Several recent CPMG studies of fast-folding mutants of the SH3 domain of the tyrosine kinase Fyn have suggested the presence of a low-populated folding intermediate where the central β-sheet is fully formed already, but not the NH2- and COOH-terminal strands β1 and β5, respectively. In the absence of β5, NOESY CPMG experiments of the fast-folding SH3 mutants N53P/V55L and A39V/N53P/V55L over a wide temperature range show the existence of an on-pathway intermediate with similar features. However, the N chemical shifts extracted for the intermediate state indicate pronounced non-native contacts between the NH2- and COOH-terminal regions not observed for other mutants. Stopped-flow measurements of the unfolding kinetics of the Fyn SH3-A57T/D62E mutant at 25°C (red) and 45°C (blue) show that the unfolding rate is predicted to follow the chevron equation: 

\[
\ln \frac{k_{\text{on}}}{k_{\text{off}}} = \ln \left( \frac{f}{f_{\text{co}} - f} \right) = \ln \left( \frac{b}{b+1} \right) = \ln \left( \frac{G_{\text{fold}}}{G_{\text{unfold}}} \right)
\]

where \(f\) denotes the fraction of folding, \(b\) is the ratio of the stability of the native state and the unfolded state, \(G_{\text{fold}}\) and \(G_{\text{unfold}}\) are the free energy of the folded and unfolded state, respectively. The unfolded state is characterized by two major contributions to the relaxation of the cross-relaxation rate constant, \(J_{\text{ex}}\), from the stopped-flow experiments are consistent with the CPMG data (Fig. 1) and report on the rate-limiting transition state between the unfolded and intermediate state (Fig. 1).

Methods and Results

Understanding of protein folding requires knowledge of the kinetics, thermodynamics, and structure of the various states that populate its folding pathway. In practice such information can be difficult to obtain. Information about these excited states is often obtained through indirect approaches using changes of temperature, pressure, denaturant concentration, and/or primary sequence. A particularly common method is the stopped-flow measurement of the folding/unfolding kinetics as a function of denaturant concentration, \(v_{\text{ex}}\), monitored by tryptophan fluorescence. For a system folding according to a simple kinetic scheme, the exchange of tryptophan and the unfolding (and the folding rate) is predicted to follow the clewson equation:

\[
\ln k_{\text{f}} = \ln \left( \frac{f}{f_{\text{co}} - f} \right) = \ln \left( \frac{b}{b+1} \right) = \ln \left( \frac{G_{\text{fold}}}{G_{\text{unfold}}} \right)
\]

where \(k_{\text{f}}\) and \(k_{\text{ex}}\) are rate constants for forward folding and exchange, respectively, extrapolated to zero denaturant, and \(f_{\text{co}}\) and \(f_{\text{ex}}\) the corresponding slopes. The clewson plot of the Fyn SH3 A39V/N53P/V55L closely follows this 2-state model (Fig. 1).

References


Fig. 1: Clewson plot of the stopped-flow folding kinetics of the Fyn SH3 A39V/N53P/V55L as a function of folding/unfolding kinetic rate constant (i.e., folded). Values of \(k_{\text{f}}\) of 0.85 and 0.52, respectively, were obtained for the clewson plots. The data are fitted to a 2-state model with high precision with \(k_{\text{f}} = 1.02 \times 10^4\) s\(^{-1}\) ± 0.03 s\(^{-1}\), \(v_{\text{ex}} = 0.008\) ± 0.002 s\(^{-1}\), and \(k_{\text{ex}} = 1.02 \times 10^4\) s\(^{-1}\) ± 0.03 s\(^{-1}\). CPMG NMR relaxation dispersion spectroscopy is extremely sensitive to chemical exchange processes on the millisecond time-scale, allowing the detection of excited states populated only to 0.5%. Quantitative analysis of the dispersion profiles yields the kinetic (exchange rates), thermodynamic (populations), and structural information in the form of chemical shift differences between the exchanging states (Fig. 1).

Table 1: Values of the parameters \(\Delta G_{\text{fs}}\) (kJ/mol) and \(\Delta H_{\text{fs}}\) (kJ/mol) in the Fyn SH3 A39V/N53P/V55L in the absence and presence of denaturant.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\Delta G_{\text{fs}})</td>
<td>15.3 ± 0.8</td>
</tr>
<tr>
<td>(\Delta H_{\text{fs}})</td>
<td>21.6 ± 0.8</td>
</tr>
</tbody>
</table>

Relative to the native state, the \(\Delta G_{\text{fs}}\) values are slightly smaller, indicating that the barriers to the folding transition are lower for the intermediate state. The values of \(\Delta H_{\text{fs}}\) are also lower, indicating that the folding transition is more enthalpically driven for the intermediate state. This is consistent with the notion that the intermediate state is more populated in the presence of denaturant, and that the barriers to the folding transition are lower for the intermediate state. The values of \(\Delta G_{\text{fs}}\) are also slightly smaller, indicating that the barriers to the folding transition are lower for the intermediate state.