Subscriber access provided by American Chemical Society

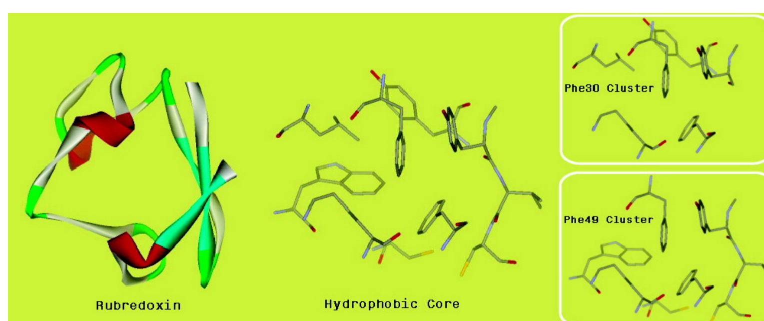
Article

Unexpectedly Strong Energy Stabilization Inside the Hydrophobic Core of Small Protein Rubredoxin Mediated by Aromatic Residues: Correlated Ab Initio Quantum Chemical Calculations

Ji Vondrek, Lada Bendov, Vojtch Klusk, and Pavel Hobza

J. Am. Chem. Soc., **2005**, 127 (8), 2615-2619• DOI: 10.1021/ja044607h • Publication Date (Web): 05 February 2005

Downloaded from http://pubs.acs.org on March 24, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 29 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

ACS Publications
High quality. High impact.

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S
ARTICLES
Published on Web 02/05/2005

Unexpectedly Strong Energy Stabilization Inside the Hydrophobic Core of Small Protein Rubredoxin Mediated by Aromatic Residues: Correlated Ab Initio Quantum Chemical Calculations

Jiří Vondrášek, Lada Bendová, Vojtěch Klusák, and Pavel Hobza*

Contribution from the Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic

Received September 6, 2004; E-mail: pavel.hobza@uochb.cas.cz

Abstract: The formation of a hydrophobic core of globular proteins is believed to be the consequence of exterior hydrophobic forces of entropic nature. This, together with the low occurrence of hydrogen bonds in the protein core, leads to the opinion that the energy contribution of core formation to protein folding and stability is negligible. We show that stabilization inside the hydrophobic core of a small protein, rubredoxin, determined by means of high-level correlated ab initio calculations (complete basis set limit of MP2 stabilization energy + CCSD(T) correction term), amounted to ~50 kcal/mol. These results clearly demonstrate strong attraction inside a hydrophobic core. This finding may lead to substantial changes in the current view of protein folding. We also point out the inability of the DFT/B3LYP method to describe a strong attraction between studied amino acids.

Introduction

Protein folding involves two critical elements, stability and specificity. The native structure of a typical protein is only 5−15 kcal/mol more stable than the unfolded state.[1] Hence, small differences in energy between multitudes of possible noncovalent interactions are summed up to provide the properly folded structure. To gain control of protein secondary and tertiary structure requires an understanding of how these noncovalent interactions provide both stabilization and specificity.[2]

Every globular and water-soluble protein has a hydrophobic core. The core is an arrangement of hydrophobic residues buried in the protein interior. The formation of a hydrophobic core, which is the driving process of protein folding in terms of energy, is connected with the existence of a folding nucleus,[3,4] a conserved region of protein which initiates the folding.[5,6] Evidence for a nucleation condensation mechanism can be found in the work of Itzhaki et al.,[7] which can be taken as one of the most important works in the field. Some forces, such as packing forces and H-bonding during protein folding in the context of the hydrophobically driven folding, are discussed in great detail by Zhu et al.[8] and Honig et al.[9]

Core formation is believed to be the consequence of exterior hydrophobic forces of entropic nature,[10,11] an example of classical hydrophobic effect[12] characterized by small contribution (repulsive or attractive) of complexation enthalpy. This, together with low occurrence of hydrogen bonds in the protein core, leads to the assumption that the energy (enthalpy) contribution of the core formation to protein folding is small or negligible.

