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Denaturation Of Lysozyme In Aqueous Urea And Degree Of Exposure

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ABSTRACT

Free energies of transfer (ΔG_t) of Lysozyme (LZ) from water to aqueous solutions of urea (4M, 6M and 8M), a protein denaturing solvent have been dissected into cavity term [ΔG_t(cav)] and interaction term [ΔG_t(int)]. The interaction free energy includes all types interactions like hard-soft, hydrogen bonding, electrostatic etc. The cavity forming free energies have been calculated using standard version of scaled particle theory (SPT) with well reported SPT parameters. It has been found that transfer free energies of cavity formation ΔG_t(cav) for Lysozyme from water to urea-water are unfovourable whereas the transfer free energies of interaction ΔG_t(int) are favourable. These results are in conformity with the reported values for the transfer of RibonucleaseA from water to urea-water. A new approach for the calculation of degree of unfolding has been proposed.

KEYWORDS

Protein denaturation;
Cavity forming free energy;
Scaled particle theory;
Degree of exposure;
Lysozyme.

INTRODUCTION

It is well known that urea denatures aqueous solution of protein most effectively. But the mechanism of denaturing action of urea is not yet clearly established. Different mechanisms have been suggested from time to time. These are specific interaction of urea with the peptide linkage[1], water structure breaking ability of urea[2], reduction of hydrophobic interaction[3,4]etc. Thermodynamical approaches using transfer thermodynamic parameters of amino acids and proteins are also well reported[4-8]. We have also reported free energy of interactions of amino acids[9,10] and proteins [11] from water to aqueous urea applying scaled particle theory.

In this study, we intend to report transfer thermodynamic parameters of lysozyme from water to aqueous urea. It is well known that when a large solute like protein is transferred from water to other solvent, the solvent molecules are to be excluded for the accommodation of protein. Thus, free energy of solvent exclusion[12] that is also termed as cavity forming free energy is to be calculated for better interpretation of transfer free energy data. Again, when a protein is transferred to aqueous urea, it undergoes denaturation, as a result its physical states like size and shape are abruptly changed. Hence interpretation of thermodynamic data assuming same standard state of solute becomes difficult. Therefore, we have proposed[9-11,13-15] to dissect the experimental free en-

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TABLE 1 : Transfer free energies (KJ/mol) of lysozyme from water to aqueous urea at 298K

D_N (Å)	D_N (Å)	Urea	$\Delta G_t(cav)N_{[N(W)\rightarrow N(U-W)]}$	$\Delta G_t(cav)_{[N(U-W)\rightarrow D(W-U)]}$	$\Delta G_t(cav)$PathIII	$\Delta G_t(expt)^{\#}$	$\Delta G_t(int)$
	24.56	4M	18.51	-9.92	8.59	-18.65	-27.24
24.74	24.46	6M	28.27	-15.60	12.67	-27.72	-40.39
	24.37	8M	38.84	-20.89	17.95	-36.28	-54.23

[#]**Ref:**[6]

ergy terms into cavity term and interaction term

$$\Delta G_t = \Delta G_t(cav) + \Delta G_t(int) \quad (1)$$

The ΔG_t(int) contains all type of soft interactions like H-bonding, electrostatic interaction, dipole-dipole interaction, hard-soft interaction etc whereas ΔG_t(cav) is the reversible work required to create a cavity for the accommodation of the solute into a solvent consisting hard solvent molecules.

We have calculated ΔG_t(cav) of Lysozyme(no. of constituent amino acids is 129) from water to aqueous urea(4M, 6M and 8M) with well-standardized and logical SPT parameters. Finally, we have obtained interaction free energy term G_t(int) for lysozyme for transfer from water to aqueous urea. The cavity energy and interaction energy of lysozyme have been compared with our earlier[11] result of ribonuclease A(no.of constituent amino acids is 124).

Again, it is well known that when a native protein is transferred from water to denaturant the side chain of amino acids are partially exposed as a result denaturation is occurred. Thus in order to account the stability of native and denatured state of protein in denaturant media it is necessary to know the degree of exposure (α) of constituent amino acids of protein. But the calculation of degree of exposure of each type of constituent amino acids of a protein is a difficult task. Generally, average degree of exposure is measured using the equation (2)[7,16].

$$\Delta G_t(expt) = \alpha \Sigma n_i [\Delta g_t(expt)]_i \quad (2)$$

where ΔG_t(expt) and $[\Delta g_t(expt)]_i$ are transfer experimental free energies of protein and amino acid side chain of type 'i', respectively, n_i is the number of 'i' type amino acids present in a protein molecule. Here, a new approach has been made to calculate the degree of exposure using the transfer free energies of interaction ΔG_t(int) of protein and transfer interaction free energies $[\Delta g_t(int]_i$ of constituent amino acids i.e.

$$\Delta G_t(int) = \alpha \Sigma n_i [\Delta g_t(int]_i \quad (3)$$

This is because protein undergoes unfolding due to interaction with the solvent molecule.

