further referred to as pure H1. Microheterogeneity of pure H1 could be demonstrated by the fact that two

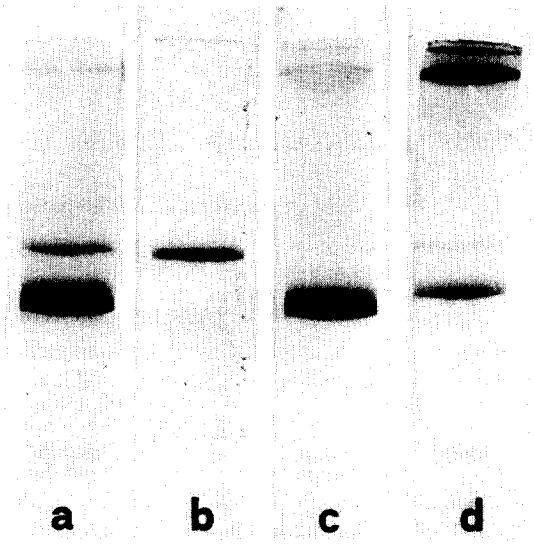

Fig.1. Electrophoretic analysis of fractions obtained by carboxymethyl-cellulose chromatography of crude H1. (a) Starting material. (b) Fraction CMC-1, identified as HMG non-histone protein. (c) Fraction CMC-2, identified as pure H1. (d) Oxidized pure H1. Migration is from top to bottom in 20% polyacrylamide gels [14].

Table 1
Amino acid composition of HMG non-histone chromosomal protein, histone H1 and histone H1 aggregation products from the insect *Ceratitis capitata*

Amino acid	Crude H1 (ref. [3]) (mol/100 mol)	Pure H1 CMC-2	HMG CMC-1	BG-1	BG-2	BG-3
Aspartic acid	5.2	4.7	17.2	4.7	4.4	4.6
Threonine	7.3	7.0	4.8	7.0	7.1	7.1
Serine	11.2	11.5	7.7	10.9	10.9	10.6
Glutamic acid	6.5	4.9	10.2	5.5	5.2	5.4
Proline	5.0	5.1	7.2	5.4	5.0	5.1
Glycine	7.6	7.5	11.4	7.9	7.2	7.1
Alanine	15.4	16.7	8.3	18.0	18.5	18.6
Cysteine	trace[a]	0.5[b]	0.0[b]	0.5[b]	0.4[b]	0.2[a]
Valine	5.7	5.2	2.3	6.5	5.7	5.6
Methionine	0.5	0.5	0.4	0.4	0.5	0.6
Isoleucine	2.3	2.8	1.8	3.0	3.2	3.5
Leucine	3.6	3.5	1.5	2.1	4.1	5.1
Tyrosine	1.3	1.7	0.8	1.3	1.7	1.8
Phenylalanine	0.8	0.8	trace	0.6	1.0	1.0
Lysine	23.0	25.1	17.6	24.4	24.0	21.5
Histidine	1.9	0.6	0.6	0.8	0.5	0.6
Arginine	2.1	1.7	8.2	1.3	1.1	1.5

[a]Determined as cysteic acid
[b]Determined as carboxymethyl cysteine

bands appeared when it was analysed by SDS–polyacrylamide gel electrophoresis. The molecular weights of these two H1 subfractions were estimated as 21 300 and 22 500, using calf thymus histone fractions as standards.

On the other hand, amino acid composition of CMC-1 (table 1) remarkably resembles those of HMG non-histone proteins [5–9]. A few minor components could also be obtained from the carboxymethylcellulose column with a low yield (less than 2% of the starting material on a basis of $A_{230\,nm}$) and they analysed like calf thymus HMG protein fractions formerly referred to as fractions D and E [8].

3.2. *Oxidative aggregation of histone H1*

The electrophoretic pattern of oxidized pure H1 is characterized by the appearance of slow-moving bands in addition to that of H1 (fig. 1d). When oxidation products were analysed by SDS–polyacrylamide gel electrophoresis (not shown), microheterogeneity of unaggregated H1 was still noticeable, and the relative intensity of subfraction bands was similar to that found in pure H1 preparations. Aggregation did not occur when aeration was carried out in the presence of 0.02 M dithiothreitol and the subsequent elimination of guanidinium chloride was accomplished by dialyzing against 0.02 M mercaptoethanol. Carboxymethylation of H1 also prevented its aggregation. These two findings suggest that aggregation might occur through intermolecular disulphide bridges.