Recent theoretical and experimental investigations of various types of noncovalent interactions have shown[13] that a rather large attraction could be gained not only from hydrogen bonding but also from other types of noncovalent interactions. Thus, the question arises of how strong are the stabilizing contributions of amino acids in a hydrophobic core. This question is of key importance for understanding the mechanism of protein folding as well as understanding protein secondary and tertiary structure.

The aim of the present work was to evaluate the stabilization energy of a model hydrophobic core based on a high-resolution X-ray structure of rubredoxin, a small soluble FeS protein (PDB code 1RB9). Stabilization energy was determined using high-level correlated ab initio calculations, specifically, as a sum of the complete basis set limit of the MP2 stabilization energy and CCSD(T) correction term.

Computational Model and Methods

Structure. Rubredoxin is a typical globular one-domain protein and contains a densely packed cluster of interacting residues centered around

(1) Branden, C.; Tooze, J. *Introduction to Protein Structure*, 2nd ed.; Garland Publishing: New York, 1999.
(2) Tatko, C. D.; Waters, M. L. *J. Am. Chem. Soc.* **2004**, *126*, 2028.
(3) Abkevich, V. I.; Gutin, A. M.; Shaknovich, E. I. *Biochemistry* **1994**, *33*, 10026.
(4) Fehrst, A. R. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 1525.
(5) Dokholyan, N. V.; Buldyrev, S. V.; Stanley, H. E.; Shakhnovich, E. I. *J. Mol. Biol.* **2000**, *296*, 1183.
(6) Shakhnovich, E. I.; Abkevich, V.; Ptitsyn, O. *Nature* **1996**, *379*, 96.
(7) Itzhaki, L. S.; Otzen, D. E.; Fehrst, A. *J. Mol. Biol.* **1995**, *254*, 260.
(8) Zhu, B.-Y.; Zhou, N. E.; Kay, C. M.; Hodges, R. S. *Protein Sci.* **1995**, *2*, 383.
(9) Honig, B.; Yang, A. S. *Adv. Protein Chem.* **1995**, *46*, 27.
(10) Tanford, C. *Science* **1978**, *200*, 1012.
(11) Rose, G. D.; Geselowitz, A. R.; Lesser, G. J.; Lee, R. H.; Zehfus, M. H. *Science* **1985**, *229*, 834.
(12) Meyer, E. A.; Castellano, R. K.; Diederich, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 1210.
(13) Müller-Dethlefs, K.; Hobza, P. *Chem. Rev.* **2000**, *100*, 143.

