SPECIFIC AGGREGATION PRODUCTS OF HISTONE FRACTIONS (PRESENCE OF CYSTEINE IN F2a1 FROM ECHINODERMS)

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1. Introduction

Frequently during the characterization of histones by gel electrophoresis, sharply defined slow moving bands are observed. These bands may correspond either to contaminating non-histone proteins or to specific aggregation products of histone fractions. In this paper some of these specific aggregation products are studied in order to obtain information on histone-histone interactions. It is found that cysteine bridges are involved in the formation of some of these aggregates, whereas in other cases specific hydrophobic interactions occur. At the same time some details of the chemical structure of the histones are elucidated. It is shown that fraction F2a1 in echinoderms contains cysteine as a constituent amino acid.

2. Materials and methods

Histone fractions were prepared as described in the literature [1–3]. Fraction F3 was further purified by dyalisis reprecipitation in ethanol. The histones obtained in this way were chemically modified by standard methods [4] when required.

The amino acid composition of the histones was determined in a Unichrom analyzer after acid hydrolysis. The results obtained coincided within experimental error with those previously published [1–3].

Electrophoresis was carried out according to the method of Chalkley and Panyim [5] slightly modified. A volume of 50 to 100 μl of sample (1 mg/ml) was used. In this way it was found that the echinoderm fractions could not be completely purified and were slightly cross-contaminated as shown in the figures presented below.

The samples to be used in electrophoresis were previously annealed at 37 °C for one hour or longer in one of the following solvents, at a concentration of 1 mg/ml:

–8 M urea, 4×10^{-3} M tris-HCl, pH = 8.8. This solvent was always freshly prepared before the experiments.

–10^{-2} M tris-HCl, pH = 9. Solid sucrose was added to this solvent before electrophoresis in order to make the solution 1 M in sucrose. Due to their high pH, these solvents will facilitate the air oxidation of cysteine. On the other hand, in some of the samples 10% mercaptoethanol was added before incubation in order to destroy disulfide bridges.

3. Results

3.1. *Fraction F3*

Electrophoresis of sea urchin F3 previously treated to induce disulfide bond formation yields a main aggregation band which corresponds to the dimer form, as shown in fig. 1. In the presence of mercaptoethanol all aggregates disappear as expected. When the cysteine residues are modified either by carboxymethylation or sulfitation, aggregation does not occur.

In the case of calf thymus, a similar result is obtained when aggregation is carried out in 8 M urea as shown in fig. 1. However, in the absence of urea further aggregation bands are apparent, most likely due to the formation of higher oligomers under these conditions. Either in the presence of mercaptoethanol or

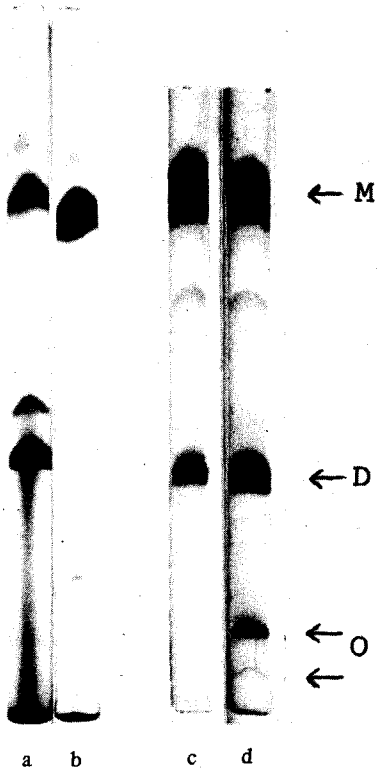

Fig. 1. Polyacrylamide gel electrophoresis of histone F3: (a) *Arbacia lixula* histone annealed for one hour before electrophoresis at 37° in 10^{-3} M tris-HCl, pH 9. The same pattern is obtained when the sample is annealed in the presence of urea; (b) ibidem, but annealed in the presence of 10% mercaptoethanol. A similar pattern is obtained with calf thymus under these conditions; (c) calf thymus histone annealed for one hour before electrophoresis at 37° in 8 M urea, 4.10^{-3} M tris-HCl, pH 8.8. The aggregation band contains several distinct subbands which indicate microheterogeneity in this histone; and (d), calf thymus histone annealed as in (a). M = monomer; D = dimer, O = oligomers (tetramer and hexamer?).

