Probing denatured-state dynamics in cytochrome *c* by electron-transfer kinetics

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A protein can fold no faster than the rate at which it can form native tertiary contacts. We have determined the rates of tertiary contact formation directly by measurements of electron-transfer (ET) kinetics in zinc-substituted, ruthenium-modified yeast cytochrome *c* under denaturing conditions. In order to examine the effect of loop size on the initial contact formation rate, we have prepared a series of cytochrome *c* mutants, each with a single surface histidine modified with pentaammine ruthenium(III). Intramolecular protein ET rates, and thus the rates of contact formation, range from ~ 100 ns to 5 μs in the presence of ~ 5.5 M guanidine hydrochloride. The variation of contact time with loop size does not agree well with predictions based on simple polymer dynamics models and shows strong dependences on the polypeptide sequence. This work confirms that denatured cytochrome *c* is not a random coil and indicates that a more sophisticated model is required for predicting tertiary contact formation dynamics. In addition, these measurements are consistent with an upper "speed limit" of ~ 100 ns for folding of cytochrome *c*.