Partial Molar Volumes of Polypeptides and Their Constituent Groups in Aqueous Solution over a Broad Temperature Range

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SYNOPSIS

The partial molar volumes of various compounds that model protein constituent groups, such as tripeptides (Gly-X-Gly, where X = Gly, Ala, Val, Leu, Ile, Pro, Met, His, Ser), homopeptides (Gly_n, n = 3, 4, 5), and simple organic analogues of amino acid side chains (methanol, acetamide, propanamide, acetic acid, propanoic acid, n-butanamine, n-butanamine nitrate, n-propylguanidine nitrate, 4-methylphenol), have been determined in aqueous solution with a vibrational densimeter in the temperature range of 5–85°C. The partial molar volumes of amino acid side chains and the peptide unit were estimated from the data obtained. Assuming additivity of component groups, the partial molar volumes of polypeptide chains of several proteins over a broad temperature range were calculated. The partial molar volume functions of four proteins (myoglobin, cytochrome C, ribonuclease A, lysozyme) were compared with those determined experimentally for the unfolded and native forms of these proteins. It has been shown that the average deviation of the calculated functions from the experimental ones does not exceed 3% over the temperature range studied.

INTRODUCTION

The correspondence between the partial molar volumes of low molecular compounds and the sum of the values for their component groups has been demonstrated a long time ago by Traube.[1,2] Later, the volume additivity was used in the calculation of the partial molar volumes of various macromolecules, in particular proteins, but only at 25°C.[3,4] For other temperatures this method has never been used, since the partial molar volumes of the constituent groups at these temperatures were unknown. On the other hand, many physical studies of proteins require knowledge of their partial molar volume over a wide temperature range. In particular, it is necessary for the determination of the partial molar heat capacity of proteins by means of a precise differential scanning microcalorimeter.[5] The experimental determination of this parameter is not always possible, since even the most sensitive vibrational densimeters require a quite considerable amount of material. In such cases, the ability to calculate the partial molar volume of a protein with a known amino acid composition would be very useful.

In this paper we report the results of the experimental determination in the temperature range of 5–85°C of the partial molar volumes of various compounds, which can serve as models of the amino acid side chains and of the peptide group. This should permit the determination of their partial molar volumes over a broad temperature range. These compounds are tripeptides (Gly-X-Gly, where X = Gly, Ala, Val, Leu, Ile, Pro, Met, His, Ser), homopeptides (Gly_n, n = 3, 4, 5), and simple organic substances (methanol, acetamide, n-propionamide, acetic acid, propanoic acid, n-butylamine, n-butylamine nitrate, n-propylguanidine nitrate, 4-methylphenol). On the basis of the data obtained, we have shown that component volume additivity is valid for proteins in the native and unfolded states over broad temperature

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range, and that this temperature range can be easily expanded by a rather simple extrapolation procedure.

MATERIALS AND METHODS

The purification of methanol, acetic acid, propanoic acid, acetamide, *n*-propionamide, *n*-butylamine, *n*-butylamine nitrate, 4-methylphenol, *n*-butylamine nitrate, nitric acid, and the solution preparation procedure for these compounds have been described earlier.[6]

The monomer units of the peptides GlyAlaGly, GlyValGly, GlyIleGly, GlyLeuGly, GlySerGly, GlyProGly, GlyHisGly, GlyMetGly were built in the conventional stepwise manner using pentafluorophenyl esters of protected amino acids as described in Ref. 7. The protected amino acids were obtained from Reanal, Hungary. The C-terminal amino acids were protected as OBut (Gly-Ser-Gly),[8] OBzl (Gly-His-Gly)[9] or OPhac[10] (the other peptides) esters through synthesis. The deprotection of the amino terminal of the growing peptide chain was carried out by cleaving the Boc group with 4 *M* HCl-dioxane and of the Z group by hydrogenation.[11] The reductive

