OSMOTIC PRESSURE STUDIES ON THE ASSOCIATION OF ACID-EXTRACTED "HISTONE IIb"

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1. Introduction

In recent years, although there have been a number of reports of the molecular weights of histones based on chemical analysis, there have been few molecular weight determinations by physical methods. The present preliminary report is concerned with osmotic pressure studies on acid-extracted "histone IIb" in solution in sodium chloride at various pH and in guanidinium chloride. These measurements show that "histone IIb" contains polypeptide chains of molecular weight 19 500 ± 500, that these chains associate to dimers, tetramers and to higher complexes and that these associated forms have a greater tendency to appear with increasing pH.

2. Materials and methods

"Histone IIb" was prepared from chicken erythrocytes by the acid-extraction procedure of Murray et al. [1]. This method has been shown to yield reproducible fractions, but has the disadvantage that the fraction called IIb is contaminated with about 5 to 10% of other histone fractions, called III and IV (ref. [1]). These latter fractions were removed by chromatography on Bio-Gel P-60 (100 to 200 mesh) and elution with 0.1 M NaCl at pH 2. Murray et al. [1] have also reported that this fraction IIb may be further separated into two components by chromatography on Bio-Gel P-60, on eluting with 0.01 N HCl. However, in our experience, this separation proved to be incomplete and could not be modified to give a complete separation on a practical scale.

The osmotic pressure (Π) measurements were made by means of a Mechrolab 503 osmometer at 25°C in 0.48 M NaCl with a buffer of constant ionic strength 0.02 (phosphate, acetate or glycine/NaCl depending upon the pH required). Solutions for osmometry were dialysed against a large excess of solvent for 24 hr. The osmometer was normally allowed to equilibrate with solvent for about 16 hr before use and was considered to be at equilibrium if it could reproduce a dialysate pressure to within ± 0.01 cm of that of the solvent. The actual procedures used were very similar to those described in recent publications [2, 3].

Protein concentrations (c in g dry protein/ml) after dialysis were determined by means of a biuret method [4] in which the optical density at 280 mμ was compared with a calibration line determined with solutions of known dry wt concentrations of protein.

3. Results and discussion

Some of the plots of Π/c against c are presented in the figures and reveal that the intercept on the Π/c axis, and so the limiting molecular weight, varies with pH (fig. 1). The curvature of each plot indicates that the apparent molecular weight of the protein varies with concentration. The contribution of Donnan effects to the observed osmotic pressure was calculated and found to be negligible, as would be expected at an ionic strength of 0.5. However the limiting slope at low c of the plots of Π/c against c at pH 5.4 and 7.5 indicate that there is a positive non-ideality term, which only appears at higher concentrations at pH 4.5

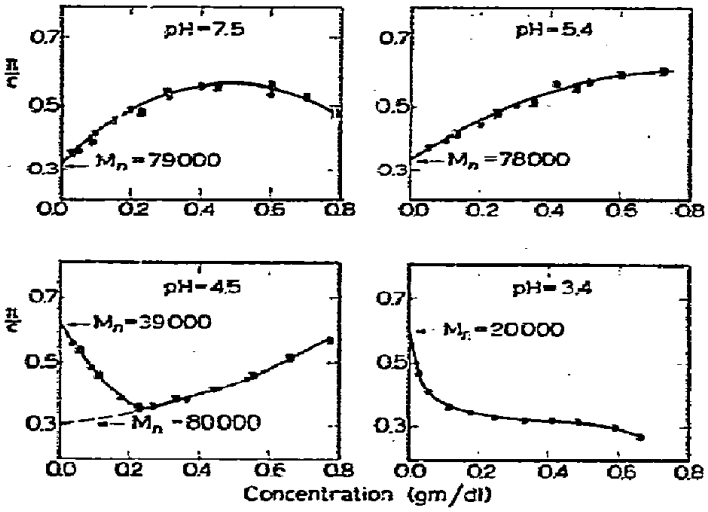

Fig. 1. Plots of π/c against c for "histone IIb" at various pH in 0.48 M NaCl and 0.2 M buffer at 25°C. Circles and squares represent distinct sets of measurements made on two different preparations of "histone IIb".

