Purification of Histone F3 by Covalent Chromatography

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The arginine-rich histone F3 has been purified by covalent affinity chromatography. By the use of activated Thiol-Sepharose 4B for the purification of cysteine containing histone F3 a highly pure protein was obtained. The simple purification procedure offers the opportunity to get larger amounts of pure histone F3 within short time.

The histones are a set of five major basic proteins which are found in combination with DNA in the chromatin of higher organisms. It is widely assumed that histones are nonspecific gene repressors and play structural roles in the chromatin. Histones have been well characterized in terms of compositions and sequence [1–5]. Although each histone from a given species is readily distinguished from the other histones of that species, homologous histones from different species appear to be remarkably similar in composition and peptide maps [6–8]. For comparative studies of histones of different species rapid methods should be available to separate a specific histone from crude histone fractions. We now describe a procedure for a convenient specific isolation of the arginine-rich histone F3 of calf thymus.

Materials and Methods

Sephadex G25 and activated Thiol-Sepharose 4B were purchased from Pharmacia Fine Chemicals Co. Uppsala, Sweden.

2,2′-Dipyridyldisulphide (Aldrich), Tris, EDTA, NaCl, cysteine (Merck), urea (Serva).

Preparation of the activated Thiol-Sepharose 4B (Sepharose-(glutathione-2-pyridyldisulphide)) was done as described by Brocklehurst *et al.* [9]. The derivative contained 1 μmol activated thiol group per 1 ml swollen gel. After use the activated Thiol-Sepharose 4B was reactivated by reduction with 20 mM dithiothreitol followed by reaction with 2,2′-dipyridyldisulphide [9].

Calf thymus histone F3 was isolated according to Johns [10] method 1. Commercially available histone F3 was obtained from Sigma Company.

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The buffer used was Tris/HCl pH 7.2 (25 °C, $I = 0.1$) which was in 0.3 M NaCl, 6 M urea and 1 mM EDTA.

Electrophoresis of histone F3 was performed according to Panyim and Chalkey [11]. Staining was achieved with Amidoblack and destaining was with acetic acid (7%).

The nomenclature used was histone H2a = IIb1 = F2a2; histone H2b = IIb2 = F2b; histone H3 = III = F3; histone H4 = IV = F2a1.

All experimental procedures were done in the cold (4 °C).

Results and Discussion

The five major components of histones consist of a very lysine-rich component F1, two slightly lysine-rich components F2a2 and F2b and two arginine-rich components F3 and F2a1. The arginine-rich histones are distinguished by their content of glycine and cysteine [1, 7, 8]. The cysteine content of F3 is species specific [12–15]. Calf thymus histone F3 consists of a main fraction which contains two cysteine residues and a minor fraction which has only one cysteine residue [16, 17]. Plant histone F3 [12], fish histone (carp) F3 [18] and the histone F3 of some rodents (rats, mice) [15] contain only one cysteine residue.

Most of the isolation procedure published for single histone fractions yield histones which are more or less contaminated. The histones as isolated by the procedure of Johns [10] were often found to be cross-contaminated [19]. Marzluft *et al.* [16] reported that histone F3 isolated according to Johns contains histone F1 and F2A1. Our observations show that F3 preparations sometimes contains F2a2 and F2A1. Several procedures, including exclusion chromatography, are reported to purify single histone fractions. Preparation of pure single histone

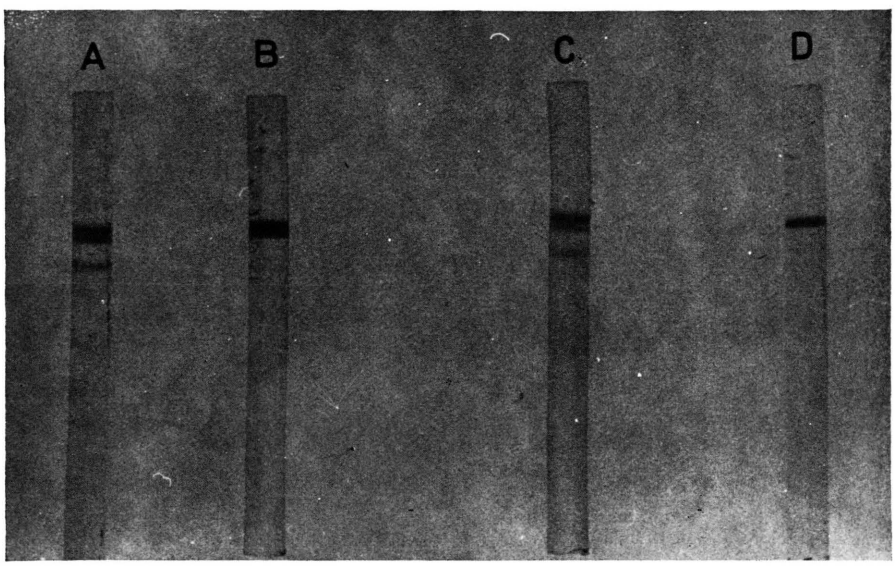

Fig. 1. Electrophoretic analyses of calf thymus histone F3 according to Panyim and Chalkey [11]. A, histone F3 isolated according to Johns [10]; B, histone F3 purified by covalent chromatography; C, histone F3 isolated according to Johns and reduced with mercaptoethanol; D, histone F3 purified by covalent chromatography and reduced with mercaptoethanol.

fractions on different Sephadex samples was reported by Starbuck *et al.*[20], and Marzluft *et al.*[16]. Bio-gel P10 columns were also used to purify single histone fractions[21, 22]. However it was reported that F3 was often contaminated with F2A2[19]. Another disadvantage is the use of long columns (3×320 cm for 1.6×350 cm) if histones are fractionated over Bio-Gel P10. Fambrough and Bonner[12] fractionated histones after column chromatography by electrophoresis.

