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Role of Solvation in the Energy Stabilization Inside the Hydrophobic Core of the Protein Rubredoxin

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There are many forces that contribute to the stability of a protein; among these are dispersion interactions, hydrogen bonding, and solvation effects. In a recent work, Vondrásek et al. estimated the in vacuo stabilization energy of the hydrophobic core of the protein rubredoxin using high level ab initio methods (Vondrásek, J.; et al. *J. Am. Chem. Soc.* **2005**, *127*, 2615). In this work, we evaluate the effects of solvation on the stability of the hydrophobic core of this protein. Solvation calculations are made using the polarizable continuum method at the MP2/aug-cc-pVDZ level of theory. It is found that, in a protein-like environment (mimicked by a continuum solvent with a dielectric constant of ∼4), the stability of rubredoxin's hydrophobic core is decreased by 40−50%. We also observed that the stabilization energy of the hydrophobic core is only slightly lower in a protein-like medium than in an aqueous one ($\Delta G^{\text{ether}} - \Delta G^{\text{water}} \approx 1.0-3.5$ kcal/mol).

Introduction

Among the forces that participate in the stabilization of protein structures, hydrophobic interactions are considered to be the most dominant.[1,2] Buried within the interior of globular proteins is a core of hydrophobic residues, which is typically believed to be formed as a result of exterior hydrophobic forces of an entropic nature.[3,4] Recent work indicates that favorable enthalpic contributions from the van der Waals interactions that occur between hydrophobic core residues may also play a vital role in protein stabilization.[5,6] In this work, we seek to clarify the role that solvation effects have in stabilizing the hydrophobic core of globular proteins.

In a recent study, Vondrásek et al. estimated the contribution of short-range interactions to the stabilization energy of a model hydrophobic core of the small protein rubredoxin using high level ab initio calculations. In this study, it was found that the enthalpic contribution of the hydrophobic core to the overall stability of the protein is significant.[5] The results of this work, obtained with RIMP2 (resolution of the identity Møller−Plesset method) calculations at the complete basis set (CBS) limit, indicate that the stabilization energy within the core of rubredoxin is on the order of 25 kcal/mol. This is a remarkable number in that it exceeds the folding free energy of most proteins by a significant amount.[7] Here, it should be noted that the calculations used to determine the hydrophobic core stabilization energy were carried out in vacuo. Several recent studies indicate that the presence of solvent has a significant effect on the interaction energies of noncovalently bound systems.[8−10] The aim of the present work is to estimate the contribution that short-range interactions make to the total stabilization energy of the hydrophobic core of the rubredoxin protein when solvation effects are taken into account.

Methodology

The following two paragraphs summarize the calculation procedures used by Vondrásek et al.

Rubredoxin is a globular one-domain protein that contains a densely packed cluster of interacting residues centered around two phenylalanines (Phe30 and Phe49) in the interior of the protein. In the calculations of Vondrásek et al., they divided this hydrophobic cluster into two smaller clusters centered around each of the central residues; these clusters are named F30 (Figure 1) and F49 (Figure 2), according to their respective central residue. The first cluster, F30, contains six amino acid residues (Phe30, Phe49, Lys46, Leu33, Tyr13, and Tyr4), while the second one contains eight residues (Phe49, Cys39, Cys6, Phe30, Lys46, Val5, Trp37, and Tyr4). The smaller clusters are further divided into chemically distinct pairs of neutral amino acid residues (modeled as methylated aminoacyl residues), with each residue in a particular cluster interacting with that cluster's central residue. The total stabilization energy of each of the subclusters was determined as the sum of the pairwise stabilization energies of a central phenylalanine with the amino acids within that subcluster.

The positions of all the heavy atoms within the amino acid systems were kept fixed at the position determined by X-ray crystallographic methods (1RB9). The hydrogen atom positions were then optimized at the B3LYP/6-31G** level. Interaction energies for the amino acid pairs were determined using the RIMP2 method along with the aug-cc-pVDZ and aug-cc-pVTZ basis sets, and the complete basis set (CBS) limit results were extrapolated from these calculations. All interaction energies were corrected for the basis set superposition error.