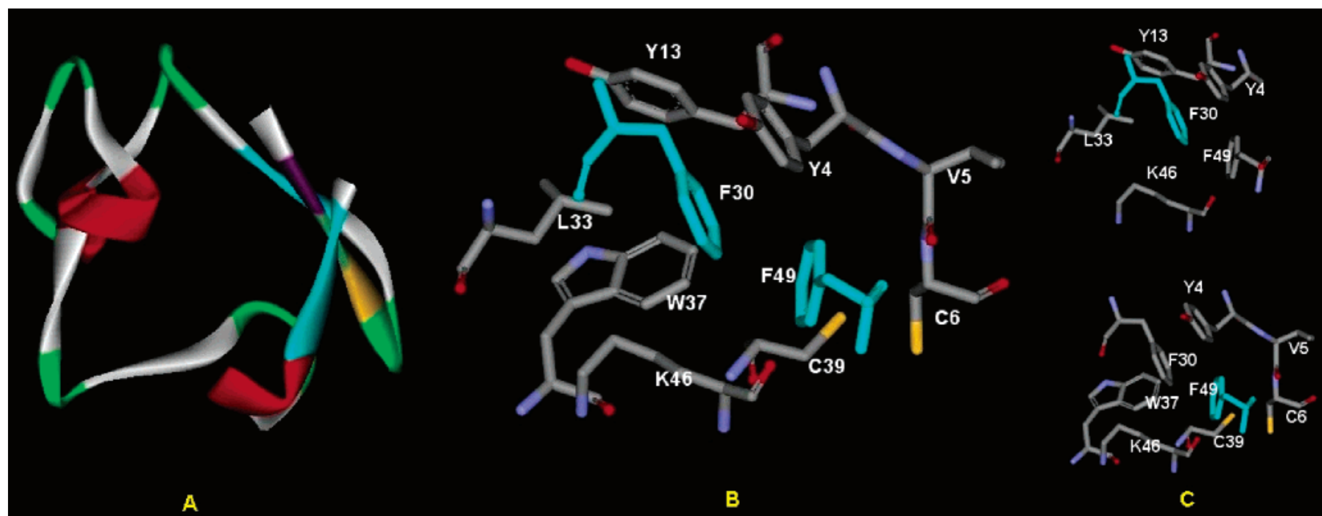


Figure 1. Rubredoxin. (A) Schematic view of the protein, (B) supercluster of F30 and F49, and (C) both subclusters individually.

two phenylalanines (F30 and F49) in the interior of the protein (Figure 1A,B). Localization of the cluster was determined by STING, a Web-based suite of programs (http://www.cbi.cnptia.embrapa.br), with distance criteria of 4.0 Å for the hydrophobic contacts of two amino acids. Water molecules are not present in the core, and thus, water is not directly participating in the core stabilization. The whole cluster was partitioned into two distinct clusters (named after the central residues, F30 and F49) and was further fragmented into well-defined, chemically distinct pairs of neutral amino acids (modeled as methylated aminoacyl residues). The central F30 and F49 phenylalanines thus interact with five (F49, K46, L33, Y13, and Y4) and seven (C39, C6, F30, K46, V5, W37, and Y4) amino acids, respectively. There is one H-bond ascribed to the F30 cluster (a classical CO···HN H-bond in the F30···L33 pair) and another two H-bonds are ascribed to the F49 cluster (a classical CO···HN H-bond in the F49···K46 pair, as well as an unusual CH···π interaction between the methyl group of the capped O terminus of V5 and the π system of the phenylalanine in the F49···V5 pair; cf. Figure 1B,C).

Interaction Energy. The heavy atom coordinates in all amino acid pairs were kept fixed at the positions from the X-ray structure (1RB9). Positions of the hydrogens were then optimized at the DFT/B3LYP/6-31G** level. The stabilization energies for all pairs of amino acids in clusters F30 and F49 were determined at the RIMP2 (resolution of identity Møller−Plesset method) level using a complete atomic orbital basis set (CBS) limit and, for a few selected pairs, also at the CCSD(T) level. It should be mentioned here that the stabilization energies of the H-bonded model and stacked clusters evaluated at the CCSD(T) and CCSDT levels were practically identical,[14] which gave full confidence in using CCSD(T) calculations.

The CCSD(T)/CBS interaction energy was approximated as

$$\Delta E_{\rm CBS}^{\rm CCSD(T)} = \Delta E_{\rm CBS}^{\rm MP2} + (\Delta E^{\rm CCSD(T)} - \Delta E^{\rm MP2})|_{\rm small\ basis\ set} \quad (1)$$

The former term was determined using the Helgaker extrapolation scheme.[15] The Hartree−Fock and correlation MP2 energies necessary for the extrapolation were determined with aug-cc-pVXZ (X = D, T) basis sets. The CCSD(T) term was calculated with a smaller basis set, 6-31G*(0.25) (exponent of d-functions changed from a standard value of 0.8 to a more diffuse one of 0.25). The use of a smaller basis set is based on the fact that the difference between the MP2 and CCSD(T) interaction energies (contrary to MP2 and CCSD(T) total energies themselves) is much less dependent on the size of the basis set, and the 6-31G*(0.25) basis set already gives satisfactory values of this difference.[16] All interaction energies were corrected for the basis set superposition error. The DFT calculations were performed using Gaussian03;[17] RIMP2 calculations were done by Turbomole,[18] and CCSD(T) calculations were performed using MOLPRO.[19]

