Loop size optimization: a new mechanism for protein stabilization

Alessia Ruggiero, Nicole Balasco, Luciana Esposito, and Luigi Vitagliano

1Institute of Biostructures and Bioimaging, C.N.R., Naples I-80134, Italy
2Dipartimento di Scienze e Tecnologie Ambientali Biologiche e Farmaceutiche, Seconda Università di Napoli, Caserta 81100, Italy

Motivation

One of the fundamental issues in both chemistry and biology is the identification of the structural determinants that dictate protein folding and stability. The decoding of the folding code of protein structures would have a major impact on native structure prediction and on de novo design. This task is particularly difficult to achieve. Unlike synthetic polymers, protein structures combine complexity, fine-tuning and marginal stability. Despite these difficulties, in recent years major progresses have been made. A very recent breakthrough in the field is represented by the discovery of Baker and colleagues that the juxtaposition of basic secondary structure elements (α-helices and β-strands) follows well-defined rules (Koga et al., 2012). These investigations identified three fundamental rules for the preferences of βℓβ (strand-loop-strand), αℓβ (helix-loop-strand) and βℓα (strand-loop-helix) structural motifs. In particular, it was shown that the chirality of βℓβ and the orientation of βℓα/αℓβ strongly depend on the loop size. In this framework, we evaluated the impact of these rules on protein structures isolated from either (hyper)thermophilic or mesophilic organisms. We used the thioredoxin (Trx) system to experimentally validate the results emerged from the statistical analyses.

Methods

Statistical surveys

Our statistical survey was based on the analyses of different structural databases made of proteins isolated from mesophilic or thermophilic organisms by assuming that the proteins of thermophilic species were on average more stable than those isolated from mesophilic ones. The adherence of these proteins to the rules identified by Baker and coworkers was evaluated.

Experiments

Wild-type E. coli Trx and a series of ad-hoc mutants were expressed and purified. The stability of these proteins was evaluated by CD spectroscopy. The structure of these variants was determined by X-ray crystallography.

Results

The statistical analyses indicate that in proteins isolated from thermophilic organisms better adhere to the Baker rules through the optimization of the size of the loop connecting secondary structure elements (Balasco et al., 2013). We then experimentally validated this mechanism using the thioredoxin isolated from...
E. coli (EcTrx), a widely characterized protein that has been used as a model in a large number of investigations (Esposito et al., 2012, Ruggiero et al., 2009). Comparative analyses of loop sizes between EcTrx and Trx isolated from hyperthermophiles suggested that the size loop connecting helix 1 (α1) to strand 2 (β2) in EcTrx could be modified to better follow the rules. Chimeric variants were therefore prepared by replacing the loop of EcTrx with the corresponding ones present in the Trx isolated from Sulfolobus solfataricus and S. tokodaii. Interestingly, although the sequences of this loop region of the two latter proteins did not display any significant similarity, their insertion in EcTrx sequence induced a remarkable stabilization of the protein (~ 12 °C). The crystallographic structure of the variant embodying the loop from the S. solfataricus counterpart shows that the loop shortens. According to the fundamental rules the shortening of this loop should favor the topology of the connected elements of the Trx fold thus explaining the stabilization of this variant. Moreover, the crystal structure indicates that the side chains of the loop residues do not form any stabilizing interaction with the rest of the protein. This explains why similar effects are obtained using the unrelated sequence of S. tokodaii. These considerations indicate that topological aspects may have an important impact on protein stability. Finally, on the basis of the these results we propose a novel protocol of protein stabilization that can be applied in a variety of different scenarios.

References