Investigation of the Effect of the NH···OC Hydrogen Bond from Cys69 to PYP Chromophore Using Novel Active-center Model Compound

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A PYP active-center model compound containing a hydrogen bond to the conjugated carbonyl oxygen was synthesized and its effect on the electronic properties of the chromophore were investigated. The intramolecular hydrogen bond induces significant change in the absorption maximum of the π–π^* transition in the chromophore.

Photoactive yellow protein (PYP) isolated from the purple sulfur bacterium *Halorhodospira halophila*[1] is considered the blue-light photosensor protein that is implicated in the negative phototaxis of the bacterium.[2,3] By other groups,[4–6] it has been reported that PYP is composed of 125 amino acids and one chromophore (4-hydroxycinnamic acid), and the chromophore is covalently bound to Cys69 of the π-loop (residues 63–78) via a thiol ester linkage and exists as a phenolate anion in E configuration in the hydrophobic core of the protein. The chemical structure and the hydrogen bond network between the chromophore and the amino acid residues (Tyr42, Glu46, and Cys69) are summarized in Figure 1a.[7] Point mutation studies on Tyr42 and Glu46 have been revealed that hydrogen bonds to the hydroxy oxygen of the chromophore regulate the electronic properties of the chromophore, and have important roles in exerting the biological function.[8–10] However, the role of the hydrogen bond from the amide NH in Cys69 to the carbonyl oxygen in the chromophore is unknown.

To investigate the role of the hydrogen bond between Cys69 and the chromophore, we designed novel model compounds (shown in Figure 1b) containing an intramolecular hydrogen bond to the carbonyl oxygen in the form of a seven-membered ring like native PYP. In order to form an intramolecular hydrogen bond stably, we chose a rigid 1,2-phenylenediamide skeleton as the linker between the hydrogen bond donor and acceptor instead of the alkyl chain. Thus, we could investigate in detail the effect of the hydrogen bond to the carbonyl oxygen on the electronic state of the chromophore by using this model compound, which leads to the elucidation of the effect of the hydrogen bond from the amide NH in Cys69 to the conjugated carbonyl group on the chromophore.

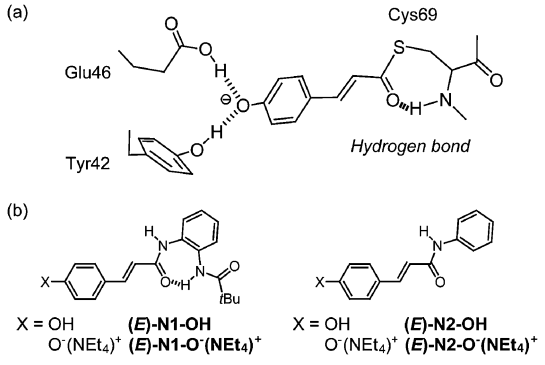

Figure 1. (a) Hydrogen-bond network of the active center in photoactive yellow protein. (b) E formed model compounds.

The amide groups of (*E*)-N**1**–OH and (*E*)-N**2**–OH were synthesized by using DCC. The deprotonation of the hydroxy groups was performed by neutralization with sodium ethoxide, and the counter cation was exchanged with tetraethylammonium cation to increase the solubility in organic solvents.

In Figure 2, the crystal structure of (*E*)-N**1**–OH is shown. The geometry of model compound (*E*)-N**1**–OH has twisted conformation. The 1,2-phenylenediamide skeleton was twisted between each amide plane and the phenylene ring, and the dihedral angles (C9–N10–C11–C12 and C11–C12–N11–C17) were 54.9(4) and 133.2(4)°, respectively. In contrast, the dihedral angle (C9–N10–C11–C12) of (*E*)-N**2**–OH without the intramolecular hydrogen bond was −169.3(1)° in the crystal structure described in Figure S1.[11] In the twisted conformation of (*E*)-N**1**–OH, the amide group (–NHCO*t*-Bu), which is the hydrogen-bond donor, is located in the vicinity of the carbonyl group contained in the plane of the extended π-electron conjugation system. Consequently, the distance between the amide nitrogen N11 and the carbonyl oxygen O2 is short (2.72(3) Å). The orientation between the amide and carbonyl groups and the short N11–O2 distance strongly suggest the presence of an intramolecular NH···OC hydrogen bond between amide proton H11 and carbonyl oxygen O2 in the crystal structure of model compound (*E*)-N**1**–OH. Intermolecular hydrogen bonds were also observed in the crystal structures of (*E*)-N**1**–OH and (*E*)-N**2**–OH. In (*E*)-N**1**–OH, two types of the intermolecular hydrogen bond are observed between the hydroxy group and the carbonyl oxygen O11, the bridging amide NH and the cyclic ether oxygen of tetrahydrofuran. In (*E*)-N**2**–OH, two intermolecular hydrogen bonds are observed between the hydroxy group and the bridging amide NH.[11] Two types (intra- and inter-) of the hydrogen bond observed in the crystal structure of (*E*)-N**1**–OH were also confirmed by IR spectroscopy. In the spectrum, two different bands were observed in the region of the amide NH stretching vibration (3231, 3260 cm^{-1}, Figure S2[11]).