Molecular Mechanics Calculations. All molecular mechanics calculations were performed using MOE (The Molecular Operating Environment), version 2004.03, software available from Chemical Computing Group Inc. (1010 Sherbrooke Street West, Suite 910, Montreal, Canada H3A 2R7; http://www.chemcomp.com).

Discussion of the Ab Initio Methods. The most common method of choice for the systems with sizes similar to the model intermolecular complexes studied in this work is either the DFT technique or the second-order Møller−Plesset (MP2) perturbation method.[20] The DFT methods provide reliable characteristics of isolated systems as well as H-bonded complexes, and due to their favorable size/CPU time ratio, they are extensively used also for biomolecular systems. The MP2 methods, covering a rather large portion of correlation energy, can be utilized not only for isolated systems and H-bonded complexes but also for stacked complexes. When used together with the resolution of identity technique (RIMP2 method[21]), a very favorable accuracy/CPU time ratio can be achieved.[22,23] The CBS limit extrapolated from the aug-cc-pVDZ and aug-cc-pVTZ energies is slightly underestimated with

(14) Pittner, J.; Hobza, P. *Chem. Phys. Lett.* **2004**, *390*, 496.
(15) Halkier, A.; Helgaker, T.; Jørgensen, P.; Klopper, W.; Koch, H.; Olsen, J.; Wilson, A. K. *Chem. Phys. Lett.* **1998**, *286*, 243.
(16) Jurečka, P.; Hobza, P. *Chem. Phys. Lett.* **2002**, *365*, 89.
(17) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, revision C.02; Gaussian, Inc.: Pittsburgh, PA, 2004.
(18) Ahlrichs, R.; Bär, M.; Häser, M.; Horn, H.; Kölmel, C. *Chem. Phys. Lett.* **1989** *162*, 165.
(19) *MOLPRO*, a package of ab initio programs designed by H.-J. Werner and P. J. Knowles, version 2002.1, R. D. Amos, A. Bernhardsson, A. Berning, P. Celani, D. L. Cooper, M. J. O. Deegan, A. J. Dobbyn, F. Eckert, C. Hampel, G. Hetzer, P. J. Knowles, T. Korona, R. Lindh, A. W. Lloyd, S. J. McNicholas, F. R. Manby, W. Meyer, M. E. Mura, A. Nicklass, P. Palmieri, R. Pitzer, G. Rauhut, M. Schütz, U. Schumann, H. Stoll, A. J. Stone, R. Tarroni, T. Thorsteinsson, and H.-J. Werner.
(20) Møller, C.; Plesset, M. S. *Phys. Rev.* **1934**, *46*, 618.
(21) Feyereisen, M.; Fitzgerald, G.; Komornicki, A. *Chem. Phys. Lett.* **1993**, *208*, 359.

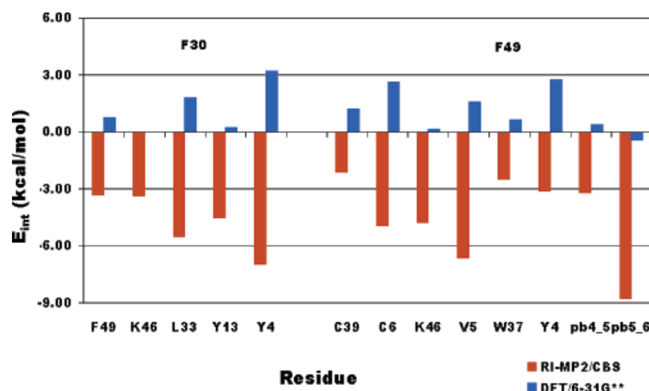


Figure 2. DFT and MP2/CBS interaction energies of F30 and F49 phenylalanines with selected amino acids from the rubredoxin core; DFT interaction energy of the F30···K46 pair is 0.

