THE MOLECULAR WEIGHT OF NUCLEOSOME PROTEIN BY LASER LIGHT SCATTERING

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1. Introduction

The present experimental evidence on the structure of the nucleosome suggests that a 'core' containing two of each of the histone molecules H2A, H2B, H3 and H4 is surrounded by 180 to 200 base pairs of DNA [1–3]. It has been claimed from cross-linking evidence that in 2 M NaCl, at pH 9.0, an octameric nuclear core can be isolated in the absence of DNA from rat liver chromatin [4]. However, examination by hydrodynamic and formaldehyde cross-linking techniques [5] of the histone core from chicken erythrocyte chromatin, extracted by similar procedures but at pH 7.4, suggest it is isolated as two heterotypic tetramers of the four histones.

Light scattering is an exceptionally suitable technique for resolution of this type of discrepancy between different forms of experimental data. It yields an absolute, weight average molecular weight over a wide concentration range, and with the availability of laser light sources and modern filtration techniques, it can be employed on small amounts of material with no dust contamination. Consequently it has been used to resolve problems relating to the conformations adopted by multi-subunit proteins, particularly in solutions of high ionic strength where small molecule binding can complicate the measurement of other molecular parameters [6].

The experimental work described in this paper was undertaken to obtain a correct molecular weight for an equimolar mixture of the four histones in solution and to determine whether the tetrameric or octameric form was present in solution. By determining the molecular weight over a wide concentration range it was also possible to investigate the possibility that a concentration dependent association of the tetramer to the octamer could be occurring.

2. Materials and methods

2.1. *Isolation of histone core*

Nuclei from chicken erythrocytes were prepared according to Shaw et al. [7] and lysed into distilled water at 0°C. Nuclei from calf thymus were spun down from a filtered homogenate in 0.075 M NaCl, 0.024 M EDTA, 25 mM sodium bisulphite, pH 8.0, and washed five times before being lysed into distilled water. The chromatin gels were extracted three times with 0.6 M NaCl, 10 mM sodium bisulphite, 12.5 mM sodium borate, pH 9.0, to release H1 and H5 which were separated by centrifugation for 1 h at 30 000 rev/min in a Beckman T30 rotor. The core protein was then dissociated from the DNA by addition of solid NaCl to 2.0 M and separated from the DNA for 12 h at 50 000 rev/min in a Beckman T50 rotor.

Core protein preparations were routinely screened in the analytical ultracentrifuge using Schlieren optics and showed a single peak corrsponding to $s_{20,w}$ of 4.3 ± 0.3, and on acid–urea polyacryl-

amide gels [8] to demonstrate the stoichiometry of H2A, H2B, H3 and H4 and lack of contamination by H1. Concentrations were determined using $E^{1\%}$ at 275.5 nm = 3.69 [9,10].

2.2. *Light scattering measurements*

Light scattering experiments were performed as described before [11] using a helium–neon laser as light source. Samples were extensively dialysed to chemical equilibrium before measurement of the light scattering or the refractive increment.

The nature of the problem requires a detailed analysis of the possible errors in the parameters in the light scattering equations. The calibration figure has an error of ± 2%. Although the instrument cannot be calibrated at ionic strengths above 1 M due to the insolubility of the ludox colloidal silica used for this purpose, and some possible unforeseeable refraction changes could be invoked to change this figure by a small amount, the instrument has also been used to study DNA molecules at ionic strengths greater than 4.0 [12] when the molecular weights obtained were identical to those obtained at low ionic strength [11,13]. The refractive index increment of the sample was measured from 0.8–5.0 mg/ml and fitted using a least squares statistical programme. The value was 0.160 ± 2%. The only other possible error in the molecular weight determination lies in the extinction coefficient used to measure concentration, being about 2%, bringing the total error in measurement to 6%.

3. Results

Figure 1 shows the relationship between apparent weight average molecular and concentration for histones prepared from chick erythrocyte chromatin. The extrapolated molecular weight is 56 500. It can be seen that a slight molecular repulsion occurs so that the apparent molecular weight becomes slightly lower at high concentrations. This virial phenomenon is common in solutions of high ionic strength and reflects an affinity of the molecules for the solvent rather than for each other [6].

It has been suggested that a concentration dependent association of the histone molecules could occur in solution leading to an octameric structure at high concentrations and a tetrameric one at low concentrations. Figure 2 shows the molecular weight of calf thymus histones over a much wider concentration range. It can be seen that up to 5.2 mg/ml, there is no indication of any molecular association occurring and the apparent molecular weight again extrapolates to that of a tetramer at 60 000.

