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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 and Biophysics and the Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pennsylvania 19104

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The mechanism by which proteins fold is under active theoretical and experimental investigation. Of particular current interest are the earliest events in folding such as secondary structure formation and hydrophobic collapse, which occur on the nanosecond to microsecond time scale. There is a need for photochemical triggers that can be used to initiate folding and conformational change on this time scale. Existing methods include electron transfer-induced folding of hemeproteins,[1] photodissociation of aromatic disulfides,[2] photodissociation of bezoinyl cages,[3] photoisomerization of diazostilbene derivatives,[4] and temperature jump experiments.[5,6] However, these techniques suffer from several limitations. Temperature jump experiments are limited to conditions where the protein undergoes a net unfolding reaction or to the folding of proteins that undergo cold denaturation. Light-initiated ligand dissociation and electron transfer-initiated folding are limited to hemoproteins. Furthermore, photodissociation of bezoinyl cages and photoisomerization of diazostilbene derivatives are not readily reversible, which precludes signal averaging of a single sample, and studies with diazostilbene and bezoinyl cages as well as disulfide derivatives have involved the preparation of cyclic structures that present highly restrained conformation states.

Proteins are known to fold or change conformations in response to a change in the charge distribution of their cofactors. Furthermore, measurements by Lockhart and Kim[7] of the interaction between monomeric α-helices and solvent-exposed dipolar groups (internal Stark effect) or a titratable group (electrostatic screening) show that the magnitude of the interaction energy between probe and partial charge on the N-terminus is significant (>1 kcal mol^{-1}) and stabilizing. Thus, we sought an aromatic amide whose electronic configuration could be rapidly switched in response to a short laser pulse. To be most applicable, this transition should be readily reversible, and it should generate a relatively inert excited state with a lifetime significantly longer than the time scale for helix formation.

Tris-bipyridyl derivatives of Ru(II) appear to fulfill these requirements (Figure 1). Upon irradiation with visible light, amide derivatives of tris-bipyridyl complexes such as [Rub$_2$m-OH]$^{2+}$ (where Ru = Ru(II); b = 2,2′ bipyridine; and m-OH = 4′-methyl-2,2′-bipyridyl-4′-carboxylic acid) undergo metal-to-ligand charge transfer (MLCT) to create an excited state which persists for ∼1−2 μs in solution under anaerobic conditions.[8] This probe typically generates a photoinduced dipole change of $\Delta\mu$ ∼5−9 D, depending on ligand substitution;[9] that is stable on the 100 ns time scale expected for helix formation. In addition, excited-state resonance Raman experiments of the methyl amide analogue ([Rub$_2$m-NHCH$_3$]$^{2+}$) indicated that the electron resides primarily on the electron-deficient ligand (m-NHCH$_3$).[10]

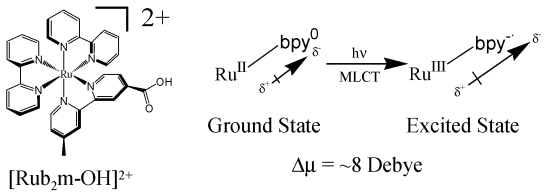

Figure 1. Tris-bipyridyl complex and the dipole moment change between ground and excited states.

Table 1. Peptides Used in Current Study

code	sequence	conformation
Fs	H_2N-AAAAA(AAARA)$_3$A-$CONH_2$	helical
Fu	H_2N-AAPAA(APARA)$_3$A-$CONH_2$	random
RuFs	[Rub$_2$m]$^{2+}$-CONH-AAAAA(AAARA)$_3$A-$CONH_2$	helical
RuFu	[Rub$_2$m]$^{2+}$-CONH-AAPAA(APARA)$_3$A-$CONH_2$	random

We therefore synthesized a series of peptides and their Rub$_2$m-modified derivatives on the basis of Kim's α-helix forming sequences (Table 1). The folding kinetics of these peptides have been extensively studied by T-jump infrared (IR) and fluorescence methods,[5] which provides an important comparison for the current study. Peptides Fs and RuFs were designed to be 50% helical at room temperature, so that the maximal change in folding was easily observable. We also prepared a nonhelical control with a helix-breaking Pro included in the sequence. Circular dichroism (CD) spectroscopy indicated that Fs and RuFs were 50% helical as expected (Supporting Information). Indeed, the incorporation of an aromatic capping group in RuFs led to a slight enhancement of the helical content. Also, as expected, peptides Fu and RuFu were essentially devoid of helical content.

