# Transfer Thermodynamics Of Some Amino Acids In A Protein Stabilizing And A Denaturant Media

✉ *Corresponding Author*

**I.Basumallick**
**Dept. Of Chemistry, Visva-Bharati,**
**Santiniketan-731235, (INDIA)**
E-mail: ibasumallick@yahoo.co.uk

*Co-Author*

**Someswar Chatterjee**
Dept. of Chemistry, Visva-Bharati, Santiniketan-731235, (INDIA)

## ABSTRACT

A comparative study on transfer free energies of some amino acids from water to aqueous urea, a protein-denaturing medium, and water to aqueous glycerol, a protein-stabilizing medium, is presented. Transfer free energies have been dissected into cavity term $[\Delta G^0_t(cav)]$, electrical term $[\Delta G^0_t(ele)]$ and interaction term $[\Delta G^0_t(int)]$. Interaction term includes all type of interaction like hard soft, hydrogen bond, hydrophobic interaction etc. Calculation of hard soft parameters of these amino acids and co solvents, by semi empirical method using standard soft ware, reveals that this type of hard soft interaction contributes significantly to the $\Delta G^0_t(int)$.
© 2007 Trade Science Inc. - INDIA

## INTRODUCTION

Much attentions[1-6] are now paid to the thermo-dynamic studies of solute-solvent interaction in protein stabilizing and denaturing media. This is because the mechanism of solvent induced folding unfolding of protein can hardly be considered as settled one. Among the denaturing solutes urea is most effective; similarly glycerol is a well-known stabilizer. Various mechanisms have been proposed to explain the protein stabilizing action of glycerol and denaturing ac-tion of urea. Some of them are: (i) weakening the inter peptide hydrogen bond[7], (ii) alternation of water structure[8,9] by urea or glycerol (iii) reduction of hydrophobic interaction[10-12], (iv) preferential hydration[13,14] of protein in aqueous urea and aqueous glyc-erol, are more attractive.

However in order to understand in depth the dy-namic of solvent perturbation of protein structure, i.e. estimation of driving force for urea induced un-folding and glycerol induced folding, it would be of interest of know the thermodynamics of solvation

# Regular Paper

of individual amino acids, the building blocks of protein, in aqueous urea and aqueous glycerol.

Time to time attention has been paid to determine the various thermodynamic properties such as molar volume[2], enthalpy of solution[1], solubility of various amino acids in aqueous urea and aqueous glycerol[7,15]. The purpose of such studies is to gain the basic aspect of amino acids solvation.

Tanford and Nozaki[7] reported free energies of some amino acids from water to aqueous urea from solubility measurement. Transfer free energies data of some amino acids in aqueous glycerol[15] are also available. But correlation of experimental transfer free energies of different amino acids with the interaction parameters like, hydrophilicity, hydrophobicity or soft hard interaction is a difficult task. This may be due to the fact that experimental transfer free energies are composite in nature and consisting of electrical free energies, cavity forming free energies and free energies of interaction. It is expected that the free energies of interaction of these amino acids will bear a good correlation with the hard-soft type interaction. Therefore, in this paper an attempt has been made to dissect experimental transfer free energies into free energies of interaction, cavity free energies and electrical free energies of transfer.

It may be mentioned that similar dissection of transfer free energies of different solutes[16-18] including amino acids[3,19] give better understanding of solute-solvent interactions.

The amino acids studied here are glycine, alanine, valine, leucine, phenylalanine in 1mol% of urea and 1mol% of glycerol.

## CALCULATION

Standard transfer free energies in mole fraction scale $\Delta G_t(\text{expt})$ is obtained by equation

$$\Delta G^0_t(\text{expt}) = \Delta G^0_t(\text{m}) - RT\ln(M_s r_w / M_w r_s) \qquad (1)$$

where M and $\rho$ stand for molar mass and density; subscripts w and s are for water and aqueous co-solvent, respectively. $\Delta G^0_t(\text{m})$ are taken from well reported literature data[15] or extrapolating the literature data[7].