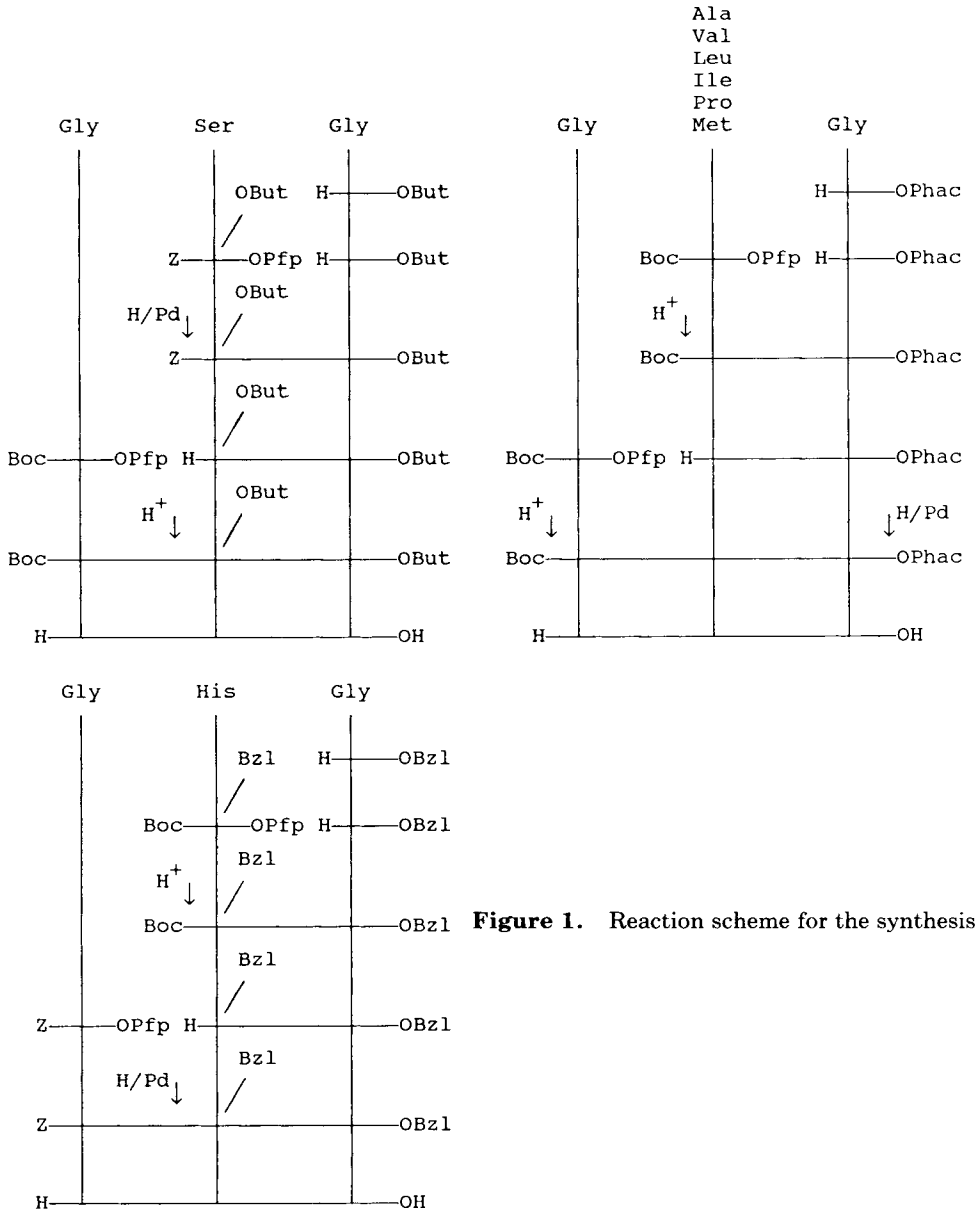

Figure 1. Reaction scheme for the synthesis of tripeptides.

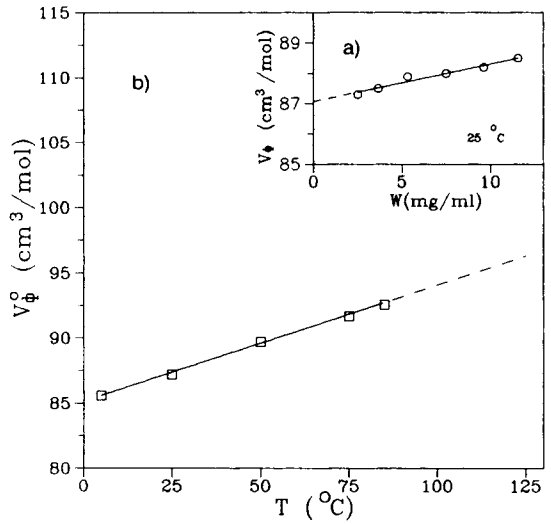

Figure 2. (a) Dependence of the apparent molar volume V_ϕ, of *n*-butylamine on concentration (W) at 25°C. (b) Temperature dependence of the partial molar volume V_ϕ^0 of *n*-butylamine. The broken extension of continuous lines represents the region of extrapolation.

