REFERENCES

Allen, R. J. L. (1940). *Biochem. J.* **34**, 856.
Astbury, W. T. (1942). *J. chem. Soc.* p. 337.
Astbury, W. T. (1947). *J. Chim. Phys.* **44**, 3.
Astbury, W. T., Reed, R. & Spark, L. C. (1948). *Biochem. J.* **43**, 282.
Bailey, K. (1942*a*). *Trans. Faraday Soc.* **38**, 186.
Bailey, K. (1942*b*). *Biochem. J.* **36**, 121.
Bailey, K. (1944). *Advances in Protein Chemistry*, **1**, 289. New York: Academic Press.
Bailey, K. (1946*a*). *Nature, Lond.*, **157**, 368.
Bailey, K. (1946*b*). *Rep. Progr. Chem.* **43**, 280.
Bailey, K., Chibnall, A. C., Rees, M. W. & Williams, E. F. (1943). *Biochem. J.* **37**, 360.
Bailey, K., Gutfreund, H. & Ogston, A. G. (1948). *Biochem. J.* **43**, 279.
Bailey, K. & Perry, S. V. (1947*a*). *Biochem. J.* **41**, xxii.
Bailey, K. & Perry, S. V. (1947*b*). *Biochim. Biophys. Acta*, **1**, 506.
Balenović, K. & Straub, F. B. (1942). *Stud. Inst. Med. Chem. Univ. Szeged*, **2**, 17.
Bergmann, M. & Niemann, C. (1937–8). *J. biol. Chem.* **122**, 577.
Chibnall, A. C., Rees, M. W. & Williams, E. F. (1943). *Biochem. J.* **37**, 354.
Davidson, J. N. & Waymouth, C. (1944). *Biochem. J.* **38**, 39.
Dubuisson, M. & Hamoir, G. (1943). *Arch. int. Physiol.* **53**, 308.
Fearon, W. R. & Foster, D. L. (1922). *Biochem. J.* **16**, 564.
Foreman, F. W. (1914). *Biochem. J.* **8**, 463.
Gale, E. F. (1945). *Biochem. J.* **39**, 46.
Hall, C. E., Jakus, M. A. & Schmitt, F. O. (1946). *Biol. Bull. Woods Hole*, **90**, 32.
Lawrence, A. S. C., Miall, M., Needham, J. & Shen, S.-C. (1944). *J. gen. Physiol.* **27**, 233.
Liubimova, M. N. & Engelhardt, W. A. (1939). *Biochimia*, **4**, 716.
Lugg, J. W. H. (1937). *Biochem. J.* **31**, 1422.
Lugg, J. W. H. (1938). *Biochem. J.* **32**, 2114.
McMeekin, T. L. & Warner, R. C. (1942). *J. Amer. chem. Soc.* **64**, 2393.
Macpherson, H. T. (1946). *Biochem. J.* **40**, 470.
Mirsky, A. E. & Pauling, L. (1936). *Proc. nat. Acad. Sci., Wash.*, **22**, 439.
Nicolet, B. H. & Shinn, L. A. (1939). *J. Amer. chem. Soc.* **61**, 1615.
Palmer, J. W., Smyth, E. M. & Meyer, K. (1937). *J. biol. Chem.* **119**, 491.
Pirie, N. W. (1936). *Brit. J. exp. Path.* **17**, 269.
Rees, M. W. (1946). *Biochem. J.* **40**, 632.
Sanger, F. (1945). *Biochem. J.* **39**, 507.
Schneider, W. C. & Klug, H. L. (1946). *Cancer Res.* **6**, 691.
Smorodinzew, I. A., Schirokow, N. W. & Zyganowa, P. W. (1933). *Biochem. Z.* **262**, 425.
Straub, F. B. (1942). *Stud. Inst. med. Chem. Univ. Szeged*, **2**, 3.
Straub, F. B. (1943). *Stud. Inst. med. Chem. Univ. Szeged*, **3**, 33.
Tristram, G. R. (1946). *Biochem. J.* **40**, 721.
Van Slyke, D. D., Hiller, A. & Dillon, R. T. (1942). *J. biol. Chem.* **146**, 137.
Weber, H. H. & Stöver, R. (1933). *Biochem. Z.* **259**, 269.

