[Contribution from the Eastern Regional Research Laboratory[1]]

Apparent Specific Volume of α-Casein and β-Casein and the Relationship of Specific Volume to Amino Acid Composition

By T. L. McMeekin, M. L. Groves and N. J. Hipp

The partial specific volumes of proteins are of great importance in determining their molecular weights by sedimentation and diffusion. The apparent specific volumes are calculated from the densities of the protein solutions and are identical with the partial specific volumes in dilute solutions. A value of about 0.750 has been reported for the partial specific volume of many proteins.[2] It has been suggested,[3,4] however, that the apparent specific volume of a protein is largely determined by the volume increments of the amino acid residues of which it is composed. The recent complete amino acid analyses of α-casein and β-casein by Gordon, *et al.*,[5] which show considerable differences in the composition of these proteins, offer the opportunity of comparing the specific volume calculated from the amino acid content with the experimentally determined specific volume.

Experimental

Casein Preparations.—The preparations were the same as those used by Gordon, *et al.*,[5] and described by Warner,[6] with the exception of β-casein, which was prepared by Hipp, *et al.*,[7] by a modification of the method devised by Warner. The β-casein was free from α-casein, as shown by electrophoresis, and contained 0.60% phosphorus and 15.3% nitrogen. The amino acid analysis of β-casein by Gordon, *et al.*, however, was made on the sample prepared by Warner. Consequently, our calculated specific volume of β-casein is based on one preparation, whereas our experimentally determined specific volume is based on another, a sample which has not been analyzed for amino acids. It is believed, however, that the electrophoretic analyses and the nitrogen and phosphorus content characterized β-casein well enough to make a valid comparison of the measurements of the two preparations.

Density Measurements.—The casein samples were dissolved in the minimum amount of dilute sodium hydroxide to give a *p*H of 6.4 to 6.9—approximately 4.6 cc. of 0.1 *N* sodium hydroxide for each gram of casein. Densities were determined at 25° in pycnometers of about 20-ml. capacity. The protein concentration was determined on aliquots of the solution by dry weight at 105°. The calculated amount of sodium present was subtracted in calculating the dry weight. The dry weight value agreed with the weight of casein used in making the solutions. Densities of solutions of whole casein were also determined in 6.66 molar urea solutions and in acid solutions of *p*H 3.1. Urea was purified by dissolving it in 70% alcohol at 40° and precipitated by chilling to −5°. The recrystallized urea was dried *in vacuo* at 50°. Casein solutions with a *p*H of 3.1 were prepared by dissolving casein in dilute sodium hydroxide and adding lactic acid rapidly with stirring until the desired *p*H was reached. In each case the density of a solution containing all the added ingredients, with the exception of the casein, was determined and used as the solvent density in calculating the apparent specific volumes.

Densities and Apparent Specific Volumes of Casein Solutions.—Table I gives the data on the densities and calculated apparent specific volumes of α-casein, β-casein and whole casein solutions. The equation of Svedberg and Chirnoaga[8] was used in calculating the apparent specific volumes: $V = [w - (1 - h)]/\rho h$, where w is weight of solvent in pycnometer, l is weight of solution, h is weight of protein, and ρ is density of solvent. No correction was made for ash because these preparations contained only small amounts of true ash.[6]

Table I

Densities and Apparent Specific Volumes of Casein Solutions

Concn. of casein g. per 100 cc.	*p*H of solution	Density of solution at 25°	Density of solvent	Apparent specific volume of solution
		Whole Casein in Alkali		
1.82	6.4	1.00247	0.99757	0.732
2.73	6.4	1.00522	.99777	.729
5.44	6.4	1.01313	.99847	.732
			Av.	0.731
		Whole Casein in Acid		
0.0[a]	3.1	.....	.....	0.730
2.07	3.1	1.00546	.99978	.725
3.31	3.1	1.01060	1.00140	.721
4.14	3.1	1.01417	1.00248	.716
		Whole Casein in 6.66 *M* Urea		
4.07	5.2	1.10557	1.0973	0.726
5.47	5.2	1.10803	1.0972	.731
6.46	5.2	1.10988	1.0972	.732
			Av.	0.730
		α-Casein		
2.67[b]	6.4	1.00507	0.99777	0.728
5.33[b]	6.4	1.01320	.99847	.725
1.81[c]	6.4	1.00248	.99757	.730
2.74[c]	6.4	1.00528	.99782	.729
4.39[c]	6.4	1.01010	.99819	.730
5.48[c]	6.4	1.01345	.99847	.728
			Av.	0.728
		β-Casein		
1.38[d]	6.9	1.00099	0.99742	0.743
2.76[d]	6.9	1.00491	.99777	.743
5.54[d]	6.9	1.01288	.99847	.741
3.51[e]	6.9	1.00723	.99797	.738
			Av.	0.741

[a] Extrapolated value. [b,c] Different preparations of α-casein. [d,e] Different preparations of β-casein.

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) Svedberg and Pedersen, "The Ultracentrifuge," Oxford Press England, 1940.

