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First received September 22nd, 1969; revised manuscript received November 10th, 1969

J. Chromatog., 46 (1970) 317–320

CHROM. 4506

Behaviour of histones in exclusion chromatography and gel electrophoresis in relation to their molecular weights

The system of gel exclusion chromatography introduced by PORATH AND FLODIN[1] with dextran gels and later extended to polyacrylamide gels has been used with success in separating fractions of the histones[2–8]. As far as the authors are aware, however, none of these investigations was carried out to establish the molecular weights in the manner described by WHITAKER[9] and ANDREWS[10] for many well-characterised proteins. This study was therefore undertaken because knowledge of this parameter is essential information for understanding the structure of deoxyribonucleohistone.

Experimental

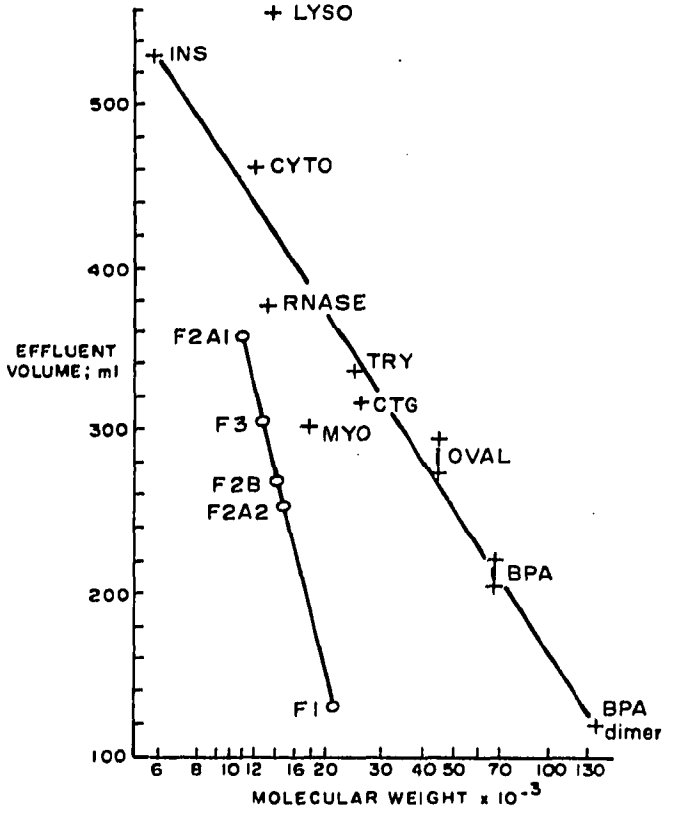
As standards, the following proteins were used (with their molecular weights given in parentheses): bovine serum albumin (67 000 and containing a little dimer); hen ovalbumin (45 000); bovine chymotrypsinogen (25 670) and trypsin (24 300); horse heart myoglobin (17 800); egg lysozyme (14 400); bovine pancreatic ribonuclease (13 700); horse heart cytochrome *c* (12 450); bovine insulin (5900) and salmine (about 4000). The following histone fractions were prepared from calf thymus (for nomenclatures see ref. 11): F1, (I); F2B, (IIb2); F2A2, (IIb1); F3, (III); and

F2A1, (IV), and the extra histone F5, (F2C) from chicken erythrocyte nuclei[12,13] kindly given by Dr. E. W. JOHNS.

The chromatography was carried out on columns (3 × 60 cm) of G-75 and G-100 dextran (Sephadex) and P-60 and P-100 polyacrylamide (Biogel) at room temperature and a flow rate of about 24 ml/h maintained by a peristaltic pump. Fractions of 3–4 ml were collected in weighed tubes and the fraction size checked by weighing every 5th or 10th tube. In any given run the weights agreed to within 0.3%. The sample, generally 1–3 mg, was dissolved in 2 or 3 ml of the solvent for application. The columns were run with 0.01 or 0.02 *N* HCl, and in some instances with 0.02 *N* HCl–0.1 *M* KCl. Acrylamide gel electrophoresis was carried out by the modification described for histones by JOHNS[14].

Results

The results of chromatography on Biogel P-100 are shown in Fig. 1 in the usual form of a semi-log plot. In 0.02 *N* HCl the results were very similar and those on Sephadex and on P-60 Biogel were also in general similar but showed poorer resolution