To account for solvation effects, the solvation contribution to the inter-residue interaction energy is estimated to be the difference between the interaction energy of the solvated system

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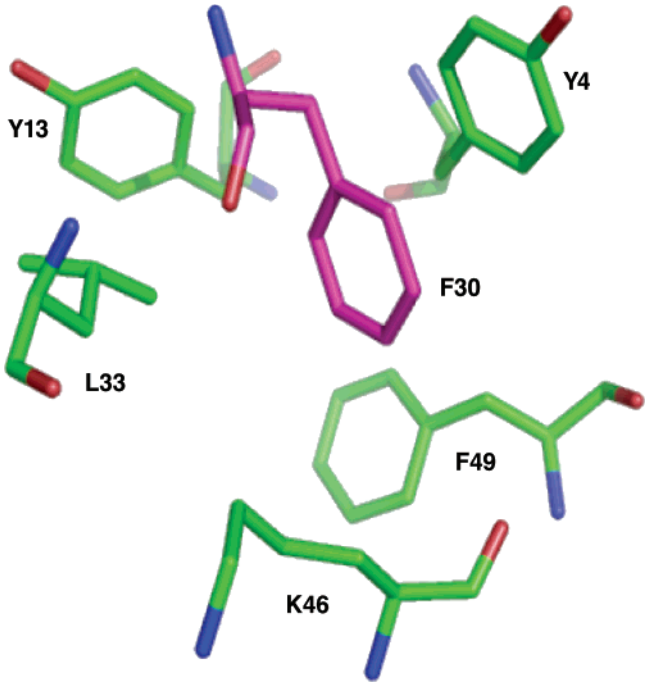

Figure 1. The F30 subcluster from the hydrophobic core of the rubredoxin protein.

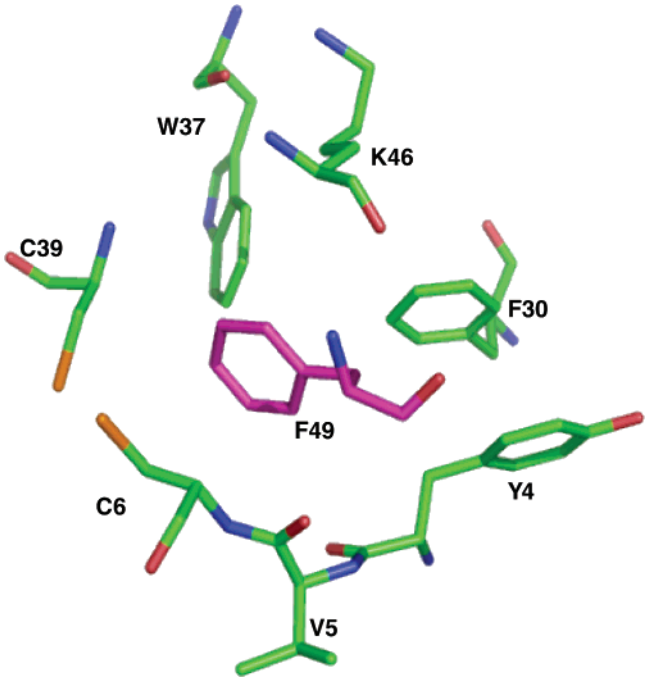

Figure 2. The F49 subcluster from the hydrophobic core of the rubredoxin protein.

and that of the system in a vacuum

$$\Delta\Delta G_{solv} = \Delta G_{solv} - \Delta E_{vac}$$

The total interaction energy in solvent is given by

$$\Delta G = \Delta E_{vac}^{CP} + \Delta\Delta G_{solv}$$

It should be noted here that ΔE_{vac}^{CP} is the interaction energy of the amino acid dimer as calculated in a vacuum and corrected for the basis set superposition error (BSSE), whereas ΔE_{vac} and ΔG_{solv} have no BSSE corrections.

The values of ΔE_{vac}^{CP} for all of the inter-residue interactions in both the F30 and F49 clusters are taken directly from the CBS limit results in the work of Vondrásek et al. The ΔE_{vac} and ΔG_{solv} values for each of the pairs are calculated at the MP2/aug-cc-pVDZ level.[11,12] Solvation effects are modeled using the polarizable continuum method (PCM),[13] a method that has been well validated and is the subject of several reviews.[14−16] To gain insight into the effects of solvation on the energetics of the hydrophobic core, we have carried out our calculations with two different solvents; the first of these is ether ($\epsilon = 4.335$), and the second is water ($\epsilon = 78.39$). Ether is used because its dielectric constant is taken to represent the overall dielectric constant in the interior of a protein.[8,17,18] All calculations were made using the Gaussian 03 suite of molecular electronic structure programs.[19]