(3) Cohn, McMeekin, Edsall and Blanchard, *J. Biol. Chem.*, **100**, Proc. xxviii (1933); This Journal, **56**, 784 (1934).

(4) Cohn and Edsall, "Proteins, Amino Acids and Peptides as Ions and Dipolar Ions," Reinhold Publ. Co., New York, N. Y., 1943.

(5) Gordon, Semmett, Cable and Morris, This Journal, **71**, 3293 (1949).

(6) Warner, *ibid.*, **66**, 1725 (1944).

(7) Hipp, unpublished results.

(8) Svedberg and Chirnoaga, This Journal, **50**, 1399 (1928).

Calculation of the Specific Volumes of Caseins from the Volumes of the Amino Acid Residues.— The method of Cohn and Edsall[4] for calculating the apparent specific volume of proteins was followed in detail. The specific volume was calculated by the equation $\bar{V}_p = \Sigma V_i W_i / \Sigma W_i$, where W_i is the per cent. by weight of the i'th amino acid residue in the protein as found by analysis, and V_i is the specific volume of this residue. The molecular weights and corresponding specific volumes of the amino acid residues were the ones given by Cohn and Edsall, with the exception of cystine. The cystine residue was considered to be cystine minus two molecules of water, resulting in a molecular weight for the residue of 204.18 instead of 222.18. Consequently, a value of 6.6 cc. was subtracted for the volume of the second molecule of water removed from cystine, giving a calculated molal volume for its residue of 128.8 cc. instead of 135.4 cc. When the molal volume of the residue is divided by the weight of the residue, a value of 0.63 is obtained for the specific volume of the cystine residue.

Phosphorus is present in casein in the form of a phosphoric acid ester of serine.[9] Values for the atomic volume of phosphorus and the oxygen atoms of phosphoric acid are not available for calculating the specific volume of serine phosphoric acid. Consequently, the molal volume of serine phosphoric acid was determined, and its residue volume in casein calculated. Serine phosphoric acid was made by the method of Levene and Schormuller,[10] as modified by Plimmer.[11] The product melted at 171.5° and contained 7.69% nitrogen and 17.1% phosphorus. Theoretical values for serine phosphate are 7.57% nitrogen and 16.76% phosphorus. Table II gives the values for the density and molal volume of aqueous solutions of serine phosphate.

TABLE II

MOLAL VOLUME OF SERINE PHOSPHATE

Concentration g. per 100 cc.	Density at 25°	Molal volume
2.002	1.00745	89.4
3.203	1.01359	89.9
4.004 (0.22 *M*)	1.01764	90.3

The value of 90.3 cc. for the molal volume of serine phosphate is used in calculating its residue volume, because the concentration of 0.22 *M* is close to the concentration of 0.25 *M*, used by Cohn and Edsall in their data on the molal volume of amino acids. In calculating the specific volume of serine phosphoric acid residue, 7.4 cc. was subtracted from 90.3 cc., and the result was divided by the molecular weight of the residue (167.07), giving a value of 0.50. The value of 7.4 cc. per mole change in volume caused by the formation of an amino acid from an amino acid residue in the protein was derived by Cohn and Edsall by adding the value of the atomic volumes of 2 hydrogen and 1 oxygen (6.6 cc.) to the calculated difference between the electrostriction and covolume effects (0.8 cc.).

Table III gives calculated values for the per cent. by volume of amino acid residues based on the amino acid analyses of Gordon, *et al.*,[5] for whole casein, α-casein and β-casein, as well as calculated values for their specific volumes.

Discussion

Several values have been reported for the partial specific volume of casein solutions. Chick and Martin[12] calculated the apparent density of the casein molecule in solution to be 1.39, or 0.720 for the apparent specific volume. Svedberg, Carpenter and Carpenter[13] gave the value of 0.750 for the partial specific volume of casein in solution. Our values for whole casein, as well as α-casein and β-casein, are between these two reported values. The accuracy of the value for the specific volume of a protein in solution is dependent on the accuracy of the method for determining the protein concentration. The concentrations of casein reported in Table I were determined in most cases by two methods—(1) by weighing the casein accurately before dissolving it and subtracting the moisture content, and (2) by determining the solids in the solution and subtracting the value for the dry weight of the material added to dissolve the casein. In all cases the values obtained by the two methods were in good agreement. The values for concentration reported in urea solutions were obtained from the weight of casein used.

The apparent specific volume of whole casein was 0.731 when it was dissolved in dilute alkali or in 6.66 *M* urea. When the whole casein was dissolved in lactic acid, the apparent specific volume varied considerably with the concentration of casein used. However, the extrapolated value of 0.730 for zero concentration is in agreement with the values obtained in alkali and urea solutions. The value of 0.728 for the apparent specific volume of α-casein is only slightly less than that for whole casein and is in fact within the experimental variation in determining the specific volume. This value, however, is in agreement with the expected value; Warner[6] has estimated that whole casein contains 80% α-casein and 20% β-casein and the value of the apparent specific volume of β-casein is 0.741.