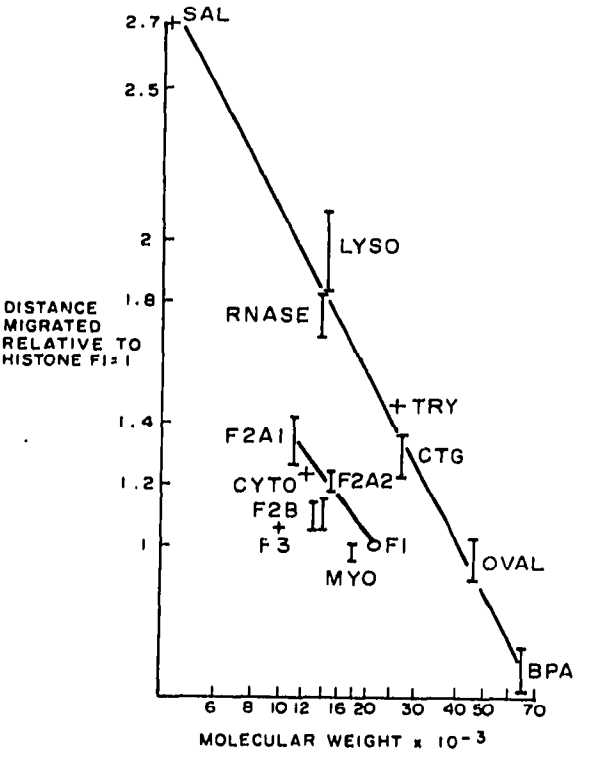


Fig. 1. Exclusion chromatography of standard proteins and histones on Biogel P-100 in 0.01 *N* HCl. Abbreviations: INS, insulin; LYSO, lysozyme; CYTO, cytochrome *c*; RNASE, ribonuclease; TRY, trypsin; CTG, chymotrypsinogen; OVAL, ovalbumin; BPA, bovine plasma albumin; MYO, myoglobin; F2A1, F3, F2B, F2A2 and F1, histone fractions. For the histone molecular weights (11 000–21 000) adopted here, see the text.

Fig. 2. Behaviour of histones and other proteins in acrylamide gel electrophoresis at pH 2.4. Abbreviations as in Fig. 1, and in addition: SAL, salmine. For the histone molecular weights (11 000–21 000) adopted here, see the text.

or a greater deviation of the standard proteins from the straight line plot. The effluent volumes of the proteins were all higher when run in 0.02 *N* HCl–0.1 *M* KCl solvent on Sephadex G-100.

A useful standard curve on P-100 was given by the following proteins: serum albumin dimer (a higher proportion of this peak could be produced by adding 10^{-3} *M* mercuric chloride to the albumin) and monomer, ovalbumin, chymotrypsinogen, trypsin, cytochrome *c* and insulin. On Sephadex G-100, serum albumin dimer was excluded, whereas cytochrome *c* and insulin were well off the line. On both columns ribonuclease and myoglobin were also well off the line under these acid conditions.

When related to these curves, the molecular weights of the histone fractions lay in the range 67 000–36 000 on Sephadex G-100 and from 125 000–22 000 on Biogel P-100, and the order of decreasing apparent weight was: F1, F5, F2A2, F2B, F3 and F2A1, though F2A2, F2B and F3 are not definitely distinguishable.

In acrylamide gel electrophoresis, the histones also migrate in a similar order, with F1 the slowest and F2A1 the fastest. They all migrate slowly, however, in comparison with several of the larger standard proteins such as ovalbumin, chymotrypsinogen or trypsin. Fig. 2 shows a semi-log plot of the molecular weight and the electrophoretic migrations, collected from many separate runs, in which all the distances are referred to histone F1 as standard.

Discussion

The histones behave anomalously in gel exclusion chromatography. None of the apparent molecular weights, ranging from 22 000 to 125 000, agree with the values from N- and C-terminal amino acid measurements[11,15], approximate though these are, or with the values from ultracentrifugal studies[16–18] or with those for fractions F2B and F2A1 now established by the amino acid sequences[19,20]. These last three methods of measurement put the whole group of histones within the range of 10 000 to 25 000 molecular weight, so that one limited result of the exclusion chromatographic studies is an indication of the relative sizes of the different fractions.

Taking the molecular weight of 21 000 as correct for histone F1 (I)[16], and 11 000 for F2A1 (IV)[19], the results here give values for F2A2, F2B and F3 in the range 15 000 to 13 000, and for fraction F5 (F2C), which was determined only on a G-100 column, a value of 18 000.

The interpretation of the observed behaviour of the histones on these columns can be considered under four headings.

Aggregation. Low ionic strength, acid conditions were chosen here to avoid the aggregation which is favoured by high salt and neutral or alkaline pH. A partial aggregation would give rise to chromatographic peaks of the monomeric forms eluting later from the columns, but this did not occur. Moreover, aggregate bands were not seen in acrylamide gel electrophoretic patterns of these histone fractions, although slow-running bands can be readily produced by prior treatment under known aggregating conditions.

Ionic interaction with the column material. Acidic group ionisation in Sephadex and also in Biogel owing to slight hydrolysis of amide groups would be strongly depressed by operation of the columns at pH near 2, as was done here. Moreover, the presence of acidic groups would lead to retardation of the basic histones, which is the opposite of the observed effect.

Hydrophobic interactions. Interactions of this sort may depend very much on amino acid sequence and conformation. However, the histones do not have a much lower content of hydrophobic amino acids, as defined by HATCH[21], than that of the standard proteins used.

Conformation. Owing to their high net positive charges and the rupture of the α-helical sections, the histones are largely unfolded under the acid conditions used. Prolyl residues produce permanent bends in polypeptide chains which would be important determinants of their behaviour in gel pores. It is noteworthy that the higher the proline content of the histone fraction, the more readily it is excluded from the gel. Thus the order of elution and the proline content (as moles %) are: F1 (9.2); F5 (6.7); F2A2–F2B–F3 (4.5–4.8–5.0) and F2A1 (1.0). No other amino acid (except to a less marked extent, alanine) shows any direct correlation with elution behaviour. Interference with the ability to penetrate the gel is also suggested in their electrophoretic mobilities. In spite of high net positive charges per unit weight, especially in fraction F1, they are retarded when compared with some larger proteins of lower net charge (Fig. 2).

This investigation has been supported by grants to the Chester Beatty Research Institute, Institute of Cancer Research, from the Medical Research Council and the British Empire Cancer Campaign for Research.

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Received November 10th, 1969