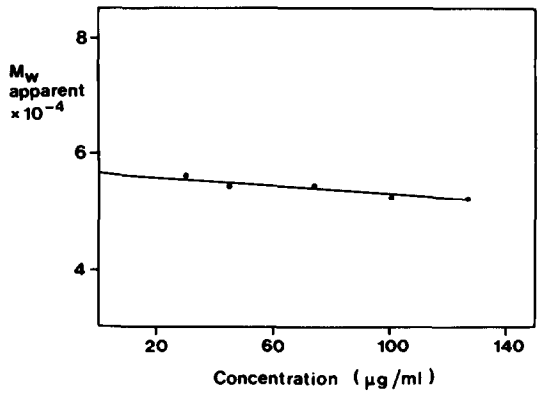

Fig.1. The relationship between apparent molecular weight and concentration for chick erythrocyte histones H2A, H2B, H3 and H4 in 2 M NaCl, 0.1 M CHES buffer pH 9. The apparent molecular weight plotted on the ordinate is the reciprocal of the well established light scattering equation $Kc/R\Theta$, where c represents concentration, $R\Theta$ the scattered light of solution over solvent at 90°, and K the optical constant. The molecular weight definition is 'apparent' because the true molecular weight is only obtained at the intercept on the ordinate where ideal conditions of infinite dilution apply.

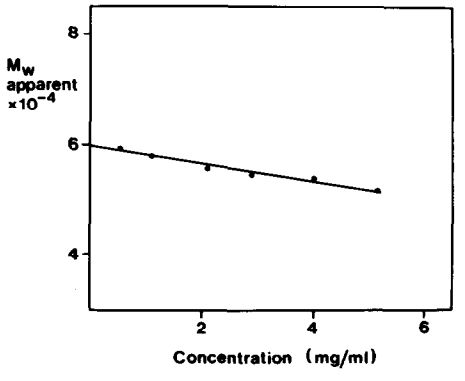

Fig.2. The relationship between apparent molecular weight and concentration for calf thymus histones H2A, H2B, H3 and H4 in 2 M NaCl, 0.1 M CHES buffer, pH 9.

4. Discussion

If the central core on a nucleosomal particle does indeed consist of eight histone molecules comprising a molecular weight of about 120 000 then it is logical to suppose that the component histone molecules have a sufficiently high degree of affinity for each other to enable an octameric form to be detected in the absence of DNA under the appropriate solution conditions. It is also logical to anticipate that in solutions of high ionic strength where the protein molecules may be effectively dehydrated, conditions close to those present in the nucleosomal particle may be created. Thus it is reasonable to expect a realignment of affinities among histone molecules in high salt compared to that found among them at physiological ionic strengths. Nonetheless a simple environmental ionic change is very different from the extensive binding force of a tightly wound strand of DNA on the outside of a nucleosome. Consequently it also remains reasonable to anticipate that the octameric conformation of histones can only exist in combination with DNA. The data in this paper do not support the suggestion that a histone octamer is present in solutions of high ionic strength [4], and lend considerable weight to the evidence that a tetrameric form of the histones is present in solution [5]. Our data are not complicated by any possible anomalies that may arise in cross-linking experiments or by the measurement of parameters such as the partial specific volume which can lead to a possible high error factor in experiments with the analytical ultracentrifuge. Within the range 0.3–5.2 mg/ml the histones from two different sources behave as tetramers. Association to octamers at concentrations above this level, or dissociation to smaller oligomers at concentrations below this level cannot be ruled out but the former would correspond to a very weak association indeed.

The nature of the tetramer is not clearly revealed by simple measurement of molecular weight as the individual histones are close to each other in size. Thus it is possible that the well-established $(H3H4)_2$ tetramer and a $(H2AH2B)_2$ tetramer may coexist in solution. However the possibility that the tetramer is composed of one molecule each of the four histones remains a strong one as it would satisfy the symmetry conditions believed to be represented in the nucleosome.

The ability of the putative octamer of histones in the nucleosome to exist as two tetramers may have a functional significance by allowing the processes of transcription and replication to occur without extensive disruption of the chromatin subparticle [14].

5. Conclusion

The molecular weight of an equimolar mixture of the four histones extracted from the nucleosome has been shown to correspond to a tetrameric complex in 2 M NaCl. We conclude that the data obtained from analytical ultracentrifugation reflect the condition of the molecules in solution more accurately than those from cross-linking of protein subunits.

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