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Thermodynamics of interaction of water vapour with 20 different poly-L-amino acids

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Abstract

The uptake of water vapour by 20 different polyaminoacids have been evaluated by an isopiestic vapour pressure technique in absence of solute at three different temperatures (22°C, 30°C and 37°C). The water vapour adsorption isotherm for different polyaminoacids in the range of water activity varying between zero and unity apparently agreed with that expected from a type III BET isotherm. From the linear BET plots, obeyed in the lower range of water activity, the BET constants n_m and Q_m for different polyamines have been evaluated. The amount of water vapour adsorbed (n_1)was calculated in moles/kg of polyaminoacids as well as in moles/mole of amino acid residue. Its value at unit water activity (Δn_1^o) has been evaluated by an extrapolation method and the results support that the multilayer adsorption of water vapour at the interface of polyaminoacids takes place. Values of Δn_1^o are strictly comparable in terms of moles per kg rather than mole per mole unit. In case of β lactoglobulin (βlg), lysozyme and BSA, theoretically obtained Δn_1^o values were observed to be considerably larger than experimental values of Δn_1^o. This indicated that amino acid residues in the polypeptide release a large amount of water due to the formation of a globular structure. Using the Bull equation in the integrated form, standard free energies, ΔG_w^o for water–polyamino acid binding interaction at two different temperatures have been evaluated. Based on the Clausius–Clapeyron equation in an integrated form, the integral enthalpy for water–polyamino acid interaction has also been evaluated.

Keywords: Isopiestic technique; Adsorption isotherm; Polyaminoacids; Free energies; Water–polyamino acid interaction

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1. Introduction

Synthetic polyamino acids play an important part in detecting the role of individual units of L-amino acids in proteins of known amino acid sequence to the total observed property of the protein. Scheraga and co-workers [1] published an interesting series of reports covering this important aspect of protein and polypeptide chemistry based on the α-helical form of a series of homopolyamino acids, e.g. polyglycine, poly L-alanine, poly L-leucine and poly L-valine. The data obtained from such a series are particularly informative with respect to the effects of hydrophobic interaction upon formation of the α-helix [2], because of the increase in size of the non-polar side chains with the ascending homologous series. However, as the size of the non-polar side chain increases, the solubility of the polyamino acid decreases.

The importance of solvent–solute interactions for the study of conformation of protein type macromolecules can be better understood if the hydration of the constituent polyamino acids are known. Hydration of a series of proteins and their constituent amino acids were measured by Kuntz and coworkers [3,4]. The NMR technique used by them indicates that there is no general correlation between the line width of the water resonance peak and the nature of the ionic side chains of the polypeptides [5]. However, the nature of helicity is clear from NMR study, as the polypeptides that are considered to exist in a random coil conformation at the room temperature exhibit sharper water lines [3] than the corresponding polypeptides in a helical conformation. The change in line width occurs over a pH range close to that expected from conventional solution studies [6]. However, tentative proposals for the hydration of poly amino acids were made by Kuntz et al. [7,8]. Glasel [9] reported deuteron magnetic spin–lattice relaxation experiments on D_2O solutions of poly L-glutamic acid, poly L-lysine, etc. He concluded that poly L-glutamic acid interacts strongly with the solvent in an ionised state (negatively charged), but monomers reveal very little interaction. The interaction of the macromolecules decreases with the increasing degree of neutralisation of the carboxylate group, and reaches zero at pH 6. It then increases rapidly, as the polymer becomes completely neutralised. Matohisar and Tatsuo [10] computed the thermodynamic properties of hydrated water associated with proteins of known three-dimensional structure and they obtained average values of hydration free energy, enthalpy and heat capacity of unfolding for every amino acid residue.

In our laboratory, for several years, the study of binding of water to powdered proteins [11–17], nucleic acids [18], surfactants [19] and powdered solids [20,21], both in the absence and presence of neutral salts, have been investigated using isopiestic vapour pressure technique. The results of such studies have been utilised for the evaluation of thermodynamic parameters related to the water–biopolymer, water–surfactant and water–powdered solid interactions. In this paper, such studies will now be extended to investigate the thermodynamic aspects of interaction of water with 20 different polyamino acids. An attempt will be made also to compare hydration of 20 different amino acid residues of poly L-amino acids with the hydration of these residues occurring in different globular proteins, possessing primary, secondary and tertiary structures in aqueous environment.

2. Materials and method

In the present investigation, 19 different poly L-amino acids, all chemicals of high quality were purchased from Sigma Chemical Company, USA. Only sample 14 from Peptide Institute, Japan was a gift from Prof S. Ikeda of Nagoya University, Japan. The names of the poly amino acids with sample number and average molecular weight (AMW) are as follows:

Cysteine obtained from Sigma was benzoylated at the side-chain sulfur atom.

(1)Poly-S-cbz L-cysteine (No P0263, AMW 5000–15 000); (2) poly L-tyrosine (No P 7887, AMW 40 000–100 000); (3)poly L-threonine (No P 8077, AMW 2000–15 000); (4) poly L-glutamine (No 8202, AMW 2000–15 000); (5) Na poly L-aspartate (No. P 6762, AMW 15 000–50 000); (6)

poly L-lysine bromide (No. P 7890, AMW 15 000–30 000); (7) poly L-arginine chloride (No. P 7762, AMW 15 000–70 000); (8) poly L-histidine chloride (No. P 2534, AMW 15 000–50 000); (9) poly L-alanine (No. P. 5517, AMW 10 000–25 000); (10) poly L-tryptophan (No. P 4647, AMW 1000–5000); (11) poly L-isoleucine (No. P 3329, AMW 5000–15 000); (12) poly L-glycine (No. P0254, AMW 15 000); (13) poly L-serine (No. P5887. AMW 5000–10 000); (14) Na poly L-glutamate (No. P4761, AMW 15 000–50 000); (15) poly L-valine (No. P3908, AMW 5000–10 000); (16) poly L-leucine (No. P5762, AMW 3000–15 000); (17) poly L-proline (No. P2254, AMW 1000–10 000); (18) poly L-phenyl alanine (No. P6886, AMW 2000–5000); (19) poly L-methionine (No. P2908, AMW 5000–15 000); and (20) poly L-asparagine (No. P8137, AMW 5000–15 000).

AR grade sulfuric acid, obtained from E. Merck, India was used. Double distilled water was used throughout the investigation.

