THE PARTIAL SPECIFIC VOLUME OF MUSCLE PROTEINS*

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SUMMARY

The partial specific volume, $\bar{v}$, of the muscle proteins: rabbit myosin, rabbit tropomyosin and *Pinna nobilis* tropomyosin has been explored by the pycnometric method as a function of solvent composition and temperature. The values determined in neutral salt solutions fit the general pattern of agreement noted by McMEEKIN AND MARSHALL between observed and calculated partial specific volumes of proteins. Since this agreement depends on neglecting electrostriction in calculating $\bar{v}$, measurements were made in a non-aqueous organic solvent, xylene, and in solutions of different pH as well as in concentrated urea. The findings obtained are consistent with the view that electrostriction exists in these proteins but is presumably being masked by a compensating factor which may well be the "excluded volume" postulated by LINDERSTRØM-LANG.

Significant variations of $\bar{v}$ with temperature over the range 5–25° were observed for the 3 proteins studied, suggesting that the evaluation of molecular weights from sedimentation–diffusion data at temperatures below room temperature should also include $\bar{v}$ measurements at the same temperature.

INTRODUCTION

The calculation of molecular weights from ultracentrifuge data requires the evaluation of the term $(1 - \bar{v}\varrho)$. Since the partial specific volume, $\bar{v}$, of most proteins is generally between 0.70 and 0.75 ml/g and the solvent density, ϱ, is normally close to unity, a 1 % error in $\bar{v}$ results in one of about 3–4 % in the factor $(1 - \bar{v}\varrho)$. For this reason it is imperative that accurate values of $\bar{v}$ be obtained.

McMEEKIN AND MARSHALL[1], from the available data on amino acid analysis, have calculated partial specific volumes for several proteins, using the specific volumes of the individual amino acid residues as suggested by COHN AND EDSALL[2]. In most of the cases studied good agreement was obtained between calculated and experimentally observed values. The success of these calculations suggested that it is correct to neglect electrostriction due to the charged side chain groups in calculating the volume of a protein from that of its constituent amino acid residues. However, both EDSALL[3] and WAUGH[4] have pointed out that electrostriction must occur in proteins since most of their acidic and basic groups are readily accessible for titration and therefore presumably also for interaction with water.

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One possible explanation for this apparent absence of electrostriction in proteins might be found in the appreciable uncertainty associated with the $\bar{v}$ values for the amino acid residues (errors of the order of $\pm$ 2 %); it may partly be chance that values were chosen which fit so well the values for the proteins. Another possible explanation comes from LINDERSTRØM-LANG's work on the very large volume contractions attending the early stages of the enzymic hydrolysis of proteins[5]. This study suggests that the framework of the native protein structure is held in a configuration which contains a certain amount of empty space in the interior of the molecule and which is inaccessible to solvent. This so-called "excluded volume" may be compensating for the effects of electrostriction. CHARLWOOD[6], using the magnetic float method, confirmed the presence of the excluded volume in edestin, serum albumin and ovalbumin and showed that it may alter in size under different experimental conditions.

The present study reports the effect of solvent composition and temperature on the $\bar{v}$ values of the muscle proteins: rabbit myosin, rabbit tropomyosin and an invertebrate tropomyosin isolated from the mollusc, *Pinna nobilis*. These proteins were chosen because in each case there is a high ratio of charge to mass ($\sim$300 charged residues/10^5 g protein) so that the effects of electrostriction should be very pronounced. In particular it was thought that these proteins might well prove to be the exception to the MCMEEKIN-MARSHALL rule of calculating $\bar{v}$ values without correcting for electrostriction.

The temperature variable was explored to test the reliability of the assumption, usually made, that $\bar{v}$ varies little with temperature within the range 0–25°. If there was any significant variation this could be a serious source of error in molecular weight determinations from ultracentrifugal data. This is particularly true with myosin whose sedimentation and diffusion properties must be determined below 18° in view of its tendency to aggregate at room temperature[7].