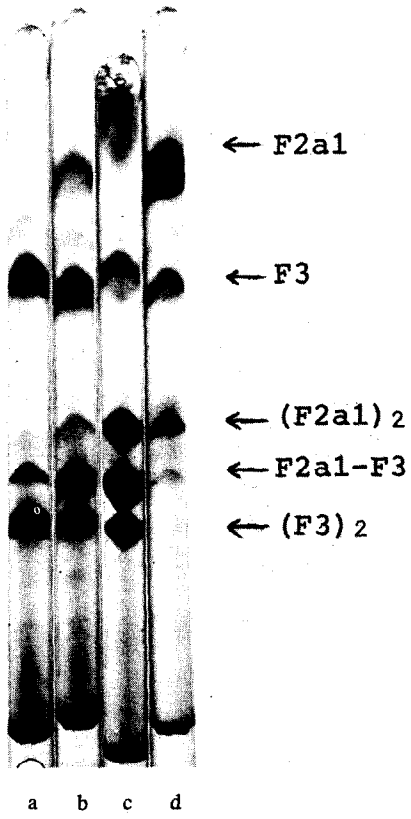

Fig. 2. Polyacrylamide gel electrophoresis of mixtures of histone fractions F2a1 and F3 from *A. lixula*. Before electrophoresis the mixtures were annealed for one day at room temperature in 8 M urea, 4.10^{-3} M tris-HCl, pH 8.8. The following ratios of F3:F2a1 were used: (a) 1:0; (b) 2:1; (c) 1:2; and (d) 0:1. From the electrophoretic pattern it can be seen that the purified fractions are slightly cross-contaminated.

when the cysteine residues are modified, no aggregation bands are observed. It is interesting to note that although oligomers are stabilized by hydrophobic interaction (as shown by their sensitivity to urea), they only appear after disulfide dimer formation (as shown by their sensitivity to mercaptoethanol).

3.2. *Fraction F2a1*

In calf thymus no specific aggregation product has been observed neither in F2a1 nor in F2a2, although after prolonged treatment the sample aggregates completely and does not penetrate the gel. Mixtures with sea urchin F3 do not show any mixed aggregates.

On the other hand, sea urchin F2a1 has a definite tendency to form dimers, as shown in fig. 2. Furthermore, when mixtures of F2a1 and F3 are annealed, mixed dimers appear. From the patterns obtained, also given in fig. 2, it is clear that F2a1 has a higher tendency to react with F3 than with itself! The disulfide nature of the bonds involved in the formation of dimers is demonstrated in fig. 3, where it is shown that all aggregates disappear in the presence of mercaptoethanol. This behaviour points out to the presence

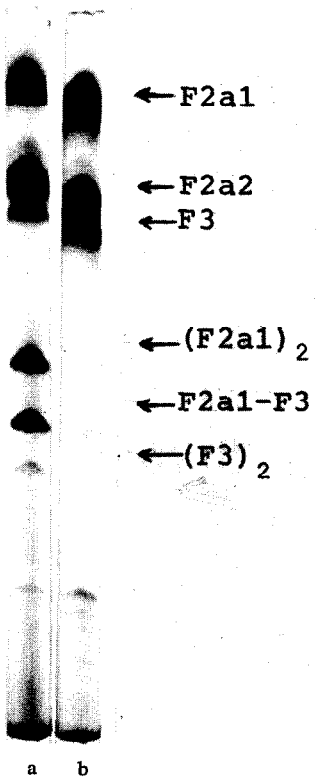

Fig. 3. Polyacrylamide gel electrophoresis of histone F2a from the sea urchin *Paracentrotus lividus*, contaminated with F3. Before electrophoresis the samples were annealed for one hour at 37° in 8 M urea, 4×10^{-3} M tris-HCl, pH 8.8; (a) in the absence of mercaptoethanol and (b) in the presence of 10% mercaptoethanol.

