J|A|C|S COMMUNICATIONS

Published on Web 01/19/2010

Activation of Fluorescent Protein Chromophores by Encapsulation

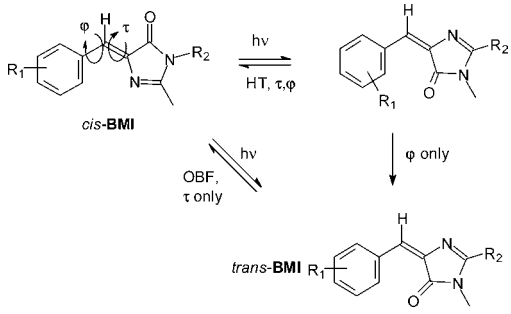
Anthony Baldridge,[†] Shampa R. Samanta,[‡] Nithyanandhan Jayaraj,[‡] V. Ramamurthy,*[,‡] and Laren M. Tolbert*[,†]

School of Chemistry and Biochemistry, 901 Atlantic Drive, Georgia Institute of Technology, Atlanta, Georgia 30332-0400, and Department of Chemistry, University of Miami, Coral Gables, Florida 33146

Received October 19, 2009; E-mail: tolbert@chemistry.gatech.edu

The chromophores in fluorescent proteins (FPs) exhibit a remarkable difference in emission quantum yield as a function of sequestration within the β-barrel. Upon denaturation, the fluorescence diminishes by 4 orders of magnitude, the result, presumably, of disruption of the restraining β-barrel.[1] The effect of sequestration has been the subject of many studies, revolving mostly around the inhibition of double bond twisting leading to isomerization through a "one-bond flip" (OBF) process. However, rotation about the formal single aromatic bond has been proposed as a deactivation mechanism.[2] The former effect is somewhat belied by both the fluorescence of some chromophores which also undergo cis/trans isomerization and the relatively modest viscosity effect. Alternatively, a combination of the two twisting modes ("hula twist" or HT) has been speculated to account for a volume conserving process within the β-barrel (see Scheme 1).[3] We were therefore intrigued with the possibility of encapsulation of the chromophore within a deep cavity cavitand, the so-called "octaacid" (OA), which forms a hydrophobic capsule-like container, of dimensions 14 Å × 7 Å upon dimerization[4] and mimics the β-barrel.

Scheme 1. Torsional Modes in Excited States of BMIs

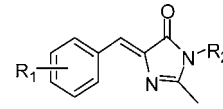


Using previously described methods,[5] we synthesized a number of model FP chromophores, i.e., benzylidene-3-methylimidazolidinones ("BMIs"), with alkyl groups on both the phenyl and imidazolidinone rings (see R_1 and R_2 in Table 1) and exposed them to buffered solutions of OA in D_2O. Complexation of the chromophores[6] is associated with upfield NMR chemical shifts compared to CD_3CN solvent, corresponding to placement of protons within the shielding region of the hydrophobic (aromatic) cavity. As we anticipated from earlier studies,[7] the *para*-alkyl derivatives exhibited strong shielding of both *para*- and *N*-alkyl groups within the cavity (see Figure 1 and Supporting Information, SI). In contrast, *meta*- and *ortho*-alkyl groups were shielded less effectively, suggesting that the para groups anchor the aryl group at one end of the capsule.

Examination of the effect of encapsulation on fluorescence led to quantum yields, relative to those in benzene, which served as a proxy for the aromatic OA interior,[8] that were at variance with expectations based upon anchoring of the aryl groups. Thus neither the *para*-alkyl group nor the *meta*-alkyl group was effective in ameliorating internal conversion, as evidenced by little change in emission quantum yield. In contrast, the *ortho*-alkyl groups increased the emission quantum yield by ca. 1 order of magnitude in the case of **9** (See Figure 2 and Table 2).

Free FP chromophores, as those in some FPs, undergo relatively efficient cis/trans isomerization.[9] Thus we were intrigued by the possible effect of encapsulation on the photochemistry as well. Indeed, all of the chromophores, which were synthesized in their initial cis configuration, readily underwent isomerization in CH_3CN to reach a photostationary state (PSS) with cis/trans ranging from ca. 40:60 to 60:40 (see Table 2). In contrast, irradiation in the OA capsule produced, in most cases, PSS favoring the less stable trans isomer, reaching ≥94% trans in the case of **1**, **9**, or **11**.

