Journal of Chromatography, 77 (1973) 443–444

CHROM. 6497

Note

The estimation of molecular weights of basic proteins (histones) by gel filtration in 6 *M* guanidine hydrochloride solution

It has recently been found that the molecular weights of basic proteins determined by gel filtration according to WHITAKER[1] and ANDREWS[2] exceed by 2–5 fold those estimated by other methods. The differences were observed by PHILLIPS AND CLARKE[3] for histones and by DEIBLER *et al.*[4] for basic myelin proteins. The same has been found by us for viscotoxins—basic proteins from mistletoe (*Viscum album* L.) exhibiting cytotoxic properties[5]. This phenomenon may be due to aggregation of basic proteins and/or differences in conformation compared with that of globular proteins.

Reduction of disulphide bonds and a strongly denaturing medium prevent aggregation and disrupt the native structure of protein into true random coils. A concentrated solution of guanidine hydrochloride exhibits denaturing properties[6]. The high ionic strength of this solution also prevents electrostatic interaction between basic proteins and Sephadex.

The method for the determination of the molecular weights of reduced proteins and non-globular polypeptide subunits by gel filtration in 6 *M* guanidine hydrochloride solution containing 2-mercaptoethanol was described by DAVISON[7] and FISH *et al.*[8]. The estimation of the molecular weights of histones was carried out by a modification of this method. The following proteins were used as standards (the molecular weights are given in parentheses): chymotrypsinogen (25 000), myoglobin (17 800) and cytochrome C (12 400) from the "Non-Enzymatic Protein M.W. Markers" kit obtained from Mann Research Laboratories, U.S.A., and lysozyme (14 300), Grade I, obtained from Sigma Chemical Co., U.S.A.

The following fractions of calf thymus histones were used as basic protein standards: F1 (21 000–22 000)[9,10], F2A2 (15 000)[3], F3 (15 324)[11], F2B (13 774)[12], F2A1 (11 280)[13], and extra histone F2C from chicken erythrocyte nuclei (16 700)[10]. All of the histone fractions were kindly supplied by Dr. E. W. JOHNS of the Chester Beatty Research Institute, London Guanidine hydrochloride (Gu-HCl) was purified by the method of NOZAKI AND TANFORD[14].

The samples of standard proteins and histones (2.5 mg/ml) were reduced with 0.1 *M* dithiothreitol (Sigma) in 6 *M* Gu-HCl solution, according to KLAUS *et al.*[15], except that no buffer was used.

The chromatography was performed on a silicone-treated glass column (1 × 40 cm) with Sephadex G-75 dextran gel, equilibrated with 6 *M* Gu-HCl, at room temperature.

Usually 0.2 ml of the solution of reduced protein (*i.e.*, 0.5 mg) was applied on to the top of the column. The flow-rate of 6 *M* Gu-HCl solution of 4.5 ml/h was main-

tained by means of a peristaltic pump. Fractions of 1 ml were collected and the effluent volume was determined by gravimetry.

The standard proteins and histones were determined spectrophotometrically at 280 and 230 nm, respectively. A fraction of effluent, collected before the emergence of protein, was used as a reference.

The column void volume (V_0) was determined with Blue Dextran 2000 (Pharmacia). The elution volume (V_e) was determined, to the nearest 0.1 ml, from an elution diagram by extrapolating both sides of the solute peak to an apex[2].