The light-induced helix formation was monitored by infrared spectroscopy.[11] Previous studies[12] have shown that solvated helices absorb around 1630 cm^{-1}, whereas disordered conformations absorb around 1650 cm^{-1}. Therefore, the excited state decay kinetics of the RuFs and RuFu peptides were probed near these frequencies. As shown (Figure 2), both peptides exhibit an instantaneous bleaching component as well as a long-lived background. Because the Ru complex is a racemic mixture of optical isomers (lambda and delta), the signal observed corresponds to that of an average of the two optical diastereomers. The instantaneous component is the result of the photoinduced Stark effect, which effectively broadens the vibrational transition and therefore causes a bleaching of the amide I′ band.[2] The long-lived component, however, is attributed to the decrease in D_2O's absorbance due to the local heating of the solvent by energies released from the excited-state decay process. Nevertheless, the increase in temperature is small (∼0.5 °C). Following the initial instantaneous bleaching, the excited

* To whom correspondence should be addressed. E-mail: (F.G.) gai@sas.upenn.edu; (D.G.M.) deweym@mail.med.upenn.edu.
[†] Department of Chemistry.
[‡] Department of Biochemistry and Biophysics and the Johnson Research Foundation.

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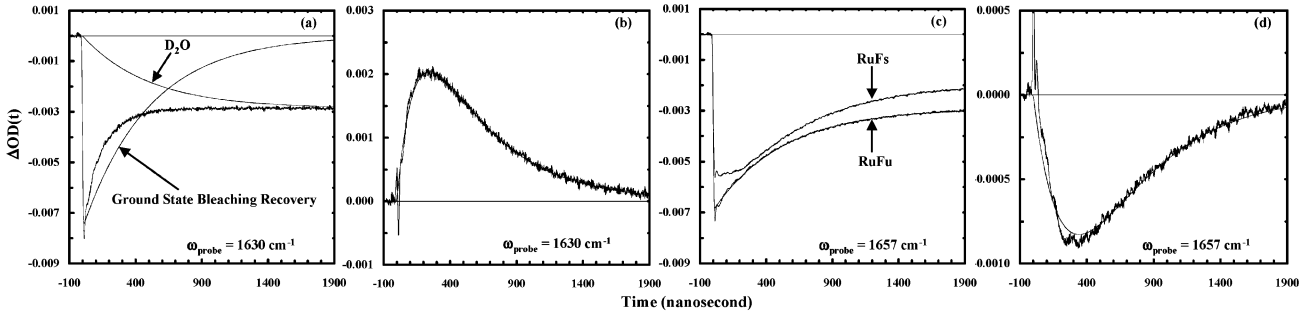

Figure 2. (a) Decay kinetics of the RuFs peptide at 1630 cm^{-1} and 30.3 °C. Also shown are the D_2O signal and bleaching recovery signal, as indicated. (b) The net helix-coil transition signal obtained from (a) and the fit to a biexponential function, that is, $\Delta OD(t) = 0.0047[\exp(-t/524\text{ ns}) - \exp(-t/140\text{ ns})]$. (c) Decay kinetics of the RuFs and RuFu peptides at 1657 cm^{-1} and 30.3 °C, as indicated. (d) The net helix-coil transition signal obtained from (c) and the fit to a biexponential function, that is, $\Delta OD(t) = -0.003[\exp(-t/524\text{ ns}) - \exp(-t/239\text{ ns})]$. The longer helix-coil transition time observed at 1657 cm^{-1} is presumably due to the poorer quality of the signal around zero time.