Table 1. Substitution Pattern of BMIs Incorporated in OA Cavitand[a]



No.	R_1	R_2	No.	R_1	R_2
1	H	Me	**10**	2-Me	n-Bu
2	2-Me	Me	**11**	2-Me	n-Pent
3	3-Me	Me	**12**	3-Me	n-Pr
4	4-Et	Me	**13**	2-Et	n-Pr
5	2,5-Me	Me	**14**	4-Me	Et
6	4-Me	Me	**15**	4-Et	n-Pr
7	2-Et	Et	**16**	4-Me	n-Pr
8	2-Me	Et	**17**	2-Me	i-Pr
9	2-Me	n-Pr			

[a] Lengths of the molecules provided in SI.

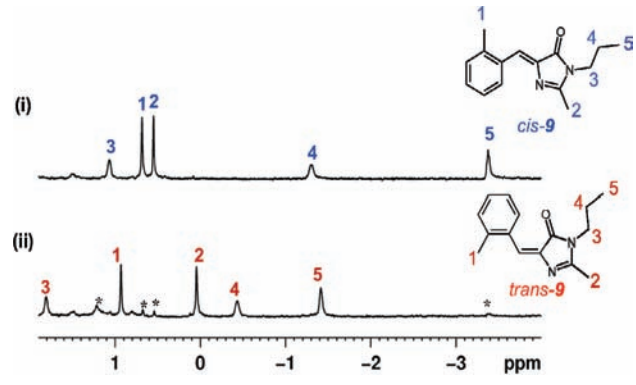


Figure 1. Partial ^{1}H NMR (500 MHz, 10 mM $NaB_4O_5(OH)_4$ in D_2O) spectra for the capsular assemblies of (i) *cis*-**9**@OA_2 and (ii) *trans*-**9**@OA_2. '*' represents residual *cis*-**9**@OA_2.

[†] Georgia Institute of Technology.
[‡] University of Miami.

10.1021/ja908870k

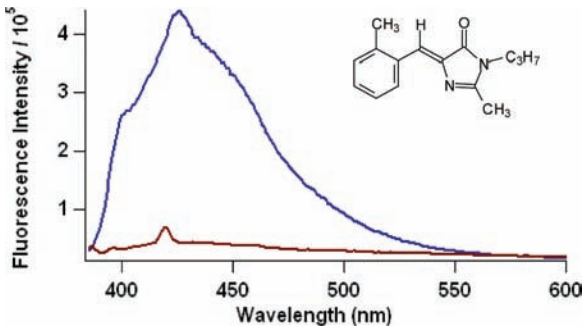


Figure 2. Fluorescence of *cis*-**9** in aerated solution (red = benzene, blue = OA).

Table 2. Isomerization Ratio,[a] T_{PSS},[b,c] and Emission Quantum Yields within OA and in Organic Solvents

	OA (cavitand)		CD_3CN	Emission	
No.	After irrad. (cis:trans)	T_{PSS}, min	After irrad. (cis:trans)	Φ_f (in OA) $\times 10^{-3}$	Φ_f (in PhH) $\times 10^{-3}$
1	5:95	6	50:50	3.27	1.40
2	22:78	8	43:57	1.35	0.98
8	22:78	8	58:42	3.20	1.30
9	6:94	2	39:61	10.0	1.47
10	24:76	10	39:61	4.90	1.09
11	2:98	10	39:61	2.16	1.19

[a] Monitored from ^{1}H NMR spectra. Irradiations were done by using a 310 nm cutoff filter. [b] Time required to reach PSS. [c] In CD_3CN T_{PSS} was invariably 10 min.

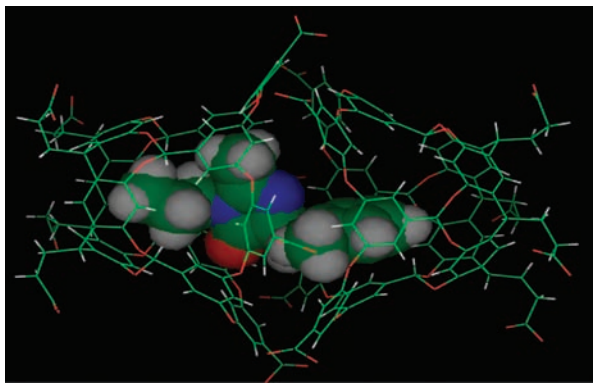


Figure 3. Fluorophore *cis*-**9** within OA dimer cavity.

