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Thermodynamic studies of solvation of a series of homologous α-amino acids in aqueous mixtures of protic ethylene glycol at 298.15K

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ABSTRACT

Standard transfer Gibbs energies, ΔG^0_t(i) of a series of homologous α-amino acids(i) like glycine (gly), dl-alanine (ala), dl - α-amino butyric acid (aba) and dl -nor- valine (n-val) from water to aqueous mixture of protic ethylene glycol (EG) with 0, 20, 40, 60, 80 and 100 wt% EG compositions have been determined by measuring solubility of these amino acids at 25^{0}C using 'formal titrimetry'. The chemical effects of the transfer Gibbs energies of the α-amino acids(i), $\Delta G^0_{t,ch}$(i) have been obtained by subtracting the cavity effects, $\Delta G^0_{t,cav}$(i),estimated by the scaled particle theory and dipole-dipole interaction effects, $\Delta G^0_{t,d\text{-}d}$(i) calculated by means of the Keesom orientation expression. $\Delta G^0_{t,ch}$(i) are dictated by decreased hydrophobic hydration(H_bH) and the superimposed increased acidity and increased dispersion effects of EG-water mixtures as compared to that in water.

KEYWORDS

Zwitter ion;
α-amino acids;
Solvation of amino acids;
Hydrophobic hydration.

INTRODUCTION

It is well known that proteins play a key role in nearly all biological processes. The basic structural units of proteins are amino acids. The side chains of these building blocks differ in size, shape, charge, hydrogen bonding capacity, hydrophobicity and chemical reactivity. Much attention has been given[1-11] to determine the various thermodynamic properties such as molar volume[1], enthalpy and entropy of solutions[3,7-9], solubility[7-10] of various amino acids in different aqua-organic media. Theoretical[4-6] as well as experimental [7-10] studies on amino acid solvation in different protein stabilizing (i.e glycerol) and denaturing media (i.e urea) were also performed. The purposes of such studies were to gain the various mechanism of amino acid solvation. Different mechanisms have been proposed to explain the protein stabilizing action of glycerol and destabilizing action of urea. Some of them are: (i) reduction of hydrophobic hydration[7-8], (ii) alternation of water structure[7-8] (iii) weakening the inter peptide hydrogen bond[11-12], (iv) preferential solvation of protein[13-15]. However, in order to gain the idea of amino acid solvation in depth we have taken the task in this paper to study the thermodynamics of solvation of a series of homologous α-amino acids with graded increase of (-CH_2-) group like glycine (gly), dl-alanine (ala), dl-α-amino butyric acid (aba) and dl-nor-valine (n-val) in aqueous mixture of protic as well as structure maker ethylene glycol containing (-CH_2-CH_2-) organic moiety and two hydroxylic groups (-OH) with stronger H-bonding ability. From this objective, we are presenting in this article the solubilities of these α- amino acids in aqueous mixtures of ethylene glycol with 0, 20,40,60,80 and 100 wt% of EG at 25^{0}C and related standard transfer Gibbs energies at 298.15K.

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EXPERIMENTAL

α-amino acids like glycine (gly) (E Merck) and dl-alanine (ala), dl-α-amino butyric acid (aba) and dl-norvaline (n-val) were used after drying in a vacuum desiccators without further purification. Ethylene glycol (EG) was purified by mixing drying agent K_2CO_3 and finally by distillation. Aqueous mixtures of co-solvent that have been used are 0, 20, 40, 60, 80 and 100 wt%. Samples, whose solubilities are to be measured were taken in stoppered glass tubes and shaken until saturated solutions were made. Then these solutions were kept in a thermostat to adjust the desired temperature i.e. 25^{0}C and to equilibrate for 2-3 days with occasional shaking and addition of solute, if required. After 2-3 days aliquots of the saturated solutions were taken in stoppered conical flasks and solubilities were measured by 'formal titration' method[7-8] after dilution with water and adding appropriate amounts of freshly neutralized formaldehyde (GR,E Merck) and then the titration was made by standard NaOH (GR,E Merck) solution with Phenolphthalein indicator. These measurements were taken at 25^{0}C. A solution was considered to attain saturation when concentrations measured at 2-days intervals agreed with the experimental error of 1-1.5%. It may change the composition of the solvents, to avoid these, fresh solvent have been used for different set. Three sets of measurements were made for all the solutes by equilibrating the solutions from both above and below the required temperature and the solubilities were found to agree to within ±1 to 1.5%.