CALCULATION

Transfer of protein from water to aqueous urea has been considered into two ways elsewhere[11]. Among the two ways one way is sum of the two path-pathI: transfer of native protein (N) from water (W) to aqueous urea (U-W) and then PathII: native protein is changed to denatured state (D) in aqueous urea as figure1.

The transfer cavity forming free energies ΔG_t(cav) along pathIII are the sum of cavity contribution due to both pathI and pathII i.e.

$$\Delta G_t(cav) = \Delta G_t(cav)N_{[N(W)\rightarrow N(U-W)} + \Delta G_t(cav)_{[N(U-W)\rightarrow D(W-U)]} \quad (4)$$

Scaled particle theory (SPT)[9-11,15] has been applied in computation of cavity forming free energy. SPT equations and solvent parameters have been reported elsewhere[9,11]. The equivalent hard sphere diameter of native(D_N) lysozyme has been calculated from the reported[17] partial specific volume data using Farrell's treatment[18]. It is well reported[19,20] that volume change in denaturation is a negative quantity. It has been assumed that volume changes due to thermal denaturation of lysozyme and ribonucleaseA[11,20] are almost same. Again as before[11] this volume change data is equated with that due to denaturation in 8M urea. The volume change due to denaturation by 4M and 6M urea are obtained by method of intrapolation.

Transfer free energies of interaction ΔG_t(int)of protein from water to aqueous urea are obtained by equation (1) and result are shown in TABLE 1.

Accordingly, transfer free energies of interaction ΔG_t(int) of protein from water to urea along PathIII would be sum of transfer free energies of interaction due to both non-exposure part and exposure part. Thus the total transfer free energies of interaction would be,

$$\Delta G_t(int) = (1-\alpha)\,\Delta G_t(int)N + \alpha \Sigma n_i [\Delta g_t(int)]_i \quad (5)$$

where α is the degree of exposure of native protein in aqueous urea, $\Sigma n_i [\Delta g_t(int)]_i$ term represent the sum of interaction free energies of transfer of all amino acids

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TABLE 2 : Constituent amino acids of lysozyme and ribonucleaseA and transfer free energies of interaction (KJ/mol) from water to 8M urea at 298K

Name	Amino acids no. present in Lsozyme	Amino acids no. present in RibonucleaseA	$[\Delta g_t(int)]^{\$}$
Glycine	12	3	1.31
Alanine	12	12	1.15
Threonine	7	10	0.22
Valine	6	9	-0.22
Proline	2	4	-0.12
Glutamine	3	7	-0.55
Histdine	1	4	-0.72
Luecine	8	2	-0.98
Methionine	2	4	-1.45
Cystein	8	8	-1.01
Aspagine	13	10	-1.15
Phenylalanine	3	3	-2.42
Tyrocine	3	0	-3.07
Tryptophan	6	6	-4.08
Isoluecine	6	3	-0.98*
Serine	10	15	-1.01*
Asparatic acid	8	5	-1.15*
Glutamic acid	2	5	-0.55*
Lysine	6	10	-0.72*
Arginine	11	4	-0.72*

$Ref[9]

*It is assumed that the interaction free energies of isolucine and luecine; serine and cystein; asparatic acids and asparagines; glutamic acids and glutamine; lysine and histidine; arginine and histidine are same as these amino acids of each pair possess similar type of side chain.

TABLE 3 : Degree of exposure of proteins in 8M urea

Protein	$\Delta G_t(int)N$ (KJ/mol)	$\Sigma n_i [\Delta g_t(int)]_i$ (KJ/mol)	$\alpha_{(calculated)}$	$\alpha_{(literature)}$ $^{\$}$
Lysozyme	-39.11	-85.79	0.32	0.21
RibonucleaseA	-41.15	-78.64	0.33	0.34

$ Ref:[6,7]

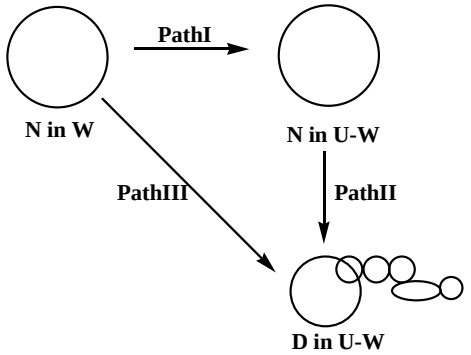

Figure 1 : Schematic representation of transfer of protein from water to aqueous urea

present in the protein molecule and $\Delta G_t(int)N$ is simply the transfer free energies of interaction of that native protein. Therefore, the first and second terms in the right hand side of the equation (5) represent the interaction free energies of transfer of the non-exposure and exposure portion of the protein molecule respectively.

The values of transfer free energies of interaction $\Delta g_t(int)$ of each amino acids from water to 8M urea are taken form well reported[9] literature data (TABLE 2) and the values of $\Sigma n_i [\Delta g_t(int)]_i$ have been shown in TABLE 3.

The $\Delta G_t(int)N$ we has been calculated from $\Delta G_t(expt)$ using usual procedure[6,7]. Using the values of $\Delta G_t(int)$, $\Delta G_t(int)N$ and $\Sigma n_i[\Delta g_t(int)]_i$ in equation (5) we have determined the value of α and results have shown in TABLE 3.