The PSS favoring trans is rationalized by the more efficient sequestration of the molecule in a more compact conformation (see Figures 1 and 3 and SI). The OA causes the chemical shifts of the N-alkyl substituents in the cis isomer to be greatly shifted upfield as a result of placement within the shielding region of the aromatic rings (see Figure 1 and SI). Upon conversion to the trans isomer, the *N*-alkyl protons are shifted downfield, while the C-2 methyl substituent is shifted upfield, reflecting a better fit when the imidazolidinone ring is not anchored through the N-alkyl group. This is further supported by the increasing trans isomer in the case of the *ortho*-Me derivative on progressing from methyl through pentyl on the imidazolidinone ring, which packs the molecule more tightly. We note, parenthetically, that while the cis isomer is the most stable one in solution, encapsulation of the chromophore drives the photostationary state toward the trans isomer, reflecting the more favorable equilibrium for encapsulation of the trans. Similarly, the slow ground-state isomerization of the chromophore also drives formation of the trans isomer, which becomes the stable form in the presence of the octaacid.

Curiously, the propensity for cis/trans isomerization does not correlate readily with internal conversion efficiency. Isomerization of stilbene-like molecules, for instance, is generally associated with formation of a twisted state which quenches fluorescence.[10] In our case, the presence of an ortho substituent inhibits internal conversion without necessarily inhibiting isomerization. We are forced to conclude that, within the OA capsule, the ortho substituent prevents ready twisting around the single bond (motion ϕ in Scheme 1). Thus when single-bond rotation is prevented, less efficient internal conversion allows either fluorescence or double-bond isomerization to take place. In solution, however, the combination of a one-bond flip with efficient single-bond twisting allows both ready isomerization and efficient internal conversion. As we have observed, in the protein a low frequency motion is largely responsible for the loss of fine structure and contributes heavily to internal conversion.[11]

MD/MM calculations (see SI) allowed location of the most efficient fluorophore **9** inside the capsule (see Figure 3), consistent with the NOESY spectra (see SI). These were not conclusive as to the barrier to isomerization in the 2-methyl case or to the preference for the trans isomer. Further studies on this issue are in progress.

Acknowledgment. We thank the National Science Foundation (CHE-0809179 and CHE-0848017) for support and Rajib Choudhury for help with Figure 3. A.B. acknowledges a fellowship from the Center for Organic Photonics and Electronics (COPE) at Georgia Tech.

Supporting Information Available: Experimental information, synthetic details, characterization, and additional spectroscopic information is provided. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Enoki, S.; Saeki, K.; Maki, K.; Kuwajima, K. *Biochemistry* **2004**, *43*, 14238–14248.
(2) Martin, M. E.; Negri, F.; Olivucci, M. *J. Am. Chem. Soc.* **2004**, *126*, 5452–5464.
(3) (a) Liu, R. S. H. *Acc. Chem. Res.* **2001**, *34*, 555–562. (b) Maddalo, S. L.; Zimmer, M. *Photochem. Photobiol.* **2006**, *82*, 367–372.
(4) (a) Gibb, C. L. D.; Gibb, B. C. *J. Am. Chem. Soc.* **2004**, *126*, 11408–11409. (b) Jayaraj, N.; Zhao, Y.; Parthasarathy, A.; Porel, M.; Liu, R. S. H.; Ramamurthy, V. *Langmuir* **2009**, *25*, 10575–10586.
(5) Lerestif, J. M.; Perrocheau, J.; Tonnard, F.; Bazureau, J. P.; Hamelin, J. *Tetrahedron* **1995**, *51*, 6757–6774.
(6) Unfortunately, use of the more hydrophilic *para*-OH group precluded complexation.
(7) Parthasarathy, A.; Kannumalle, L. S.; Ramamurthy, V. *Org. Lett.* **2007**, *9*, 5059–5062.
(8) Porel, M.; Jayaraj, N.; Kannumalle, L. S.; Maddipatla, M. V. S. N.; Parthasarathy, A.; Ramamurthy, V. *Langmuir* **2009**, *25*, 3473–3481.
(9) (a) He, X.; Bell, A. F.; Tonge, P. J. *FEBS Lett.* **2003**, *549*, 35–38. (b) Henderson, J. N.; Ai, H.-W.; Campbell, R. E.; Remington, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 6672–6677. (c) Andresen, M.; Stiel, A. C.; Trowitzsch, S.; Weber, G.; Eggeling, C.; Wahl, M. C.; Hell, S. W.; Jakobs, S. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 13005–13009.
(10) (a) Waldeck, D. H. *Chem. Rev.* **1991**, *91*, 415–36. (b) Saltiel, J.; Waller, A S.; Sears, D. F. Jr. *J. Am. Chem. Soc.* **1993**, *115*, 2453–65.
(11) Stavrov, S. S.; Solntsev, K. M.; Tolbert, L. M.; Huppert, D. *J. Am. Chem. Soc.* **2006**, *128*, 1540–1546.

JA908870K