Bull and coworkers [22,23] had previously developed the isopiestic vapour pressure method for the quantitative measurement of hydration of proteins. The method has been used by Chattoraj and co-workers for the study of hydration of powdered proteins like BSA [11,12,24], haemoglobin [24], casein [25], etc. They have further utilised this method for the study of hydration of alumina [20], silica [20], barium sulfate [21] and powdered surfactants [19]. In the present study also, the same isopiestic method was used for the study of hydration of different poly-L-amino acids.

Before use, all the PAA's were completely dried by keeping them in a vacuum desiccator, containing concentrated H_2SO_4. The moisture content of PAA was determined by drying a given weight of the sample at 105°C in a vacuum drier at 105°C for 24 h until constant weight was attained. For PAA samples containing polar non-ionic and ionic side chain residues, adsorption experiments were carried out at lowest values of P/P_o, and the samples at the end of the experiments were then dried in the same weighing bottle at 105°C for 24 h to obtain the correct weight of the dry PAAs free of water.

For the study of hydration of PAA's, in absence of solute, a definite weight (W_{PAA}) of dry polyamino acid was taken in a previously weighed specially designed weighing bottle. The lid of the weighing bottle was taken out and the bottle containing the powdered sample was allowed to float on a H_2SO_4 solution, called the reference solution, taken in a desiccator which was evacuated appropriately, and kept in an air-thermostat for 5 days at a fixed temperature (accuracy ± 0.1°C). The reference solution was frequently stirred for 5 days until a state of isopiestic equilibrium between the hydrated sample in the bottle and the reference solution was reached. The sample bottle was taken out and weighed, from which the moles (n_1) of water vapour adsorbed per kilogram of dry poly L-amino acid was calculated. The concentration of H_2SO_4 in the reference solution at equilibrium was estimated titrimetrically and the corresponding value of relative humidity (p/p^o) was obtained from the standard table [26]. The standard deviation (S.D.) of measurement of n_1 was found not to exceed 3%.

3. Results and discussion

Naturally occurring polypeptides and proteins of various types result from the polymerisation of 20 different amino acids. These amino acids are differentiated by the 20 different side chain (R) groups and the amino acid residues are linked to each other by peptide bond (–NHCO–). In the case of proline, linkage with other amino acid residues occur through the pyrolidine group. Twenty different side chains of the amino acid residues occurring in a protein molecule may be broadly divided into three groups: (a) hydrophobic groups; (b) hydrophilic or polar, but non-ionic groups; and (c) strongly hydrophilic cationic and anionic groups. Organic formulae of side chain groups are present in Tables 1–3. The peptide link –NHCO– present in between two amino acid residues in the polypeptide chain is known to have negligible attraction for water [28,29]. The end groups of a polypeptide chain also contain either COO^- or NH_3^+, but if the chain contains more than 100–1000 amino acid residues, the effect of hydration of the end groups

may be neglected in comparison to the hydration of a large number of R groups present in the chain. Thus, the hydration of the side chain residues are major factors for protein–water or polypeptide–water interactions.

In Figs. 1–3, values of n_1 for different polyamino acids have been plotted against water activities a_1 (equal to p/p_o according to Raoult's law) at several temperatures. At a fixed temperature, values of n_1 for each polyamino acid increase slowly at first with increase in a_1 from zero, but as the water activity exceeds the value 0.90, the value of n_1 sharply increases with further increase of a_1 close to unity. This indicates that the adsorption of water molecules in this region of a_1 on the surface of each type of polyamino acid is multimolecular in nature. In the lower range of a_1, BET Eq. (1) is found to be obeyed by each polyamino acid:

$$\frac{a_1}{n_1(1-a_1)} = \frac{1}{n_m K_B} + \frac{K_B - 1}{n_m K_B} a_1 \quad (1)$$

The linear plot of the left side of this equation against a_1 in the low-pressure range is shown in Fig. 4. The shape of each isotherm apparently agrees with that expected from type III BET isotherm [27]. From the slope and intercept of the

Table 1
Values of M_R, n_m, n_m^R, Q_m and n_1 for hydration of poly L-amino acids with non-polar R group at $T = 303$ K

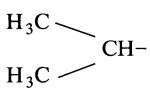
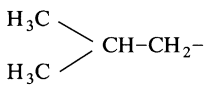
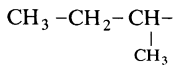
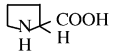
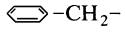
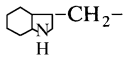
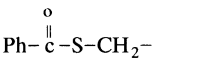
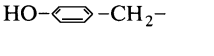
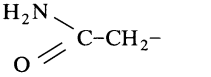
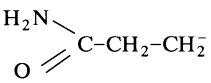
Sample	M_R Mol. wt of amino acid residue	Molecular formula of side chain (R) of amino acid residue	n_m Moles of H_2O/kg of poly L-amino acid	n_m^R Moles of H_2O/Mole of amino acid residue	Q_m kJ/mole	n_1 at $a_1 = 0.92$	
						Moles of H_2O/kg of poly L-amino acid	Moles of H_2O/mole of amino acid residue
Poly L-Alanine	71.1	H_3C-	1.94	0.138	0.441	11.7	0.831
Poly L-Valine	99.1		0.860	0.080	3.44	6.05	0.60
Poly L-Leucine	113		0.540	0.054	0.261	4.20	0.475
Poly L-Isoleucine	113		0.636	0.072	0.629	4.80	0.543
Poly L-Proline	97.1		3.36	0.32	0.948	10.1	0.981
Poly L-Phenylalanine	147		0.350	0.050	2.44	1.30	0.191
Poly L-Tryptophan	186		0.314	0.058	2.25	2.75	0.512
Poly L-Methionine	131	$CH_3-S-CH_2-CH_2-$	0.380	0.040	1.10	4.40	0.577

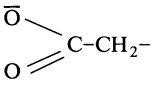
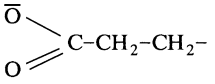
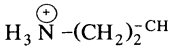
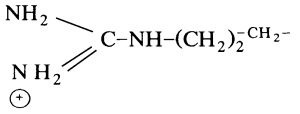
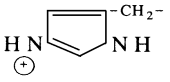
Table 2
Values of M_R, n_m,n_m^R, Q_m and n_1 for hydration of poly L-amino acids with uncharged polar R group at $T = 303$ K