respect to the physically more justified CBS limit obtained from the aug-cc-pVTZ and aug-cc-pVQZ energies, but this underestimation is rather small (0.2−0.4 kcal/mol).[23] Higher-order correlation energy terms are important and should be included. In the CCSDT[24,25] calculations, all single, double, and triple and also part of quadruple and hextuple electron excitations are determined iteratively (i.e., up to the infinitive perturbation order). The CCSDT energies are very close to energies obtained from the full configuration interaction calculations and are thus approaching the true nonrelativistic energies. Though in the CCSD(T) calculations the triple excitations are determined only at the fourth perturbation order, the interaction energies, as mentioned in the text, are practically identical and the CCSD(T) method represents a method of choice even for larger biomolecular clusters.

Results and Discussion

The total stabilization energy of both clusters was determined as the sum of the pairwise stabilization energies of a central phenylalanine with the amino acids in its neighborhood. These energies were first determined at the frequently used DFT/B3LYP/6-31G** level. It should be mentioned here that DFT calculations are now widely used for biomolecular purposes and are even adopted in ab initio molecular dynamic simulations of the Car−Parinello-type. Figure 2 shows that 11 of 12 DFT pair interaction energies are repulsive, and the 12th one is only very slightly attractive. The DFT picture is thus consistent with the expected nature of interactions in a hydrophobic core with a low occurrence of hydrogen bonds. All pair interactions are either repulsive or negligible.

However, is this conclusion correct? It is evident in Figure 1 that the aromatic rings of the central phenylalanines are in contact with the aromatic and aliphatic side chains of the neighboring amino acids. These contacts should be stabilized by London dispersion energy. The theoretical description of London energy is difficult, and only highly accurate correlated ab initio calculations with extended basis sets are adequate in this case.[13]

Therefore, the calculations should be performed at the highest possible level, excluding the traditional problems of ab initio quantum chemical calculations, that is, the incompleteness of the AO basis set and insufficient amount of correlation energy covered.

(22) Jurečka, P.; Nachtigall, P.; Hobza, P. *Phys. Chem. Chem. Phys.* **2001**, *3*, 4578.
(23) (a) Hobza, P.; Šponer, J. *J. Am. Chem. Soc.* **2002**, *124*, 112802. (b) Šponer, J.; Jurečka, P.; Hobza, P. *J. Am. Chem. Soc.* **2004**, *126*, 10142. (c) Jurečka, P.; Hobza, P. *J. Am. Chem. Soc.* **2003**, *125*, 15608.
(24) Čížek, J. *J. Chem. Phys.* **1966**, *45*, 4256.
(25) Čížek, J. *Adv. Chem. Phys.* **1969**, *14*, 35−89.

Table 1. Pair of Interaction Energies (in kcal/mol) of the Selected Residues Clustered around F30[a]

	RIMP2			ΔCCSD(T)[b]	CCSD(T)/CBS
residue	aug-cc-pVDZ	aug-cc-pVTZ	CBS	6-31G*(0.25)	
F49	−3.1	−3.3	−3.3	−/0.6	
K46	−3.1	−3.3	−3.4	0.3/0.2	−3.10
L33	−4.9	−5.3	−5.5	0.5/0.2	−5.00
Y13	−4.2	−4.4	−4.5	0.6/0.4	−3.90
Y4	−6.5	−6.8	−7.0	−/1.7	
sum	−21.8	−23.2	−23.7		

[a] Compare Figure 1. [b] First number is the correction for whole modeled residue; second number is the correction for side chain only (side chain modeled from C_β atom).