Table II Comparison of the Obtained Partial Molar Volumes of Some Organic Compounds and Peptides at 25°C with Literature Values

	V_ϕ^0 ($cm^3 \cdot mol^{-1}$)		
Compound	This Work	Literature	Ref.
Methanol	38.1	38.68	14
		38.05	15
Acetic acid	51.8	51.6	16
		51.9	17
Propanoic acid	67.8	67.9	17
Acetamide	56.0	55.60	18
		55.82	19
n-Propionamide	71.3	71.54	19
		71.47	20
n-Butylamine	87.4	88.8	21
		89.9	22
Triglycine	112.9	111.81	23
		112.1	24
Tetraglycine	151.1	149.6	24
		149.7	23
Pentaglycine	189.5	187.1	23

cleavage of the OPhac and OBzl groups was made with Zn dust in 50% acetic acid in water as described in Ref. 12 or by means of hydrogenation.[11] HCl-dioxane (4 M) was used for the reductive cleavage of the *tert*-butyl type protecting groups.[8] The synthetic route is summarized in Figure 1. The procedures of peptide purification, analysis, solution preparation, and determination of peptide concentration in solution were the same as described in Ref. 6.

The procedures of the purification of horse heart cytochrome C, sperm whale myoglobin, bovine pancreatic ribonuclease A, and hen egg white lysozyme were the same as reported in Ref. 13. The unfolded states for these proteins were obtained[13] by the removal of a heme group, and/or by reducing and carboxyamidomethylating cysteine residues and acidifying the solution. All measurements of the solution density were carried out in 10 mM glycine or phosphate buffer solutions in the acidic pH region.

Table I Partial Molar Volumes V_ϕ^0 ($cm^3 \cdot mol^{-1}$) for Some Amino Acid Side-Chain Analogues in Aqueous Solution

	Temperature (°C)						
Substance	5	25	50	75	100[a]	125[a]	σ
Methanol	38.0	38.1	38.4	38.9	39.6	40.5	0.2
Acetic acid	49.9	51.8	54.1	56.5	58.8	61.1	0.4
Propanoic acid	65.8	67.8	70.3	72.8	75.2	77.8	0.5
Acetamide	55.0	56.0	57.2	58.5	59.7	60.9	0.4
n-Propionamide	69.3	71.3	72.8	75.2	77.4	78.2	0.4
4-Methylphenol	99.1	100.3	101.8	103.3	104.8	106.3	1.1
n-Butylamine	85.6	87.4	89.6	91.9	94.1	96.3	0.5
n-Propylguanidine	121.0	127.1	131.1	133.9	136.3	137.8	0.7
n-Butylamine nitrate	112.2	114.9	118.5	122.0	125.5	129.1	0.7
Nitric acid	28.9	31.1	33.1	34.3	35.3	35.8	0.5
n-Butylamine (ionized)	83.3	83.8	85.4	87.7	90.2	93.3	1.2
n-Propylguanidine (ionized)	92.1	96.0	98.0	99.6	100.0	102.0	1.2

[a] Extrapolated values, σ standard deviation.

Table III Partial Molar Volumes V_ϕ^0 ($cm^3 \cdot mol^{-1}$) of Some Peptides in Aqueous Solution at Various Temperatures

Peptide	Temperature (°C)						
	5	25	50	75	100[a]	125[a]	σ
Gly-Gly-Gly[b]	110.2	112.9	114.8	116.7	118.8	120.5	0.7
Gly-Gly-Gly-Gly[b]	149.6	151.1	153.3	155.2	157.0	158.6	1.0
Gly-Gly-Gly-Gly-Gly[b]	188.6	189.5	191.2	192.9	194.6	196.3	1.4
Gly-Gly-Gly[c]	114.0	115.5	117.6	119.7	121.8	123.9	0.8
Gly-Ala-Gly[c]	127.9	132.4	138.1	143.6	149.2	154.8	0.8
Gly-Val-Gly[c]	163.8	168.2	173.7	179.0	184.6	189.9	0.9
Gly-Ile-Gly[c]	167.6	171.3	175.7	180.3	184.7	189.4	1.1
Gly-Leu-Gly[c]	178.1	182.8	188.2	193.4	199.4	204.8	1.0
Gly-Ser-Gly[c]	130.3	133.1	136.8	140.3	143.7	147.4	0.9
Gly-His-Gly[c]	168.2	169.2	171.1	172.9	174.7	176.5	1.1
Gly-Pro-Gly[c]	128.2	130.7	132.5	135.0	137.5	140.1	0.9
Gly-Met-Gly[c]	176.4	177.3	178.2	179.5	180.8	182.1	1.1

[a] Extrapolated values.
[b] In pure water as a solvent.
[c] In 0.5 *M* sodium acetate buffer, pH 4.0, as a solvent; σ standard deviation.