Sample	M_R Mol. wt of amino acid residue	Molecular formula of side chain (R) of amino acid residue	n_m Moles of H_2O/kg of poly L-amino acid	n_m^R Moles of H_2O/Mole of amino acid residue	Q_m kJ/Mole	n_1 at Moles of H_2O/kg of poly L-amino acid	$a_1 = 0.92$ Moles of H_2O/mole of amino acid residue
Poly L-Glycine	57.1	H–	2.23	0.127	0.442	8.67	0.495
Poly L-Serine	87.1	$HO-CH_2-$	2.39	0.208	0.397	10.1	0.88
Poly L-Threonine	101	$CH_3-CH(OH)-$	3.31	0.335	5.79	22.0	2.22
Poly L-Cbz-Cysteine	107		0.093	0.019	4.85	0.325	0.067
Poly L-Tyrosine	163		2.04	0.337	1.02	6.50	1.06
Poly L-Asparagine	114		8.06	0.340	0.780	35.6	0.406
Poly L-Glutamine	128		5.78	0.740	0.401	144	18.4

linear plot, the BET constants n_m and K_B for different polyamino acids have been evaluated at 30°C. Here, n_m, equal to moles of water bound to free groups of PAAs directly for formation of a saturated monolayer, has been evaluated for different polymer systems. These values are included in Tables 1–3. Also K_B is equal to $e^{-Q_m/RT}$, where Q_m is equal to heat of adsorption for monolayer formation. Values of Q_m, evaluated from the monolayer plot, are also included in Tables 1–3.fig 4

The values of molecular weight M_R for different side chain groups, included in Tables 1–3, are different from each other so that by dividing n_m by $1000/M_R$, values of n_m^R moles of water adsorbed per mole of residue at monolayer saturation for different systems have been evaluated (vide Tables 1–3). It has been observed that nearly 1 mole of water is bound per mole of side chain groups of basic and acidic amino acid residues (vide Table 3), in agreement with the proposition by Pauling [30] earlier for different hydrophilic groups of protein at monolayer saturation. From Table 2, however, it is noted that, except for the glutamine residue, values of n_m^R for uncharged polar residues are significantly less than unity (vide Table 2). Based on Bull's [22] data on the isopiestic hydration of various proteins, Pauling [30] has proposed that, for uncharged polar side chain groups also, n_m^R should be close to unity. The present results on polyamino acids do not agree with this viewpoint.

Table 3
Values of M_R, n_m,n_m^R, Q_m and n_1 for hydration of poly L-amino acids with ionic polar R group at $T = 303$ K

Sample	M_R Mol. wt of amino acid residue	Molecular formula of side chain (R) of amino acid residue	n_m Moles of H_2O/kg of poly L-amino acid	n_m^R Moles of H_2O/mole of amino acid residue	Q_m kJ/mole	n_1 at Moles of H_2O/kg of poly L-amino acid	$a_1 = 0.92$ Moles of H_2O/mole of amino acid residue
Na-Poly L-Aspartate	137		10.0	1.37	0.466	80.0	11.0
Na Poly L-Glutamate	150		8.73	1.31	0.209	79.3	11.9
Poly L-Lysine Bromide	209		3.75	0.784	0.512	46.0	9.61
Poly L-Arginine Chloride	193		5.75	1.11	0.409	24.0	4.62
Poly L-Histidine Chloride	174		4.57	0.793	0.693	43.0	7.46

According to Pauling [30], the hydrophobic sidechain group should not significantly adsorb any water molecule for monolayer formation. Our values, presented in Table 1, indicate that, except for the proline residue, values of n_m^R are less than 0.10, in qualitative agreement with the viewpoint of Pauling [30]. For proline, n_m^R is 0.32, which indicates that this residue behaves exceptionally because it contains a pyrolidine group. Since n_m^R values for hydrophobic side chains are not negligible, therefore, additive values of n_m^R for total non-ionic polar group plus hydrophobic group present in protein may be close to unity. Only in this manner will Pauling's analysis of protein hydration at monolayer state become valid.

In Tables 1 and 2, values of Q_m, representing the heats of adsorption of H_2O at monolayer saturation for hydrophobic residues of poly L-valine, poly L-phenyl-alanine and polar uncharged poly-S-cbz L-cysteine, are relatively high. For poly L-proline and poly L-tyrosine, the values of Q_m are moderate in magnitude, whereas for other polar and non-ionic side chains, the energies of adsorption are relatively low. However, in all cases, values of Q_m are of the same order of magnitude. All values of Q_m are expected to be close to or less than heat of evaporation of liquid water [27], so that monolayer points are not sharply exhibited in water vapour isotherm for different polyamino acids (vide Figs. 1–3). For this reason, the shape of each BET isotherm assumes the expected shape for type III rather than type II BET isotherms [27].

Let us now focus our attention on comparative values of n_1 for different polyamino acids above 0.90 water activity, when multilayer adsorption of

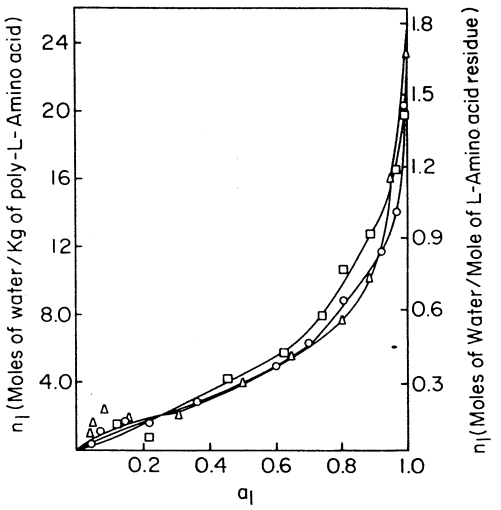


Fig. 1. Plot of η_1 vs. a_1 for poly-L-alanine at (□) 22°C, (○) 30°C, (△) 37°C.

