Anomalous Behaviour of Bovine Serum Albumin in Electrophoresis on Non-Denaturing Polyacrylamide Gel

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The most frequently used methods of molecular-weight determination are gel filtration by column chromatography (Andrews, 1965, 1970) and analytical ultracentrifugation (Archibald, 1947; Van Holde & Baldwin, 1958; Martin & Ames, 1961; Yphantis, 1964).

Shapiro *et al.* (1967) reported a method of determining molecular weights by electrophoresis on polyacrylamide gel of proteins treated with sodium dodecyl sulphate. The following year a new polyacrylamide-gel electrophoresis method was described that did not require denaturation of the proteins with sodium dodecyl sulphate and that used standard analytical apparatus and solutions (Hedrick & Smith, 1968). This system was based on previous work by Smithies (1962), Raymond & Nakamichi (1964), Tombs (1965) and Ingram *et al.* (1967), variously using starch and polyacrylamide gels. These authors found a reasonable correlation between molecular weight and gel 'limiting pore size'.

The method developed by Hedrick & Smith (1968) detects size, charge and molecular-weight differences between macromolecules. The system depends on running marker proteins of known molecular weight on gels of different acrylamide concentrations, and hence different porosities, and measuring their electrophoretic mobilities relative to a marker dye front. By plotting $\log(\text{relative mobility})$ against corresponding gel concentration a 'negative-slope' linear relationship may be obtained. A plot of negative-slope values of a range of markers against their respective molecular weights (determined by other methods) produces a calibration curve from which the molecular weight of a protein giving a known negative slope may be obtained. Hedrick & Smith (1968) showed that proteins of identical molecular size but different charge give parallel negative-slope plots, but proteins of different molecular size but similar charge give non-parallel plots intersecting at very low gel concentrations. Proteins of different size and charge produce non-parallel plots not necessarily intersecting at low gel concentrations.

Using this method Hedrick & Smith (1968) found that protein molecular weights between 50 000 and 500 000 could be determined at least as accurately as by any other method. Advantages are that proteins under examination are not chemically denatured, only

small samples are required and these need not be pure provided that their positions on the gel can be determined, and running times are conveniently short with conventional analytical polyacrylamide-gel systems.

This non-denaturing system has recently been used to determine the molecular weight of the 12S subunit of the coat protein of foot-and-mouth-disease virus by using bovine serum albumin and its polymers as molecular-weight markers (Talbot & Brown, 1972).

To make the method as simple as possible in operation a suitable marker protein system would afford savings in time, manipulations and cost. Bovine serum albumin may serve as such a system since it is possible to detect not only the monomer but also a number of its polymers depending on size of sample applied and the sensitivity of the protein stain. Under the conditions used in the present experiments polymers up to the septamer were occasionally, and up to the pentamer always, detectable, thus giving a molecular weight range 67 000–335 000. To check the validity of this albumin polymer system, proteins of known molecular weights were run on the same gel batches to provide a comparison. The proteins used were ovalbumin (mol.wt. 47 000; Tomimatsu, 1965), haemoglobin (mol.wt. 64 500; Hill *et al.*, 1962), bovine serum albumin (mol.wt. 67 000; Loeb & Scheragen, 1956), yeast alcohol dehydrogenase (mol.wt. 150 000; Hayes & Velick, 1954), bovine liver catalase (mol.wt. 240 000; Samejima & Yang, 1963), urease (mol.wt. 483 000; Sumner *et al.*, 1938) and ferritin (mol.wt. 560 000; Canovas & Stanier, 1967).

The polyacrylamide-gel system used is based on that recommended by Shandon Scientific Co. Ltd., London N.W.10, U.K. The components of the system were: (1) tris (36.3 g), 1 M-HCl (48 ml), *NNN'*-tetramethylethylenediamine (0.92 ml) and water to 100 ml; (2) acrylamide (30 g), methylenebisacrylamide (0.8 g), $K_3Fe(CN)_6$ (0.015 g) and water to 100 ml; (3) ammonium persulphate (0.28 g/100 ml of water); (4) water; reservoir buffer: glycine (28.8 g), tris (6 g) and water to 1000 ml.