F3 is the only histone which contains cysteine residues. This gives the opportunity to make use of affinity chromatography which allows the separation of proteins containing thiol groups from proteins lacking thiol groups. Several methods are available for separating thiol containing material from other material (for discussion of these methods see Brocklehurst *et al.*[9] an references therein). We used in our experiment activated Thiol-Sepharose 4B as described by Brocklehurst *et al.*[9] which is commercially available. Activated Thiol-Sephanose 4B is a mixed disulphide formed between 2,2′-dipyridyldisulphide and glutathione coupled tho CNBr-activated Sepharose 4B. Activated Thiol-Sepharose 4B reacts with solutes containing thiol groups with the formation of a mixed disulphide and the release of 2-thiopyridone. The solute is thus covalently linked to the stationary phase from which it can be eluted by the addition of reducing agents, *e. g.* cysteine.

Fresh prepared histone F3 (200 mg) isolated according to Johns[10] was dissolved in 15 ml 8 M urea. The sample was centrifuged to remove undissolved protein and applied to a column (1.5×30 cm) with 13 g activated Thiol-Sephanose 4B equilibrated with Tris HCl buffer ($I = 0.1$) pH 7.2 (25 °C) (0.3 M NaCl, 6 M urea, 1 mM EDTA). Elution with the equilibration buffer was achieved at a slow flowrate (15 ml/h). Ultraviolet absorption was detected at 343 nm where 2-thiopyridone is known to absorb. The presence of 2-thiopyridone indicates that thiol containing protein has reacted with the gel. This procedure was repeated twice with batches of crude histone F3 of 200 mg in 15 ml 8 M urea. After the third sample of crude histone F3 had passed, the column was washed another 3 hours with the equilibration buffer to remove the histones which do not contain thiol groups and which might have aggregated with histone F3. From the ultraviolet absorption of the 2-thiopyridone at 343 nm the amount of thiol group containing material is calculated taking an extinction coefficient of 7×10^3 for 2-thiopyridone[23]. The covalently bound histone F3 was removed from the gel with 30 mM cysteine dissolved in the elution buffer. Cysteine, 2-thiopyridone and the buffer material were removed by gel filtration on Sephadex G10 equilibrated in 0.1 M acetic acid. 2-Thiopyridone and the urea are well separated by gel filtration on Sephadex G10 whereas cysteine is not. It seems that cysteine is more strongly bound to the histone F3 than the other buffer material. Cysteine was removed by dialyzing the eluate from the G10 column against several changes 0.1 M acetic acid. The protein was lyophilized. The actual yield was 215 mg whereas the yield calculated by the 2-thiopyridone absorption at 343 nm was 235 mg.

The total capacity of the activated Thiol-Sepharose 4B is approx. 15 mg of SH-group containing protein per ml swollen gel. The histone F3 reacted with only 5 – 6 mg protein/ml swollen gel which indicates that some of the active groups are inaccessible for the SH-groups of the protein.

The pure histone F3 was analysed with polyacrylamide gel electrophoresis. Fig. 1 * shows the electrophoretic pattern of histone F3 as isolated by Johns and the sample purified by affinity chromatography. The F3 fraction as isolated by the method of Johns contains some material of the other histone fractions most probably histone F2a2 and F2A1. The two cysteine residues in calf thymus histone F3 can form intra- and intermolecular disulphide bridges[12, 15]. The purified sample has about 15 – 20% of intramolecularly oxidized histone F3 which moves faster than the reduced form and disappears on the addition of mercaptoethanol. Under the conditions of the isolation no dimers are formed by intermolecular disulphide bridges as the electrophoretic pattern shows. Purification of commercially available calf thymus histone F3 (Sigma) was also tried by the procedure described above. Only very small amounts reacted with the gel. This can be circumvented by activating the histone F3 fraction in 10 M urea with mercaptoethanol. The thiol-reagent is removed on Sephadex G10, equilibrated with deaerated buffer (Tris/HCl pH 7.2, 0.3 M NaCl, 6 M urea, 1 mM EDTA) and directly from this column applied to the activated Thiol-Sepharose 4B column which was equilibrated with the same

* Fig. 1 see Plate on page 72 b.

buffer. Elution of the protein was done with cysteine as described above. The activated Thiol-Sepharose 4B can be reactivated [9] and used for another purification procedure of histone F3.

Conclusions

The described purification procedure allows to isolate highly pure histone F3 in larger quantities and offers the opportunity for comparative studies of histone F3 preparations of different tissue and different species. The immobilized histone F3 might also be used in binding studies, affinity chromatography and especially in studies which envolve the interaction of histone F3 with the other histones and the interaction with DNA.

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