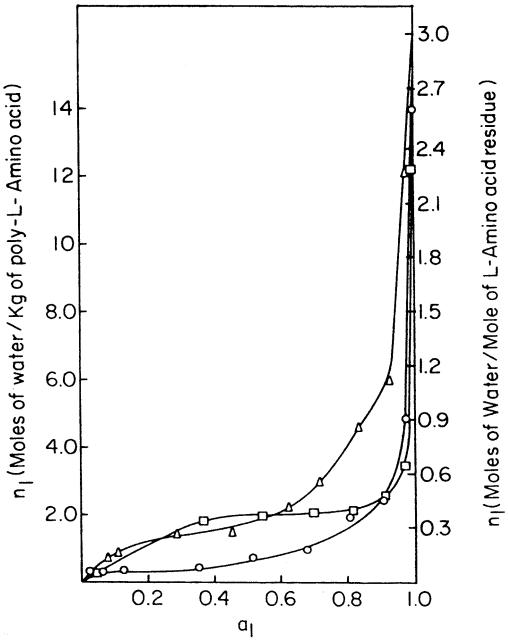


Fig. 2. Plot of η_1 vs. a_1 for poly-L-tryptophan at (□) 22°C, (○) 30°C, (△) 37°C.

water vapour at the interface of polyamino acids take place. Above $a_1 = 0.90$, n_1 sharply increases

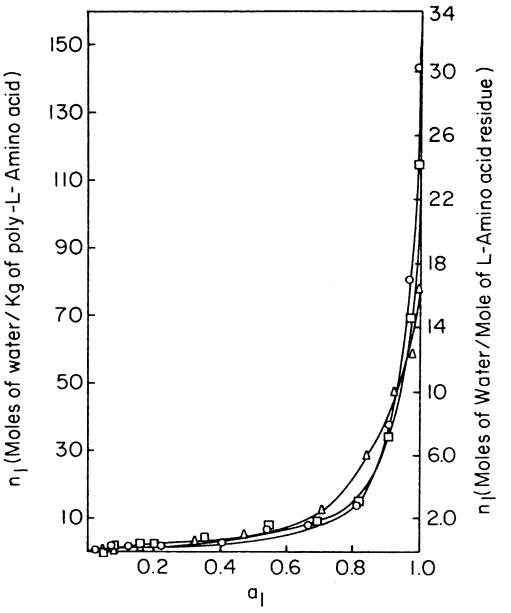


Fig. 3. Plot of η_1 vs. a_1 for poly-L-lysine buomide at (□) 22°C, (○) 30°C, (△) 37°C.

with an increase of a_1. In all cases, values of n_1 extrapolated to unit water activity have been evaluated [11]. Such extrapolated values of n_1 (termed as Δn_1^o) in moles per kg as well as mole per mole of amino acid residues are presented in Tables 4–6 and 10 for critical comparison.

It is found from Table 4 that, even for amino acid residues containing hydrophobic non-polar groups, Δn_1^o in mole/unit varies from 1 to 6. These values are 7–88-fold higher than that of n_m^R as a result of a large increase in hydration in the range of a_1 varying between 0.90 and 1.0. Values of Δn_1^o in mole per mole basis stand in the following order of amino acid residues:

isoleucine > methionine > tryptophan > proline > phenylalanine > alanine > leucine > valine.

It may be pointed out that molecular weight of amino acid residues are different, so that the extensive quantity Δn_1^o is strictly comparable in terms of moles per kg rather than mole per mole unit. Values of Δn_1^o in moles of water per kg of polyamino acid stands in the order:

Table 4
Values of Δn_1^o and $-\Delta G_W^o$ for hydration of poly L-amino acids with non-polar R group at $T = 303$ K

Sample	$\Delta n_1{}^o$		$-\Delta G_W{}^o$	
	Moles of H_2O/kg of Poly L-amino acid	Moles of H_2O/mole of amino and residue	kJ/kg of poly L-amino acid	kJ/mole of amino acid residue
Poly L-Alanine	22.1	1.57	27.0	1.92
Poly L-Valine	11.5	1.14	8.10	0.803
Poly L-Leucine	13.0	1.47	3.90	0.441
Poly L-Isoleucine	55.0	6.23	13.4	1.52
Poly L-Proline	23.4	2.27	10.1	0.981
Poly L-Phenyl Alanine	14.0	2.06	1.80	0.264
Poly L-Tryptophan	15.0	2.79	6.58	1.23
Poly L-Methionine	26.6	3.49	4.50	0.590

isoleucine > methionine > proline > alanine > tryptophan > phenylalanine > leucine > valine.

A comparison of the order of Δn_1^o in mole per mole unit is of interest as a physical concept, but, in terms of mole per kg, is possibly right from a thermodynamic standpoint. This will be clarified later on.

Values of Δn_1^o in mole per mole unit for polar but non-ionic residues of polyamino acids given in Tables 5 and 10 will be now considered. Values of Δn_1^o for these types of side chains vary from 1 to 60 moles of H_2O per mole residue. From Table 5, it is also noted that, except for poly L-threonine and poly L-glutamine, the extent of variation of Δn_1^o is exactly similar to that observed for amino acid residues containing exclusively hydrophobic groups. Thus, the secondary hydration of the side chain polar groups may possibly have a major contribution from multilayer adsorption of water molecules on the surface of PAA powder. The order of polyamino acids in terms of Δn_1^o in mole per mole basis stands thus:

glutamine > theronine > asparagine > serine > tyrosine > glycine > cbz-cysteine.

In terms of moles per kg of amino acid residue, this order is again observed to be slightly different from the above mentioned order of Δn_1^o (vide Table 5). It may be pointed out that although the hydration of residues at $a_1 \to 1$, containing polar uncharged groups are similar in order to that of

Table 5
Values of Δn_1^o and $-\Delta G_W^o$ for hydration of poly L-amino acids with uncharged polar R group at $T = 303$ K

Sample	$\Delta n_1{}^o$		$-\Delta G_W{}^o$	
	Moles of H_2O/kg of Poly L-amino acid	Moles of H_2O/mole of amino acid residue	kJ/kg of poly L-amino acid	kJ/mole of amino and residue
Poly L-Glycine	18.4	1.05	24.6	1.40
Poly L-Serine	17.2	1.50	2.10	0.183
Poly L-Threonine	200	20.2	51.4	5.20
Poly L-Cbz-cysteine	2.75	0.57	1.88	0.390
Poly L-Tyrosine	9.00	1.47	22.2	3.62
Poly L-Asparagine	82.0	9.36	32.8	3.75
Poly L-Glutamine	465	59.6	122	15.6

Table 6
Values of Δn_1^o and $-\Delta G_W^o$ for hydration of poly L-amino acids with ionic polar R group at $T = 303$ K

Sample	Δn_1^o		$-\Delta G_W^o$	
	Moles of H_2O/kg of poly L-amino acid	Moles of H_2O/mole of amino acid residue	kJ/kg of poly L-amino acid	kJ/mole of amino acid residue
Poly L-Aspartate	410	56.2	122	16.7
Na-poly L-Glutamate	176	26.4	130	19.5
Poly L-Lysine Bromide	83	17.3	47.4	9.91
Poly L-Arginine Chloride	170	32.8	46.9	9.04
Poly L-Histidine Chloride	185	32.1	59.9	10.4

the residues, containing hydrophobic groups. (vide Tables 4 and 5), the range of values of n_m^R involving monolayer hydration are widely different in two cases (vide Tables 1, 2, 4 and 5).

