# INTERACTION OF RENATURED HISTONES f3 AND f2a1

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## SUMMARY

Ultracentrifuge measurements show that the individual histones f2a1 and f3 interact to form tetramers in solution. The conditions which facilitate renaturation are discussed.

Two recent papers (1,2) reported the existence of tetramers $(f2a1)_2(f3)_2$ in histone solutions prepared by the salt extraction procedures of van der Westhuyzen and von Holt (3).

In this paper we report that $(f2a1)_2(f3)_2$ tetramers may be formed from separate solutions of f2a1 and f3. The ability to form such tetramers has little or nothing to do with how the histones are prepared; it has to do only with how they are handled after preparation.

## MATERIALS AND METHODS

Histones f3 and f2a1 were purified by the method of Ruiz-Carrillo and Allfrey (4) and Mauritzen et al.(5). They were electrophoretically pure (6) (Fig. 1) and the amino acid analyses agreed with the known sequences (7-9). Prior to measurements histone f3 was reduced by the method of Ruiz-Carrillo and Allfrey (4) and chromatographed on G-25 equilibrated with 0.01 M HCl $1.0 \times 10^{-3}$ dithiothreitol to remove urea Tris. Histone f2a1 was simply dissolved in water. Concentrations of the histone stock solutions were determined from the absorbance at 275.5 nm (10,11). Molar extinction coefficients of $4.04 \times 10^3$ and $5.4 \times 10^3$ $cm^{-1}M^{-1}$ were used for histones f3 and f2a1.

The aggregation of histones requires salt (11-14). To avoid aggregation therefore it is important that histone complexes be prepared by mixing the histones before adding salt. To this end we first mixed equimolar solutions of f2a1 in

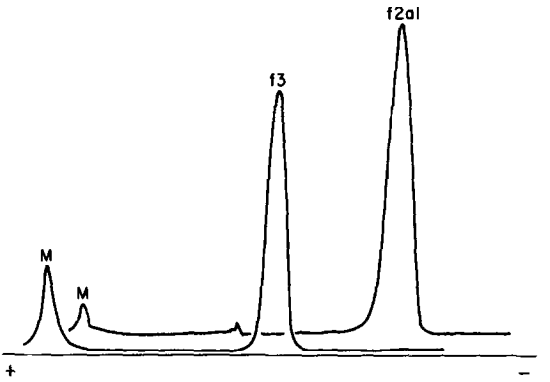


Figure 1. Densitometer patterns of the individual histones f3 and f2a1 used for the measurements; M refers to the marker protein, bovine serum albumin. The electrophoretic conditions are those of Panyim and Chalkley (6). The small irregularity in the f2a1 tracing is due to a scratch in the gel.

water, and reduced f3, and then added buffer. Solutions were dialyzed at 7° for at least 18 hrs against the solution buffer before centrifuging.

Sedimentation measurements were performed as described by Yphantis using a Beckman Model E ultracentrifuge (15). The data were analyzed with a program written by Dr. Robert Dyson. A value of $\bar{v}$ = .74 was calculated from the amino acid analyses (16).

## RESULTS

Sedimentation equilibrium measurements of 1:1 mixtures of f2a1 and f3, to which salt has been added, indicate that a tetramer is formed. Figure 2 gives data for mixtures in the acetate buffer system of Roark et al. (2) and data for the NaCl-phosphate buffer system of Kornberg and Thomas (1). Similar results are obtained at 9 mM and 12.5 mM phosphate, the solution conditions usedin other studies reported from this laboratory (11). $M_w$ is somewhat lower than the tetramer value of 53,200 calculated from the sequences (7-9). However, the tetramer is highly charged and Donnan effects would be expected to lower the apparent molecular weight (17).