The experimental transfer free energies have been dissected into cavity part, electrical part and inter-

action part as equation (2)

$$\Delta G^0_t(\text{expt}) = \Delta G^0_t(\text{cav}) + \Delta G^0_t(\text{ele}) + \Delta G^0_t(\text{int}) \qquad (2)$$

Scaled particle theory has been applied earlier[3,20] in computation of cavity forming free energies of various type of solute as well as zwitter ionic solute in a wide variety of pure and mixed solvent using the following equation

$$\Delta G_t(\text{cav}) = G_c + RT\ln(RT/V) \qquad (3)$$

where

$$G_c = RT[-\ln(1-Z) + \{3X/(1-Z)\}D + \{3Y/(1-Z)\}D^2 + \{9X^2/4(1-Z)^2\}D^2]$$

$$Z = \pi N_A (z_1 a^3 + z_2 b^3) / \sigma V$$
$$X = \pi N_A (z_1 a^2 + z_2 b^2) / \sigma V$$
$$Y = \pi N_A (z_1 a + z_2 b) / \sigma V$$
$$V = M/\rho$$

In this expression $N_A$ is Avogadro's number, $z_1$ and $z_2$ are the mole fraction of water and urea or glycerol, respectively. 'D', 'a' and 'b' are the hard sphere diameter of amino acids, water and urea or glycerol, respectively. $\Delta G_t(\text{cav})$ represents the differences $_s\Delta G_t(\text{cav}) - _w\Delta G_t(\text{cav}) = (_sG_c - _wG_c) + RT\ln(V_w/V_s)$. Hard sphere diameters of a mino acids are taken from well reported literature data[3] otherwise have been calculated from reported partial volume data[21] and using Farrell's treatment[22]. In the calculation of $\Delta G^0_t(\text{cav})$ from water to aqueous glycerol(1mol%) the hard sphere diameters: 2.76 Å[3,20] and 4.94 Å[20,23] for water and glycerol, respectively; the densities: 0.9973gm/cc[3,20] for water and 1.0103gm/cc[20] for glycerol, have been used and results are displayed in TABLE 1. $\Delta G^0_t(\text{cav})$ from water to 1mol% urea are taken by extrapolating the literature data[19] and are shown in TABLE 2.

When amino acids are transferred from water to aqueous binary the dielectric constant of media change appreciably, so that free energy of transfer due to electrostatic effect $\Delta G^0_t(\text{ele})$ has been calculated using Scatchard and Kirkwood expression[3] of zwitter ion as shown in equation (4)

$$\Delta G^0_t(\text{cav}) = k_2(1/D_2 - 1/D_1) \qquad (4)$$

Where $D_1$ and $D_2$ are the dielectric constants of water and co-solvent, respectively, and $k_2$ is constant. The deduction of the value of $k_2$ have been discussed elsewhere[3]. $\Delta G^0_t(\text{ele})$ from water to 1mol% glycerol has been calculated using the dielectric constants 78.74[3,20] and 77.19[20] for water and 1mol% glycerol, respectively, and the result is 0.174KJ/mol. Trans-

**Regular Paper**

**TABLE 1: Transfer free energies (KJ/mol) of amino acids from water to 1mol% glycerol at 298K**

| Amino acids | Hard sphere diameter(A) | $\Delta G^0_t$(cav) | $\Delta G^0_t$(ele) | $\Delta G^0_t$(int) | $\Delta G^0_t$(expt) |
|---|---|---|---|---|---|
| Gly | 3.85 | -0.052 | | 0.133 | 0.235 |
| Ala | 4.20 | -0.047 | | 0.148 | 0.275 |
| Val | 5.00 | -0.034 | 0.174 | 0.178 | 0.318 |
| Lue | 5.40 | -0.028 | | 0.085 | 0.255 |
| Ph-ala | 5.60 | -0.024 | | 0.050 | 0.200 |