In Table 6, values of Δn_1^o for residues containing ionic side chain groups in mole per mole, as well as mole per kg unit, are presented. Values of Δn_1^o in these cases vary from 17 to 56, compared to the range of monolayer hydration n_m^R shown to be close to unity (vide Table 6). The order of amino acid residues in terms of values of Δn_1^o in mole per mole unit stands as:

aspartate > glutamate > arginine > histidine > lysine.

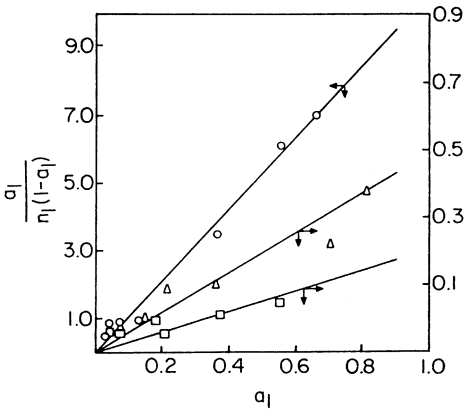


Fig. 4. BET plot for (○) poly-L-cbz-cystine, (△) poly-L-Alanine, (□) poly-L-histidine chloride at 30°C.

The order will be different, if Δn_1^o is expressed in terms of moles per kg of PAA (vide Table 6).

Klotz [29] suggested that water bound to protein at a high humidity region could assume an ice like structure induced by the non-polar parts of the amino acid residues. Bull and Breese [22] suggested that all the water adsorbed by proteins at 0.92 water activity strongly interacts with the biopolymer. The interaction above this relative humidity was regarded by them as weak. The weakly-held water can be very easily squeezed out of the protein glass formed at and beyond $a_1 = 0.92$. This excess water may be regarded as water of crystallisation of protein crystals. They have measured water uptake by several proteins at 0.92 water activity (vide Table 11). A plot of the uptake against the effective number of hydrophilic groups (carboxylic, amino, etc.) was made by these workers. From these results, they concluded that each hydrophilic group of protein could be complexed with six molecules of water. However, the amino groups of asparagine and glutamine occurring in protein do not complex in this manner. Furthermore, each amino group present in protein may break up the structure of six molecules of water-bound or complexed with neighbouring carboxyl, amino or imino groups of the polypeptide chain.

In Tables 1–3, values of n_1 at 0.92 water activity for 20 different amino acids are presented. It is noted with interest that moles of water associated per mole of hydrophobic residue vary from 0.20 to 0.80. For proline, however, n_1 is equal to

1. It appears that the hydration of hydrophobic non-polar amino acid residues have increased significantly from monolayer values n_m^R. On the other hand, values of n_1 at 0.92 water activity for non-ionic polar residues are considerably small compared to 6.0 moles/mole of polyamino acid residue, as expected for different proteins studied by Bull et al. [23]. For poly L-tyrosine, poly L-threonine and poly L-glutamine, values of n_1 are 1, 2 and 18, respectively, on a mole per mole basis, whereas for other side chains, n_1 is less than unity. Therefore, the structure-breaking effect of an amide group of glutamine in protein is absent in poly L-glutamine. Furthermore, n_1 values for most other cases are less than unity, but to some extent, greater than n_m^R.

At 0.92 water activity, n_1 for polyaminoacids with ionic side chain groups in mole/mole basis vary from 5 to 12, which is not unexpected. However, Bull's contention that these values should be 6 is not quantitatively valid for polyamino acids.

From all these discussions, it may be concluded that the hydration of side chain groups of polyamino acids may be broadly divided into three stages. These three stages are primary hydration in monolayer state, secondary hydration at $a_1 =$ 0.92 and tertiary hydration at unit water activity. At a_1 equal to zero, the intermolecular and intra-chain attraction within macromolecules forming solid powder, in all cases, must be very high. Upon exposing these particles made of ionic residues to water vapour at lower values of a_1, the intermacromolecular attraction in the bulk phase and the surface free energy at the solid–air interface will be reduced. Due to this, all ionic groups, along with their counter-ions (in the form of ion-pairs), will gradually appear from the bulk to the interface for monolayer hydration. At the completion of primary hydration, each ion-pair may adsorb one molecule of H_2O from the vapour state so that n_m^R in mole/mole unit becomes close to unity.

When the solid particles made of hydrophobic residues are similarly exposed to water vapour, the rigid particles with a small exposed area of the hydrophobic interface may adsorb only four molecules of H_2O, so that values of n_m^R will be quite low in most cases. However, for PAAs with polar non-ionic residues, a major fraction of the polar group will be transferred from the bulk to the surface of the particle, and so n_m^R will increase due to increase in water binding sites. The values of n_m^R are still less than unity (Table 2).

At the stage of secondary hydration ($a_1 = 0.92$), more H_2O molecules from the condensed vapour phase will be adsorbed by PAAs containing ionic groups. The ion-pairs begin to dissociate at this stage, so that 5–12 molecules of H_2O per residue may be bound to the residue and their counter-ions (Table 3).

At $a_1 = 0.92$, values of n_1 on a fixed and rigid surface of non-polar residues will increase with the formation of multilayers (vide Figs. 1 and 2 for polyalanine and polytryptophan). Under the same condition for PAAs with polar residues, more water binding sites will remain exposed so that values of n_1 will vary between 0.5 and 18 (except CBZ derivative of polycysteine) and adsorbed water molecules form multilayers.

In the tertiary state of hydration, values of Δn_1^o for ionic residues are large (vide Table 6) so that multilayer formation has largely been extended. Such increases in the values of Δn_1^o for polar residues are moderate, whereas they are minimal for non-polar residues (Tables 4 and 5).

Δn_1^o includes all types of H_2O molecules packed in primary, secondary and layers, thus forming an inhomogeneous surface phase. As in the case of water adsorption by different proteins, the plot of water activity gradient da_1/dn_1, against n_1 for different PAAs are found to be different. Only at a_1 equal to unity does the activity gradient da_1/dn_1 become zero, so that water bound to PAA may remain in equilibrium with that of the inhomogeneous bulk phase of water.