Table 2. Pair of Interaction Energies (in kcal/mol) of the Selected Residues Clustered around F49[a]

	RIMP2		
residue	aug-cc-pVDZ	aug-cc-pVTZ	CBS
C39	−1.7	−2.0	−2.1
C6	−4.4	−4.8	−5.0
F30	−3.1	−3.3	−3.3
K46	−4.0	−4.6	−4.8
V5	−5.6	−6.4	−6.7
Y37	−2.3	−2.4	−2.5
Y4	−2.7	−3.0	−3.1
sum	−23.8	−26.5	−27.5

[a] Compare Figure 1.

Inspecting the RIMP2/CBS interaction energies (the lower part of Figure 2 and Tables 1 and 2), we found a very surprising picture. All 12 pairs of interaction energies were negative (i.e., stabilizing), and the stabilization energies were relatively high (for six pairs, even higher than 4.5 kcal/mol at the CBS limit). Especially important were the F30···Y4 and F49···V5 pairwise interactions with stabilization energies of about 7 kcal/mol. The first pair is stabilized by the interaction of the two aromatic rings, and the structure corresponds to a parallel-displaced structure of a benzene dimer. F49···V5 interaction is of a different nature. Due to the fragmentation procedure, the pair contains the CH···π contact instead of the π···π contact present in the real system (interaction of π electrons of phenylalanine and a peptide bond). As we will see later, the very large stabilization energy of the present system (F49···V5) only approaches the stabilization energy of an alternative model containing a phenylalanine ring and a peptide bond. The F30···Y4 and F49···V5 pairs clearly illustrate the stabilization role of the amino acid aromatic ring and show that strong stabilization (comparable or even higher than H-bonding) can originate from dispersion attraction without the presence of any classical H-bond.

Stabilization in the remaining 10 pairs is significant, as well, and supports the idea that the structural motifs of aromatic rings and aliphatic chains also contribute considerably to the stability of the system. It also supports a conclusion of Loladze et al.[26] that packing of nonpolar groups in the protein interior is favorable and is largely defined by a favorable enthalpy of van der Waals interactions. The CCSD(T) correction term, the calculation of which is extremely time demanding, is, in the

(26) Loladze, V. V.; Ermolenko, D. N.; Makhatadze, G. I. *J. Mol. Biol.* **2002**, *320*, 343.

cases of F30···K46, F30···L33, and F30···Y13, rather small (contrary to the case of stacked DNA base pairs), and the CBS limit of the MP2 stabilization energy can thus serve as a measure of true stabilization. Using this conclusion, we can state that both F30 and F49 clusters are described sufficiently on the RIMP2/CBS level.

The total stabilization of F30 with the surrounding amino acids amounts to nearly 24 kcal/mol and, in the case of F49, nearly 28 kcal/mol (Tables 1 and 2). The average stabilization of phenylalanine with one partner is thus more than 4 kcal/mol. This is very high stabilization, equivalent to hydrogen bonding, and is definitely far from the expected negligible or even repulsive interaction energy. These energies are very comparable to the above-mentioned energy difference between the folded and unfolded states of protein (5−10 kcal/mol), further underlining the biological significance of this stabilization.

For residues in the F30 cluster, the interaction of side chains without the backbone atoms (e.g., starting with C_β atom of side chain) was also considered. The smaller size of these systems allowed us to evaluate the CCSD(T) correction (second term of eq 1) coming from the amino acid side chains themselves. As long as the inter-amino acid contact of the residues with F30 was mediated mainly by the side chains, it also helped us to get an approximate view of the effect of correlation energy covered by CCSD(T) in the case of the complexes exceeding our computational capabilities. The energies are also presented in Table 1.

There is just one case where the CCSD(T) correction for the side chain interaction exceeds 1 kcal/mol: the F30···Y4 interaction. It shows that the RIMP2/CBS interaction energy of −7 kcal/mol is overestimated by around 1.7 kcal/mol. Because the amino acids are in contact by their side chains only, it is plausible to consider the correction for the interaction of side chains as a correction for the interaction of whole amino acids.