Table IV Experimentally Measured Values of the Partial Volumes of Cytochrome C, Lysozyme, Ribonuclease, and Myoglobin in the Native (N) and Unfolded (U) States at Various Temperatures

Temperature (°C)	V_ϕ^0 (Units)	Cytochrome C		Lysozyme		Ribonuclease		Myoglobin	
		N	U[a]	N	U[b]	N	U[b]	N	U[a]
5	cm^3/mol	—	—	10210	10270	9540	9860	12830	12520
	cm^3/g	—	—	0.713	0.696	0.697	0.697	0.721	0.728
10	cm^3/mol	8650	8230	—	—	—	—	—	—
	cm^3/g	0.703	0.703	—	—	—	—	—	—
15	cm^3/mol	—	—	10190	10270	9730	9800	12890	12680
	cm^3/g	—	—	0.712	0.696	0.711	0.693	0.724	0.737
25	cm^3/mol	8610	8370	10280	10580	9830	9900	13070	12760
	cm^3/g	0.700	0.715	0.718	0.716	0.718	0.700	0.734	0.742
35	cm^3/mol	—	—	10420	10780	9930	10000	13070	12850
	cm^3/g	—	—	0.728	0.730	0.725	0.707	0.734	0.747
40	cm^3/mol	8790	8480	—	—	—	—	13140	12930
	cm^3/g	0.715	0.725	—	—	—	—	0.738	0.752
45	cm^3/mol	—	—	10450	10880	10010	10150	—	—
	cm^3/g	—	—	0.730	0.737	0.731	0.718	—	—
50	cm^3/mol	9000	—	—	—	—	—	—	—
	cm^3/g	0.732	—	—	—	—	—	—	—
55	cm^3/mol	—	—	10590	10880	10080	10220	—	—
	cm^3/g	—	—	0.740	0.737	0.736	0.723	—	—
60	cm^3/mol	9130	8520	—	—	—	—	—	—
	cm^3/g	0.742	0.728	—	—	—	—	—	—
65	cm^3/mol	—	—	10910	11100	10160	10720	—	—
	cm^3/g	—	—	0.762	0.752	0.742	0.758	—	—
75	cm^3/mol	9260	8660	—	—	—	—	—	—
	cm^3/g	0.753	0.740	—	—	—	—	—	—

[a] Apo form.
[b] With carboxyamidomethylated cysteine residues.

The solution density was measured by a vibrational densimeter DMA 60/602 (Anton Paar, Austria) in the temperature range of 5–85°C. The apparent molar volume V_ϕ was calculated from the density of the solution ρ and the density of the solvent ρ_0 by the equation

$$V_\phi = \frac{M}{W} \cdot \left(1 - \frac{\rho}{\rho_0} + \frac{W}{\rho_0}\right) \qquad (1)$$

where M is the molar mass of the solute and W is the concentration of the studied substance in the solution. The partial molar volume at infinite dilution V_ϕ^0 was obtained by linear extrapolation of the apparent molar volume V_ϕ to zero concentration (Figure 2b). For the temperatures above 85°C, the partial molar volume was found by extrapolation (Figure 2a).

RESULTS

The partial molar volumes of methanol, acetic acid, propanoic acid, acetamide, *n*-propionamide, 4-methylphenol, *n*-butylamine, *n*-butylamine nitrate, *n*-propylguanidine nitrate, and nitric acid determined in our experiments in the temperature range of 5–125°C are presented in Table I. The partial molar volumes of *n*-propylguanidine and *n*-butylamine cations were calculated from the V_ϕ^0 values of the corresponding nitrates and that of nitric acid.

A comparison of the partial molar volumes of the simple organic compounds at 25°C obtained in this work with those reported in literature (Table II) shows that, in all cases, the deviation does not exceed an experimental error of the order of 0.8%. This permits us to assume that our data for all other temperatures are of comparable reliability.

The partial molar volumes of the peptides are presented in Table III. A comparison of the temperature dependencies of the partial molar volume of Gly_3 in pure water and in 0.5 *M* $NaCH_3COO$ pH 4.0 buffer solution shows a notable difference. This difference may be caused by the Na^+ and CH_3COO^- counterions that are attracted by the charged groups of the peptide molecule. Such an explanation follows from the study of the transfer of peptides from pure water to an aqueous NaCl solution.[25] It was shown that this process is accompanied by a significant volume increase caused by the interaction of Na^+ and Cl^- with the charges at the ends of the peptide molecule.

The measured partial molar volumes for Gly_3, Gly_4, and Gly_5 at 25°C were compared with the available literature data (Table II). The comparison shows a reasonable agreement between the present results and those published earlier.

