Available online at www.sciencedirect.com
ScienceDirect
Chemical Physics Letters 444 (2007) 71–75
CHEMICAL PHYSICS LETTERS
www.elsevier.com/locate/cplett

Photophysics and applications in biosensor for 4′-*N*,*N*-dimethylamino-2-*trans*-styryl-(6-chloroquinoline)

Shun-Li Wang *, Ju-Mei Lin

Department of Applied Chemistry, National Chia Yi University, Chia Yi 600, Taiwan, ROC

Received 22 May 2007; in final form 26 June 2007
Available online 30 June 2007

Abstract

A push–pull compound with intramolecular charge transfer (ICT) characteristic, 6-Cl-2-StQ-NMe_2, was synthesized in this work. The emission maxima of 6-Cl-2-StQ-NMe_2 correlates excellently with the static polarity of the environment. In an aqueous solution, 6-Cl-2-StQ-NMe_2 was observed to form an 'H type' aggregate in the ground state, but deaggregation occurred in the excited state. We find that 6-Cl-2-StQ-NMe_2 can be used as a biosensor. Quantitative analysis showed that a linear relationship exists when bovine serum albumin (BSA) concentration is between 40 μg/ml and 5000 μg/ml. While monitoring the conformational change of BSA, the denaturation reaction was observed when the temperature exceeded 60 °C.

1. Introduction

It is important for biochemists to search for various ways in detecting proteins with high sensitivity in qualitative and quantitative analysis [1]. Fluorescence is the most sensitive and easily available method to study intermolecular interactions. Incorporation of a fluorescent molecule into proteins will change its emission properties during the recognition event and produce a dramatic fluorescence spectral change [2]. A good fluorescent biosensor should possess high fluorescence quantum yield and display obvious spectral change for reflecting their microenvironment.

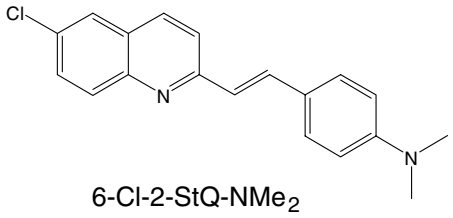
For compounds with intramolecular charge transfer (ICT) characteristic, the dipole moment of the excited state are much higher than that of the ground state, so the formation and emission of the ICT state will be heavily affected by the environment. In previous works, we have studied the photophysics of push–pull styryl systems [3–5]. With ICT characteristic, these compounds displayed high sensitivity to the environment and to intermolecular interactions. Therefore, it is worth to test the ICT compounds as a biosensor. There are two benefits using ICT compounds as a biosensor in proteins [6,7]. First, they are neutral and hydrophobic, so they incorporate easily with protein macromolecules. Second, their emission maxima correlate excellently with the static polarity of the environment. However, the applications of ICT compounds are often complicated due to the aggregation of these molecules in aqueous media [8].

It is common for hydrophobic organic molecules to form various kinds of molecular aggregates in water [9]. In these aggregates, the relative mutual orientation of the molecules creates a microenvironment that is dramatically different from those in bulk phase. Thus one can observe an obvious change in the excited photophysics upon formation of an aggregate [10–13].

In this Letter, a push–pull compound, 4′-*N*,*N*-dimethylamino-2-styryl-(6-chloroquinoline) (6-Cl-2-StQ-NMe_2) possessing ICT characteristic was synthesized, as shown in Scheme 1. We then studied the photophysics of 6-Cl-2-StQ-NMe_2 in aqueous and mixed aqueous-organic solutions and explored the application of this compound as a biosensor.

* Corresponding author. Fax: +886 5 2717901.
E-mail address: wangshunli@mail.ncyu.edu.tw (S.-L. Wang).

0009-2614/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.cplett.2007.06.122



Scheme 1.