**TABLE 2: Transfer free energies (KJ/mol) of amino acids from water to 1 mol% urea at 298K**

| Amino acids | Hard sphere diameter(A) | $\Delta G^0_t$(cav) | $\Delta G^0_t$(ele) | $\Delta G^0_t$(int) | $\Delta G^0_t$(expt) |
|---|---|---|---|---|---|
| Gly | 3.85 | 0.030 | | 0.075 | 0.011 |
| Ala | 4.20 | 0.041 | | 0.067 | 0.014 |
| Val | 5.00 | 0.071 | -0.094 | -0.007 | -0.030 |
| Lue | 5.40 | 0.087 | | -0.052 | -0.059 |
| Ph-ala | 5.60 | 0.096 | | -0.135 | -0.133 |

ferred electrical free energy change of amino acids from water to 1mol% urea, -0.094KJ/mol, has been taken from the extrapolating result of literature data[19].

Transfer free energies of interaction $\Delta G^0_t$(int) values have been calculated from experimental free energies of transfer after subtracting the cavity forming free energy and electrical effect i.e.

$$\Delta G^0_t(int)=\Delta G^0_t(expt)-\Delta G^0_t(cav)-\Delta G^0_t(ele) \quad (5)$$

The values of $\Delta G^0_t$(int) from water to 1mol% glycerol and from water to 1mol% urea are shown in TABLE 1 and TABLE 2, respectively.

It is assumed that, the hard-soft interaction between amino acid and co-solvent is guided by difference between the hardness($\eta$) of corresponding molecule. According to the HSAB principle, it may be considered lower the difference of hardness higher will be the hard-hard interaction between two molecules. The hardness of the molecules has been calculated using the following operational definition as Pearson[23].

**TABLE 3: $\in_{HOMO}$, $\in_{LUMO}$ and hardness ($\eta$) of amino acids and co-solvents.**

| Amino acids/ co-solvents | $\in_{HOMO}$ (au) | $\in_{LUMO}$ (au) | Hardness ($\eta$)(ev) |
|---|---|---|---|
| Gly | -0.381 | 0.008 | 5.27 |
| Ala | -0.386 | 0.011 | 5.38 |
| Val | -0.379 | 0.007 | 5.23 |
| Lue | -0.380 | 0.006 | 5.23 |
| Ph-ala | -0.361 | 0.001 | 4.91 |
| Glycerol | -0.402 | 0.092 | 6.70 |
| Urea | -0.395 | 0.012 | 5.52 |
| Water | -0.451 | 0.125 | 7.8 |

$$\eta = (I-A)/2 \quad (6)$$

where I and A are the ionization potential and electron affinity of the molecule. According to Koopmar's theorem[23] $-\in_{HOMO}=I$ and $-\in_{LUMO}=A$.

The energies of HOMO and LUMO of different amino acids and co-solvents have been calculated by semiemperical method using arguslab 4.0[24, 25] software and results are shown in TABLE 3.

**RESULT AND DISCUSSION**

Figure 1 shows that the variation experimental free energies transfer of amino acids from water to 1mol% glycerol and from water to 1mol% urea with the hard sphere diameter of amino acids.

The positive values of $\Delta G^0_t$(expt) indicate all amino acids are destabilized in glycerol and the destabilization is not regular with the size of hydrophobic side chain of amino acids. On the other hand, in 1mol% urea all amino acids, except gly and ala, are stabilized and the stabilization increases with the size of hydrophobic part of amino acids but destabilization of gly and ala are anomalous on this account.

