The Key to the Stabilisation of the Hydrophobic Core of Rubredoxin

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Introduction

The protein thermostability has many important consequences to their possible use in the industry as well as for the explanation of protein evolution. For the study of thermostability, one needs to find an appropriate protein model. Rubredoxin family includes several small proteins ranging from mesophilic to hyperthermophilic ones. It has been argued whether the difference in their stability is caused by the addition of the salt bridges or by the more favorable packing of the hydrophobic core in the thermophilic rubredoxins [1,2].
Here we have tried to increase the thermostability of the thermophilic rubredoxin 1brf by replacing phenylalanine residues situated in its hydrophobic core by naphtylalanine residues, which have stronger dispersion interactions and higher solvation enthalpy.

General approach

Rubredoxin structure from *Pyrrococcus furiosus* was taken from http://rcsb.org - PDB ID: 1BRF. OPLS-AA/L force field was modified to include naphtylalanine (NPA) residue. There are 2 phenylalanine (PHE) residues in the hydrophobic core in positions 29 and 48. The protein was modified by all possible substitutions. Every NPA residue has 2 different rotamers hence there are 8 possible substitutions. We have then performed molecular dynamics simulations of the substituted protein in explicit water. We have taken snapshots from simulation in 20 ps intervals. Snapshots have been then optimized at MM level and interaction energy matrices (IEM) were calculated by PM6-DH method.

Computational details

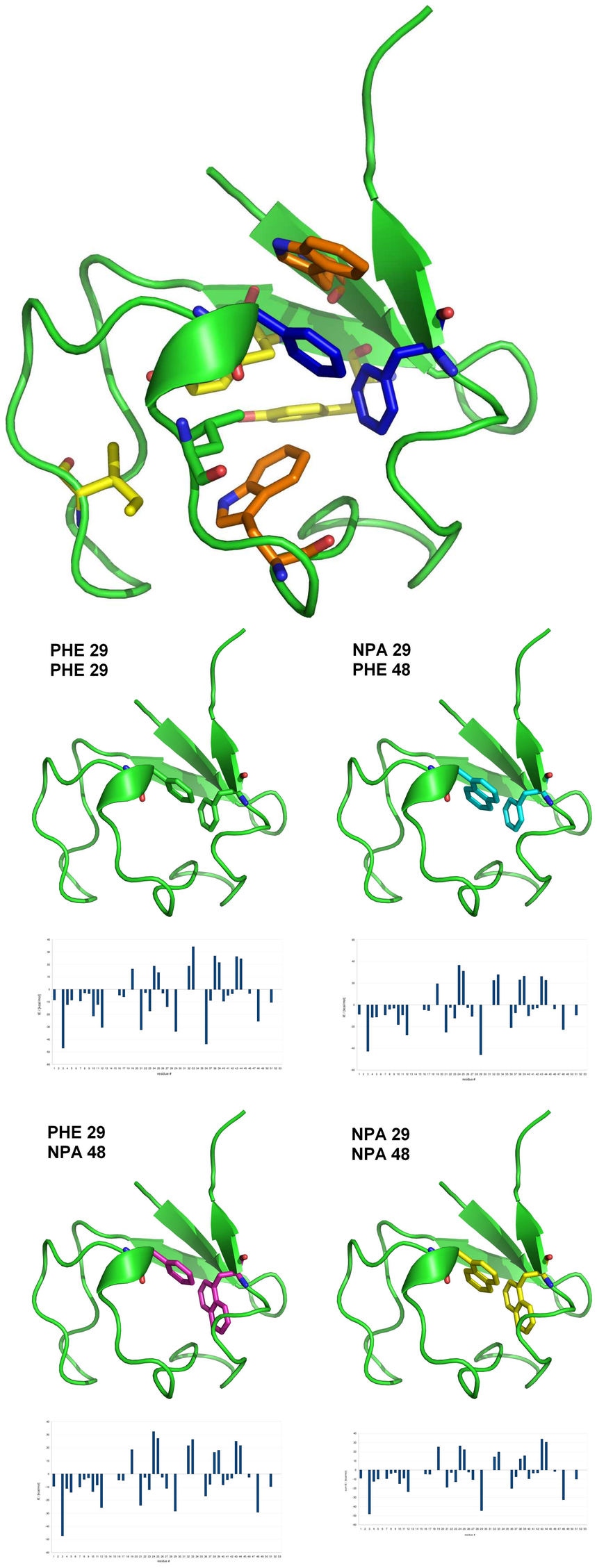
Molecular dynamics simulations were performed with parameters as follows:

- opls-aa/l (naphtylalanine written by self)
- 100 ps prerun.
- 2 ns simulation (0.002 ps step)
- NPT ensemble
- 300 K, Nosé-Hoover thermostat
- Berendsen pressure coupling
- Cut off on electrostatics
- Gromacs 3.3 program package

IEM was calculated using this protocol:

- energy minimization with opls-aa/l force field
- truncation of residues at Cα
- addition of hydrogens to complete valence shell
- calculation of pairwise interaction energies between residues with PM6-DH method using MOPAC 2007 code

Sum of interaction energies of a particular residue with other residues suggests how the residue participates on stabilisation of protein. These were averaged over all optimized structures.



Results & Discussion

- **Sum of the mean interaction energy of NPA 29 is lower than PHE 29 by 15 kcal/mol and of NPA 48 is lower than PHE 48 by 10 kcal/mol.**
- **The mean interaction energies between other residues changed after substitution and are more influential on the change of stabilisation energy.**
- **Radii of gyration strongly depend on rotameric form of substituted residue. RoG of more stable protein with substituted PHE 29 has RoG lower than the other and even lower than 1BRF itself.**
- **The stabilisation energy was increased by 45 kcal/mol by subsitution of PHE 29 and by 70 kcal/mol by substitution of both PHE residues. In the case of substituted residue PHE 48, we have already calculated one rotamer, which shows destabilisation by 20 kcal/mol**
- **The RMSD of IEM calculations is aproximately 10% of the particular value.**

Perspectives

- Calculate IEM at MM level (OPLS), QM calculations of PHE/NPA with other residues.
- Include solvation effects and calculate the enthalpy change (in water solution) of the replacement of PHE residues by NPA residues.
- Calculate IEM for other proteins (including mesophilic) from rubredoxin family.
- Try to improve thermostability for another proteins (currently working on mesophilic rubredoxin 1rb9) and to compare to experimental data.

References

[1] Berka, K., Hobza, P., Vondrášek, J., 2009. Analysis of energy stabilization inside the hydrophobic core of rubredoxin. ChemPhysChem 10 (3), 543-548.
[2] Vondrášek, J., Kubař, T., Jenney, Adams, M. W., Kožíšek, M., Černý, J., Sklenář, V., Hobza, P., 2007. Dispersion interactions govern the strong thermal stability of a protein. Chemistry - A European Journal 13 (32), 9022-9027.

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