EXPERIMENTAL

Myosin was prepared from rabbit muscle by the procedure of MOMMAERTS AND PARRISH[8] which incorporates the essential features of the methods of SZENT-GYÖRGYI[9] and of PORTZEHL, SCHRAMM AND WEBER[10]. It was crystallized by dilution of freshly prepared solutions with water and the precipitates were dialyzed against water at 0° until the protein swelled to a gel. This dialysis was continued at 4° for 1 week. The salt-free gel was dried over P_2O_5, first in the cold, then *in vacuo* at room temperature, and finally after being powdered at 110° *in vacuo* over P_2O_5 until its weight was constant. Heating was thus avoided until essentially all water had been removed. When such preparations were dissolved in 0.5 *M* KCl buffered with phosphate at pH 7 to a total ionic strength of 0.6 they were found to approach complete purity as judged by ultracentrifugal analysis.

Tropomyosin was prepared from rabbit muscle by BAILEY's method[1] and stored at 0° as an ethanol–ether dried powder. Material prepared this way was shown to be electrophoretically homogeneous at pH 7.

Some measurements are also reported on invertebrate tropomyosin samples kindly supplied by Dr. K. BAILEY who prepared them from the whole adductors of *Pinna nobilis* by the ethanol method[12]. These samples gave good electrophoretic and analytical criteria of purity[13].

Stock protein solutions of a definite concentration were made up by direct weight of the dry protein in whatever solvent system was being explored. These were always checked interferometrically by measuring the refractive index difference between solution and solvent. This was in fact the only satisfactory method for determining protein concentrations in concentrated urea solutions. Before this could be done, it was necessary to determine the specific refractive index increments of the proteins in the principal solvent systems used. For this purpose a Phoenix precision differential refractometer was used and this was calibrated by means of data for KCl solutions at 5461 Å[14,15]. Refractometer measurements at 5780 Å and parallel dry weight experiments led to the figures shown in Table I.

TABLE I

SPECIFIC REFRACTIVE INCREMENTS AT 5780 Å AND 25°

Protein	*Solvent system*	*Specific refractive increment* (g^{-1} cm^3)
Rabbit tropomyosin	KCl–phosphate buffer (pH 7, I = 0.6)	0.188 ± 0.001
	8 *M* urea in KCl–phosphate buffer (pH 7, I = 0.6)	0.155 ± 0.001
Pinna tropomyosin	KCl–phosphate buffer (pH 7, I = 0.6)	0.188 ± 0.001
	8 *M* urea in KCl–phosphate buffer (pH 7, I = 0.6)	0.156 ± 0.001
Rabbit myosin	KCl–phosphate buffer (pH 7, I = 0.6)	0.189 ± 0.001

Solutions of the muscle proteins were measured in capped pycnometers of about 10 ml capacity which were calibrated with air-free distilled water. Weighings were made on a Bunge balance and a Sartorius semi-micro balance (sensitivity 0.01 mg) and great care was taken to duplicate weighing conditions. Partial specific volumes were calculated from the equation[16]:

$$1 - \bar{v}\varrho = \frac{1 - w_2}{m} \cdot \frac{dm}{dw_2} \qquad (1)$$

where m is the mass of solution, w_2 is the weight fraction of the protein (0.01 × the concentration by weight) and ϱ is the density of the solution.

In the case of the density measurements in xylene, protein, which had been dried at 110° *in vacuo*, was weighed by difference into the pycnometer and immediately covered with the organic solvent which had been previously dried. In all, the protein was exposed to the atmosphere no longer than 10 to 15 sec. The partly filled pycnometer was then evacuated to remove dissolved and occluded gases and to facilitate complete wetting of the protein.

Temperature regulation was achieved through the use of an Aminco mercury-in-glass thermoregulator model No. 4204. Electrical contact was made as the mercury column reached the upper platinum contact wire. The water bath contained a centrifugal type pump which circulated the bath fluid past a 700 W low-lag immersion heater. A Beckman thermometer was used as a quick check on temperature fluctuations and these were steady to ± 0.01° during measurements.

