Light Induced Helix Formation

Cheng-Yen Huang,[†] Shu He, [‡] William F. DeGrado,[‡] Dewey G. McCafferty*[,‡] and Feng Gai*[,†]

[†]Department of Chemistry and [‡]Department of Biochemistry & Biophysics and the Johnson Research Foundation, University of Pennsylvania, Philadelphia, PA 19104

Supporting Information

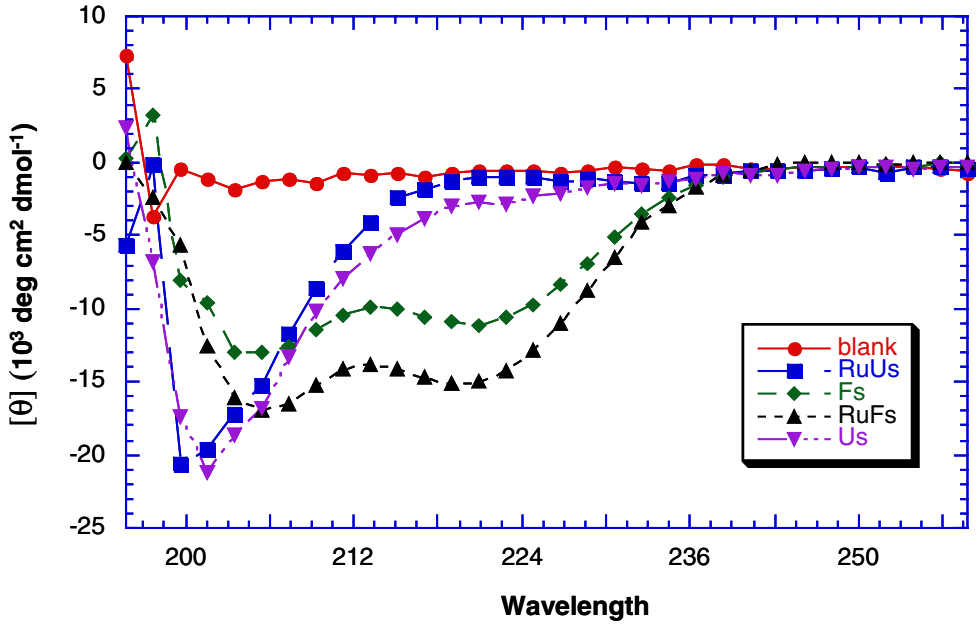


Figure 1. CD spectra of the peptides used in current study.

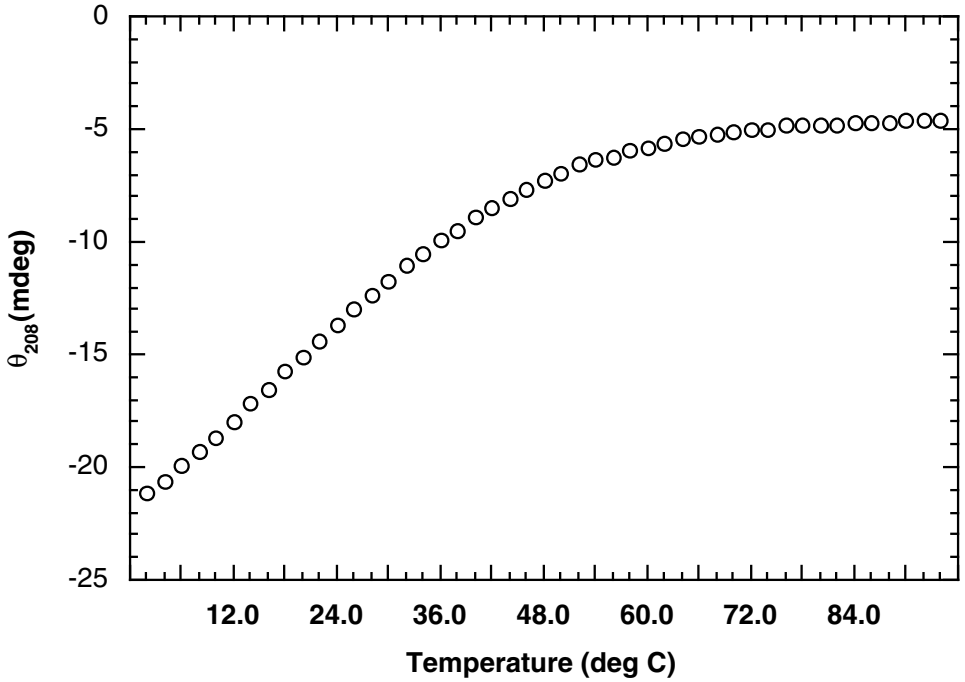
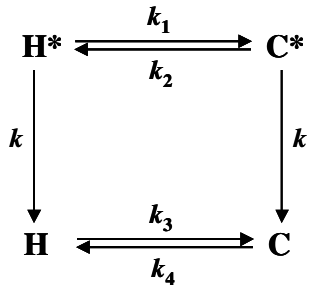