2. Experimental

Compound 6-Cl-2-StQ-NMe_2 was prepared by following the literature procedure [3]. 6-Chloroquinaldine and 4-*N*,*N*-dimethylaminobenzaldehyde in acetic anhydride were refluxed for 1.5 h in the presence of the catalyst $ZnCl_2$. The solid product was purified by column chromatography and re-crystallized from methanol. Bovine serum albumin (type A-7906) was purchased from Sigma. All the solvents were Uvasol grade from Merck or spectrophotometric grade from Aldrich and were used as received. UV–vis absorption spectra were recorded on a Hitachi U-3010 spectrometer and fluorescence spectra were obtained using a Hitachi F-4500 fluorescence spectrometer.

3. Results and discussion

The fluorescence spectra of 6-Cl-2-StQ-NMe_2 in a series of solvents are shown in Fig. 1. Absorption maxima are not sensitive to the solvent polarity; however, the fluorescence spectra display a bathochromic shift in polar solvents. This is a typical phenomenon in an intramolecular charge transfer (ICT) state. The ground state and excited-state dipole moments of 6-Cl-2-StQ-NMe_2 are 2 D and 15 D, respectively, which were calculated from Stokes shifts and solvent polarity function [3].

To become an excellent biosensor, the emission maxima need to be highly affected by the polarity of the environment. The relationship between the emission maxima of 6-Cl-2-StQ-NMe_2 to different solvent polarity (the Kirkwood function, $f_\varepsilon = (\varepsilon - 1)/(2\varepsilon + 1)$) is shown in Fig. 2. A good linear correlation exists between the emission maxima (cm^{-1}) and the solvent polarity functions. It is important to note that the solvents used in Fig. 2 include protic solvents and aprotic solvents. Therefore, the emission maxima of 6-Cl-2-StQ-NMe_2 only respond to the media polarity and is not influenced by hydrogen bonding between solvent molecules and 6-Cl-2-StQ-NMe_2 [14].

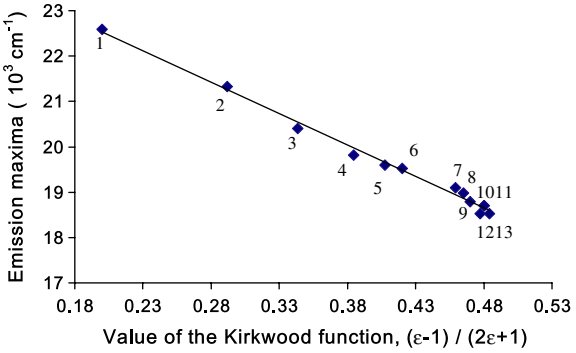


Fig. 2. The relationship between the emission maxima of 6-Cl-2-StQ-NMe_2 and solvent polarity function (the Kirkwood function, $f_\varepsilon = (\varepsilon - 1)/(2\varepsilon+1)$) (1, cyclohexane; 2, dibutyl ether; 3, ether; 4, EA; 5, THF; 6, CH_2Cl_2; 7, 1-butanol; 8, acetone; 9, ethanol; 10, DMF; 11, CH_3CN; 12, methanol; 13, DMSO) (EXC = 400 nm).

To use 6-Cl-2-StQ-NMe_2 as a biosensor, it is necessary to know its photophysics in aqueous solution. For apolar molecules in aqueous solution, aggregation is easily observed because of the hydrophobic interactions. Since the aggregate of organic compounds often display different photophysics, it is important to study the fluorescence of 6-Cl-2-StQ-NMe_2 in an aqueous solution.