Temperatures below 20° were achieved using a cooling coil which operated from a 0.25-h.p. hermetically sealed condensing unit. In order to maintain ± 0.01° accuracy at the lower temperatures, the thermoswitch operating the condensing unit was set

below the desired temperature and the immersion heater, controlled by a Bronwill contact thermometer and electronic relay, was used to obtain fine temperature adjustments.

RESULTS AND DISCUSSION

The partial specific volumes of the muscle proteins in the various solvents and at the different temperatures were determined by plotting the mass, m, of a given volume of protein solution against the weight fraction, w_2, of protein. Fig. 1 shows a typical plot of the data obtained for rabbit tropomyosin in KCl–phosphate buffer (pH 7, I = 0.6) at 25 ± 0.01°. The slope of the line, dm/dw_2, was drawn according to the method of least squares and when used in equation (I) gave a value of 0.736 ± 0.002. A complete list of the v values obtained for the muscle proteins as a function of solvent composition is given in Table II. These values are estimated to be accurate to ± 0.002 and were independent of protein concentration in all the cases studied.

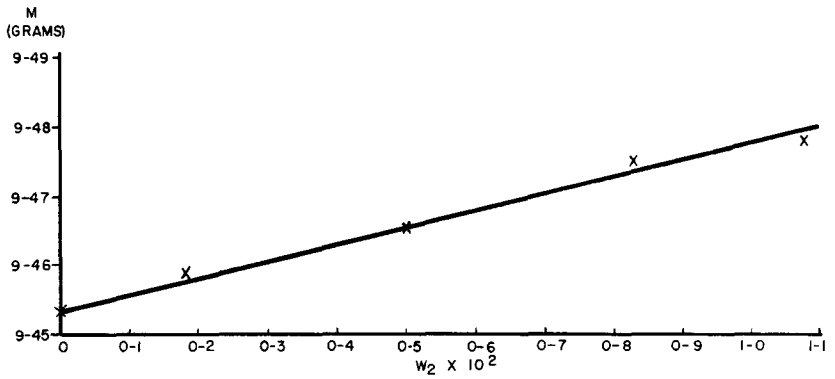

Fig. 1. Plot of m (mass of a given volume of solution of rabbit tropomyosin in phosphate-buffered KCl of pH 7, ionic strength = 0.6 for which the solute weight fraction is w_2) against w_2.

The $\bar{v}$ values for rabbit tropomyosin in water at pH 7 and in the neutral salt solutions (Expts. 2 and 3 of Table II) agree very favorably with the calculated value of 0.739[11]. Good agreement between experimental and theoretical $\bar{v}$ values was also found when neutral salt solutions of both *Pinna* tropomyosin and rabbit myosin were examined pycnometrically (compare Expts. 8 and 9 of Table II with the calculated value[17] of 0.729 and Expt. 13 with the value[11] of 0.735). This suggests that with these proteins no serious error is incurred in evaluating $\bar{v}$ from the specific volumes of their constituent amino acid residues without taking into account the effects of electrostriction.

However, that electrostriction is occurring in aqueous solutions of the muscle proteins is shown by the experiments carried out in xylene. Comparison between the values of $\bar{v}$ in water or neutral salt solution and in xylene would indicate a volume constriction of solvent water of 0.040 ml, 0.030 ml and 0.028 ml for rabbit tropomyosin, *Pinna* tropomyosin and rabbit myosin respectively. One can also calculate the degree of electrostriction from the number of ionizing groups in the molecule[18], assuming a volume decrease of 20 ml per charge pair per mole[3]. The results of this calculation are summarized in column 3 of Table III from which it is seen that