TABLE 1 and TABLE 2(third column) shows, unlike the experimental free energies the cavity forming free energies of transfer for all amino acids from water to glycerol are favorable while cavity effects are unfavorable during the transfer of amino acids from water to 1mol% urea. It may be noted that the sign of $DG^0_t$(cav) indicates it is easy to create a cavity in glycerol than that of water while it is reverse in

**Bio**CHEMISTRY
*An Indian Journal*

# Regular Paper



**Figure 1: Variation of transfer free energies $\Delta G^0_t$(expt) of amino acids from water to aqueous binary with hard sphere diameter of amino acids at 298K**



**Figure 2: Variation of transfer free energies $\Delta G^0_t$ (cav) of amino acids from water to aqueous binary with hard sphere diameter of amino acids at 298K**
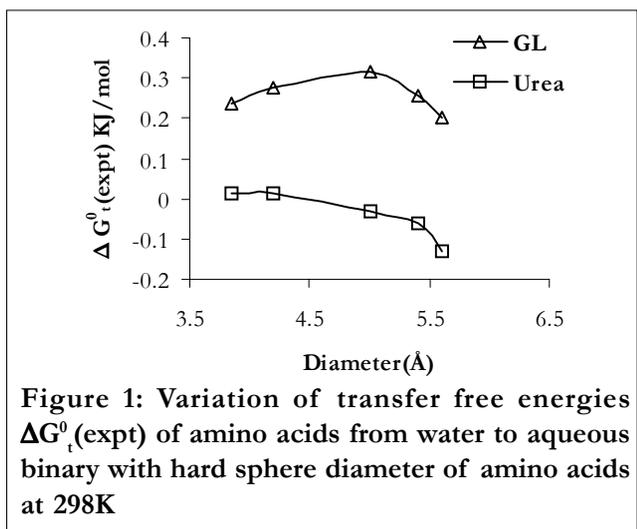
urea. Figure 2 shows that transfer cavity forming free energies increase with the hard sphere diameter of amino acids in both co-solvents.

Recently, Graziano[25] explained that the sign of $\Delta G^0_t$(cav) depends on diameter and density of the co solvent. These two parameters affect $\Delta G^0_t$(cav) in opposite way i.e. cavity forming free energy increases with decrease in hard sphere diameter and increase in number density of co-solvent. The hard sphere diameter and number density of both urea and glycerol are higher than water but during transfer to aqueous glycerol the increasing effect of hard sphere diameter predominates while number density of aqueous urea prevails, rendering unfavorable $\Delta G^0_t$(cav). Admittedly, authenticity of this cavity calculation will be guided by the uncertainties that are incorporated via solute and solvent diameter and densities of the solvent. Previously[19], for the sake of satisfaction we have calculated cavity term varying the solute diameter to ±0.2A and solvent diameter to ±0.05A but these variation does not affect the physical reliability of the result of SPT calculation. However, in this present calculation the solute and solvent parameters have been taken from standard literature data or have been derived from literature data using well-established relation.

The electrical effect during transfer of amino acids from water to aqueous glycerol and aqueous urea are just reverse to cavity effect. The $\Delta G^0_t$(ele) from water to 1mol% glycerol of all amino acids is unfavorable due to lower dielectric constant of aqueous glycerol while higher dielectric constant of aqueous urea electrically favors the transfer of amino acids
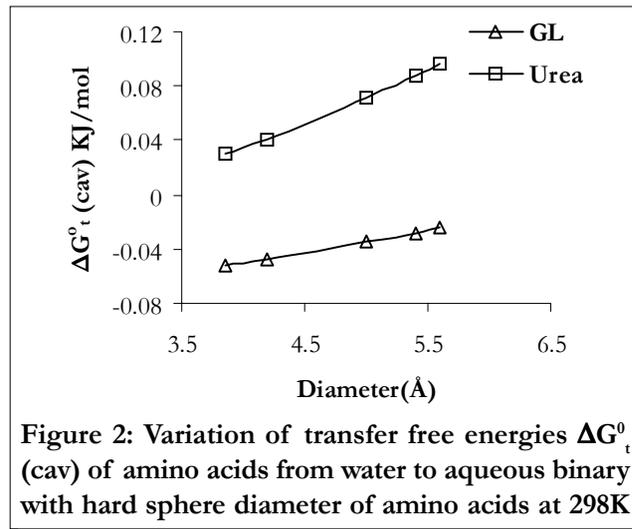
from water to 1mol% urea.