Figure 2. Thermal denaturation of RuFs peptide.

(a) Excited State Decay Kinetics of a Two-State System



For the above two-state system, its excited state decays according to following equations:

$$\mathbf{H^*(t)} = \frac{1}{(k_1 + k_2)}\left[\left(k_1\mathbf{H^*(0)} - k_2\mathbf{C^*(0)}\right)\exp(-\alpha t) + k_2\left(\mathbf{H^*(0)} + \mathbf{C^*(0)}\right)\exp(-\beta t)\right] \quad (1)$$

$$\mathbf{C^*(t)} = \frac{1}{(k_1 + k_2)}\left[\left(k_2\mathbf{C^*(0)} - k_1\mathbf{H^*(0)}\right)\exp(-\alpha t) + k_1\left(\mathbf{H^*(0)} + \mathbf{C^*(0)}\right)\exp(-\beta t)\right] \quad (2)$$

Where, $\alpha = k_1+k_2+k$, $\beta = k$, H*(0) and C*(0) are the populations of H* and C* at time zero. So, the net population change of H*, due to the equilibrium shift taking place at the excited state, is then

$$\Delta\mathbf{H^*(t)} = \left(\mathbf{H^*(0)} + \mathbf{C^*(0)}\right)\left[\exp(-\beta t) - \exp(-\alpha t)\right] \quad (3)$$

Therefore, the observed optical density change at a specific probing frequency due to the net helix formation can be modeled by a bi-exponential function, namely,

$$\Delta\mathbf{OD(t)} = \mathbf{A}\left[\exp(-\beta t) - \exp(-\alpha t)\right] \quad (4)$$

Here, the amplitude A is a constant determined by the pump energy, the absorbance of the sample at the pump wavelength, the infrared absorbance of the peptide at the probing frequency. It can be shown that this amplitude, A, is related to the stability change of the helical structure induced by the coupling between the Ru complex dipole and the helical dipole, i.e.,

$$\mathbf{A} = \mathbf{A_0}\frac{\left(\mathbf{K_{eq}} - \mathbf{K'_{eq}}\right)}{\left(1 + \mathbf{K_{eq}}\right)\left(1 + \mathbf{K'_{eq}}\right)} \quad (5)$$

Where A_0 is a constant; $\mathbf{K_{eq}}$ and $\mathbf{K'_{eq}}$ are the ground-state and excited-state equilibrium constants for unfolding, respectively. Using the relationship $\Delta G = -RT\ln(K_{eq})$, we can get

$$\mathbf{K'_{eq}} = \mathbf{K_{eq}}\exp\left(-\frac{\Delta\Delta\mathbf{G}}{\mathbf{RT}}\right) \quad (6)$$

Substituting equation (6) into equation (5) yields

$$\mathbf{A} = \mathbf{A_0}\frac{\mathbf{K_{eq}}\left(\mathbf{1} - \exp\left(-\frac{\mathbf{\Delta\Delta G}}{\mathbf{RT}}\right)\right)}{\left(1 + \mathbf{K_{eq}}\right)\left(1 + \mathbf{K_{eq}}\exp\left(-\frac{\mathbf{\Delta\Delta G}}{\mathbf{RT}}\right)\right)} \quad (7)$$

(b) Dipolar Interaction Energy:

The dipolar interaction energy[1] between two electric dipoles that are aligned head to tail is

$$\mathbf{E} = -\frac{2\mu_1\mu_2}{4\pi\varepsilon r^3} \qquad (8)$$

where r is the distance between the two dipoles. If E = 0.15 kcal/mol, μ_1 = 8 Debye, and r = 2 nm, $\varepsilon = 7.08 \times 10^{-11}$ $C^2/N\ m^2$ (see ref. 7), equation (8) yields μ_2 = 50 Debye.