RESULTS

Solvent parameters of EG-Water solvent systems are listed in TABLE 1. The solubilities (S) of amino acid (on molal scale) are listed in TABLE 2. The molal solubilities in the aqueous ethylene glycol as well as in water are used to compute standard free energies of transfer DG^0_t (m) using the equation 1[15-19].

$$\Delta G_t^0(m) = RT\ln(S_W / S_s) \quad (1)$$

Where the subscripts w and s are for water and aqueous EG respectively. It is assumed that the ratio of activity coefficients of these amino acids in these solvents mixtures is unity. Standard transfer free energies in mole fraction scale $\Delta G_t^{0,c}$ (i) is calculated by the equation 2.

$$\Delta G_t^{0,c}(i) = \Delta G_t^0(m) - RT\ln(M_s / M_W) \quad (2)$$

Where M_s and M_w refer to the molar mass of cosolvent and reference solvent, water respectively. ΔG_t^0(i) are listed in TABLE 2. Now ΔG_t^0(i) may be ascribed as the sum of the following terms (assuming dipole-induced dipole term to be negligible)

$$\Delta G_t^0(i) = \Delta G_{t,cav}^0(i) + \Delta G_{t,d-d}^0(i) + \Delta G_{t,ch}^0(i) \quad (3)$$

Where $\Delta G^0_{t,cav}$(i) stands for the transfer free energy contribution of the cavity effect involving the creation of cavities for the species in water and aquo-organic solvents and $\Delta G^0_{t,d-d}$(i)stands for the dipole-dipole interaction effect involving interaction between dipolar zwitter-ionic amino acid and solvent molecules. While $\Delta G^0_{t,ch}$(i) includes all other effects such as those arising from acid-base or short range dispersion interaction, hydrophilic (H_lH) or hydrophobic (H_bH) hydration and structural effects. $\Delta G^0_{t,cav}$(i)values were computed by the use of scaled particle theory[20-22] assuming the solutes and solvent molecules as equivalent hard sphere models as dictated by their respective diameters. (vide TABLE 2)

The equations[20-22] used for cavity calculation are as follows:

$$\Delta G_t^0(cav) = G_c + RT\ln(RT / V) \quad (4)$$

where $G_c = RT(-\ln(1-Z) + 3X.D/(1-Z) + 3Y.D^2/(1-Z) + 9X^2.D^2/(2(1-Z)^2))$

$$Z = (\Pi N_A / 6V)(Z_1 a^3 + Z_2 b^3)$$

$$X = (\Pi N_A / 6V)(Z_1 a^2 + Z_2 b^2)$$

$$Y = (\Pi N_A / 6V)(Z_1 a + Z_2 b); V = M / d$$

In this expression N_A is Avogadro's number, M and d are the molar mass and the density of the solvents respectively, Z_1 and Z_2 are the mole fraction of water and co-solvent respectively. D is the hard sphere diameter of amino acids, a, is that for water taken as 2.74A^0 and b, is that for co-solvent. Finally $\Delta G^0_{t,cav}$(i)represents the difference.

$$_s\Delta G_t(cav) - _w\Delta G_t(cav) = (_sG_c - _wG_c) + RT\ln(V_w / V_s).$$

For the calculation of $\Delta G^0_{t,cav}$(i)the required solvent parameters are taken from TABLE 1.

Here $\Delta G^0_{t,d-d}$(i) was calculated as per equation 5.

$$\Delta G_{t,d-d}^0(i) = (_s\Delta G_{d-d}^0(i) - _w\Delta G_{d-d}^0(i)) \quad (5)$$

by means of Keesom orientation expression[23]. And for $_s\Delta G^0_{d-d}$(i) in a solvent, 's', as given herewith,

$$_s\Delta G_{d-d}^0(i) = -(8\Pi/9)N^2\mu_s^2\mu_x^2\sigma_{sx}^{-3}(kT)^{-1}V_x^{-1} = A/TV_s;$$

$$where A = -(8\Pi/9)N^2\mu_s^2\mu_x^2\sigma_{sx}^{-3}(k)^{-1} and V_s = M_s / d_s$$