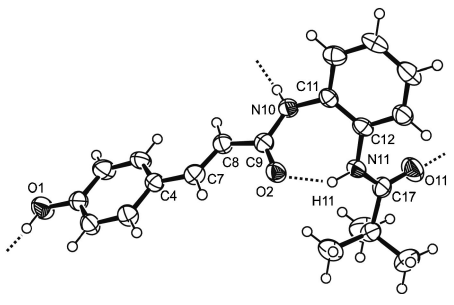

Figure 2. Crystal structure of the model compound (*E*)-N**1**–OH.

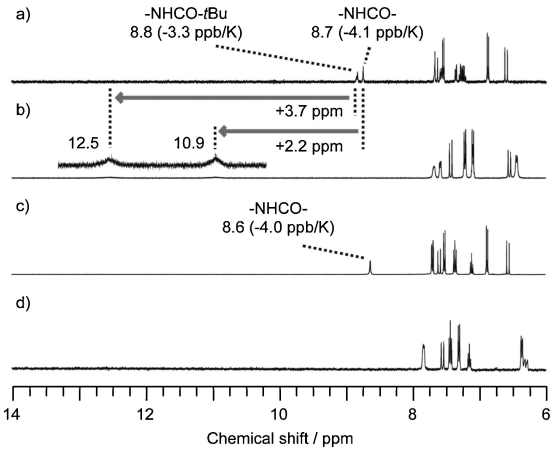

Figure 3. ^{1}H NMR spectra of the model compounds (a) (*E*)-N**1**–OH, (b) (*E*)-N**1**–O$^-$(NEt$_4$)$^+$, (c) (*E*)-N**2**–OH, and (d) (*E*)-N**2**–O$^-$(NEt$_4$)$^+$ in acetonitrile-d_3 at 243 K.

These studies in the solid state indicate the formation of the intramolecular NH···OC hydrogen bond to the conjugated carbonyl oxygen in the model compound (*E*)-N**1**–OH. Thus, we tried to confirm the formation of the intramolecular NH···OC hydrogen bond in solution. ^{1}H NMR spectra of the model compounds in acetonitrile-d_3 at 243 K are shown in Figure 3. In ^{1}H NMR spectra of phenolate anions [(*E*)-N**1**–O$^-$(NEt$_4$)$^+$) and (*E*)-N**2**–O$^-$(NEt$_4$)$^+$)] in acetonitrile-d_3, no amide NH signal was observed at 303 K, but two broad amide NH signals of (*E*)-N**1**–O$^-$(NEt$_4$)$^+$) were observed at 243 K. An amide NH signal of the intramolecular hydrogen-bond donor (–NHCO*t*-Bu) in (*E*)-N**1**–OH was observed at 8.8 ppm and its temperature coefficient was -3.3 ppb/K, which is smaller than those of the bridging amide NH signals (–NHCO–) in (*E*)-N**1**–OH (-4.1 ppb/K) and (*E*)-N**2**–OH (-4.0 ppb/K). The results suggest that the intramolecular NH···OC hydrogen bond established in the solid state is also formed in solution. In addition, an amide NH signal of the intramolecular hydrogen-bond donor in the phenolate anion state (*E*)-N**1**–O$^-$(NEt$_4$)$^+$) was observed at 12.5 ppm, which is shifted to the lower field compared with that of the phenol state (*E*)-N**1**–OH (8.8 ppm). This significant low field shift (+3.7 ppm) of the amide NH signal indicates that the intramolecular NH···OC hydrogen bond is formed more stably in the phenolate anion state (*E*)-N**1**–O$^-$(NEt$_4$)$^+$) because the negative charge on the conjugated carbonyl oxygen atom is increased by deprotonation of the hydroxy group.