Figure 3 shows the variation of transfer interaction free energies with hard sphere diameter of amino acids. The positive values of $\Delta G^0_t$(int) indicate all amino acids are destabilized in aqueous glycerol and the destabilization curve shows a maximum for valine. $\Delta G^0_t$(int) from water to aqueous urea gradually decreases with increase in the hard

Sphere diameter of amino acids. Like experimental transfer free energies, the transfer free energies of interaction indicates phenylalanine are most stabilized in aqueous urea but the destabilization of glycine and alanine are now regularized with the size of hydrocarbon side chain. The unfavorable and favorable observed interaction energies may be explained in terms of hard soft interaction between amino acids-water, amino acids-co-solvent and water co-solvent. The differences of hardness(Figure 4) indicate that the interactions of urea-amino acids are stronger hard-hard type while water-amino acids are weaker hard-soft type. Therefore, during transfer of amino acids from water to aqueous urea the favorable interaction energies seem to be partly guided by hard soft interaction.

The addition of glycerol to amino acids solution in water the weaker hard-soft interaction of water-amino acids converted into three weaker hard-soft interactions i.e. water-amino acids, water-glycerol and amino acids-glycerol. These weaker hard soft interactions in aqueous glycerol lead the unfavorable free energies of interaction on transfer. It is interesting to note that, with respect to hard soft interaction the anomalous most favorable and least unfavorable interaction energies of phenylalanine in aqueous urea
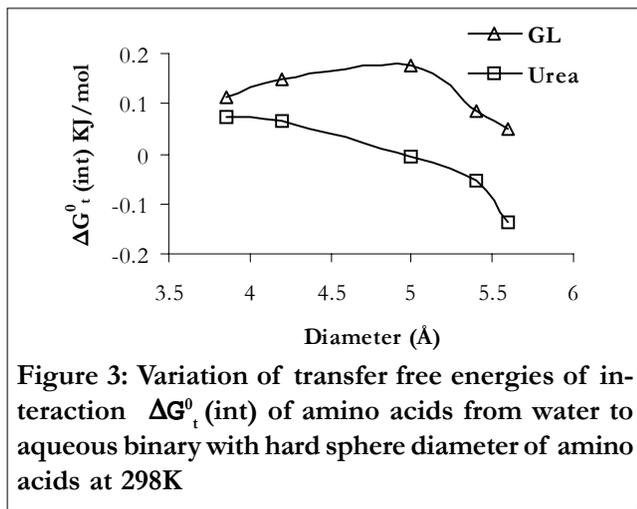
**Figure 3: Variation of transfer free energies of interaction $\Delta G^0_t$ (int) of amino acids from water to aqueous binary with hard sphere diameter of amino acids at 298K**
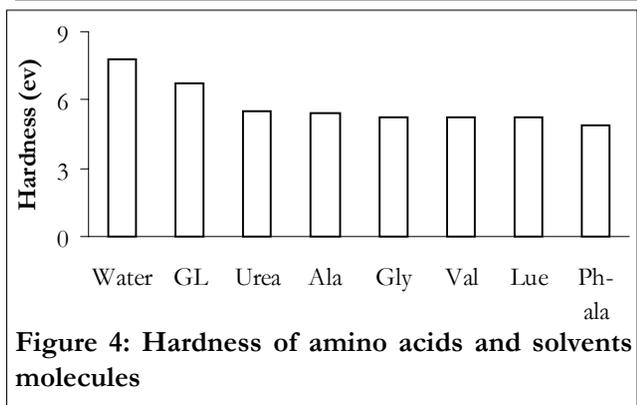


**Figure 4: Hardness of amino acids and solvents molecules**

and in aqueous glycerol, respectively, the other interaction like hydrophobic hydration due to its larger hydrophobic side chain, are to be further considered.