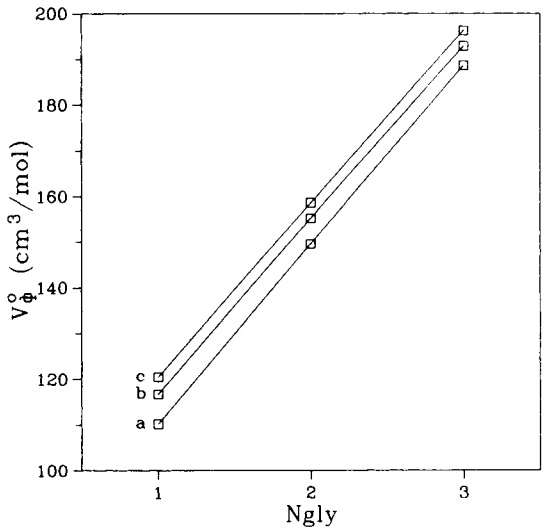

Figure 3. Dependence of the partial molar volume of glycine homopeptides on the number of glycyl units N_{gly}, in the peptide molecule at various temperatures: (a) 5°C, (b) 75°C, and (c) 125°C. The lines have been drawn by the least-square calculation.

The partial molar volumes of cytochrome C, myoglobin, lysozyme, and ribonuclease in the native and unfolded states determined in our experiments are listed in Table IV for the temperature range of 5–75°C. The difference between the partial volumes of proteins in the native and unfolded states is at the limit of the experimental error and cannot be considered to be significant. According to many authors (see for a review Ref. 26), this difference in general is so small that it is unobservable by a direct volumetric method and can be only judged by the influence of pressure on protein stability.

DISCUSSION

From the data on the partial molar volumes of homopeptides one can determine the partial molar volume of the glycyl unit -CH_2CONH-. Figure 3 represents the dependence of the partial molar volumes of homopeptides on the number of glycine units N_{gly} in the molecule. The linearity of this dependence in the temperature range studied means that the glycyl unit contributes additively to the partial volume of the peptide molecule. The slope of the plot of V_ϕ^0 vs N_{gly} at any given temperature corresponds to the partial volume contribution of a single glycyl unit at this temperature.

The partial molar volume of the peptide unit, the -CHCONH- group, can be easily obtained from the temperature dependence of the partial molar volume of the $-CH_2CONH-$ group:

$$V_\phi^0(\text{-CHCONH-}) = V_\phi^0(\text{-}CH_2CONH\text{-}) - V_\phi^0(H) \quad (2)$$

where $V_\phi^0(H)$ is the partial molar volume of the H atom.

The partial molar volumes of the amino acid side chains $V_\phi^0(-R)$ can be calculated from the partial molar volumes of tripeptides and organic analogues by the following equations:

$$V_\phi^0(-R) = V_\phi^0(\text{Gly-X-Gly}) - V_\phi^0(\text{Gly-Gly-Gly}) + V_\phi^0(H) \quad (3)$$

for the tripeptides and

$$V_\phi^0(-R) = V_\phi^0(A) - V_\phi^0(H) \quad (4)$$

for the organic analogues.

The calculation of the V_ϕ^0 values for the protein constituent groups by Eqs. (2)-(4) requires knowledge of the partial molar volume of the hydrogen atom $V_\phi^0(H)$. The latter can be determined using, for example, the following obvious expression:

$$V_\phi^0(CH_3OH) - V_\phi^0(H) = V_\phi^0(\text{Gly-Ser-Gly}) - V_\phi^0(\text{Gly-Gly-Gly}) + V_\phi^0(H) \quad (5)$$

which gives for the partial molar volume of the hydrogen atom

$$V_\phi^0(H) = \frac{V_\phi^0(CH_3OH) - V_\phi^0(\text{Gly-Ser-Gly}) + V_\phi^0(\text{Gly-Gly-Gly})}{2} \quad (6)$$

Table V Temperature Dependence of the Partial Molar Volumes of Amino Acid Side Chains and of the Peptide Unit[a]

Group or Side Chain of Amino Acid Residue	Temperature (°C)					
	5	25	50	75	100	125
Gly	10.9	10.3	9.6	9.2	8.9	8.5
Asn	44.1	45.7	47.8	49.3	50.8	52.4
Asp	39.0	41.5	44.5	47.3	49.9	52.6
Gln	58.4	61.0	63.2	66.0	68.5	69.7
Glu	54.9	57.5	60.7	63.6	66.3	69.3
Lys	74.7	77.1	80.0	82.7	85.2	87.8
Lys[b]	72.4	73.5	75.8	78.5	81.3	84.8
Tyr	88.2	90.0	92.2	94.1	95.9	97.8
Arg[b]	81.2	85.7	88.4	90.4	92.1	93.5
Phe	82.0	86.3	92.0	97.9	102.6	107.9
Thr	44.2	44.7	45.9	47.6	50.9	54.6
Pro	36.0	35.8	34.1	33.7	33.5	33.2
Met	73.3	72.1	70.2	69.0	67.9	66.7
His	65.1	64.0	63.1	62.4	61.8	61.1
Ala	24.8	27.2	30.1	33.1	36.3	39.4
Val	60.7	63.0	65.7	68.5	71.7	74.5
Leu	75.0	77.6	80.2	82.9	86.5	89.4
Ile	64.5	66.1	67.7	69.8	71.8	74.0
Ser	27.1	27.8	28.8	29.7	30.7	32.0
Trp[c]	110.9	110.9	110.9	110.9	110.9	110.9
CysH[c]	40.5	40.5	40.5	40.5	40.5	40.5
$CysCH_2CONH_2$[d]	73.7	75.9	78.7	80.6	82.4	84.4
-CHCONH-	28.3	28.0	28.6	28.9	29.0	29.4