In order to test this possibility, the aggregated forms of H1 and the remaining unaggregated H1 were separated by Bio-Gel chromatography, as shown in fig.2. Table 1 gives the amino acid composition of fractions BG-1, BG-2 and BG-3 from Bio-Gel chromatography. Fraction BG-3 was aerated as described under Materials and methods for 16 h, but no aggregation was detected, indicating that the remaining unoxidized H1 is actually unable to further aggregate. On the other hand, a sample of fraction BG-2 (4 mg) was dissolved in 1 ml 6.0 M guanidinium chloride, 0.3 M Tris, pH 8.3 and reduced with 0.02 M dithiothreitol. After eliminating guanidinium chloride by dialyzing against 0.02 M mercaptoethanol, recovered protein run as a single band in 20% polyacrylamide gels, its mobility being identical to that of pure H1.

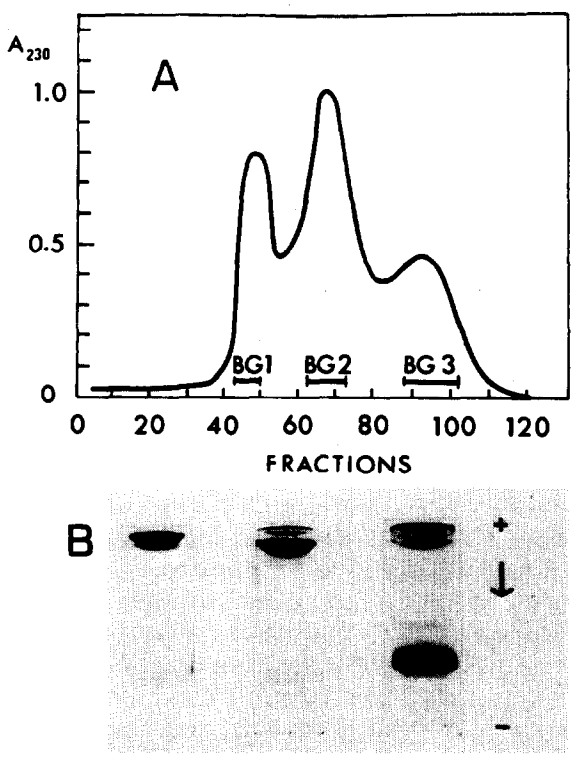

Fig.2. (A) Bio-Gel P-100 chromatography of oxidized pure H1. (B) Electrophoretic analysis of fractions BG-1 (left), BG-2 (middle) and BG-3 (right). The electrophoretic pattern of starting material is given in fig.1d. Migration is from top to bottom in 20% polyacrylamide gels [14].

4. Discussion

It appears from the evidence presented here that *Ceratitis capitata* H1 aggregation implies the formation of an intermolecular disulphide bridge. Actually, its prevention by carboxymethylation or by the use of reducing agents, as well as the disaggregation of fraction BG-2 after treating it with dithiothreitol strongly support the above assumption. This unique property of fly H1 seems to be interesting, although it is not possible to decide whether or not thiol-disulphide interconversion occur under in vivo conditions. If it were, aggregation of H1 might have some important biological role, as preliminary evidence shows that complexes between aggregated H1 and DNA melt some 10°C higher than those formed with whole unoxidized H1 [17].

Fraction BG-3 is unable to form disulphide bridges and, taking into account that both H1 subfractions can be oxidized to a similar extent, we can guess that ability to aggregate depends neither upon the sole presence of cysteine nor upon primary structure differences. A reasonable hypothesis might be that aggregation is controlled by covalent modification of some residue placed in the environment of the cysteinyl residue. The multiplicity of aggregated forms, evidenced by both gel electrophoresis and Bio-Gel chromatography, would then arise from the differences in molecular weights of H1 subfractions.

On the other hand, fraction CMC-1 appears to be an HMG non-histone chromosomal protein; its composition and electrophoretic mobility bear considerable resemblance, although not identity, with those of calf thymus HMG1 or HMG2 [8]. A similar protein has been reported to occur in *Drosophila,* although its amino acid analysis has not been given [18]. The identification of the other minor fractions obtained from the carboxymethyl-cellulose column remains unclear, and we have to emphasize that some of the former HMG fractions are, in fact, degradation products as it has been recently pointed out [19].

Acknowledgements

This work has been supported by a grant from the Comisión Asesora de Investigación Científica y Técnica (Spain). We are very indebted to professor A. M. Munico for constant advice and encouragement and we also thank Drs J. M. Fernández-Sousa and J. Perera for their valuable suggestions and Mr G. González de Buitrago for amino acid analysis.

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