Here N stands for Avogadro's number, μ_s and μ_x

are the dipole moment of solvent and amino acid molecules, respectively, (see TABLE 2), σ_{sx} is the distance at which the attractive and repulsive interactions between the solvent and solute molecules are equal and is generally equal to ½(σ_s+σ_x), where, σ_s and σ_x are the hard sphere diameter of solvent and solute molecules respectively. And μ_s and μ_s for such mixed binary solvent system are computed with the variation of mole fraction of the co-solvent as done by Graziano[24]. Following Kim et al.[25] and Marcus[23] in order to get $\Delta G^0_{t,d\text{-}d}(i)$ term on mole fraction scale the quantity was again multiplied by the term X_{s1}, where,

$$X_{s1}=X_s(\mu_s/\sigma^3_s)/(\mu_w/\sigma^3_w) \quad (6)$$

which is the real mole fraction contribution due to dipole-dipole interaction[25]. $\Delta G^0_{t,cav}(i)$and $\Delta G^0_{t,d\text{-}d}(i)$ are subtracted from $\Delta G^0_t(i)$ to get $\Delta G^0_{t,ch}(i)$ of amino acids and all these values are shown in TABLE 2. The values of $\Delta G^0_t(i)$ and $\Delta G^0_{t,ch}(i)$ are illustrated in figures 1,4 and 5.

DISCUSSION

Figure 1 shows the variation of $\Delta G^0_t(i)$ with mol% of EG, in EG-H_2O mixtures. $G^0_t(i)$ values of amino acids , indicate that except nor-valine all the other amino acids (glycine, dl-alanine and dl-α-amino butyric acid) are almost destabilized in EG-H_2O mixtures. Glycine is destabilized more or less regularly upto 100 mol % EG with sharp increase of $\Delta G^0_t(i)$ values . In case of alanine, it is stabilized most in around 10 mol% EG and then become destabilized upto 100 mol% almost sharply. On the other hand dl-α-amino butyric acid become destabilized up to 100 mole % of EG with slight decrease of $\Delta G^0_t(i)$ values at around 50 mol% EG. But only dl-nor-Valine is stabilized upto 100 mol % EG almost regularly with maximum stability in around 50 mol% EG. The stability order is about$\Delta G^0_t(i)$ (dl-n-Valine) > $\Delta G^0_t(i)$ (dl-Ala.) > $\Delta G^0_t(i)$ (dl-aba) > $\Delta G^0_t(i)$ (Gly.).

On the other hand unlike $\Delta G^0_t(i)$, $\Delta G^0_{t,cav}(i)$ for all four amino acids become progressively negative (Figure 2), therefore their stability is due to creation of cavity after transforming from water to EG is favorable and the order is as follows, dl-n-val. > dl-aba. >dl-ala. >gly. Negative values of $\Delta G^0_{t,cav}(i)$ indicates that it is easy to create a cavity in EG than that of water. Here $\Delta G^0_{t,cav}(i)$ values are guided by hard sphere diameter of solute and solvent and density of the solvent mixtures. The hard-sphere diameter and number density of EG is greater than that of water and during transfer to aqueo-

TABLE 1: Values of solvent parameters (mean mol. Weight (M_S), density(d_s), dielectric constant(ε_s), dipole-moment(μ_s), diameter(σ_s) of the H_2O+EG system at 298.15K

Wt % EG	10^3M_s (kgmol^{-1})	10^3ds (kg.m^{-3})	ε_S	μ_s (Debye)	σ(Å)
0	18.01	0.9970	78.46	1.834	2.740
20	21.01	1.0220	72.80	1.864	2.850
40	25.15	1.0480	66.88	1.906	3.004
60	31.36	1.0720	59.60	1.969	3.230
80	41.67	1.0940	49.50	2.074	3.615
100	62.07	1.1099	37.67	2.280	4.370

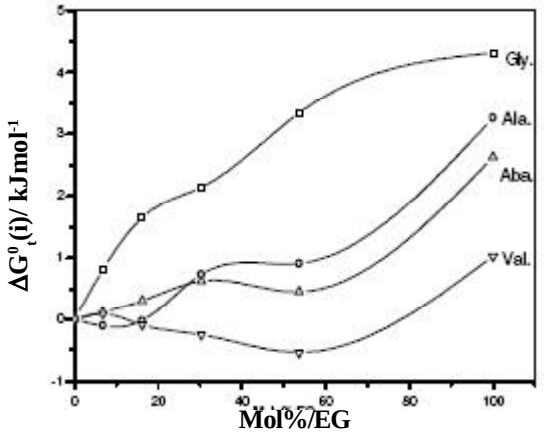

Figure 1: Variation of $\Delta G^0_t(i)$ of glycine, dl-Alanine, dl-α-amino butyric acid and dl-nor-Valine with mole% EG in aq- Ethylene glycol mixture at 298.15K