TABLE II

PARTIAL SPECIFIC VOLUME OF MUSCLE PROTEINS MEASURED AT 25° AS A FUNCTION OF SOLVENT COMPOSITION

Protein	*Experiment number*	*Protein treatment*	$\bar{v}$
Rabbit tropomyosin	1	Solution of isoelectric protein in H_2O—pH adjusted to 7 with 0.01 *N* NaOH	0.732 ± 0.002
	2	Dialyzed in 0.5 *M* KCl–0.035 *M* Na_2HPO_4, 0.015 *M* KH_2PO_4 (pH 7, I = 0.6)	0.736
	3	Dialyzed in 1 *M* KCl–0.035 *M* Na_2HPO_4, 0.015 *M* KH_2PO_4 (pH 7, I = 1.1)	0.739
	4	Dry protein covered with xylene	0.772
	5	Solution of isoelectric protein in H_2O adjusted to pH 2 with 0.01 *N* HCl	0.752
	6	Dialyzed in 8 *M* urea and KCl–phosphate buffer (pH 7, I = 0.6)	0.728
	7	Initially exposed to 8 *M* urea and KCl–phosphate buffer (pH 7, I = 0.6) then dialyzed against KCl–phosphate buffer (pH 7, I = 0.6) alone until free of urea	0.734
		Theoretical value calculated from BAILEY's amino acid composition figures[11]	0.739
Pinna tropomyosin	8	Dialyzed in 0.5 *M* KCl–0.035 *M* Na_2HPO_4, 0.015 *M* KH_2PO_4 (pH 7, I = 0.6)	0.730
	9	Dialyzed in 1 *M* KCl–0.035 *M* Na_2HPO_4, 0.015 *M* KH_2PO_4 (pH 7, I = 1.1)	0.733
	10	Dry protein covered with xylene	0.762
	11	Dialyzed in 8 *M* urea and KCl–phosphate buffer (pH 7, I = 0.6)	0.727
	12	Initially exposed to 8 *M* urea and KCl–phosphate buffer (pH 7, I = 0.6) then exhaustively dialyzed against KCl–phosphate buffer (pH 7, I = 0.6) alone until free of urea	0.731
		Theoretical value calculated from BAILEY's amino acid composition figures[17]	0.729
Rabbit myosin	13	Dialyzed in 0.5 *M* KCl–0.035 *M* Na_2HPO_4, 0.015 *M* KH_2PO_4 (pH 7, I = 0.6)	0.732
	14	Dry protein covered with xylene	0.760
		Theoretical value calculated from BAILEY's amino acid composition figures[11]	0.735

excellent agreement is obtained between the calculated and experimentally observed electrostriction.

The fact that this electrostriction is not manifesting itself in the observed $\bar{v}$ values may be due to its being masked by a compensating factor such as the "excluded volume" suggested by LINDERSTRØM-LANG's work[5]. Table III also includes estimates of the excluded volume obtained by taking the observed $\bar{v}$ and subtracting the calculated $\bar{v}$ less the calculated electrostriction. The excluded volumes so deduced correspond to 4–5 % of the observed partial molal volumes for the 3 muscle proteins; furthermore, they agree very favorably with both the calculated and experimentally observed electrostrictions. These values are consistent with recent optical rotatory dispersion studies on the muscle proteins which suggest that the greater part of their molecules is folded in the form of an α-helix[19,20]. Such extensive folding would no doubt render a small portion of the total volume of the molecule inaccessible to solvent[4].

TABLE III

ELECTROSTRICTION AND EXCLUDED VOLUMES OF THE MUSCLE PROTEINS

Protein	*Number of charged groups/ 10^5 g protein (after* TRISTRAM[18]*)*	*Calculated electrostriction (ml/g)*	*Experimentally observed electrostriction (ml/g)*	*Calculated* $\bar{v}$	*Experimentally observed* $\bar{v}$	*Calculated excluded volume (ml/g)*
Rabbit tropomyosin	376	0.038	0.040	0.739	0.736*	0.035
Pinna tropomyosin	315	0.032	0.030	0.729	0.732	0.035
Rabbit myosin	270	0.027	0.028	0.735	0.732	0.024

* Mean value of $\bar{v}$ for rabbit tropomyosin in water and in the 2 neutral salt solutions.

Expt. 5 of Table II lists $\bar{v}$ for rabbit tropomyosin in acid solution (pH 2) as 0.752, a value considerably larger than the 0.732 observed in water at pH 7. At pH 7 most of the carboxyl groups in the protein are charged while at pH 2 they are neutralized by reaction with protons. This elimination of part of the electrostriction corresponds to an increase in volume of 11 ml for each proton bound by the protein[21,22]. The expansion due to neutralization has been calculated for rabbit tropomyosin as 0.025 ml/g which agrees very well with the experimentally determined value of 0.020 ml/g.