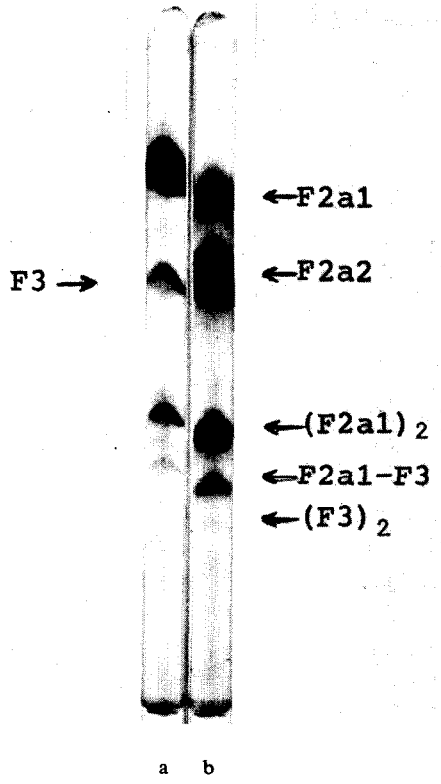

Fig. 4. Polyacrylamide gel electrophoresis of histones annealed for one day at room temperature in 8 M urea, 4×10^{-3} M tris-HCl, pH 8.8: (a) F2a1 from *A. lixula* sperm; and (b) F2a from the haemal system of sea cucumber *Holothuria tubulosa*.

of cysteine in this histone. This has been confirmed by amino acid analysis of the aminoethylated protein, which has been found to contain 1.1% of the aminoethylcysteine on a mole basis. A similar amount of cysteic acid is found in this protein after reaction with performic acid. The aminoethylated protein is unable to form aggregates.

It might be thought that the presence of cysteine is a peculiarity of sperm F2a1. However, as shown in fig. 4, a similar aggregation behaviour is found in fraction F2a from a somatic tissue of the echinoderm *Holothuria tubulosa*, prepared as described elsewhere [6]. Therefore it can be concluded that the presence of cysteine is a feature typical of this fraction in the echinoderms studied, both in somatic and germinative tissues.

In all cases, both with F3 and F2a1, aggregation was never complete even after a prolonged treatment of the protein. This result indicates that part of the histone is unable to form a dimer, perhaps due to the occurrence of naturally blocked cysteine groups or, more likely, to deterioration of some of the cysteine residues during the isolation and purification of histones.

4. Discussion

The experiments suggest that fraction F3 contains a single cysteine group capable of disulfide bond formation in the various organisms studied here. This cysteine group can form disulfide bridges which originate histone dimers. These bridges do not seem to

occur generally *in vivo*, since freshly isolated histones do not contain significant amounts of dimer, although they appear when the nuclei have been handled at a pH (e.g. pH = 8) which favours disulfide bond formation. As judged by their electrophoretic mobility and amino acid composition, histones F3 from calf thymus and sea urchin sperm are very similar [3]. However the slight differences which are present have a physicochemical significance, since the calf thymus histone is susceptible to form higher oligomers, whereas either sea urchin or pea F3 [7] only form dimers. Furthermore the calf thymus fraction shows significant microheterogeneity, which may be due to differences in amino acid sequence, modification of residues, or both. No microheterogeneity is detectable in the echinoderm histones studied.

One of the most interesting features of the experiments reported is the finding that histone F2a1 contains cysteine in echinoderms, although the overall amino acid composition is very similar to the analogous fraction from calf thymus [3]. The presence of this amino acid in this histone, known to have a remarkable evolutionary stability [8], indicates that in echinoderms either this fraction has a special biological function or the substitution of cysteine occurs in a region without a critical role.

Cysteine may occur in this histone due to a mutation which took place early in the evolution of echinoderms and was later conserved. However, since the echinoderms are a rather primitive zoological group, it is also possible that cysteine has been conserved from a more primitive ancestor. Cysteine would have been lost in more evolved organisms, such as calf and pea. If this was the case, one may than ask about the possibility that histones F3 and F2a1 have evolved from a common precursor, since both contain cysteine in echinoderms. As a matter of fact, the sequence results presently available [9] indicate that this is probable, since six amino acids coincide in the 19 amino acid terminal peptide. Furthermore significant sequence homologies occur in F3 peptide T(m)-Ia [9] and residues 13–19 or 41–45 in F2a1 [8]. The same happens with peptide T(m)-IIb1 and residues 26–34.

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