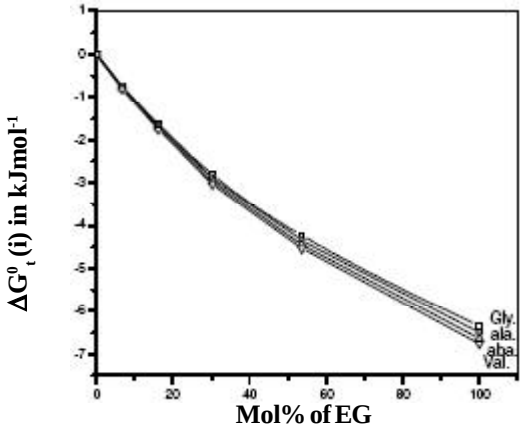

Figure 2: Variation of $\Delta G^0_{t,cav}(i)$ of glycine, dl-Alanine, dl-α- amino butyric acid and dl-nor-Valine with mole % EG in aq-Ethylene glycol mixtures at 298.15K

EG these parameters leads to favorable$\Delta G^0_{t,cav}(i)$.The above stability order is also in accordance to the increase in hard sphere diameter of amino acids from glycine to dl-n-valine (TABLE 2)

Also from the values of $\Delta G^0_{t,d\text{-}d}(i)$ (Figure 3) it is found that $\Delta G^0_{t,d\text{-}d}(i)$ values are positive for all amino

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TABLE 2 : Gibbs energies of transfer i.e. $\Delta G^0_t(i)$, $\Delta G^0_{t,cav}(i)$, $G^0_{t,dd}(i)$, $\Delta G^0_{t,ch}(i)$ of glycine, dl-alanine, dl-amino butyric acid and dl-nor valine from water to aq-Ethylene glycol in different compositions at 298.15K (on mole fraction scale in kJ mol^{-1})

Wt%ofsolvent(EG)	Mole % of co-solvent	Solubility(m) molkg^{-1}	$\Delta G^0_t(i)$ kJmol^{-1}	$\Delta G^0_{t,cav}(i)^a$ kJmol^{-1}	$\Delta G^0_{t,dd}(i)^a$ kJmol^{-1}	$\Delta G^0_{t,ch}(i)$ kJmol^{-1}
		Glycine				
0	0	3.34(3.33©)[b]	0	0	0	0
20	6.8	2.07	0.805	-0.750	1.22	0.335
40	16.2	1.23	1.648	-1.636	5.09	-1.806
60	30.3	0.81	2.140	-2.813	12.30	-7.35
80	53.7	0.36	3.3443	-4.240	22.20	-14.52
100	100	0.17	4.315	-6.357	33.10	-22.428
		dl-Alanine				
0	0	1.80(1.85©)[b]	0	0	0	0
20	6.8	1.61	-0.105	-0.773	1.00	-0.332
40	16.2	1.30	-0.021	-1.684	4.29	- 2.627
60	30.3	0.77	0.731	-2.899	10.30	-6.67
80	53.7	0.54	0.906	-4.353	18.70	-13.44
100	100	0.14	3.264	-6.510	28.00	-18.226
		dl-Amino butyric acid				
0	0	2.19(2.199 ©)[b]	0	0	0	0
20	6.8	1.79	0.119	-0.793	0.875	0.037
40	16.2	1.39	0.299	-1.726	3.74	-1.715
60	30.3	0.98	0.619	-2.974	9.00	-5.407
80	53.7	0.79	0.448	-4.451	16.30	-11.401
100	100	0.22	2.629	-6.646	24.50	-15.225
		dl-Nor-Valine				
0	0	0.677(0.683©)[b]	0	0	0	0
20	6.8	0.56	0.089	-0.811	0.779	0.121
40	16.2	0.50	-0.086	-1.763	3.33	-1.653
60	30.3	0.43	-0.249	-3.039	8.02	-5.230
80	53.7	0.36	-0.534	-4.536	14.50	-10.498
100	100	0.13	1.024	-6.762	21.80	-14.014

[a]**The required diameter and other solvent parameters of EG and its aqueous mixtures are taken from Ref.[23].The required diameter of glycine, alanine, amino butyric acid and nor-valine are 5.64, 6.16, 6.58 and 6.92A^0, respectively, as given in Ref.[7]. Dipole-moment values of α-amino acids are 15.7D for glycine, 15.9D for alanine and 16D for amino butyric acid and nor-valine[26]. 'b' indicate ref.[7].**

acids, with the order, gly. > dl-ala. > dl-aba. >dl- n-val.