[a] All values are given in $cm^3 \cdot mol^{-1}$.

[b] Ionized form.

[c] Calculated from the data on amino acid reported in Ref. 27.

[d] Partial molar volume of carboxyamidomethylated cysteine was calculated as $V_\phi^0(CysH) - V_\phi^0(H) + V_\phi^0(Asn)$.

The partial molar volume of the H atom, calculated in this way, is given in Table V. At 25°C our value, 10.3 $cm^3\ mol^{-1}$, agrees well with that obtained earlier by Hoiland,[27] 10.6 $cm^3\ mol^{-1}$. This permits us to conclude that the values of $V_\phi^0(H)$ obtained by us for all other temperatures are also reliable.

Using Eqs. (2)-(4), one can calculate the partial molar volumes of all protein contituent groups over the temperature range examined. The values obtained are listed in Table V. Table V also includes the values of the partial molar volumes of the Thr and Phe side chains that we had determined earlier,[28,29] using ethanol and toluene as analogues. In Table VI our results on the partial molar volumes of amino acid side chains are compared with those calculated from the partial molar volumes of amino acids[30] and from dipeptides[31] at 25°C. The comparison shows that, in most cases, there is a reasonable agreement between the two sets of data obtained on molecules with and without charges. At the same time, temperature dependencies of the partial molar volume of the amino acid side chains obtained in this work differs from those calculated from the data on amino acids[32] obtained for the temperature range of 15–55°C. This difference may be described by the influence of the charges in amino acids. Such an explanation follows, on the one hand, from the fact that influence of charges in tripeptides is largely reduced,[25] and on the other hand, there are not charges at all in most simple organic compounds used by us as models for the amino acid side chains.

As seen from Table V, the partial molar volumes of amino acid side chains depend on temperature in a very different way (see also Figure 4). They are decreasing functions for Gly, His, and Pro, and increasing functions with various slopes for the other amino acid residues. One can assume that the different temperature dependencies are caused by different contributions of various groups to the amino acid residues (charged, polar, nonpolar). However,

Table VI Partial Molar Volumes of Protein Constituent Groups at 25°C Obtained in This Work and Reported in the Literature V_ϕ^0 ($cm^3 \cdot mol^{-1}$), van der Waals Volumes[a] of These Groups V_{vw} ($cm^3 \cdot mol^{-1}$), and Their Packing Densities ξ

Side Chain of Amino Acid Residue	Partial Molar Volume		V_{vw}	$\xi = V_{vw}/V_\phi^0$
	This Work	Literature		
Asn	45.7	44.2,[b] 44.2[c]	32.5	0.711
Asp	41.5	38.8[b]	28.0	0.675
Gln	61.0	61.4[b]	42.7	0.700
Glu	57.5	56.4[b]	38.2	0.664
Lys	77.1	75.8[b]	47.8	0.621
Tyr	90.0	90.3[b]	60.3	0.670
Phe	86.3	89.0,[b] 89.5[c]	55.4	0.645
Thr	44.7	43.9,[b] 42.6	28.5	0.638
Pro	35.8	49.7[b]	27.3	0.763
Met	72.1	72.3[b]	44.9	0.623
His	64.0	66.2[b]	45.0	0.703
Ala	27.2	27.5,[b] 26.4[c]	13.7	0.504
Val	63.0	57.8,[b] 56.1[c]	34.1	0.541
Leu	77.6	74.6,[c] 73.7[c]	44.4	0.572
Ile	66.1	72.5[b]	44.4	0.672
Ser	27.8	27.7,[b] 27.0[c]	18.3	0.658
Gly	10.3	10.7[d]	3.4	0.330
Trp	110.9	111.1[b]	72.7	0.656
Cys	40.5	40.7[b]	25.0	0.617
-CHCONH-	28.0	27.7[e]	25.9	0.925

[a] V_{vw} values are taken from Ref. 33.