(c) Ground-State Bleaching Recovery and D_2O Signals:

To reveal the helix-coil transition kinetics at the excited state, the photobleaching signal and D_2O's absorbance change due to local heating must be subtracted from the measured decay kinetics. Laser flash photolysis experiments show that both RuFs and RuFu peptides exhibit single-exponential excited-state decay kinetics with identical lifetime (data not shown). Thus, the bleaching recovery signal can be determined by the following function, $\Delta OD_B(t) = A_B*\exp(-t/\tau_D)$; where A_B is the initial amplitude and τ_D is the excited-state decay lifetime which can be determined by fitting the IR signal of RuFu peptide to a single-exponential function. Equally, the D_2O signal can be determined by a similar function, i.e., $\Delta OD_D(t) = A_D*[1-\exp(-t/\tau_D)]$; where A_D is a constant (because thermal cooling has a time constant much greater than 2 μs, ΔOD_D does not show any significant decay even at the longest delay time (~2 μs) used in this study). To determine A_B and A_D, the IR signal of RuFs peptide was modeled first by a double-exponential function, i.e., $\Delta OD(t) = C*\exp(-t/\tau_1) + D*\exp(-t/\tau_2) + E$; where C, D, and E are constants. And then the amplitudes of A_B and A_D were calculated based on the following relationships: $A_B = C + D + E$, and $A_D = E$.

(d) Helix-Coil Transition at the Excited State:

It can be shown that if there is no helix-coil transition process taking place at the excited state, the observed decay signal of the RuFs peptide should follow single-exponential kinetics (only population decay). Otherwise, the decay signal of the RuFs peptide should exhibit double-exponential behavior with $\tau_1 = \tau_D$, and $1/\tau_2 = 1/\tau_D + 1/\tau_{HC}$; where τ_{HC} is the helix-coil relaxation time constant (see above). Figure 3 shows the fits to either a single- or double-exponential function. Clearly, the fit to a double-exponential function results in more random residuals, indicating that indeed the helix-coil transition process takes place at the excited state.

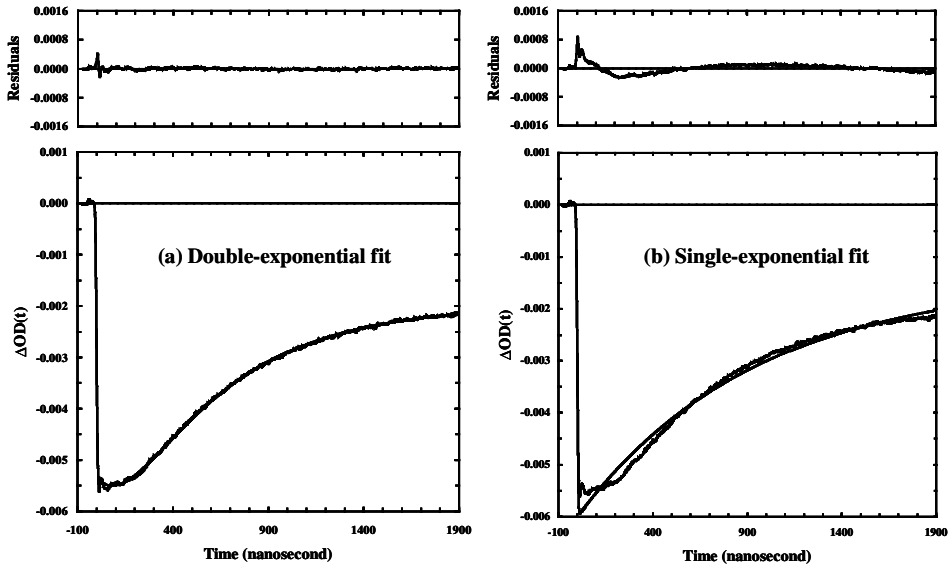


Figure 3. Decay signal observed at 1657 cm^{-1} and fits to a single- and double-exponential function plus a background, as indicated.

[1] McHale, J. L., *Molecular Spectroscopy*; Prentice-Hall: Upper Saddle River, 1999; p 60.