$\Delta G^0_{t,d\text{-}d}(i)$ values increase with the increase of dipole-moment of solute (i. e. amino acids) and co-solvent (i.e. EG) and it also decrease with the increase of hard-sphere diameter of solute and co-solvent. During transfer from water to water-EG mixtures these parameters lead to $\Delta G^0_{t,d\text{-}d}(i)$ values for amino acids as like the above order.

As we know $\Delta G^0_t(i) = \Delta G^0_{t,cav}(i) + \Delta G^0_{t,d\text{-}d}(i) + \Delta G^0_{t,ch}(i)$, ignoring dipole-induced dipole interaction, and the chemical contributions of free energy of solvation, $\Delta G^0_{t,ch}(i)$ can be obtained by subtracting $\Delta G^0_{t,cav}(i)$ and $\Delta G^0_{t,d\text{-}d}(i)$from $\Delta G^0_t(i)$. Here in such solute-solvent system chemical interactions may be involved are of different types; i.e. acid-base type interaction, H-bonding interaction, Hydrophobic hydration (H_bH), hydrophilic hydration, hard-soft interaction, dispersion

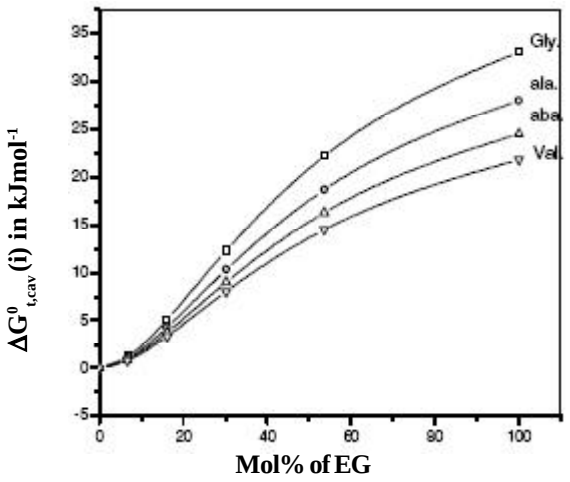

Figure 3: Variation of $\Delta G^0_{t,d\text{-}d}(i)$ of glycine, dl-Alanine, dl-α-amino butyric acid and dl-nor-Valine with mole% EGin aq-Ethylene glycol mixtures at 298.15K

interaction etc.

Figures 4 and 5 shows the variation of $\Delta G^0_{t,ch}$ (i) of amino acids with mole % of co-solvent, EG. From this profile it is observed that all the solutes, i.e. amino acids are stabilized in the aq-EG solvent system with the order: $\Delta G^0_{t,ch}$ (n-val.)> $\Delta G^0_{t,ch}$ (aba.)> $\Delta G^0_{t,ch}$ (ala.) >$\Delta G^0_{t,ch}$ (gly.), with slight distortion in water rich region.

Similar but reverse type of destabilization order has been noticed in aquo-2-PrOH and in aquo-ACN by Kundu and co-workers[7]. Besides this, all the amino acids are stabilized with the above as well as similar order in GL-water mixtures[8]. Moreover, solubility studies of amino acids and peptides in water reflected that solubility decreases with increasing number of hydrocarbon (-CH_2-) groups in homologous series of different organic compounds.