Results

The interaction energies for all of the residue pairs considered in this work in a vacuum, water, and ether are given in Figure 3 and in Table 1. Here, it can clearly be seen that the introduction of solvent always results in a decrease of the interaction energies of these amino acid pairs. The Phe30···Leu33, Phe30···Tyr13, Phe49···Lys46, Phe49···Cys39, and Phe49···Cys6 pairs each have interaction energies in ether that are no more than half as strong as their interaction energies in a vacuum. Another conclusion that can be drawn from these data is that, in almost every case, the attraction between these residue pairs is stronger in ether than in water. In particular, the Phe30···Leu33 and Phe49···Val5 pairs exhibit interaction energies in water that are only 50 and 55% as strong as their respective interaction energies in ether. Exceptions to this rule are the Phe30···Tyr13 pair, which is slightly more stable in water than in ether, and the Phe30···Phe49 and Phe49···Trp37 pairs, which have approximately the same interaction energies in both solvents. The Phe30···Tyr4 pair represents the strongest interaction among all residue pairs considered here; this can be attributed to the attraction between the aromatic groups of these residues, whose conformation corresponds to the parallel-displaced conformation of the benzene dimer.[20,21] The interaction between these two residues exhibits a relatively small amount of destabilization (destabilization of 1.3 and 1.4 kcal/mol in ether and water, respectively). The second strongest interaction occurs between Phe49 and Val5. The strength of this interaction can be attributed to the presence of a CH···π interaction. This interaction is weakened significantly when

TABLE 1: Pair Interaction Energies (kcal/mol) of Selected Residues from the Core of Rubredoxin

residue	vacuum	ether	water
	F30 Cluster		
Phe49	−3.3	−2.3	−2.3
Lys46	−3.4	−1.7	−1.6
Leu33	−5.5	−2.2	−1.1
Tyr13	−4.5	−2.1	−2.2
Tyr4	−7.0	−5.7	−5.6
sum	−23.7	−14.0	−12.8
	F49 Cluster		
Phe30	−3.3	−2.3	−2.3
Lys46	−4.8	−2.1	−1.6
Cys39	−2.1	−0.6	−0.4
Cys6	−5.0	−2.4	−1.8
Val5	−6.7	−3.8	−2.1
Trp37	−2.5	−1.8	−1.8
Tyr4	−3.1	−1.8	−1.1
sum	−27.5	−14.7	−11.2