[b] Calculated from the data on the partial molar volumes of amino acids[30] by the equation $V_{p,\phi}^0(-R) = V_{p,\phi}^0(X) - V_{p,\phi}^0(Gly) + V_{p,\phi}^0(H)$, where $V_{p,\phi}^0(H) = 10.3\ cm^3 \cdot mol^{-1}$.

[c] Calculated by addition of the partial molar volume of hydrogen atom $V_\phi^0(H)$ to the values reported in Ref. 31.

[d] Calculated from the data of Hoiland[27] as $V_\phi^0(-H) = V_\phi^0(-CH_3) - V_\phi^0(-CH_2-)$.

[e] Calculated from the data taken from Ref. 23 as $V_\phi^0(-CH_2CONH-) - V_\phi^0(-H)$.

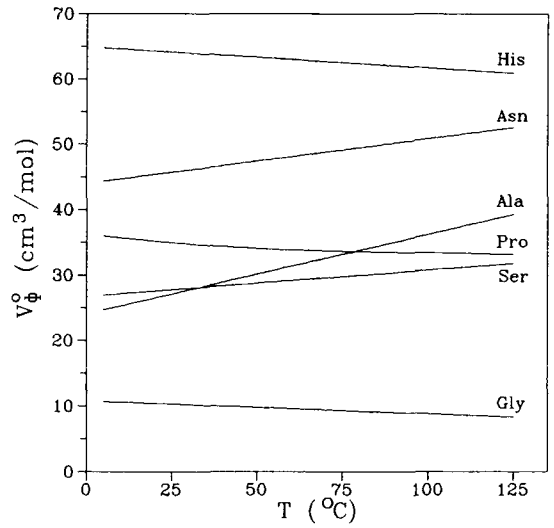

Figure 4. Temperature dependence of the partial molar volumes of the side chains of some amino acid residues.

we have not succeeded in resolving these effects by far.

Table VI also gives the van der Waals volumes enclosed by the van der Waals envelope of a given molecule V_{vw}.[33] As seen, these volumes are significantly smaller than the actual volumes of space occupied by a given molecule in aqueous solution V_ϕ^0. The ratio of these two volumes $\xi = V_{vw}/V_\phi^0$, which could be regarded as a parameter specifying the packing density of a solute molecule in solution, varies from 0.3 to 0.9, as predicted by the scaled particle theory.[33,34]

The values obtained for the partial molar volumes of protein constituent groups can be used for the calculation of the partial molar volumes of proteins in a wide temperature range, with the assumption that all these groups contribute additively to the total volume:

$$V_\phi^0(\text{cal}) = (N-1)\cdot V_\phi^0(\text{-CHCONH-}) + \sum_{i=1}^{N} V_\phi^0(-\text{R}_i) \quad (7)$$

where N is the number of amino acid residues in the protein sequence, V_ϕ^0(-CHCONH-) is the contribution of a peptide unit, and $V_\phi^0(-\text{R}_i)$ is the contribution of the ith amino acid side chain. Then, the

Table VII Comparison of Temperature Dependencies of the Partial Molar Volumes of Cytochrome C, Myoglobin, Lysozyme, and Ribonuclease A Obtained Experimentally for the Native $V_{\phi,N}^0$(exp), and Unfolded States $V_{\phi,U}^0$(exp), with Those Calculated by Eq. (7) V_ϕ^0(cal)[a]

	Temperature (°C)					
Protein	5	25	50	75	100	125
Cytochrome C						
$V_{\phi,N}^0$(exp)[b]	8090	8310	8590	8850	9120	9390
$V_{\phi,U}^0$(exp)[b]	8200	8350	8550	8750	8950	9150
V_ϕ^0(cal)	8640	8750	8990	9230	9460	9720
Myoglobin						
$V_{\phi,N}^0$(exp)[b]	12430	12610	12820	13040	13260	13480
$V_{\phi,U}^0$(exp)[b]	12540	12760	13030	13310	13590	13860
V_ϕ^0(cal)	12800	12990	13360	13710	14050	14430
Lysozyme						
$V_{\phi,N}^0$(exp)[b]	10110	10330	10600	10870	11150	11420
$V_{\phi,U}^0$(exp)[b]	10380	10620	10910	11210	11500	11800
V_ϕ^0(cal)	10500	10690	11000	11280	11550	11850
Ribonuclease						
$V_{\phi,N}^0$(exp)[b]	9610	9800	10040	10280	10520	10770
$V_{\phi,U}^0$(exp)[b]	9700	9960	10290	10620	10950	11280
V_ϕ^0(cal)	9950	10110	10420	10700	10970	11290

[a] All values are expressed in cm$^3\cdot$mol^{-1}.