The absorption and emission spectra of 6-Cl-2-StQ-NMe_2 in various ratios of methanol–aqueous solutions are shown in Fig. 3. The absorption maxima displayed a blue-shift when the ratio of aqueous in the mixed solution was increased, but the emission maxima exhibited opposite behavior except in a pure aqueous solution. It is obvious to

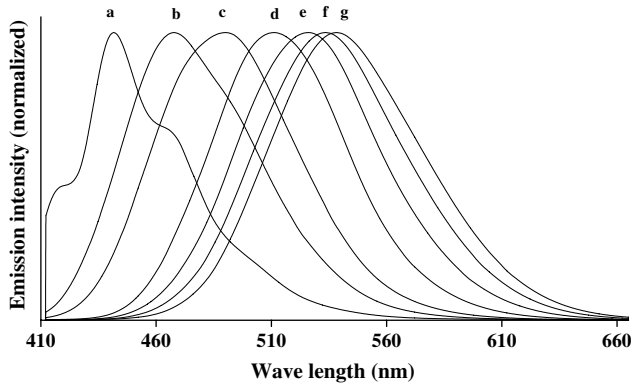


Fig. 1. The emission spectra of compound 6-Cl-2-StQ-NMe_2 in different solvents (EXC = 400 nm): (a) cyclohexane; (b) di-butyl ether; (c) ether; (d) CH_2Cl_2; (e) acetone; (f) CH_3CN; (g) DMSO.

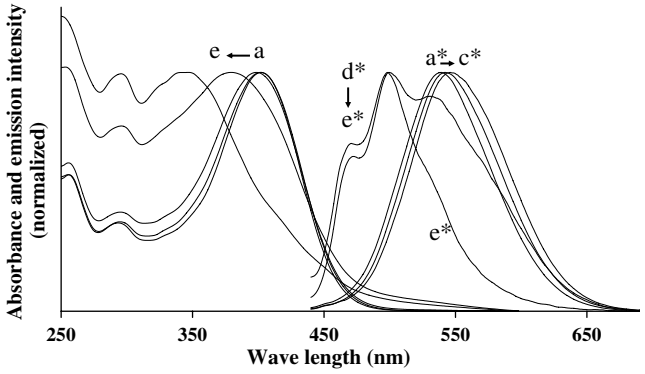


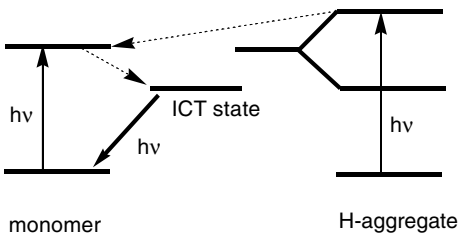
Fig. 3. The absorption and emission spectra of compound 6-Cl-2-StQ-NMe_2 (1.0×10^{-5} M) in different mixed solvents (v/v_0) between methanol and water. (a) Methanol 100%, (b) methanol 80%, (c) methanol 60%, (d) methanol 40%, (e) methanol 20% (a, b, c, d, e are absorption spectra; a*, b*, c*, d*, e* are emission spectra; EXC = 400 nm).

find a 'jump' in the blue-shift of the absorption maxima when the ratios of water is higher than 80%, and the emission maxima displays a sudden blue-shift instead of red-shift. Since the polarity of water is higher than methanol, it is reasonable to observe a red-shift in the emission maxima when the content of water is increased (from 540 nm to 550 nm). The discontinuity of the emission maxima in a high-water content solution was due to the formation of aggregates because the poor solvation of water to the hydrophobic organic molecules. The behavior of the blue-shift for compound 6-Cl-2-StQ-NMe_2 in the absorption maxima after aggregation corresponds to the formation of an 'H' aggregate [15].

It is important to note two interesting things from Fig. 3. First, the absorption and the emission maxima of the aggregates were located at 342 nm and 499 nm, respectively. The huge Stokes shift (9200 cm^{-1}) cannot be explained simply by a pure ICT emission. Second, the fluorescence of the aggregates displayed a fine structure, but the monomer exhibited a structureless emission in the polar solvent (Fig. 1).