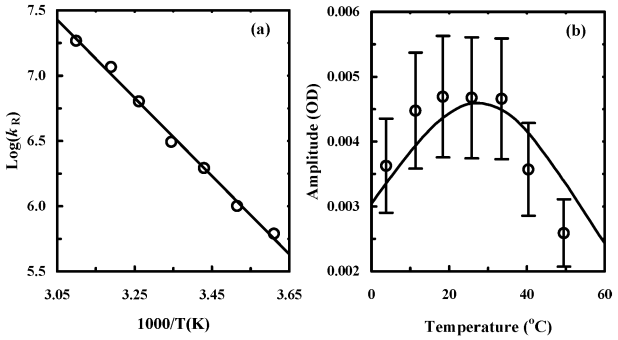

Figure 3. (a) Arrhenius plot of the observed helix-coil transition rate constant, k_R. (b) The net helix-coil transition amplitude at 1630 cm^{-1} versus temperature. The solid line is the fit to eq 7 in the Supporting Information.

state of the RuFu peptide is found to decay following first-order kinetics with a time constant of ~524 ns at 30.3 °C (Figure 2c), which increases to ~652 ns at 3.9 °C. However, due to the concomitant helix-coil transition process, the decay kinetics of the RuFs peptide show complex behavior. The net helix-coil transition signal (Figure 2b,d) of the RuFs peptide was obtained by subtracting the ground-state bleaching recovery signal as well as the D_2O signal, as indicated (Figure 2a). Both signals were constructed from a single-exponential function with a time constant that is equal to that of the RuFu peptide and with amplitudes determined from the instantaneous bleaching component and the long-lived background (D_2O). As expected, the resulting helix-coil transition signal exhibits a positive absorbance at 1630 cm^{-1} (Figure 2b) and a negative absorbance at 1657 cm^{-1} (Figure 2d), indicative of a net helical content increase at the excited state.

It can be shown (Supporting Information) that the helix-coil transition signal can be described by a biexponential function if this transition is assumed to be a two-state process, although this assumption is not rigorously valid.[13] The two rate constants are $\alpha = k_R + k$ and $\beta = k$, respectively, where k is the excited-state decay rate constant, and $k_R = k_1 + k_2$ is the helix-coil transition rate constant. Thus, fitting the helix-coil transition signal to a biexponential function with one rate constant being fixed to the population decay rate constant of the RuFu peptide allows us to obtain the helix-coil relaxation rate constant, k_R. In the temperature range of 4−50 °C, we found that this rate constant follows the Arrhenius relationship with $E_a = 13.5$ kcal/mol (Figure 3a). This is entirely consistent with our earlier studies.[6] In addition, the amplitude of the helix-coil transition signal indicates how much the helical conformation is stabilized by the photoinduced charge-transfer process, which generates an electric dipole that is roughly in parallel with the helical dipole but with the negative end residing at the N-terminus of the helix. Fitting the amplitudes of the helix-coil transition signals (Figure 3b) to eq 7 in the Supporting Information yields $\Delta\Delta G = 0.15 \pm 0.05$ kcal/mol, favoring the helical conformation. If the dipole moment of the excited-state Ru complex is assumed to be 8 D larger than that of the ground state, such a stabilization factor corresponds to a helical dipole moment of 50 D (Supporting Information), which is consistent with the helical dipole moment estimated using 3.5 D/residue.[14]

In summary, incorporation of $[Rub_2m\text{-}OH]^{2+}$ at the N-terminus of the Fs peptide enhances its stability by ~0.15 kcal/mol through the mechanism of dipole−dipole coupling at the excited state. Therefore, photoinduced charge generation at a well-controlled and specific location provides a convenient means to trigger helix-coil transition on nanosecond or even faster time scales, which complements other fast initiation methods.

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Supporting Information Available: CD spectra of peptides used in this study, excited decay kinetics of a two-state system (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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