Molecular Weight of Tropomyosin from Rabbit Muscle

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(*Received* 22 *January* 1948)

In the preceding paper (Bailey, 1948) the preparation and properties of tropomyosin are described. The present paper deals with the determination of its molecular weight by three methods: osmotic pressure, sedimentation-diffusion and amino-acid analysis. The partial specific volume and density of the dry protein have also been determined.

METHODS AND RESULTS

Tropomyosin samples. Two preparations have been examined: sample *A*, identical with that used for amino-acid analysis and shown to be electrophoretically homogeneous (Bailey, 1948), and sample *B*, for which there were only analytical criteria for purity.

Osmotic pressure. Measurements were carried out at 0° by the method of Adair (1925) in buffer of composition 0·2M-KCl, 0·0133M-Na_2HPO_4, 0·0267M-NaH_2PO_4, pH 6·5. Protein concentration was determined by the micro-Kjeldahl method, taking 16·7% as the N content of the protein.

Following the procedure of Adair & Robinson (1930), the ratio of osmotic pressure (cm. of water) to protein concentration (P/C) is plotted against C (Fig. 1); concentration is expressed as g./100 ml. solvent, and is obtained by employing the determined partial specific volume (see below) of 0·71. It will be seen that P/C varies considerably with C, whereas for some proteins (e.g. lactoglobulin, ovalbumin, serum albumin) the variation is small over a similar range of concentration, provided that C is expressed as g./100 ml. solvent. This anomaly is probably due either to a thermal interaction between the particles or to a statistical interaction due to their asymmetry. Extrapolation of P/C to zero concentration gives a value of 2·63, indicating a molecular weight of 88,000.

Sedimentation-diffusion data. The sedimentation constant was obtained by examination in the Svedberg oil-turbine ultracentrifuge at Oxford using the method of Philpot (1938). The concentrations of the protein were determined refractometrically, assuming a specific refractive increment of 0·00180; the solvent was as above, but contained NaCl instead of KCl. The speed was 1010 rev./sec.

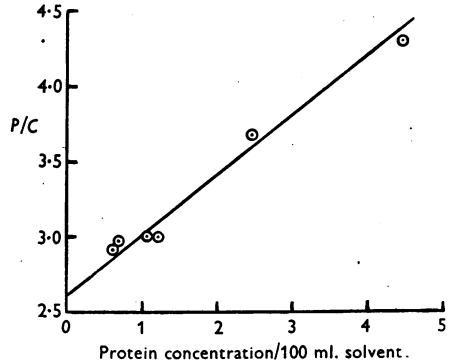

Fig. 1. Osmotic pressure/protein concentration (P/C) as a function of C (rabbit tropomyosin). P in cm. water; C in g./100 ml. of solvent. Buffer: 0·2 M-KCl, 0·0133 M-Na_2HPO_4, 0·0267 M-NaH_2PO_4, pH 6·5, 0°.

The diffusion constant was determined by two different methods. In method 1, measurements were carried out in a cell similar to that of Lamm & Polson (1936) and boundaries were observed by the Philpot (1938) optical system. The diagrams were enlarged photographically and the diffusion constant D calculated from the formula $D=\sigma^2/2t$, where σ represents half the distance between inflexion points, and t the time in sec. The position of inflexion points was located by dividing the maximal height of the curve by $\sqrt{e}$. Two runs were carried out, one at 0·7% protein concentration and one at 1·2%; the solvent was that of the osmotic pressure measurements. Four photographs of the boundary were made during each run, and mean values of D were derived from the four curves of each experiment. Method 2 is essentially new and is described elsewhere (Coulson, Cox, Ogston & Philpot, 1948).

The sedimentation diagram for sample A (Fig. 2) showed a single homogeneous component of s_{20} (corr.) $2{\cdot}60\times10^{-13}$. Integration of the areas of the boundary in the diagram gave 103% recovery of the refractive increment (Philpot, 1939; Johnston & Ogston, 1946), showing that this boundary includes the whole of the sedimenting material. Sample B was likewise homogeneous.