RESULT AND DISCUSSION

In pathI, protein is transferred from water to aqueous urea as native state. During such transfer the cavity diameter of solute is unaltered but diameter of solvent is changed. Along pathII, since native protein is changed to denatured state in aqueous urea the cavity diameter alters due to denaturation. Thus cavity forming free energies along these paths may helps to understand the role of cavity energies towards denaturation by urea.

For the transfer of native lysozyme from water to aqueous urea along pathI the cavity forming free energies(Figure 2) are unfoavourable. These unfavourable transfer cavity forming free energies indicate it is harder to create a cavity for the accommodation of native protein in aqueous urea than in water. But, along

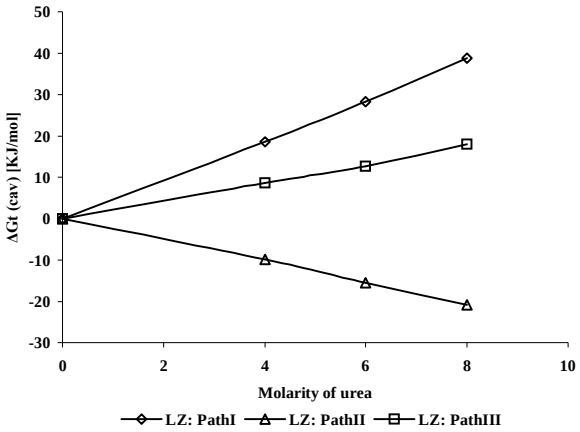

Figure 2 : Transfer cavity forming free energies of lysozyme (LZ) from water to aqueous urea at 298K

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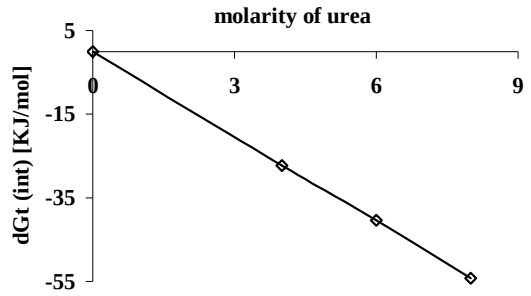

Figure 3 : Transfer free energies of interaction of lysozyme(LZ) from water to aqueous urea at 298K

pathII the fovourable transfer cavity forming free energies are due to decrease of solute(protein) diameter. Finally, the effective cavity contribution toward the transfer of lysozyme from water to aqueous urea along pathIII, which is given as equation (4), is unfavourable. It is interesting to note that the similar results have been reported [11] for the transfer of ribonucleaseA from water to aqueous urea.

The analysis of cavity equation indicate that cavity forming free energies values are more sensitive to the hard sphere diameter and number density of solvent than the hard sphere diameter of solute. However, we have followed a standard procedure[9-11,13-15,18] to calculate the equivalent hard sphere diameter of native and denatured lysozyme and used the well reported[9,11] values of hard sphere diameter and density of urea.

The transfer interaction free energies ΔG_t(int) (Figure 3), which are obtained after subtracting the cavity free energies from experimental transfer free energies, actually operate during denaturation process. Like the transfer free energies of interaction of ribonucleaseA[11] the ΔG_t(int) values of lysozyme for pathIII indicate interaction between protein and solvent in urea-water favours the denaturation. It may be noted that the denaturing action of urea has been studies using contact interaction model[12]. The study of contact interaction model by Schellman[12], shows that the values of total interaction as indicated from virial coefficient of lysozyme is negative in urea as denaturing media.

Thus the observed favourable interaction free energies and unfavaourable cavity energies of lysozyme in urea-water are in consistency with the results obtained from contact interaction model. Thus, both in terms of concept and prediction the usefulness of calculation of ΔG_t(cav) for transfer of protein to a solvent where protein structure is perturbed is established from present study.

As we have mentioned the experimental transfer free energies ΔG_t(expt) of denaturation are rationalized after subtracting the cavity part. Thus, it seems that the use of interaction free energies ΔG_t(int) may logical to calculate the degree of exposure. The transfer free energies of interactions Δg_t(int) (TABLE 2), which are also free from cavity part and electrostatic interaction part have been used to calculate the degree of exposure. These free energies of interaction are mainly guided by hydrophobicity of side chain. TABLE 3 shows the values of degree of exposure(α) of proteins in aqueous urea. The agreement between the calculated and reported values of α additionally support the dissection study of experimental free energies into cavity part and interaction part and also the proposed approach for the calculation of degree of exposure of protein in denaturing media.

In conclusion, it may be stated that experimental free energy of transfer of protein from water to aqueous urea need to be dissected into cavity part and interaction part to gain an idea about the effective interaction experienced by the protein in urea as a denaturant media. Admittedly, the dissection of favourable interaction free energy in terms of its various component such as hydrophobic interaction, H-bonding, electrostatic interaction, hard-soft interaction is to be considered for further insight.

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