Analysis of dissection studies reveals that like the experimental free energies the various driving forces of solvation of amino acids in aqueous urea and aqueous glycerol are work in opposite way. Cavity effect favors the transfer of all amino acids from water to aqueous glycerol while it is harder to create a cavity for accommodation of any amino acid in aqueous urea than that of water. Again, due to lower dielectric constant the amino acids are destabilized in aqueous glycerol but transfer of amino acids is electrically favorable from water to higher dielectric aqueous urea media. Like the experimental transfer free energies the interaction free energies of transfer reveal the amino acids are comparatively more stabilized in aqueous urea than in aqueous glycerol. These transfer free energies of interaction are partly guided by hard soft interaction.

## REFERENCES

[1] K.P.Prasad, J.C.Ahluwalia; Biopolymers, **19**, 273 **(1980)**.
[2] T.S.Banipal, G.Singh, B.S.Lark; J.Solution.Chem., **30**, 657 **(2001)**.
[3] P.Das, S.Chatterjee, I.Basumallick; J.Chin.Chem.Soc., **51**, 1 **(2004)**.
[4] S.Li, X.Hu, R.Lin, W.Sang, W.Fang; J.Solution.Chem., **30**, 365 **(2001)**.
[5] G.I.Makhatadze; J.Phys.Chem., **103**, 4781 **(1999)**.
[6] J.P.Chatterjee, I.N.Basumallick; J.Chem.Soc.Faraday Trans I, **84**, 2697 **(1988)**.
[7] Y.Nozaki, C.Tanford; J.Biol.Chem., **238**, 4074 **(1963)**.
[8] G.M.Blackburn, T.H.Lelley, E.Walmsley; J.Chem.Soc. Faraday Trans I, **76**, 915 **(1980)**.
[9] I.N.Basumallick, K.I.Kundu; Can.J.Chem., **58**, 78 **(1980)**.
[10] C.Tanford; Adv.Protein.Chem., **24**, 1 **(1970)**.
[11] M.Roseman, W.P.Jenks; J.Am.Chem.Soc., **97**, 631 **(1975)**.
[12] D.O.V.Alonoso, A.K.Dill; Biochemistry, **30**, 5974 **(1991)**.
[13] S.N.Timasheff; Annu.Rev.Biophys.Biomol.Struct., **22**, 67 **(1993)**.
[14] K.Gekko, S.N.Timasheff; Biochemistry, **20**, 4677 **(1981)**.
[15] K.Gekko; J.Biochem., **90**, 1633 **(1981)**.
[16] N.Nandi, I.N.Basumallick; J.Phys.Chem., **94**, 2537 **(1990)**.
[17] N.Nandi, I.N.Basumallick; J.Phys.Chem., **97**, 3900 **(1993)**.
[18] N.Nandi, I.N.Basumallick; Z.Physik.Chem., **173**, 179 **(1991)**.
[19] S.Chatterjee, I.Basumallick; J.Chin.Chem.Soc. (In Press)
[20] S.P.Rudra, B.P.Chakroborty, K.K.Kundu, I.N. Basumallick; Z.Physik.Chem., **150**, 211 **(1986)**.
[21] Y.Harpaz, M.Gerstein, C.Chothia; Structure, **2**, 641 **(1994)**.
[22] J.T.Edward, P.G.Farrell; Can.J.Chem., **53**, 2965 **(1975)**.
[23] R.G.Pearson; Ingo.Chem., **27**, 734 **(1988)**.
[24] M.A.Thompson, G.K.Schenter; J.Phys.Chem., **99**, 6374 **(1995)**.
[25] M.A.Thompson; J.Phys.Chem., **100**, 14492 **(1996)**.
[26] G.Graziano; J.Phys.Chem., **105**, 2632 **(2001)**.