In 8 *M* urea $\bar{v}$ for rabbit tropomyosin and *Pinna* tropomyosin was found to be 0.728 and 0.726 respectively (Expts. 6 and 11 of Table II). These values are 1–1.5 % lower than the corresponding values for these two proteins in the same solvent system in the absence of urea. This decrease in volume is consistent with the view that in urea some unfolding of the protein molecule is taking place thereby eliminating a portion of the excluded volume and facilitating penetration by the solvent. The total volume contraction observed is 440 ml/mole with rabbit tropomyosin and 675 ml/mole with *Pinna* tropomyosin. Volume contractions of the same order of magnitude have been observed to accompany the destruction of the native configuration in other proteins[6,21,23,24].

It seemed of some importance to determine whether the observed changes in $\bar{v}$ in 8 *M* urea were reversible. Accordingly, solutions of *Pinna* tropomyosin and rabbit tropomyosin were dialyzed exhaustively against KCl–phosphate buffer (pH 7, I = 0.6) until free of urea and then studied pycnometrically. The $\bar{v}$ values of rabbit

and *Pinna* tropomyosin were 0.734 and 0.731 respectively, in good agreement with 0.736 and 0.730 for the same proteins taken up in KCl–phosphate buffer directly. This suggests the essential reversibility of the urea treatment of these proteins, insofar as the criterion of pycnometry is concerned. A similar conclusion based on optical rotation and viscosity studies has been reached with *Pinna* tropomyosin[20].

The variation of $\bar{v}$ with temperature is summarized in Table IV.

TABLE IV

THE PARTIAL SPECIFIC VOLUME OF MUSCLE PROTEINS AS A FUNCTION OF TEMPERATURE

All measurements carried out in solvent medium of 0.5 *M* KCl, 0.035 *M* Na_2HPO_4, 0.015 *M* KH_2PO_4 (pH 7, I = 0.6).

Protein	*Temperature of measurement (C)*	$\bar{v}$
Rabbit tropomyosin	5	0.724
	15	0.730
	25	0.736
Rabbit myosin	5	0.720
	15	0.725
	25	0.732
Pinna tropomyosin	5	0.719
	15	0.725
	25	0.730

The data show that with the 3 proteins considered there is a variation of 1.5–1.7 % between $\bar{v}$ at 5° and $\bar{v}$ at 25°. Since errors in $\bar{v}$ are essentially multiplied by a factor of 3 in the final calculation of molecular weights, the above variations would result in errors of the order of 5 % in the molecular weight values deduced from the Svedberg equation. The results of these measurements therefore cast some doubt on the validity of the assumption that $\bar{v}$ varies little with temperature and suggest that the evaluation of molecular weights from sedimentation and diffusion data at temperatures below room temperature should also include $\bar{v}$ measurements at the same temperature.

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ON THE CONVERSION OF FRUCTOSE TO GLUCOSE BY GUINEA PIG INTESTINE

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SUMMARY

Inverted intestinal sacs of the guinea pig, but not of the rat, convert fructose to glucose during its passage through the intestinal wall. An exchange of ^{14}C-activity between C-1 and C-6 of the glucose formed from [1-^{14}C]fructose under these conditions has been observed. Fructokinase and glucose-6-phosphatase have been found to be present in the intestinal mucosa of the guinea pig and it has been confirmed that the latter enzyme is absent in the same tissue of the rat. These observations lead to the conclusion that the conversion of fructose to glucose by the guinea pig intestinal mucosa occurs by the same mechanism as that operating in the liver, involving fructose-1-phosphate and triose phosphates as intermediates. It is suggested that the inability of rat intestine to convert fructose to glucose is due to the absence of glucose-6-phosphatase.

INTRODUCTION

It has been recognized for some time that a conversion of fructose to glucose occurs during intestinal absorption of fructose[1–4]. The mechanism involved in this conversion

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