Global and Local Structural Changes of Protein Unfolding

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Summery: In the mean-field Ising model, the folding-unfolding behavior of a protein is regarded as an ensemble of units reduced from, presumably, peptide bonds or amino acid residues. Units of similar thermodynamic properties are further classified into groups that are related to, for instance, α-helices or β-sheets. Units of the same group are assumed to unfold collectively, whereas those of different groups may undergo either sequential or coupled unfolding. The introduction of unfolding groups facilitates the description of non-collective local structural changes experimentally observed via a multi-group unfolding. We incorporate denaturants and temperature effects into the free energy expression of the protein upon dissolution in a specific environment at thermal equilibrium, and a model protein, *cytochrome c*, was examined. Results indicate that there are at least four unfolding groups induced unfolding of *cytochrome c*: two are related to the prosthetic heme group, whereas another two groups are α-helices and global nearly group, which largely account for global changes in protein morphology. The extracted thermodynamic parameters, on the basis of the Ising model, can closely predict unfolding behaviors of the proteins in compounded denaturing environments.

Keywords: *cytochrome c*; ising model; mean filed approximation; multi-group unfolding

Introduction

The Ising model with a mean field approximation developed by Lin's group can describe both kinetics and thermodynamics of protein folding-unfolding influenced by denaturant effects[1–3] or calorimetric change of enthalpy, as well as the unfolding in atomic force measurements,[4,5] via a microscopic point of view. The model displays the unfolded fraction as $f_u^\ell = \{1+\exp[\frac{2\varepsilon^\ell + 4J^\ell(1-2f_u^\ell)}{k_B T}]\}^{-1}$, where the unfolding free energy $\Delta G^\ell(C_m, T) = 2\varepsilon^\ell$ is determined by $\varepsilon^\ell \equiv \Delta\varepsilon_m^\ell C_m + \Delta\varepsilon_T^\ell(T - T_{1/2}^\ell)$.[6,7] The Ising model provides a simple yet coherent description on the multi-group unfolding behaviors of the protein not only at global but also local scales via considering multi-groups unfolding as a summation behaviors of the individual correlated groups $f_u = \sum_\ell \gamma_\ell f_u^\ell$. In this study, the unfolded *cytochrome c* was introduced by presence of urea (0 to 10 M) as well as changes in temperature (295 to 363 K). The global morphology of *cytochrome c* was observed via small-angle X-ray scattering (SAXS), the local behaviors such as heme group and the secondary structure were observed by UV-Vis absorption and circular dichroism (CD).

Results and Discussion

To consistently extract parametric values $2\Delta\varepsilon_T$, $2\Delta\varepsilon_m$, and $T_{1/2}$, we have jointly fitted the three urea-dependent f_u profiles together with the three temperature-dependent f_u profiles, as listed in Table 1.

The results show unfolding of α-helices is closely related to the global morphology

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Table 1.
Fitted thermodynamic parameters of *cytochrome c* in urea- and temperature-induced unfolding.

Group	γ	$2\Delta\varepsilon_m$ (kcal mol^{-1} M^{-1})	$2\Delta\varepsilon_T$ (kcal mol^{-1} K^{-1})	$T_{1/2}$ (K)	ΔG_o(293K) (kcal mol^{-1})
Heme	0.011	-0.92 ± 0.2	-0.138 ± 0.015	333 ± 11	5.9 ± 0.6
Heme	0.029	-1.03 ± 0.10	-0.138 ± 0.015	346 ± 5	7.3 ± 0.8
α-helices	0.12	-1.30 ± 0.21	-0.167 ± 0.026	354 ± 1	10.2 ± 0.8
Global-nearly	0.84	-1.30 ± 0.21	-0.167 ± 0.026	359 ± 5	10.9 ± 0.9

change in the unfolding process. In contrast, the $f_{u,abs}$ profile exhibits two-step features, characteristic of a two-group unfolding at lower temperatures. The γ values suggest that the global structural change with minor contributions from Heme and α-helices, and dominant contribution from the global-nearly group observed by SAXS. We have further extracted the thermal unfolding free energy $\Delta G_o(T) = 2\Delta\varepsilon_T(T - T_{1/2})$ for each group. With the denaturant and temperature effects incorporated in the free energy expression, we may predict the unfolded fraction of *cytochrome c* at arbitrary temperature and/or urea concentrations. The calculated f_u profile using thermodynamic parameters given in Table 1 matches well with the independent SAXS observation of thermally induced unfolding of *cytochrome c* in 6 M urea, as shown in Figure 1. This urea concentration is expected to result in decreased ΔG and hence a shift of the unfolded fraction profile by $\Delta T = \Delta\varepsilon_m C_m/\Delta\varepsilon_T = 47$ K from the denaturant-free counterpart.

Conclusions

Combining SAXS and spectroscopic techniques, we have demonstrated that a recently developed mean-field Ising model provides an adequate basis for the quantitative description of the unfolding behaviors of *cytochrome c* induced by changes in temperature and urea concentration. A multi-group unfolding behavior of *cytochrome c* was observed. Thermodynamic parameters extracted from simple denaturing processes, on the basis of the Ising model, can be used to predict unfolding behaviors of the proteins in compounded denaturing environments. Integrated local and global structure information improves the understanding of the protein folding-unfolding mechanism.

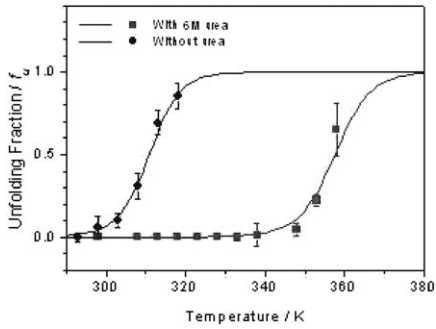

Figure 1.
Two temperature dependent profiles (solid curves) displayed together with the SAXS results (scattered points) demonstrate the unfolding fraction without and with 6 M urea of *cytochrome c*. Both calculated unfolding fraction profiles share the same thermodynamic parameters in Table 1. The profile of 6 M urea indicates a nice prediction for the SAXS observation.

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