Kuntz and co-workers [3] performed an interesting study of hydration of a variety of proteins and their constituent amino acids, using proton NMR technique. The method involved rapid freezing of the protein solution in liquid nitrogen, followed by equilibriation at the temperature of the experiment (-35°C). The studies were extended to a wide range of polypeptides in a variety of conformational modes to determine the extent of hydration of each peptide residue. The

experiment was carried out at standard condition of −35°C and pH 6–8, with few exceptions. All the solutions contained the polypeptides at a concentration of 5–10% in 0.01 mol dm^{-3} KCl with the pH adjusted with KOH or HCl. The degree of hydration was calculated with the assumption that all residues in polypeptide chain are exposed to solvent and there was no buried residue. All amino acids with ionised side chains are heavily hydrated, e.g. Arg, His, Lys, Glu, Asp and non-polar proline residues contained 3–8 moles of water per mole of amino acid residue, but these values are found to be much lower than that predicted by our isopiestic method [vide Δn_1^o (mole/mole) in Tables 4–6] carried out at 30°C in the absence of neutral salt.

In isopiestic experiments carried out at 30°C, both primary, secondary and tertiary hydration of water to protein were measured in the present case at the state of thermodynamic equilibrium. The water, in part, may remain frozen at −35°C and only tightly-bound water molecules attached to PAAs are estimated by NMR in a frozen state. This is possibly the reason that the extents of hydration measured by NMR are indeed quite low. Bull and Breese [22,23] as well as Chattoraj and Bull [18] had noted that the magnitudes of hydration of proteins and nucleic acids evaluated by the isopiestic method at 25°C and at $a_1 \to 1$ are considerably higher than those measured by calorimetric studies at −7°C [33].

The standard free energy change (ΔG_W^o) due to the hydration of PAAs as a result of the change of water activity from zero to its standard value of unity is calculated from the Bull's equation [23,31]:

$$\Delta G_W^o = -RT\int_{a_1=0}^{a_1=1} \frac{n_1}{a_1}\,\mathrm{d}a_1. \qquad (2)$$

From the graphical evaluation of the area under the curve obtained by plotting n_1/a_1 as a function of a_1 between the limits $a_1 = 0$ to $a_1 = 1$, the value of the integral for each polyamino acid and, hence, ΔG_W^o has been evaluated. ΔG_W^o has been expressed in kJ per kg of PAA and not in kJ per mole of residue, for strict comparison of the affinity of water molecules of different polyamino acids. These are given in Tables 4–6. It is interesting to note that ΔG_W^o for all 20 polyamino acids are negative in sign. This means that the hydration of dry PAAs is thermodynamically spontaneous process at the standard condition.

It is noted from Table 6 that the magnitude of ΔG_W^o are very high for polyamino acids with ionic side chains, since the affinity for water molecules of ionic R groups is very high. ΔG_W^o stands in the following order for PAAs with ionic side chains:

glutamate > aspartate > histidine > lysine
≅ arginine.

ΔG_W^o for PAAs containing non-polar side chain groups are significantly lower in magnitude than that of ionic groups. ΔG_W^o values of these polyamino acids stand in the order (vide Table 4).

alanine > isoleucine > proline > valine
> tryptophan > methionine > leucine
> phenylalanine.

In Table 5, the values of ΔG_W^o for polyamino acids containing side chain with polar non-ionic groups stand in the order:

glutamine > threonine > asparagine > glycine
> tyrosine > serine > cbz-cysteine.

Furthermore, the value of ΔG_W^o for glutamine is comparable to that of glutamate. Values of $-\Delta G_W^o$ for threonine are similar to that of lysine and arginine. On the other hand, ΔG_W^o values for serine and cbz-cysteine are comparable to those of phenylalanine and many other polyamino acids containing hydrophobic groups.

In Table 7, values of ΔG_W^o and Δn_1^o for a few PAAs measured at different temperatures have been presented. From this table, it appears that values of ΔG_W^o and Δn_1^o depend, to some extent, on temperature. From these data, the average value of $(\Delta G_W^o)_{av}$, equal to $1/2[(\Delta G_W^o)_1 + (\Delta G_W^o)_2]$ for an average temperature $1/2(T_1 + T_2)$, can be evaluated using an additivity rule.

The integral enthalpy of hydration of different polyamino acids have been calculated using the

Table 7
Values of $(\Delta G_W^o)_{av}, (\Delta H_W^o), T_{av}(\Delta S_W^o)$ and (ΔS_W^o) for hydration of poly L-amino acids with non-polar R group

Sample	T_{av} Temp (K)	$-(\Delta G_W{}^o)_{av}$ kJ/kg of poly L-amino acid	$(\Delta H_W{}^o)$ kJ/kg of poly L-amino acid	Tav $(\Delta S_W{}^o)$ kJ/kg of poly L-amino acid	$(\Delta S_W{}^o)$ kJ/kg/K of poly L-amino acid
Poly L-Alanine	299	25.4	−283	−258	−0.863
Poly L-valine	299.5	13.4	443	456	1.52
Poly L-Leucine	299.5	7.70	309	316	1.05
Poly L-Isoleucine	299	13.5	−723	−710	−2.37
Poly L-Proline	299.5	16.1	574	590	1.97
Poly L-phenyl Alanine	299.5	9.1	31.1	33.2	0.111
Poly L-Tryptophan	299	7.80	−132	−124	−0.415
Poly L-Methionine	299.5	20.8	10.1	14.6	0.048

integrated form of the Clausius–Clapyron equation [21]:

$$\Delta H_W^o = -\frac{RT_1T_2}{T_2 - T_1}\int^{\Delta^{n_1^o}} \ln\frac{a_2}{a_1}\,\mathrm{d}n_1. \quad (3)$$

Here a_1 and a_2 stand for water activities at temperatures T_1 and T_2 for the same value of n_1 for a pair of temperatures T_1 and T_2. Values of ΔH_W^o for different PAAs at average temperature T_{av} equal to $1/2(T_1 + T_2)$ have been evaluated using Eq. (3).

The average entropy change ΔS_W^o for the integral process of hydration of PAAs are calculated using the thermodynamic relation:

$$\Delta G_W^o = \Delta H_W^o - T_{av}\Delta S_W^o \quad (4)$$

Values of $(\Delta G_w^o)_{av}, \Delta H_w^o$ and ΔS_w^o at different values of T_{av} are presented in Tables 7–10 for polyamino acids belonging to hydrophobic, non-ionic polar and ionic side chain groups.

From Table 8, we note with interest that ΔH_W^o for glycine is almost zero, so that hydration of glycine is totally controlled by entropy. ΔH_W^o, as well as $T_{av}\Delta S_W^o$ for other polyamino acids, may be positive or negative in magnitude. The magnitude of ΔH_W^o and $T_{av}\Delta S_W^o$ are also close to each other and the range of magnitude variation of these quantities are significantly wide and the signs of these quantities may be positive of negative.