The several values of s and D corrected to 20° and to a water basis are given in Table 1; molecular weights were calculated by the usual formula $M=\frac{RTs}{D(1-\bar{V}\rho)}$ using a value of 0·71 for the partial specific volume ($\bar{V}$). The mean value from these data is 92,700, somewhat higher than that by osmotic pressure. It should be noted, however, that there is a marked variation of D (and probably also of s) with concentration; strictly, the molecular weight should be estimated from the extrapolated values at zero concentration.

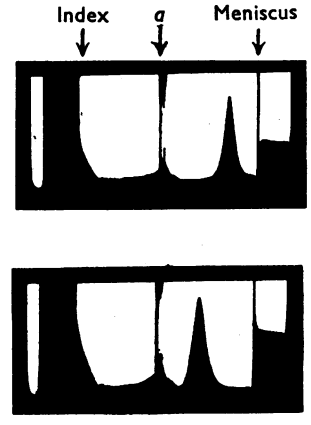

Fig. 2. Sedimentation diagrams of rabbit tropomyosin, 0·6%, 35 and 65 min. after reaching full speed. The shadow marked a is due to aberrant cell washer, which did not interfere with the sedimentation process.

Table 1. *Molecular weight of rabbit tropomyosin from sedimentation-diffusion data in solutions* $\mu=0{\cdot}267$

Sample*	Protein concentration (g./100 ml.)	s_{20} (corr.) $\times10^{13}$	D_{20} (corr.) $\times10^{7}$	Mol. wt.
A	1·20	—	1·78	—
	0·70	—	2·32	—
	0·60	2·60	(2·43)†	89,500
B	0·665	2·51	2·22	94,500
	0·635	(2·55)‡	2·26	94,300
				Mean **92,700**

* D for sample A by method 1; sample B, method 2 (see text).

† By extrapolation of values for 1·2 and 0·7% protein concentration to 0·6%.

‡ By interpolation of values for 0·60 and 0·665% protein concentration.

Molecular weight from the histidine content. Since histidine may be determined accurately (Macpherson, 1946), and since the amount in tropomyosin (0·85 g./100 g. protein) is sufficiently small, the minimal molecular weight multiplied by some small whole number should lead to a reliable value for the true figure. The minimal molecular weight thus obtained (18,180), multiplied by factors of 4, 5 and 6, gives the values 72,700, 90,900 and 109,100 respectively. Of these, only the middle value approaches those found by other methods.

Mean molecular weight. The mean molecular weight, derived from osmotic pressure, sedimentation-diffusion (mean value) and analysis, is 90,500. Using this value and an average diffusion constant of $2{\cdot}35\times10^{-7}$ (probable value at 0·6% protein concentration), D_0, the diffusion constant of a spherical

molecule of similar molecular weight, is found to be $7{\cdot}2 \times 10^{-7}$, giving a frictional ratio $D_0/D = f/f_0 = 3{\cdot}1$. This value, as far as can be ascertained, is much greater than any recorded for a protein of comparable molecular weight, though ideally it should be calculated from the value of D at zero concentration. As in the case of other proteins, the calculation of asymmetry from the frictional ratio is complicated by the uncertain magnitude of the water of hydration. Taken in conjunction with physical and X-ray evidence (Bailey, 1948; Astbury, Reed & Spark, 1948), the high value of f/f_0 indicates in a qualitative manner the pronounced asymmetry of the molecule.

Partial specific volume and density of dry protein. Determinations of partial specific volume ($\bar{V}$) have been made both in water and in salt solutions, using 25 ml. density bottles equilibrated at 20·8°±0·1. Corrections were applied for buoyancy and for the small amount of ash in the protein. In salt-free medium the viscosity is so high that it was necessary to evolve a special technique: the isoelectric protein (dried in ethanol, ether and *in vacuo*) was weighed into the bottle and a calculated volume of N/70 NaOH added to give a final pH of 6·5. Within 24 hr. the protein had swollen to a viscous sol from which air bubbles were removed by light centrifuging. Distilled water was now added to capacity and the stopper inserted. Since there was no admixture of protein with the upper water layer, the loss of liquid in this latter operation does not incur loss of protein. A correction was applied for the contribution of Na ions to the density of the medium.