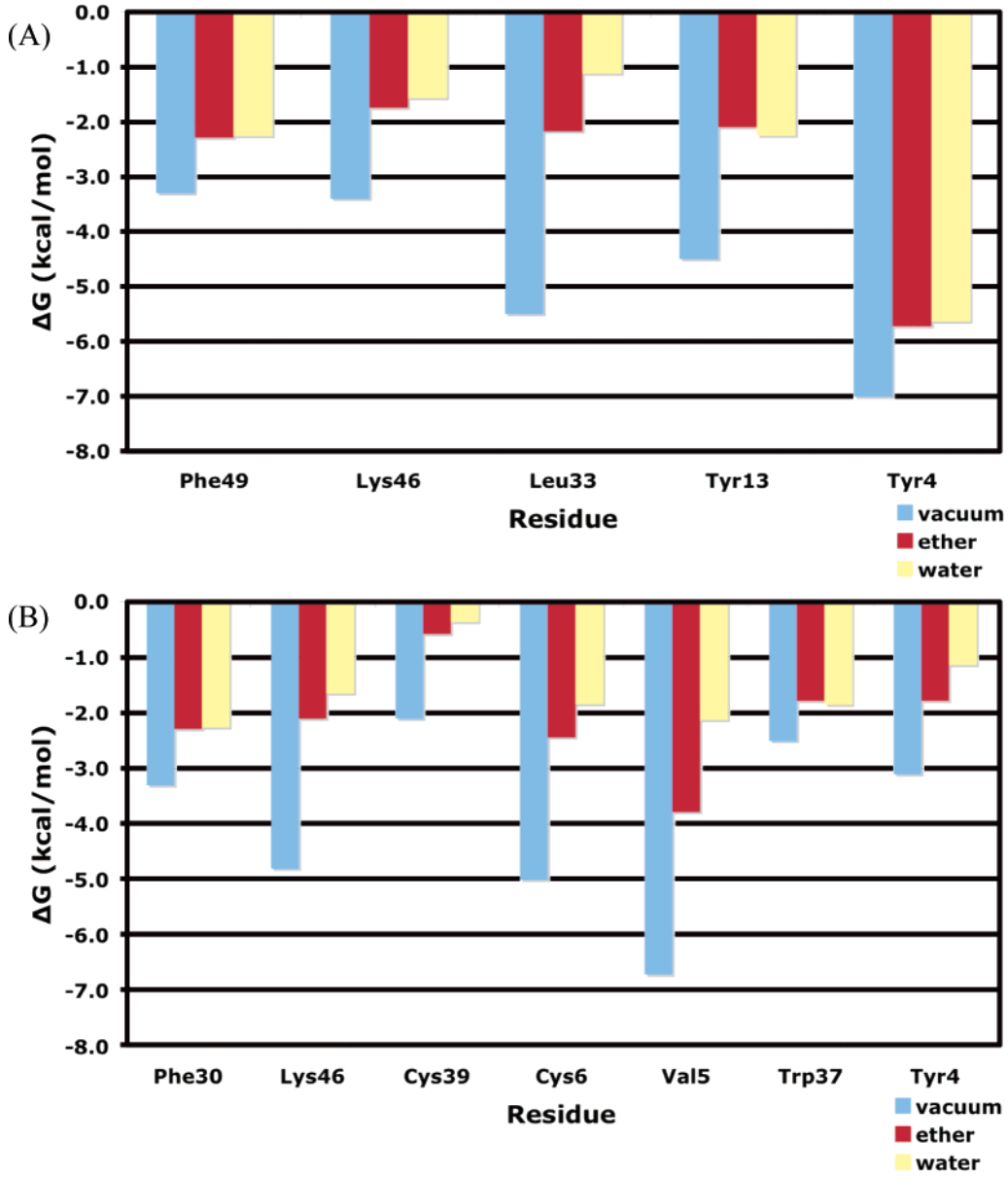

Figure 3. Interaction energies (kcal/mol) of Phe30 with residues from the F30 cluster (A) and Phe49 with residues from the F49 cluster (B) (here, in vacuo results are actually given as ΔE_{vac}^{CP}).

solvent is introduced (by 2.9 and 4.6 kcal/mol in ether and water, respectively).

It can be seen in Table 1 that the total stabilization energy of the subclusters decreases significantly upon introduction of solvent. For the F30 cluster, the total stabilization energy decreases from −23.7 kcal/mol in a vacuum to −14.0 and −12.8 kcal/mol in ether and water, respectively. The F49 cluster is destabilized from −27.5 to −14.7 kcal/mol in ether and to −11.2 kcal/mol in water. In the ether environment, which is meant to simulate an environment similar to one found in the interior of a globular protein, the total stabilization energies of the rubredoxin core subclusters are greatly decreased (by 9.7 and 12.8 kcal/mol for F30 and F49, respectively). One interesting quantity that can be estimated from the calculated data is the degree to which the hydrophobic subclusters are stabilized in a hydrophobic medium compared to an aqueous environment, that is, $\Delta G^{ether} - \Delta G^{water}$. It was found that these subclusters are only slightly more stable in ether than they are in water, with differences of −1.2 kcal/mol for the F30 subcluster and −3.5 kcal/mol for the F49 subcluster.

Conclusions

The results of our study, along with those of Vondrásek et al., indicate that short-range interactions between amino acid side chains are among the forces that contribute to the stability of rubredoxin. Our results also point toward the fact that solvation plays a pronounced role in the overall stability of rubredoxin's hydrophobic core. The introduction of solvent resulted in an unfavorable increase in the binding energy (corresponding to a decrease in stability) for each of the amino acid dimers considered here. The stability of the F49 subcluster is reduced by 47 and 59% upon solvation with ether and water, respectively. Given this large effect, it seems that solvation effects should be considered when dealing with inter-residue interactions whenever possible. Indeed, in the present case, the inclusion of solvation effects brings the computed results into better register with the observed folding free energies of proteins. Moreover, while the fragments studied here do have large interactions in the gas phase, when this is juxtaposed with solvation effects, the net effect is an overall weak stabilization of the individual portions of the hydrophobic core of rubredoxin.

Taking into account the results of the present work and those obtained in other studies,[8−10] it seems that solvation effects are likely to be important for all systems in which noncovalent bonding occurs.

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Supporting Information Available: Table showing the values of ΔE_{vac}^{CP}, ΔE_{vac}, ΔG_{solv}(water), and ΔG_{solv}(ether) for each of the pairwise residue interactions. This material is available free of charge via the Internet at http://pubs.acs.org.

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