In Fig. 5, plot of ΔH_W^o against ΔS_W^o for different PAAs is found to be linear, which means that entropy–enthalpy compensation effect is in operation for hydration of 20 different side chain groups of poly L-amino acids.

Table 8
Values of $(\Delta G_W^o)_{av}, \Delta H_{Wav}^o T_{av}(\Delta S_W^o)$ and (ΔS_W^o) for hydration of poly L-amino acids with uncharged polar R group

Sample	T_{av} Temp (K)	$(\Delta G_W{}^o)_{av}$ kJ/kg of poly L-amino acid	$(\Delta H_W{}^o)$ kJ/kg of poly L-amino acid	Tav $(\Delta S_W{}^o)$ kJ/kg of poly L-amino acid	$(\Delta S_W{}^o)$ kJ/kg/K of poly L-amino acid
Poly L-Glycine	308	24.6	0	−24.6	−0.08
Poly L-Threonine	299	54.8	57.8	113	0.378
Poly-L-cbz-cysteine	299	1.84	−7.66	−5.82	−0.019
Poly L-Tyrosine	299	15.2	453	468	1.57
Poly L-Asparagine	299.5	133	1050	1080	3.60
Poly L-Glutamine	299	57.4	−591	−526	−1.76

Table 9
Values of $(\Delta G_W^o)_{av}$, $\Delta H_W^o T_{av}(\Delta S_W^o)$ and (ΔS_W^o) for hydration of poly amino acids with ionic polar R group

Sample	T_{av} Temp (K)	$(\Delta G_W{}^o)_{av}$ kJ/kg of poly L-amino acid	$(\Delta H_W{}^o)$ kJ/kg of poly L-amino acid	Tav $(\Delta S_W{}^o)$ kJ/kg of poly L-amino acid	$(\Delta S_W{}^o)$ kJ/kg/K of poly L-amino acid
Na-poly L-Aspartate	299	133	-84.6	−23.7	−0.079
Na-poly L-Glutamate	308	107	3350	3457	11.2
Poly L-Lysine Bromide	299	48.1	199	219	0.734
Poly L-Arginine Chloride	299	58.6	−667	−608	−2.03
Poly L-Histidine Chloride	299	63	−504	−441	−1.47

The study of hydration of different globular proteins were made by Bull and Breese [22,23] extensively using isopiestic experiment at 25°C. Isopiestic hydration of BSA [32] was measured by Dutta, Hazra and Chattoraj at 30°C at various values of water activity. The values of $(n_1)_e^p$ at 92% relative humidity for different proteins from all these experiments are included in Table 11. The molecular weights and amino acid compositions of these proteins [33–36] are all known. Moles of water $(n_1)_{th}^P$ for these proteins can be computed from Eq. (5):

$$(n_1)_{th}^P = \Sigma N_i^{AA}(n_1)_i \quad (5)$$

Here N_i^{AA} stands for number of ith amino acid residues present in one molecule of globular protein. Values of N_i^{AA} for 20 different amino acids occurring in a globular protein molecule can be obtained from its amino acid composition. Values of $(n_1)_i$ for 20 different L-amino acid residues are included in Tables 1–3. Values of $(n_1)_{th}^P$ for different proteins at 30°C are given in Table 11. Neglecting slight difference in temperatures, the magnitudes of $(n_1)_{th}^P$ are observed to be significantly larger than experimental values of n_1^P, i.e. $(n_1)_e^P$. However, the order of $(n_1)_e^P$ and $(n_1)_{th}^P$ are surprisingly same. One can, thus, conclude from this that significant dehydration of amino acid residues will occur: (i) when different amino acids are arranged in sequence forming a primary sequence in a polypeptide chain; (ii) when the polypeptide chain thus formed undergoes α-helix or β-sheet conformation through hydrogen bond formation between different-NHCO-groups, thus forming secondary structure; (iii) when the polypeptide chain with secondary conformation undergoes a significant interaction between side chain groups leading to the tertiary folding of polypeptides; and (iv) when several polypeptides with tertiary folding specifically associate to form a quarternary structure. Thus, structure formation in protein from amino acids leads to large dehydration from the biopolymer as expected.

Values of $(\Delta n_1^o)_e^P$ for β-lactoglobulin [23] and lysozyme [37] at 25°C and BSA [32] at 30°C have also been estimated from isopiestic experiments. These values are 1592, 400 and 2210 moles of H_2O per kg of β-lactoglobulin, lysozyme and BSA, respectively. Replacing $(n_1)_{th}^P$ and $(n_1)_i$ in Eq. (5) by $(\Delta n_1^o)_{th}^P$ and $(\Delta n_1^o)_i$ respectively, values of $(\Delta n_1^o)_{th}^P$ are calculated to be 5432, 2192 and 8325 moles of H_2O per kg of βlg, lysozyme and BSA, respectively (Table 11). $(\Delta n_1^o)_{th}^P$ values are observed to be considerably larger than experimental values of $(\Delta n_1^o)_e^P$, which again indicate that amino acid residues in polypeptides release a large amount of water due to the formation of globular structure.

Values of standard free energy change $(\Delta G_w^o)_e^P$ for βlg [22], lysozyme [37], at 25°C and BSA [32] at 30°C, computed from Bull's equation [Eq. (3)] are 1530, 477 and 1230, respectively. Replacing $(n_1)_{th}^P$ and $(n_1)_i$ in Eq. (5) by $(\Delta G_w^o)_{th}^P$ and $(\Delta G_w^o)_i$ respectively, the theoretical value of standard free energy change for different proteins $(\Delta G_w^o)_{th}^P$ can be estimated. Here $(\Delta G_W^o)_i$ refers to standard free energy-change values of different amino acid

Table 10
Values of Δn_1^o, $-\Delta G_W^o$, $-(\Delta G_W^o)_{av}$, ΔH_W^o, $T_{av}(\Delta S_W^o)$ and ΔS_W^o for hydration of poly L-amino acids