In salt solutions an accurate salt concentration was obtained by adding either NaCl or K_2SO_4 to a dialyzed sol and diluting to 100·0 ml. Samples were then transferred to the density bottle. Protein concentration was determined both by dry weight and by N content, and experiments in which values disagreed by more than 1% were discarded. All samples were measured by weight and not by volume.

The mean value of $\bar{V}$ (Table 2) is 0·71; that calculated by summation of amino-acid residues listed in the previous paper (Bailey, 1948) is 0·735. Because of this discrepancy the details for the determination of $\bar{V}$ have been given at length. It seems clear that the assumption of $\bar{V}$ in the calculation of molecular weights may on occasion give rise to serious error, though it is true that in other cases the calculated values of $\bar{V}$ agree well with the observed (Cohn & Edsall, 1943). It is possible that the discrepancy is confined to proteins with large amounts of acid and base groups, and due either to an intense electrostriction of the molecule itself, or, more probably, to electrostriction of the water of hydration.

Table 2. *Partial specific volume* ($\bar{V}$) *and density* (ρ) *of dry tropomyosin*

Medium	$\bar{V}$	ρ
Water	0·708	—
Water	0·703	—
NaCl (1·0 M)	0·704	—
K_2SO_4 (0·064 M)	0·715	—
Paraffin	—	1·276
Paraffin	—	1·278
Xylene	—	1·280
Mean	**0·71**	**1·28**

The density of tropomyosin after drying at 100° *in vacuo* over P_2O_5 is given in Table 2. In these experiments, measurements were carried out using paraffin or xylene as displacing medium, removing entrapped air bubbles from the protein by evacuating after immersion of protein in the medium. It will be noted that there is a very large discrepancy between the 'apparent density' of the protein in solution ($1/0{\cdot}71 = 1{\cdot}41$) and the determined value for the dry protein (1·28). Similar discrepancies, though not so large, exist in other proteins; the density of dry lactoglobulin (McMeekin & Warner, 1942) is 1·26 and the 'apparent density' from the value of $\bar{V} = 0{\cdot}754$ (Pedersen, 1936) is 1·33.

SUMMARY

1. Tropomyosin from rabbit skeletal muscle is entirely homogeneous in the ultracentrifuge.

2. The molecular weight in salt solutions ($\mu = 0{\cdot}267$) is found to be 88,000 by osmotic pressure and 92,700 by sedimentation-diffusion; the value derived from the histidine content is 90,900 and the mean of all values, 90,500.

3. The osmotic pressure and diffusion constant (and thus probably the sedimentation constant) are markedly dependent upon protein concentration. At the lowest concentration investigated (0·6%), the frictional ratio is 3·1, indicating a very asymmetric molecule.

4. The partial specific volume (0·71) is lower than that calculated from amino-acid residues (0·735).

REFERENCES

Adair, G. S. (1925). *Proc. Roy. Soc.* A, **108**, 627.
Adair, G. S. & Robinson, M. E. (1930). *Biochem. J.* **24**, 1864.
Astbury, W. T., Reed, R. & Spark, L. C. (1948). *Biochem. J.* **43**, 282.
Bailey, K. (1948). *Biochem. J.* **43**, 271.
Cohn, E. J. & Edsall, J. T. (1943). *Proteins, Amino Acids and Peptides.* New York: Reinhold Publishing Company.
Coulson, C. A., Cox, J. T., Ogston, A. G. & Philpot, J. St L. (1948). *Proc. Roy. Soc.* A, **192**, 382.
Johnston, J. P. & Ogston, A. G. (1946). *Trans. Faraday Soc.* **42**, 789.
Lamm, O. & Polson, A. (1936). *Biochem. J.* **30**, 528.
McMeekin, T. L. & Warner, R. C. (1942). *J. Amer. chem. Soc.* **64**, 2393.
Macpherson, H. T. (1946). *Biochem. J.* **40**, 470.
Pedersen, K. O. (1936). *Biochem. J.* **30**, 961.
Philpot, J. St L. (1938). *Nature, Lond.*, **141**, 283.
Philpot, J. St L. (1939). *Biochem. J.* **33**, 1707.