Components (1)–(4) were mixed in the volume proportions 1:2:4:1 and the acrylamide concentration was lowered by diluting the acrylamide solution with water. A range of gel concentrations from 4 to 7.5% (w/v) could thus be covered, those above 7.5%

(w/v) being achieved by progressive substitution of acrylamide solution for component (4).

An analytical apparatus (Shandon Scientific Co. Ltd.) was used. Samples were mixed with an equal volume of 10% (w/v) sucrose in water containing Bromophenol Blue (0.001%, w/v), and 26 μ l of each mixture was applied to the gels. Samples were run into the gels at a constant current of 2mA/gel increased to 4mA/gel when the marker dye had penetrated several millimetres.

At the end of the run the gels were removed from their tubes, stained in a solution of 0.05% (w/v) Coomassie Brilliant Blue R in 7% (v/v) acetic acid for 1h and then de-stained in 7% (v/v) acetic acid alone. For haemoglobin and ferritin no staining was necessary. Mobilities of proteins relative to the dye front were measured with a clear plastic rule; positions could be determined quite readily to ± 0.5 mm. The experiment was repeated three times and the results were averaged (Table 1). The best-fitting line through the points, its negative slope and the correlation coefficient were determined for each protein by using a Diehl Sigmatron pre-programmed electronic calculator. As reported by Hedrick & Smith (1968), the deviation of markers from the best line was less than 5%. The results fell into two distinct groups, namely the albumin system and the other markers (Fig. 1). The results obtained by Hedrick & Smith (1968) in their study of albumin polymers with different gel concentrations also show this anomaly. They quote negative-slope values for monomer, dimer and trimer, but used only the monomer value as one of the many markers on their calibration curve. Their negative-slope values for the dimer and trimer (molecular weights 134000 and 201000) give mole-

cular-weight values of 205000 and 320000 when determined by using the calibration curve constructed with their other marker proteins. The values for the molecular weights of the dimer and trimer obtained in my experiments are 215000 and 320000. Hedrick & Smith (1968) also quote negative-slope values for dimer and trimer of ferritin that fall significantly below the best line through the protein marker points.

These results indicate that albumin polymers do not behave in direct accord with their molecular weights in this system but appear to be of greater size, possibly because of steric effects. Bovine serum albumin and its polymers would thus seem to be unsuitable for use as molecular-weight markers since they would be expected to give too low a molecular weight for an unknown protein. Talbot & Brown (1972) obtained, however, a molecular weight of 265000 for the 12S subunit of the coat protein of foot-and-mouth-disease virus when measured against their albumin calibration curve, and this agreed with the values obtained by other workers using different methods of molecular-weight determination. From the calibration curve in Fig. 1 a molecular weight of 265000 against albumin is 430000 against the other markers and 445000 when the calibration curve of Hedrick & Smith (1968) is used. The observation that other methods of molecular-weight determination yield values that agree with the value obtained by electrophoresis on non-denaturing polyacrylamide gel suggests that the 12S subunit of this virus coat protein is polymeric and that it behaves in the same anomalous way during electrophoresis as do albumin polymers. This conclusion about the polymeric nature of the 12S subunit of this virus coat protein agrees

Table 1. *Electrophoretic mobilities of different proteins in gels containing different concentrations of acrylamide*

Values for R_m , the mobility of a protein relative to the mobility of Bromophenol Blue, are expressed as the log function $100 \times \log(100 R_m)$. The values quoted are the means of three experiments.

