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published in

*From Computational Biophysics to Systems Biology (CBSB07),
Proceedings of the NIC Workshop 2007,*
Ulrich H. E. Hansmann, Jan Meinke, Sandipan Mohanty,
Olav Zimmermann (Editors),
John von Neumann Institute for Computing, Jülich,
NIC Series, Vol. 36, ISBN 978-3-9810843-2-0, pp. 185-188, 2007.

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<http://www.fz-juelich.de/nic-series/volume36>

Folding and Structure Prediction of Proteins Containing Disulfide Bridges

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A computational study of the proteins 1KVG and 1WQE containing disulfide bridges is presented. The simulation method bases on the protein force field PFF02 and the basin hopping technique. Both proteins were folded correctly from extended conformations with inclusion of a constraining potential.

1 Introduction

Computational prediction of tertiary structure of proteins with high accuracy on the basis of the primary structure requires development of transferable protein force fields as well as powerful optimization methods. Particularly, proteins with disulfide bridges connecting cysteine residues represent a major challenge for computational biophysics. Recently, plenty of proteins have been folded to their native conformations within experimental resolution using all-atom protein force fields and stochastic optimization methods¹⁻³. However, there are only few works that study the folding behavior of proteins with disulfide bonds, e.g., by means of molecular dynamics^{4,5}, conformational space annealing with a united-residue force field⁶, lattice models⁷, topology-based approach⁸, distance geometry⁹, neural networks¹⁰ and the island model¹¹. In this paper, we report results of protein folding simulations for proteins containing disulfide bridges: the β -hairpin 1KVG and the potassium channel blocker 1WQE. In particular, we will focus on an approach with inclusion of additional binding potential to the free energy of the protein.

2 Methods

All-atom force fields PFF01^{1,2} and PFF02¹² have been developed recently to describe the internal free energy of proteins. Besides α -helical proteins, the PFF02 enables correct description of β -sheet regions and allows unbiased comparison of results for proteins of the two types. To search for the free energy minimum, corresponding to the protein native conformation, the basin hopping technique is employed as outlined in previous work³.

To promote formation of disulfide bridges a constraining Morse potential $V_{SS}(r) = -E_0 [(1 - e^{-\beta(r-r_0)})^2 - 1]$ was considered, where r_0 is the equilibrium distance between the sulfur atoms of cysteine residues forming a disulfide bridge, E_0 is the energy corresponding to r_0 , and β is the spacial extent of the potential. The choice of Morse potential is motivated by the fact that disulfide bridges are covalent bonds. In what follows, $r_0 = 2 \text{ \AA}$ and $\beta = 1 \text{ \AA}^{-1}$.

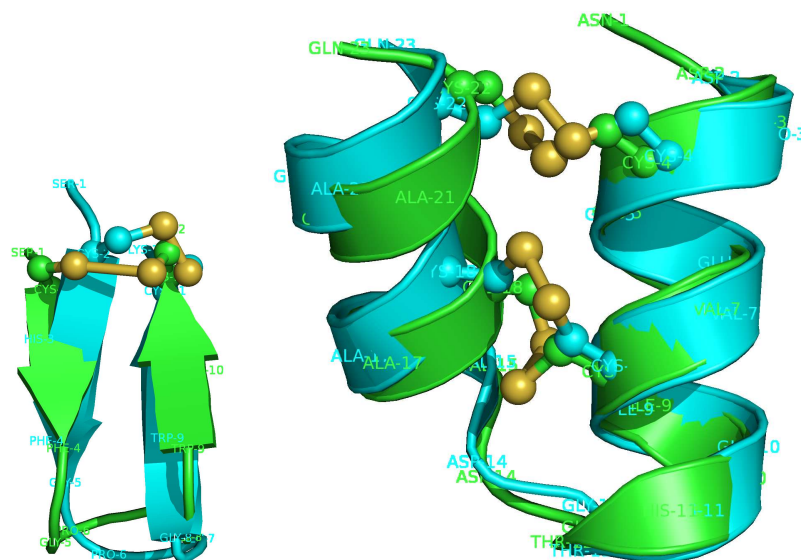


Figure 1. Cartoons of the folded proteins 1KVG (left) and 1WQE (right), shown in green, superimposed on the corresponding native structures shown in cyan. Sulfur atoms of cysteine residues forming disulfide bridges are depicted as spheres in golden color.

3 Results and Discussion

Simulations of thirty independent runs were performed for different values for E_0 starting from the extended structure, i.e. all backbone dihedral angles were set to 180° . Prediction accuracy of the results was assessed by comparing the simulated structures with natural structures determined by NMR. This comparison can be visualized overlaying the simulated structure on the reference, as shown in Fig. 1. In addition, the root mean square deviation of the backbone (RMSDb), the disulfide bond lengths and secondary structure contents are summarized in Table 1.

The α -helical 1WQE could be folded correctly to near-native conformations with and without constraining potential. However, the use of constraints increases the efficiency of the stochastic search for the global minimum significantly¹³. The other protein, 1KVG, could be folded to native conformation only in presence of the constraining potential. Turning off the constraints, the simulation of 1KVG ends up in all runs with an unstructured coil conformation. As can be seen in Table 1 the inclusion of constraining potential stabilizes the native β -sheet structure. For both proteins, the larger values for E_0 tighten the disulfide bonds while smaller values yield better overall structure.

4 Concluding Remarks

A constraining Morse potential was adopted to take into account disulfide bonds in proteins. For all proteins studied, inclusion of the constraining potential resulted in improved

E_0 [kcal/mol]	RMSDb	Res. Nr / r_{SS} [Å]		Sequence / secondary structure
1KVG		2-11		SCHFGPLGWVCK
natural	—	2.1		CEEEETTEEEEC
0	2.1	3.3		CBCBTTTBSCBC
2 [†]	2.1	2.9		CEEEETTEEEEC
5	2.3	2.7		CEEESSSSEEEEC
1WQE		4-22	8-18	NDPCEEVCIQHTGDVKACEEACQ
natural	—	2.0	2.0	CCHHHHHHHHHTCCHHHHHHHHC
0	2.1	3.3	5.5	CHHHHHHHHHHTCCHHHHHHHHC
2 [†]	1.9	2.8	2.9	CHHHHHHHHHHTCCHHHHHHHHC
5	4.4	2.7	2.7	CHHHHHHSCSSTTTCHHHHHHHHC

Table 1. Characteristics of the folded conformations in comparison with the natural conformations. In columns 3 and 4 the distances r_{SS} between the sulfur atoms of specified cysteine residues are given. Cases denoted by [†] are shown in Fig. 1.

RMSDb values and distances between cysteine sulfur atoms compared to constraint-free simulations. It was demonstrated that the constraining potential can be decisive for correct folding and prediction of the three-dimensional structure.

Acknowledgments

Grant of computing time by project CampusGrid at the Research Center Karlsruhe is gratefully acknowledged.

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