The calculated specific volumes of the caseins based on the amino acid analyses of Gordon, *et al.*,[5] and the specific volumes of the amino acid residues are in good agreement with the experimentally determined specific volume in each case. These results confirm the hypothesis that the apparent specific volume of a protein is essentially determined by the volume of amino acid residues. These results indicate that if the value for the ap-

(9) Lipmann, *Biochem. Z.*, **262**, 3 (1933).

(10) Levene and Schormuller, *J. Biol. Chem.*, **105**, 547 (1934).

(11) Plimmer, *Biochem. J.*, **35**, 461 (1941).

(12) Chick and Martin, *Biochem. J.*, **7**, 92 (1913).

(13) Svedberg, Carpenter and Carpenter, THIS JOURNAL, **52**, 241 (1930).

TABLE III

SPECIFIC VOLUMES OF CASEINS CALCULATED FROM THE VOLUMES OF THE AMINO ACID RESIDUES

	Amino acid residues, %			Specific volume of amino acid residue, V	Per cent. by volume of amino acid residue: VW		
	Whole casein	α-Casein	β-Casein		Whole casein	α-Casein	β-Casein
Glycine	2.05	2.13	1.82	0.64	1.31	1.36	1.17
Alanine	(2.39)	(2.95)	(1.36)	.74	1.77	2.19	1.00
Serine	2.82	2.40	3.90	.63	1.78	1.52	2.45
Phosphoserine	4.61	5.33	3.25	.50	2.30	2.66	1.63
Threonine	4.16	4.16	4.33	.70	2.91	2.91	3.03
Valine	6.09	5.33	8.63	.86	5.24	4.58	7.42
Leucine	7.93	6.81	10.00	.90	7.14	6.13	9.00
Isoleucine	5.26	5.52	4.74	.90	4.73	4.97	4.27
Proline	9.54	6.92	13.50	.76	7.25	5.26	10.26
Phenylalanine	4.46	4.10	5.17	.77	3.43	3.16	3.98
Methionine	2.46	2.20	2.99	.75	1.85	1.65	2.24
Cystine	0.29	0.37	....	.63	0.18	0.23	....
Tryptophan	1.09	1.46	0.59	.74	0.81	1.08	0.44
Tyrosine	5.68	7.30	2.88	.71	4.03	5.18	2.05
Histidine	2.74	2.56	2.74	.67	1.84	1.72	1.84
Arginine	3.67	3.85	3.05	.70	2.57	2.70	2.13
Lysine	7.18	7.80	5.69	.82	5.89	6.39	4.67
Aspartic acid	6.14	7.27	4.24	.60	3.69	4.36	2.54
Glutamic acid	4.92	5.01	5.62	.66	3.25	3.30	3.71
Glutamine	14.65	14.65	14.65	.67	9.81	9.81	9.81
Total	98.13	98.12	99.15		71.78	71.16	73.64
Per cent. by volume of amino acid residues .... $\Sigma V_i W_i$					71.78	71.16	73.64
Per cent. by weight of amino acid residues .... ΣW_i					98.13	98.12	99.15
Specific volume, calculated .... $\Sigma V_i W_i / \Sigma W_i$					0.731	0.725	0.743
Specific volume, observed (Table I)					.731	.728	.741

parent specific volume of a protein as determined does not agree reasonably well with the value calculated from the specific volumes of the amino acid residues a redetermination should be made of either the apparent specific volume or the amino acid content. Thus Putnam, Lamanna and Sharp[14] have calculated from the volumes of the amino acid residue that the apparent specific volume of botulinus antitoxin is 0.738, while Kegeles[15] found 0.755 for this protein. Here the variation between the calculated value and the observed value appears to be greater than might be expected. A value of 0.746 for the apparent specific volume of β-lactoglobulin may be calculated from the amino acid analysis of Brand, *et al.*[16] This value is in good agreement with the determined value of 0.751 reported by Pedersen[17] and confirmed by Brand and Kassell.[18] (The value of 6.1% for the isoleucine content of β-lactoglobulin reported by Smith and Greene[19] was used instead of Brand's value of 8.4%.)

(14) Putnam, Lamanna and Sharp, *J. Biol. Chem.*, **176**, 401 (1948).

(15) Kegeles, THIS JOURNAL, **68**, 1670 (1946).

(16) Brand, Saidel, Goldwater, Kassell and Ryan, *ibid.*, **67**, 1524 (1945).

Summary

Values for the apparent specific volume of whole casein, α-casein and β-casein have been calculated from density determinations and from the volumes of the amino acid residues. The values calculated by the two methods are in good agreement, indicating that the apparent specific volume of a protein is largely determined by the volume of its amino acid residues.

PHILADELPHIA 18, PA. RECEIVED APRIL 5, 1949

(17) Pedersen, *Biochem. J.*, **30**, 961 (1936).

(18) Brand and Kassell, *J. Biol. Chem.*, **145**, 365 (1942).

(19) Smith and Greene, *ibid.*, **172**, 111 (1948).