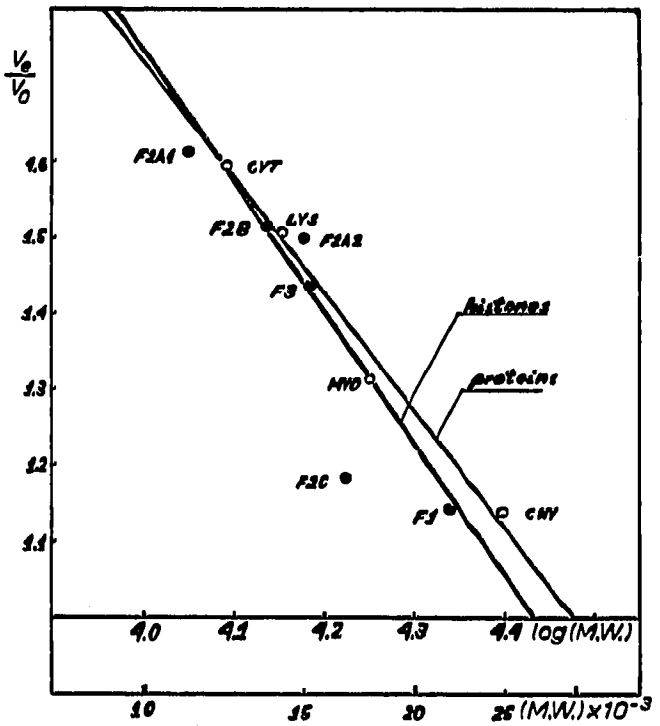

Fig. 1. Plot of relative elution volume against the logarithm of the molecular weight for standard proteins and histones.

Fig. 1 shows plots of the relative elution volume (V_e/V_0) against the logarithm of the molecular weight for standard proteins and histones, obtained under the described conditions.

The two curves are almost but not exactly coincident. The differences between the molecular weights for histones obtained from both calibrations range from zero for histone F2A1 to 7% for histone F1. This partial non-coincidence may be due, among other reasons, to the assumption that the molecular weights used for the histone fractions are correct, although exact molecular weights were calculated from the amino acid sequences only for F3, F2B and F2A1.

The V_e/V_0 value obtained for histone F2C is considerably less than that expected for the molecular weight of 16 700 given by JOHNS[10]. PHILLIPS AND CLARKE[3] and also DIGGLE AND PEACOCKE[16] reported molecular weights for the F2C fraction

of about 18 000 and 20 800 (as chloride), respectively. The molecular weights reported for other histone fractions by various workers are much less divergent. This was the reason why we did not use the V_e/V_0 value of the F2C fraction in calculating the calibration curve. A molecular weight of 21 100 (S.D. ±800) was estimated by us for the F2C fraction from the calibration curve for histones.

In spite of the above disadvantage, the technique described seems to be useful for the estimation of molecular weights of basic proteins.

Department of Drugs Technology and Biochemistry, Technical University, Gdańsk 6 (Poland)

J. M. WOYNAROWSKI
J. KONOPA

1 J. R. WHITAKER, *Anal. Chem.*, 35 (1963) 1950.
2 P. ANDREWS, *Biochem. J.*, 91 (1964) 222.
3 D. M. P. PHILLIPS AND M. CLARKE, *J. Chromatogr.*, 46 (1970) 320.
4 G. E. DEIBLER, R. E. MARTENSON AND M. W. KIES, *Biochim. Biophys. Acta*, 200 (1970) 342.
5 J. KONOPA AND R. RAJEWSKA, unpublished data.
6 C. TANFORD, *Advan. Protein Chem.*, 23 (1968) 121.
7 P. F. DAVISON, *Science*, 1961 (1968) 906.
8 W. W. FISH, K. G. MANN AND C. TANFORD, *J. Biol. Chem.*, 244 (1969) 4989.
9 D. C. TELLER, J. M. KINKADE AND R. D. COLE, *Biochem. Biophys. Res. Commun.*, 20 (1965) 739.
10 E. W. JOHNS, private communication.
11 R. J. DE LANGE, J. A. HOOPER AND E. L. SMITH, *Proc. Nat. Acad. Sci. U.S.*, 69 (1972) 882.
12 K. IWAI, K. ISHIKAVA AND M. HAYASAKI, *Nature (London)* 2226 (1970) 1056.
13 R. J. DE LANGE, D. M. FAMBROUGH, E. L. SMITH AND J. BONNER, *J. Biol. Chem.*, 243 (1968) 5906.
14 Y. NOZAKI AND C. TANFORD, *Methods Enzymol.*, 2 (1967) 715.
15 G. KLAUS, D. NITECKI AND J. GOODMAN, *Anal. Biochem.*, 45 (1972) 286.
16 J. H. DIGGLE AND A. R. PEACOCKE, *FEBS Lett.*, 18 (1971) 138.

First received September 11th, 1972; revised manuscript received November 16th, 1972