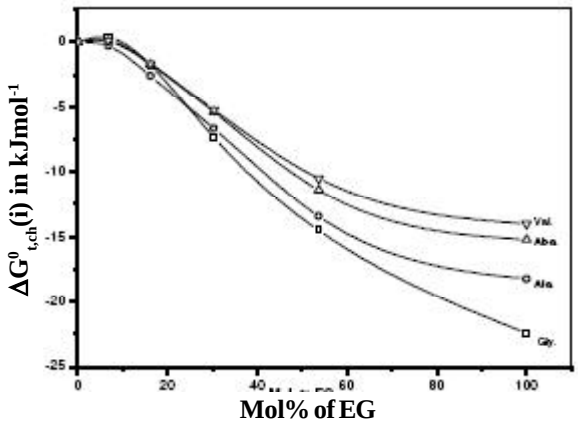

Figure 4: Variation of $\Delta G^0_{t,ch}$ (i) of glycine, dl-Alanine, dl-α- amino butyric acid and dl-nor-Valine with mole % EG in aq-Ethylene glycol mixture at 298.15K

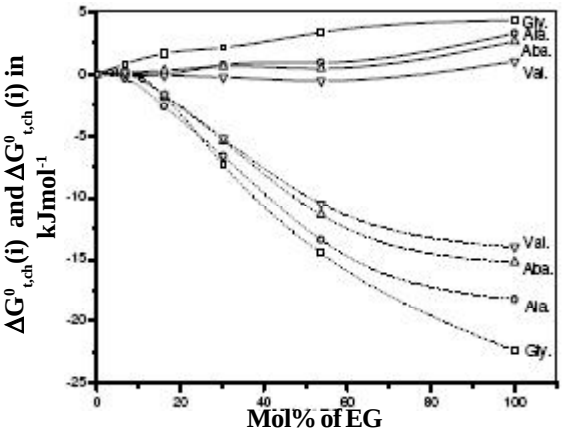

Figure 5: Variation of ΔG^0_t(i) (—) and $\Delta G^0_{t,ch}$ (i) (…..) of glycine, dl-Alanine, dl- α- amino butyric acid and dl-nor-Valine with mole % EG in aq-Ethylene glycol mixture at 298.15K

It is also supported that the solvation number[27-28] of the amino acids in water being expected to be high (TABLE 2) due to zwitter-ionic nature of amino acids ($RH^{\pm}$) having two solvation sites.

Here, the observed destabilization order of $RH^{\pm}$ is not only the effect of decrease solvation number of amino acids in the binary mixtures with increase EG-content in water; but also due to immobilization of the solvent molecules by solutes due to dipole-dipole, dipole-induced dipole, dispersion forces etc. It is worth noting that, $\Delta G^0_{t,ch}$ (i) composition profiles of these amino acids in aquo-EG exhibits inflections (more for glycine) of water rich region (above 4 mol% EG) which is due to predominant structural effect of EG in the aqua-EG mixtures (complex formation between EG and water at maximal molar fraction[29] of EG i. e. above 4 mol% EG).

In fact $\Delta G^0_{t,ch}$ (i) values of α-amino carboxylic acids ($RH^{\pm}$) here, are also guided by hydrophilic hydration (H_1H) of the zwitter-ionic head, $RC(NH_3^+)COO^-$ group of $RH^{\pm}$ molecules and relative H_1H and acidity-basicity effect of the co-solvent, EG. Ethylene glycol – water mixtures become more acidic with increase of EG content and more acidity affect the $RCOO^-$ part. On the other hand these mixtures become less basic with increase of EG content and it affect NH_3^+ part. Moreover, EG is a reducer of hydrophobic hydration, H_bH (due to its structure making propensity), as expected from the opposing effects of H_bH induced by the hydrocarbon skeleton(-CH_2-CH_2-) of the molecule and hydrophilic hydration (H_1H) as induced by 2-OH groups of the EG molecule.

Also EG (4.37Å) is larger in size than water (2.74Å). Therefore with the increase in EG content in the solvent mixtures the dispersion interactions as well as soft-soft interactions for the involved amino acids with the increased size of the $RH^{\pm}$ molecules, gradually increased, and these interactions contribute to the observed stabilization.

Therefore hydrophobic hydration (H_bH) effect of the hydrocarbon part (-CH_2-CH_2-) as induced by structure making (SM) propensity of EG as well as involved increased dispersion interaction effects of the co-solvent molecules (EG) with the increased size of the $RH^{\pm}$ molecules are also the guiding factors.

Finally, the observed increasing positive values of $\Delta G^0_{t,ch}$ (i) ($RH^{\pm}$) (from gly. to n-val.) and their relative order in aqueous mixtures of SM and more acidic EG

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indicates that the involved increasingly reduced H_bH-effect of EG overcomes, the opposing increased acidity and dispersion effects of co-solvent, EG.

In conclusion, from our observation it may be stated that ethylene glycol will perform as a good stabilizer of structure of amino acids as well as proteins.

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