In light of that, we did not evaluate the CCSD(T) correction term in the case of complex F49. The RIMP2/CBS values alone are, as written above, sufficient.

We have further used the RIMP2/CBS calculated stabilization energy for each pair of amino acids as a standard for the stabilization energies calculated by the empirical force fields frequently used in protein modeling (i.e., AMBER-parm94, parm99, ff02, ff03, CHARMm22, MMFF94, Engh-Huber, OPLS-AA, TAFF, and Rule). The situation with empirical potentials is more favorable than that for DFT (Figure 3). Three of the potentials (Charmm22, Engh-Huber, and TAFF) follow the trends of the reference method, but only qualitatively.

A more detailed examination into the structure of the F49 cluster revealed another way to treat interacting residues by keeping the existing peptide bonds (PB). In proximity to the central F49 aromatic side chain, two PBs (Y4···V5 and V5···C6) exist and can be alternatively considered as partners for the central phenylalanine (cf. Figure 4). One of these PBs (V5···C6) is parallel to the plane of the F49 side chain, while the other PB (Y4···V5) is perpendicularly oriented. The parallel arrangement is known to provide considerable stabilization energy.[27,28]

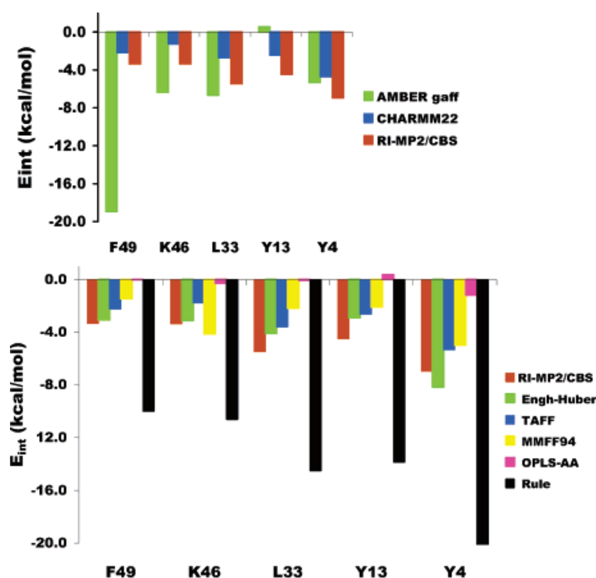


Figure 3. Comparison of the empirical interaction energies in cluster F30 evaluated by several force fields with the RIMP2/CBS results.

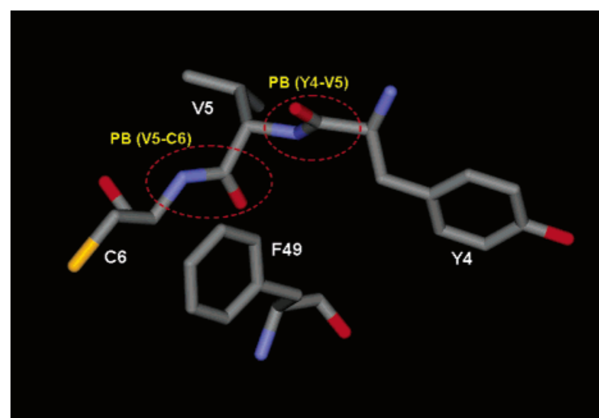


Figure 4. Two peptide bonds in proximity of the aromatic ring of the F49 residue. Dashed ellipses indicate both peptide bonds, PB(Y4−V5) and PB-(V5−C6), for which a stabilization energy was evaluated.