Protein	Acrylamide in gel ...	100 $\times$ log(100 R_m)						Negative slope	Correlation coefficient
		4.0%	5.5%	7.0%	8.5%	10.0%	11.25%		
Ovalbumin	—	—	199	192	182	177	—	5.35	0.9960
Haemoglobin	—	178	169	159	149	133	—	6.35	0.9996
Alcohol dehydrogenase	—	177	163	149	133	—	—	9.73	0.9994
Catalase	173	155	140	120	101	—	—	11.93	0.9990
Urease	190	154	126	90	—	—	—	21.87	0.9988
Ferritin	187	159	121	85	54	—	—	23.43	0.9987
Bovine serum albumin									
Monomer	—	—	194	184	172	162	—	7.57	0.9990
Dimer	—	194	178	161	143	126	—	11.78	0.9990
Trimer	—	183	162	139	114	95	—	15.44	0.9996
Tetramer	—	173	144	116	86	63	—	19.18	0.9999
Pentamer	—	—	128	94	58	—	—	23.33	0.9999

Fig. 1. Molecular-weight calibration curves

Negative-slope values for a number of marker proteins (●) and of the albumin monomer and polymers (○) were determined by electrophoresis on polyacrylamide gels containing different concentrations of acrylamide as described in the text; these values are plotted against their respective molecular weights.

with the suggestion made by Talbot & Brown (1972) that it is a complex molecule containing three molecules of each of three different proteins whose aggregate molecular weight is 270000.

In the present case use of the albumin calibration curve for determination of the molecular weight of the 12S subunit of the coat protein of foot-and-mouth-disease virus gives the correct value. This would seem to be due to similar anomalous behaviour of the albumin polymers and the 12S subunit during electrophoresis on non-denaturing polyacrylamide

gel. It is also of interest, as noted above, that polymers of ferritin also behave anomalously (Hedrick & Smith, 1968), but in this case they behave as though they were molecules of lower molecular weight than expected.

It is clear that care is needed both in the choice of marker proteins for, and interpretation of results obtained from, molecular-weight determinations by electrophoresis on non-denaturing polyacrylamide gel.

- Andrews, P. (1965) *Biochem. J.* **96**, 595–606
 Andrews, P. (1970) *Methods Biochem. Anal.* **18**, 1–53
 Archibald, W. J. (1947) *J. Phys. Colloid. Chem.* **51**, 1204–1214
 Canovas, J. L. & Stanier, R. Y. (1967) *Eur. J. Biochem.* **1**, 289–300
 Hayes, J. E. & Velick, S. F. (1954) *J. Biol. Chem.* **207**, 225–244
 Hedrick, J. L. & Smith, A. J. (1968) *Arch. Biochem. Biophys.* **126**, 155–164
 Hill, R. J., Konigsberg, W., Guidotte, G. & Craig, L. C. (1962) *J. Biol. Chem.* **237**, 1549–1554
 Ingram, L., Tombs, M. P. & Hurst, A. (1967) *Anal. Biochem.* **20**, 24–29
 Loeb, G. I. & Scheragen, H. A. (1956) *J. Phys. Chem.* **60**, 1633–1644
 Martin, R. G. & Ames, B. N. (1961) *J. Biol. Chem.* **236**, 1372–1379
 Raymond, S. & Nakamichi, M. (1964) *Anal. Biochem.* **7**, 225–232
 Samejima, T. & Yang, J. T. (1963) *J. Biol. Chem.* **238**, 3256–3267
 Shapiro, A. L., Viñuela, E. & Maizel, J. V. (1967) *Biochem. Biophys. Res. Commun.* **28**, 815–820
 Smithies, O. (1962) *Arch. Biochem. Biophys.* **98**, Suppl. 1, 125–131
 Sumner, J. B., Gralén, N. & Eriksson-Quensel, I. (1938) *J. Biol. Chem.* **125**, 37–44
 Talbot, P. & Brown, F. (1972) *J. Gen. Virol.* **15**, 163–170
 Tombs, M. P. (1965) *Anal. Biochem.* **13**, 121–132
 Tomimatsu, Y. (1965) *Biochim. Biophys. Acta* **95**, 521–534
 Van Holde, K. E. & Baldwin, R. L. (1958) *J. Phys. Chem.* **62**, 734–743
 Yphantis, D. A. (1964) *Biochemistry* **3**, 297–317