SAXS of the chemically denatured states of homologous dihydrofolate reductases.

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Introduction

Dihydrofolate reductases (DHFR) are complex α/β proteins where the mixed 8-stranded β-sheet is flanked on either side by two α-helices. The complex doubly-wound β-sheet topology is composed of an adenosine-binding domain and a larger loop subdomain.

The thermodynamic and kinetic folding mechanisms of DHFR from *Escherichia coli* (ec) [1-3], human (hs) and *Lactobacillus casei* (lc) [Wallace and Matthews, manuscript in preparation] have been studied using a variety of biophysical techniques. The proposed kinetic folding mechanism for all three DHFR homologues is surprisingly complex; development of the β-sheet topology dominates the fast folding events, and domain folding and reorganization events occur later in folding and provide access to the native state through multiple parallel folding channels.

Small-angle X-ray scattering (SAXS) studies were performed to characterize and compare the structural properties (size and shape) of the chemically denatured state for three DHFR homologues. These studies aimed to understand the role that the conformation of the urea-denatured state of DHFR plays in determining the folding pathway.

Results and Discussion

SAXS-detected equilibrium unfolding of ecDHFR, lcDHFR and hsDHFR were investigated as a function of denaturant at 15°C, pH 7.4. The low solubility of native hsDHFR and aggregation of partially (un)folded forms in the transition region for lcDHFR prevented a thermodynamic evaluation of the unfolding reaction for these two systems. However, for ecDHFR the urea-induced equilibrium unfolding was consistent with the two-state thermodynamic model (N⇆U) previously elucidated [1-3].

Guinier and Krakty [4] analysis were performed on the X-ray scattering curves. Further, the scattering data was analyzed using the pair-distance distribution function, P(r) [5]. Figure 1 shows representative (a) Kratky plots and (b) distribution functions for native and 5.9 M urea-denatured ecDHFR.

The calculated radius of gyration (R_g) from the Guinier analysis for folded ecDHFR and lcDHFR was found to be ~ 16 Å. This value agrees with the predicted value from the crystallographic coordinates for ecDHFR and lcDHFR using CRYSOL [6]. Further, the monophasic and symmetrical P(r) distributions with maximum dimensions (d_{max}) of 50 Å and R_g of 17 Å are consistent with values expected for the native state.

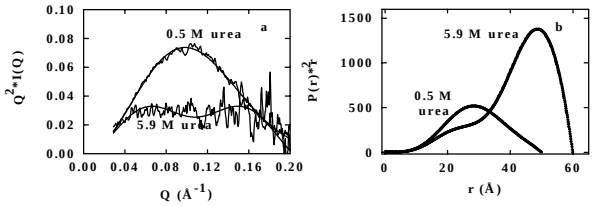

Figure 1: (a) Kratky plot and (b) P(r) distribution of native (0.5 M urea) and denatured (5.9 M urea) ecDHFR.

The Guinier and Kratky analysis of the scattering curves and the P(r) distributions for the 6M urea-denatured state(s) of all three homologues of DHFR revealed that the unfolded state is relatively compact with some random coil component. Three lines of evidence support this observation: (1) the calculated R_g values from the Guinier plot was 25 Å, which is much less that the 60 Å predicted for a polypeptide chain of this length, (2) the Kratky plot exhibited a plateau in the profile at low angles and a decrease at higher angles and (3) the P(r) is biphasic. In the P(r) distribution, the small peak located at ~ 30 Å suggests the presence of residual native-like structure and a larger peak at ~ 50 Å indicates an extended, unfolded polypeptide chain. Further the R_g calculated from the P(r) distribution is also low, consistent with that obtained from the Guinier analysis.

Conclusion

Solution X-ray scattering of the urea-denatured state of DHFR has highlighted the presence of native-like residual structure. To delineate the origin of this residual structure and its role in determining the folding pathway of DHFR, subsequent SAXS experiments will focus on circular permutant and double mutant variants of ecDHFR, as well as the guanidine hydrochloride-induced unfolded state.

References

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