[b] The values were obtained by linear least-square fitting of the experimentally measured values listed in Table IV. The $V_{\phi,N}^0$(exp) values of cytochrome C and myoglobin do not include the partial volume of a heme group, which, according to our estimate, is equal to 400 cm$^3\cdot$mol^{-1}.

values of V_ϕ^0(cal) obtained by Eq. (7) refer to the partial molar volume of proteins in the unfolded state with all amino acid residues fully exposed to the solvent, and with the Lys and Arg side chains in ionized forms.

The partial molar volumes for polypeptide chains of four proteins (myoglobin, cytochrome C, ribonuclease, and lysozyme), calculated by Eq. (7), are presented in Table VII and in Figure 5. The amino acid compositions of these proteins were taken from Ref. 35. As it has been pointed out in the results section, the partial molar volumes of model compounds were measured experimentally for the temperature range from 5 to 85°C. Above this temperature, the values of V_ϕ^0 were obtained by extrapolation. Thus, the values of the partial molar volume of protein polypeptide chains for the temperature range from 85 to 125°C, calculated by Eq. (7), have a conditional meaning. This is indicated in Figure 5 by dashed lines.

The calculated values of partial molar volumes for the above-mentioned polypeptide chains of proteins can be compared with those obtained experimentally for the unfolded polypeptide chain (Table VII, Figure 5). As seen, there is a reasonable agreement between the experimental and calculated values. The average deviation between them does not exceed 3%. This means that the partial volume function calculated by Eq. (7) successfully describes the temperature dependence of the partial molar volumes of proteins in the unfolded state. It is interesting that this function describes the volume of the native protein as well. This is because the difference in volumes between the native and unfolded states of proteins is small. The astonishing fact has not yet found a plausible explanation but the following consideration may help in its understanding.

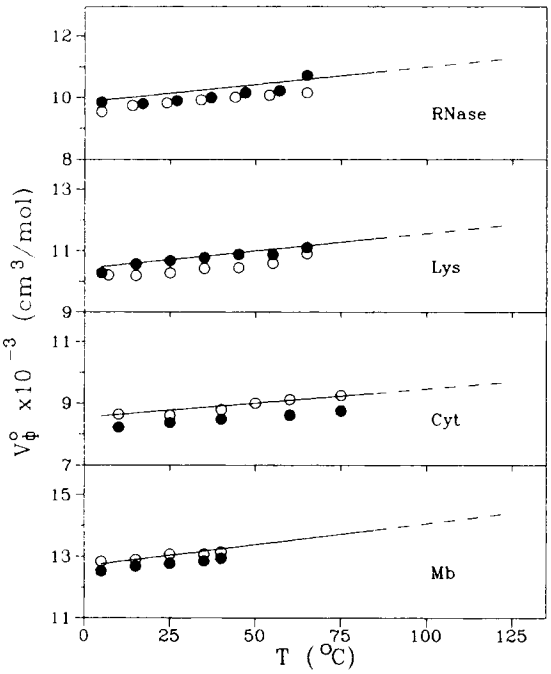

Figure 5. Temperature dependence of the partial molar volumes for ribonuclease A, lysozyme, cytochrome C, and myoglobin. The solid lines indicate the calculated values, the dashed extension of the solid lines indicate the extrapolated values. Open and solid circles indicate experimentally measured values for the native and unfolded states, respectively.

Using the van der Waals volumes of amino acid residues, given in Table VI, one can easily calculate by Eq. (7) the van der Waals volumes of the unfolded polypeptide chains: 9496 $cm^3 \cdot mol^{-1}$ for apomyoglobin, 6379 $cm^3 \cdot mol^{-1}$ for apocytochrome C, 7965 $cm^3 \cdot mol^{-1}$ for carboxymethylated lysozyme, and 7581 $cm^3 \cdot mol^{-1}$ for carboxymethylated ribonuclease. Thus, in all cases, as can be expected, the van der Waals volumes V_{vw} of the polypeptide chains are smaller than the partial volumes V_ϕ^0. For the parameter $\xi = V_{wv}/V_\phi^0$ we get 0.744, 0.764, 0.734, and 0.761 for apomyoglobin, apocytochrome, lysozyme, and ribonuclease, respectively. It is most interesting that these values are very close to the packing densities of native proteins that, according to Richards,[36,37] are in the narrow range from 0.70 to 0.78. Thus, it is not surprising that protein unfolding is not accompanied by significant changes in volume. What is surprising is the fact that the packing density of the native protein is so close to the packing density of the unfolded polypeptide chain in aqueous solution.

The practical significance of the proposed method for the calculation of the partial molar volumes of proteins by a simple summation of the volume contributions of individual amino acid residues is evident: it provides an opportunity to calculate the temperature dependence of the partial molar volume for any protein with a known amino acid composition over a broad temperature range.

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