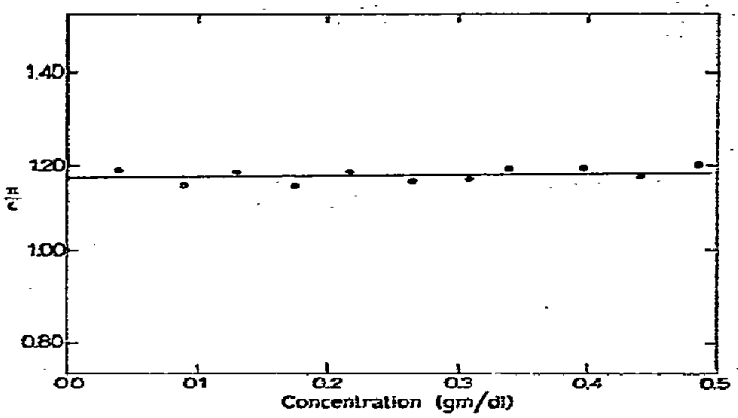

Fig. 2. Plot of π/c against c for "histone IIb" in 5.2 M guanidinium chloride at 25°C.

and not at all at pH 3.4 when other effects apparently predominate. Non-ideality must therefore be taken into account, as in sedimentation studies [5] on the histone fraction I (F1). At this stage, certain preliminary inferences about the association behaviour of "histone IIb" may be drawn from the plots, although complete analyses, such as those of Adams [6], will ultimately be necessary.

The intercept on the π/c axis shows that, at low concentrations at pH 7.5, histone IIb has a M_n = 79 000. As the concentration at first increases, the value of π/c rises, and this must be the contribution of non-ideality, but at higher c it falls and this can only be the result of association.

The plot of π/c against c for pH 5.4 is very similar in general form to that at pH 7.5 (and so are those for intermediate pH's, not shown here): the intercept corresponds to M_n = 78 000, but the curvature at high c is less and so the tendency to associate must also be less than at pH 5.4 than at 7.5.

The intercept on the π/c axis for pH 4.5 shows a limiting M_n of 39 000 at infinite dilution, but the initial slope under these circumstances is steeply negative which indicates that strong association is occurring at low c. The plot passes through a minimum with the expected positive slope appearing at high c when non-ideality effects must again be predominating. Extrapolation back to zero c of this rising part of the curve indicates that it could represent the π/c against c plot of a molecule of $M_n \approx 80\,000$, which is consistent with the predominance of a dimerisation of the unit of 39 000 mol. wt at low c.

The plot for pH 3.4 has an intercept corresponding to $M_n \doteq 20\,000$ and again initially a steep negative slope, which can only be the result of association apparently to a species of M_n = 40 000, which is the value of RTc/π at the inflexion point, although this curve clearly needs more detailed analysis. Beyond this inflexion point the slope again becomes more negative which suggests the onset of yet further association.

In order to determine the molecular weight of the smallest associating unit the osmotic pressure was measured in 5.2 M guanidinium hydrochloride (fig. 2). This shows directly that the smallest chains present have a molecular weight of 19 500 and, additionally, that the 5.2 M guanidinium chloride suppresses association and non-ideality effects.

The osmotic pressure plots therefore show that this "histone IIb" has a fundamental chain length corresponding to a molecular weight of 19 500 ± 500, which readily associates to dimers at pH 3.4, that these dimers associate to tetramers at pH 4.5, and that the tetramers themselves can form higher complexes at high concentrations at pH 5.4 to 7.5.

The histone fraction studied has been denoted as "IIb", according to the nomenclature of Rasmussen,

Murray and Luck [7]. Two subfractions, IIb_1 and IIb_2 have been distinguished [7] within "IIb"; these have been identified [8] respectively as fractions F2a2 and F2b in the nomenclature of Butler, Johns and Phillips [8] and it is only on these latter fractions so designated that end-group determinations have been reported by Butler et al. (table 6 of ref. [8]), when figures of 15–20 000 and 21–24 000, respectively, are quoted on the basis of C-terminal group analysis. Further studies and cross-comparisons will be needed, although it seems increasingly likely that the molecular weights of the unbroken chains of a number of histone fractions are around 20 000.

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