Sample	T Temp (K)	$\Delta n_1{}^o$ Moles of H_2O/mole of amino acid residue	$-\Delta G_W{}^o$ kJ/kg of poly L-amino acid	T_{av} (K)	$-(\Delta G_W{}^o)_{av}$ kJ/kg of poly L-amino acid	$(\Delta H_W{}^o)$ kJ/kg of poly L-amino acid	$T_{av}(\Delta S_W{}^o)$ kJ/kg of poly L-amino acid	$(\Delta S_W{}^o)$ kJ/kg/K of poly L-amino acid
Poly	295	1.48	23.7	299	25.4	−283	−258	−0.863
L-Alanine	303	1.57	27.0	306.5	32.2	−64.6	−32.4	−0.106
	310	1.82	37.3					
Poly	295	4.25	13.6	299	13.5	−723	−710	−2.37
L-Isoleucine	303	6.23	13.4	306.5	18.4	−213	−195	−0.636
	310	2.83	23.4					
Poly	295	2.50	9.01	299	7.8	−132	−124	−0.415
L-Tryptophan	303	2.79	6.58	306.5	10.7	279	290	0.946
	310	3.00	14.9					
Poly	295	22.2	58.2	299	54.8	57.8	113	0.378
L-Threonine	303	20.2	51.4	306.5	47.2	−832	−785	−2.56
	310	18.6	42.9					
Poly	295	0.414	1.79	299	1.84	−7.66	−5.82	−0.019
L-Cbz-Cysteine	303	0.570	1.88	306.5	2.17	30.6	32.8	0.107
	310	1.24	2.45					
Poly	295	2.04	8.17	299	15.2	453	468	1.57
L-Tyrosine	303	1.47	22.2	306.5	58	−308	−289	−0.943
	310	1.39	93.7					
Poly	295	55.1	143	299	133	−591	−526	−1.76
L-Glutamine	303	59.6	122	306.5	108	−1787	−1722	−5.62
	310	54.4	93.7					
Na-poly	295	54.5	118	299	120	−84.6	−35.4	0.118
L-Aspartate	303	46.8	122	306.5	124	−123	−69.5	−0.227
	310	42.2	126					
Poly	295	23.4	48.8	299	48.3	199	219	0.734
L-Lysine	303	17.4	47.4	306.5	55.8	−54.7	−29.9	−0.098
Bromide	310	32.2	64.2					
Poly	295	29.1	70.2	299	58.6	−667	−608	−2.03
L-Arginine	303	32.8	46.9	306.5	51.8	168	220	0.718
Chloride	310	38.0	56.7					
Poly	295	29.2	66.1	299	63	−504	−441	−1.47
L-Histidine	303	32.1	59.9	306.5	64.8	323	388	1.27
Chloride	310	26.0	69.7					

Table 11
Theoretical and experimental values of $\Delta n_1^o, n_1$ and ΔG_W^o for hydration of different proteins[a1,b1,c1]

Sample	$\Delta n_1{}^o$ (Moles of H_2O/mole of protein)		n_1 at $a_1 = 0.92$ (Moles of H_2O/mole of protein)		$-\Delta G_W{}^o$ (kJ/mole of protein)	
	$(\Delta n_1{}^o)_e{}^P$	$(\Delta n_1{}^o)_{th}{}^P$	$(n_1)_e{}^P$	$(n_1)_{th}{}^P$	$(-\Delta G_W{}^o)_e{}^P$	$(\Delta G_W{}^o)_{th}{}^P$
β-Lactoglobulin	1592	5430	654	1790	1530	2110
Lysozyme	400	2190	197	525	477	536
Bovine serum albumin	2210	8320	956	2590	1230	2590
α-Chymo-Trypsinogen	–	748	374	673	–	748
Bovine-ribonuclease	–	1629	248	500	–	577
Horse heart cytochrome-*c*	–	1236	287	355	–	589
Bovine insulin	–	493	75	154	–	214

[a] Experimental values of all the data of the above proteins except BSA are at 25°C.
[b] Theoretical values of the all data of the above proteins are calculated for 30°C.
[c] Experimental values of all the data of BSA are at 30°C.

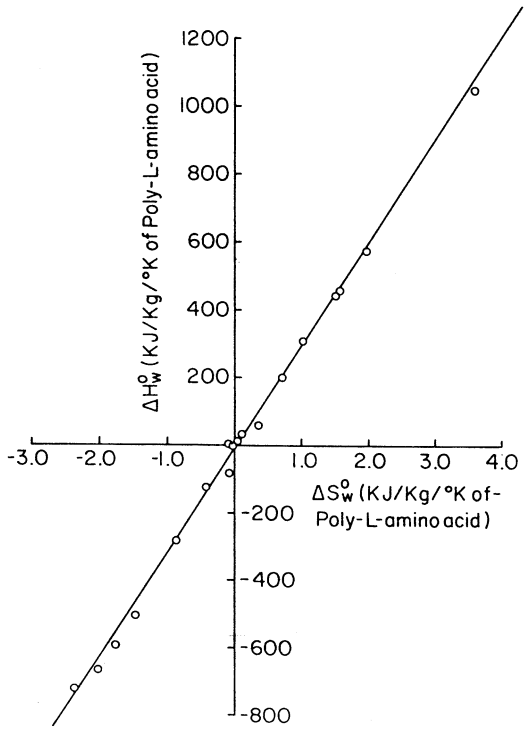


Fig. 5. ΔH_w^o vs. ΔS_w^o plot for 20 different poly-L-amino acids.

residues in kJ/kg unit given in Tables 4–6. $(\Delta G_w^o)_{\text{th}}^{\text{P}}$ values for βlg, lysozyme and BSA are 2110, 536 and 2590 kJ/kg of protein, respectively. $(\Delta G_w^o)_{\text{e}}^{\text{P}}$ values are considerably less than $(\Delta G_w^o)_{\text{th}}^{\text{P}}$.

We have already mentioned that the primary layer of water attached to proteins (and studied from BET analysis of the hydration data) may depend on the tight binding of water to polar and ionic groups of the biopolymer. The strong binding of water, forming the primary water layer of a polypeptide chain, is, in all probability, least affected when the extended chain containing different amino acid residues arranged in primary sequence is converted to a globular structure due to secondary, tertiary and quaternary folding of the biopolymer. All the polar groups in such folding states are in contact with water in the bulk phase. However, a major fraction of hydrophobic groups may be dehydrated and buried in the protein structure so that the hydrophobic hydration of hydrophobic groups of primary water layer is significantly reduced due to folding at p/p_o equal to 0.92 and unity. Further water-forming secondary and tertiary layers of amino acid residues in a polypeptide chain will be grossly reduced due to the folding of the biopolymer chain to form globular protein structure so that $(\Delta n_1^o)_{\text{exp}} \ll (\Delta n_1^o)_{\text{theoretical}}$.

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