Table 3. Pair of Interaction Energies (in kcal/mol) of the Two Peptide Bonds, PB(Y4−V5) and PB(V5−C6) with F30

complex	RIMP2 aug-cc-pVDZ	RIMP2 aug-cc-pVTZ	RIMP2 CBS	ΔCCSD(T) 6-31G*(0.25)	CCSD(T)/CBS
F49−PB (Y4−V5)	−3.0	−3.1	−3.2	0.4	−2.8
F49−PB (V5−C6)	−7.9	−8.5	−8.8	0.6	−8.2

We calculated the F49 interactions with these two PBs (each PB modeled as *N*-methylformamide), and the resulting CCSD(T)/CBS interaction energies (Table 3) amounted to −8.2 and −2.8 kcal/mol, respectively. The stabilization energy of the former motif (parallel orientation of the PB and an aromatic ring) is surprisingly high, even higher than that for hydrogen bonding, and sheds new light on the role of peptide bonds in the stabilization of protein structures. Let us only add here that the CCSD(T) correction term is repulsive (+0.6 kcal/mol), but the repulsion is only modest in comparison with that known in stacked structures of DNA base pairs.[29]

(27) Toth, G.; Watts, C. R.; Murphy, R. F.; Lovas, S. *Proteins: Struct., Funct., Genet.* **2001**, *43*, 373.
(28) Duan, G.; Smith, W. H.; Weaver, D. F. *Int. J. Quantum Chem.* **2002**, *90*, 669.
(29) Šponer, J.; Hobza, P. *Collect. Czech. Chem. Commun.* **2003**, *68*, 2231.

To show that both fragmentation procedures are justified, we evaluated the size of the possible errors originating from various fragmentations. We calculated the interaction of F49 at the RIMP2/aug-cc-pVDZ level with the whole neutral tripeptide Y4−V5−C6 (i) and compared it to the results obtained for F49 interacting with three separated amino acids (Y4, V5, and C6) (ii) and for F49 interacting with two separated PBs (Y4···V5 and V5···C6) (iii). In the first case (i), we obtained a stabilization energy of 11.9 kcal/mol for the whole neutral Y4···V5···C6 complex. In the second case (ii), we obtained an energy of 12.7 kcal/mol (the sum for Y4, V5, and C6; see RIMP2/aug-cc-pVDZ results in Table 2). In the third case (iii), we obtained and energy of 10.8 kcal/mol (see Table 3). These results show that the difference between the three approaches is negligible, in a range of only 0.8 kcal/mol, and prove that both fragmentation methods are justified.

Conclusions

The present results show a complete failure of the DFT calculations, which are not even able to describe the attraction between central phenylalanines and neighboring amino acids. The results also fully support a known, but commonly ignored, fact that DFT methods cannot be recommended for simulating systems where London dispersion interactions play a major role.

The results presented here clearly demonstrate further the substantial attraction inside a hydrophobic core. This attraction, originating in London dispersion energy between aromatic rings or between an aromatic ring and an aliphatic chain, is comparable to classical H-bonding. Moreover, residues of aromatic nature can participate in several strong interactions at once, which may be crucial for the role of key residues in establishing small world networks inside a protein.[30]

Consequently, the current view on the nature of protein secondary and tertiary structure stabilization and, especially, the origin and nature of protein folding should thus be modified. Hydrophobic nature of a protein core implies that hydrophobic interactions can initiate the folding process. Present results indicate a decisive role of stabilization energy (enthalpy). Eventual consequences are that the energy (enthalpy) rather than hydrophobicity (entropy) can play a significant role during the early stage of protein folding.

Acknowledgment. We thank Pavel Jungwirth and Alex Wlodawer for critical reading and comments on the manuscript. This work is a part of the Research Project Z4055905 and was supported by a grant from the Grant Agency of the Academy of Sciences of the Czech Republic (Grant A400550510).

Supporting Information Available: Computational and additional data as well as figures and *xyz* coordinates of interacting pairs. This material is available free of charge via the Internet at http://pubs.acs.org.

JA044607H

(30) Vendruscolo, M.; Paci, E.; Dobson, C. M.; Karplus, M. *Nature* **2001**, *409*, 641.