Finally we performed UV–vis absorption spectroscopy of the model compounds [(*E*)-N**1**–OH, (*E*)-N**1**–O$^-$(NEt$_4$)$^+$), (*E*)-N**2**–OH, and (*E*)-N**2**–O$^-$(NEt$_4$)$^+$)][11] to reveal the effect of the intramolecular NH···OC hydrogen bond to the carbonyl oxygen on the electronic state of the chromophore. The absorption maxima assigned to the π–π^* transition of the chromophore using MOPAC program were summarized in Table 1. The π–π^* transition of the model compound is located around 300 nm in the phenol state and around 410 nm in the phenolate anion state, respectively. Deprotonation of the chromophores leads to the significant red-shift at both calculated and experimental results. These results indicate the changes in the π-electron conjugation between the phenol and phenolate anion states. In both the phenol and phenolate anion states, the absorption maximum of the

Table 1. The π–π^* transitions of E formed model compounds in acetonitrile at 303 K

	λ_{max}/nm	
	–OH	–O$^-$(NEt$_4$)$^+$
(*E*)-N**1**	305	418
(*E*)-N**2**	301	415

π–π^* transition in (*E*)-N**1** is red-shifted (+4 and +3 nm, respectively) compared with that of (*E*)-N**2**. Since the configuration similarity between the chromophore parts (containing the carbonyl group, C9) of (*E*)-N**1** and (*E*)-N**2** is observed in crystal structures (Figures 2 and S1[11]), we concluded that the intramolecular NH···OC hydrogen bond contributes to the extension of π-electron delocalization along the skeleton of the chromophore. Therefore, the spectral red-shift is considered to be due to the formation of the intramolecular NH···OC hydrogen bond.

From this work, it was revealed that the intramolecular NH···OC hydrogen bond to the conjugated carbonyl oxygen influences the π-conjugation system of the chromophore. It is suggested that the NH···OC hydrogen bond from the amide NH in Cys69 to the conjugated carbonyl group contributes to the extension of π-electron delocalization and regulates the electronic state and the color of the chromophore in the active-center of PYP. As shown in this work, the design of a detailed model is crucial for understanding the chemical mechanism of protein functions. Our novel model revealed the important role of the hydrogen bond in the active-center of PYP. In future work, further advances to understand the reaction mechanism of the PYP photocycle will be achieved by using more detailed model compounds which have a thiol ester linkage, Z configuration, and so on.

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References and Notes

1 T. E. Meyer, *Biochim. Biophys. Acta* **1985**, *806*, 175.
2 M. A. Cusanovich, T. E. Meyer, *Biochemistry* **2003**, *42*, 4759.
3 K. J. Hellingwerf, J. Hendriks, T. Gensch, *J. Phys. Chem. A* **2003**, *107*, 1082.
4 M. Baca, G. E. O. Borgstahl, M. Boissinot, P. M. Burke, D. R. Williams, K. A. Slater, E. D. Getzoff, *Biochemistry* **1994**, *33*, 14369.
5 W. D. Hoff, P. Düx, K. Hård, B. Devreese, I. M. Nugteren-Roodzant, W. Crielaard, R. Boelens, R. Kaptein, J. van Beeumen, K. J. Hellingwerf, *Biochemistry* **1994**, *33*, 13959.
6 M. Kim, R. A. Mathies, W. D. Hoff, K. J. Hellingwerf, *Biochemistry* **1995**, *34*, 12669.
7 G. E. Borgstahl, D. R. Williams, E. D. Getzoff, *Biochemistry* **1995**, *34*, 6278. (PDB entry: 2phy)
8 U. K. Genick, S. Devanathan, T. E. Meyer, I. L. Canestrelli, E. Williams, M. A. Cusanovich, G. Tollin, E. D. Getzoff, *Biochemistry* **1997**, *36*, 8.
9 Y. Imamoto, H. Koshimizu, K. Mihara, O. Hisatomi, T. Mizukami, K. Tsujimoto, M. Kataoka, F. Tokunaga, *Biochemistry* **2001**, *40*, 4679.
10 S. F. El-Mashtoly, M. Unno, M. Kumauchi, N. Hamada, K. Fujiwara, J. Sasaki, Y. Imamoto, M. Kataoka, F. Tokunaga, S. Yamauchi, *Biochemistry* **2004**, *43*, 2279.
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