Continuous variation measurements clearly show that histones f2a1 and f3 interact maximally at a molar ratio of 1:1 (18). Furthermore they permit an estimation of the uncomplexed histone that is in equilibrium with the complex. If one assumes a monomer-tetramer equilibrium, an association

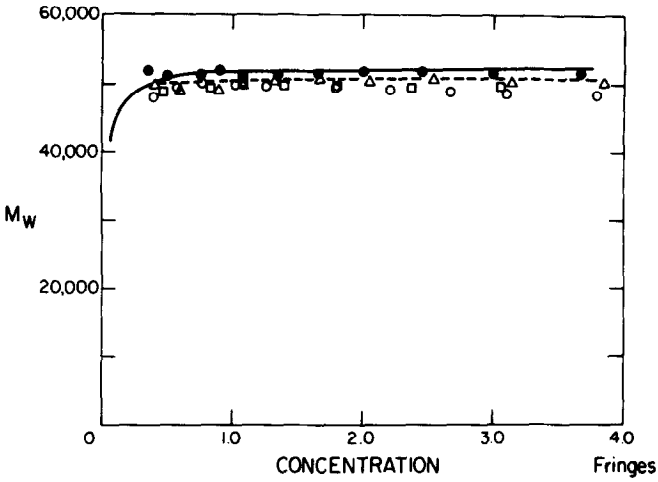


Figure 2. Weight average molecular weight of 1:1 f2a1, f3 as a function of histone concentration. Weight average molecular weight at 0.05 M NaOAc, 0.05 M $NaHSO_3$, 0.02% $NaN_3$, 3 x $10^{-4}$ M dithiothreitol, pH 5.0 and 20°: • 0.25 mg/ml, 34,000 rpm; x 0.55 mg/ml, 34,000 rpm; □ 0.25 mg/ml, 40,000 rpm. Weight average molecular weight at 0.025 M sodium phosphate, 0.15 M NaCl, 0.01 M $NaHSO_3$, 3 x $10^{-4}$ M dithiothreitol, pH 7.0 and 20°; o 0.55 mg/ml and 34,000 rpm. The solid line is the theoretical curve for a monomer-tetramer equilibrium with an association constant of 0.7 x $10^{21}$ $M^{-3}$ (18).

constant of 0.7 x $10^{21}$ $M^{-3}$ may be calculated (18). Using this value we show a theoretical curve of $M_w$ in Fig. 1 (solid line).

Roark et al. (1) used a two species plot to support a model of dimer-tetramer equilibrium. We may note, though, that the data of Roark et al. will also satisfy a monomer-tetramer two species plot, with perhaps even greater precision. However, the range of molecular weights covered in the two species plot was small and the amount of monomer and dimer must remain indeterminate at the present time.

We have found that f2a1 and f3 complex with histones other than each other (10,18) and such a competition would show up in a sedimentation run as an apparent weakening of the f2a1-f3 complex. Furthermore, the existence of non-interacting proteins would also show up as an apparent weakening. For these reasons it is important to use very pure histones to study complexing.

Our sedimentation results indicate a stronger $(f2a1)_2(f3)_2$ complex than

the results of Roark et al. However, although our histones were very pure (Fig. 1), we still cannot necessarily ascribe this discrepancy to differences in the purity of the samples. It might have a different origin.

We have observed that some sediment forms at the bottom of the centrifuge tube at the higher histone loading concentrations ($\geq$65 mg/ml), and the amount of sediment increases with histone concentration. However, in the solution, the histone shows no complexing greater than tetramer. Also, light scattering measurements at low concentration (0.25 mg/ml) show that there is no aggregation of the mixed histones after 24 hrs at room temperature (18). Histones f2a1 and f3 themselves do, of course, aggregate, and the kinetics and extent of the aggregation are highly dependent upon histone concentration, salt concentration and temperature (11-14,19).

## DISCUSSION

Denatured enzymes can be renatured (20) and DNA can be renatured (21). It should, therefore, not be too surprising that histones can be renatured. However, just as with enzymes and nucleic acids, the ability to do so depends on using appropriate solution conditions which, in turn, rests on a knowledge of the physical properties of the histones. The following considerations are critical 1.) In dilute histone solutions, upon the addition of salt, aggregation is orders of magnitude slower than the histone folding (11-13). 2.) The number of histone molecules undergoing the fast change is the same if measured either by observing the CD of the peptide planes or the polarization of fluorescence of the tyrosines (11-13). This is precisely what would be expected if part of a histone molecule underwent a highly cooperative specific folding when salt was added. 3.) The folding is independent of histone concentration while the aggregation depends strongly on the concentration. 4.) Upon cross-complexing, aggregation is blocked (10,18). 5.) Aggregation is a sensitive function of temperature and lowering the temperature inhibits aggregation (11,14,19).

All of these features suggest that histone complexes are best formed

by working with dilute histone solutions at low temperature, adding salt only after the histones are mixed. In the present work we have found that the use of low temperatures was not necessary.

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