

Table 1. Equilibrium and kinetic data for folding of MLAc

Parameters	Equilibrium	Kinetics	
		Far-UV CD (%)	Fluorescence (%)
$[\text{urea}]_{1/2}$	$2.3 \pm 0.1 \text{ M}$	—	—
m_{UN}	$-13.5 \pm 1 \text{ kJ/mol,M}$	—	—
$\Delta G_{\text{UN}}(\text{H}_2\text{O})$	$31 \pm 1 \text{ kJ/mol}$	—	—
k_{burst}	—	$>1,000 \text{ s}^{-1}$ (50)	$>1,000 \text{ s}^{-1}$ (20)
k_{fast}	—	—	$0.34 \pm 0.01 \text{ s}^{-1}$ (30)
m_{fast}	—	—	$1.5 \pm 0.05 \text{ kJ/mol,M}$
k_{IN}	—	$0.032 \pm 0.002 \text{ s}^{-1}$ (50)	$0.029 \pm 0.002 \text{ s}^{-1}$ (50)
m_{IN}	—	$-1.22 \pm 0.08 \text{ kJ/mol,M}$	$-1.39 \pm 0.08 \text{ kJ/mol,M}$
k_{unf}	—	$0.04 \pm 0.01 \text{ ms}^{-1}$ (100)	$0.04 \pm 0.01 \text{ ms}^{-1}$ (100)
m_{unf}	—	$3.97 \pm 0.11 \text{ kJ/mol,M}$	$3.85 \pm 0.09 \text{ kJ/mol,M}$
$\Delta G_{\text{IN}}(\text{H}_2\text{O})$	—	$16.5 \pm 0.5 \text{ kJ/mol}$	$16.1 \pm 1.0 \text{ kJ/mol}$
$m_{\text{IN}(\text{eq})}$	—	$-5.19 \pm 0.19 \text{ kJ/mol,M}$	$-5.24 \pm 0.17 \text{ kJ/mol,M}$

Equilibrium unfolding (at pH 7.5 and 20°C) is two-state, whereas the simplest mechanism that explains the kinetic-folding data for MLAc is $I_{\text{burst}} \leftrightarrow U \leftrightarrow I \leftrightarrow N$. $\Delta G_{\text{IN}}(\text{H}_2\text{O})$ was calculated as $RT \ln(k_{\text{IN}}/k_{\text{unf}})$ and $m_{\text{IN}(\text{eq})}$ as $m_{\text{IN}} - m_{\text{unf}}$; the Tanford β_1 value was calculated as $m_{\text{UI}(\text{eq})}/m_{\text{UN}} = (m_{\text{UN}} - m_{\text{IN}(\text{eq})})/m_{\text{UN}}$ to be 0.61 ± 0.03 . For the various kinetic phases, relative amplitude changes are given in parentheses after the rate constants. Standard deviations are derived from the fits to the data (Figs. 3 and 5A). For the equilibrium parameters, CD and fluorescence data were averaged. —, not applicable.