There are two types of aggregates: H-aggregates where molecules are arranged in a head-to-head direction and are characterized by blue-shifted absorption bands with respect to those of isolated molecule, and J-aggregates where the molecules are arranged in a head-to-tail direction and display a red-shift of the UV absorption maximum compared to individual molecules. In the ground state, 6-Cl-2-StQ-NMe_2 possesses a slightly dipole moment; therefore, the π-interaction between the two quinoline rings favor a H-type aggregation in an aqueous solution [16]. However, a larger charge separation occurs in the excited state, which disfavors the H-aggregate. This leads to the breakup of the aggregate in the excited state, and yields an excited-state monomer pair. A rigid environment exists in the monomer pair restraining the solvent relaxation around the fluorophore and produces a structured emission spectrum. The mechanism of the excited-state deaggregation reaction was proposed in Scheme 2.

From the point of physiology, serum albumin is one of the most important blood proteins [17]. The amino acid sequence of bovine serum albumin (BSA) consists of 582 residues with 17 disulfide bridges, 1 free thiol group at residue 34 (in the polypeptide chain portion that is not restrained by disulfide bonds), and 2 tryptophan residues [18]. The tryptophan residues are a fluorescent group and can be used for monitoring the concentration and conformation change of BSA. However, the low fluorescence yield of BSA leads to the application intrinsic fluorescence of BSA become inefficient. Here, we try to explore the possibility of 6-Cl-2-StQ-NMe_2 as a nonvalent protein probe for BSA [19,20].



Scheme 2.

The fluorescence spectral changes of 6-Cl-2-StQ-NMe_2 before and after the addition of BSA are shown in Fig. 4. 6-Cl-2-StQ-NMe_2 displayed a very weak structured fluorescence (from aggregate) with the maxima located at 499 nm in an aqueous solution, but the fluorescence intensity increased and maxima shifted to 468 nm after the addition of BSA. In using any of the fluorescence signals to monitor the content of the protein, it is important to realize that there may be changes in the absorption spectra as well. The difference in absorption probably contributes some errors in the fluorescence intensity change. Therefore, we used the ratios of the emission intensities to monitor the content of BSA. The relationship between the ratios of the emission intensities (468 nm/499 nm) and the BSA concentrations was inserted into Fig. 4. This plot shows a linear relationship when the BSA concentration was between 40 μg/ml and 5000 μg/ml.

Beside the quantitative analysis of BSA concentrations, we also explored the possibility of using 6-Cl-2-StQ-NMe_2 for monitoring the conformational change of BSA. BSA is a well-known globular protein that aggregates into macromolecular assemblies. At room temperature, the tertiary structure of BSA is well defined and stabilized. As temperature increases, some molecular regions become accessible to new intermolecular interactions, producing new tertiary structures through disulfide and noncovalent bonds [21–24]. The disruption of the native conformation with concomitant loss of biological activity is known as denaturation [25].

The normalized fluorescence spectra of 6-Cl-2-StQ-NMe_2 incorporated into BSA in different temperature are

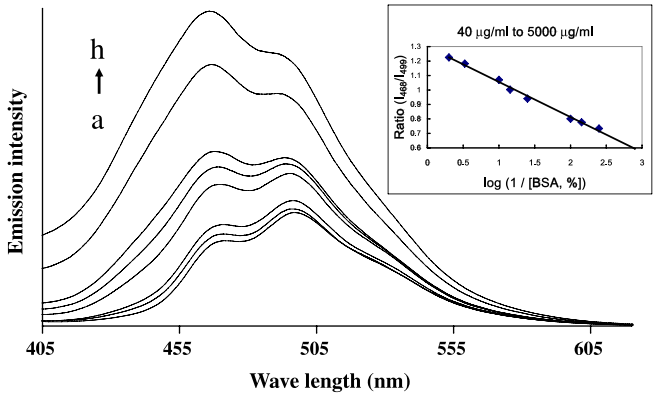


Fig. 4. The emission spectra of compound 6-Cl-2-StQ-NMe_2 (2.0×10^{-5} M) as a function of BSA concentration in aqueous solution (EXC = 350 nm): curve a–h correspond to 0.004%, 0.007%, 0.01%, 0.04%, 0.07%, 0.1%, 0.3% , 0.5% BSA (W/W_0), respectively. Insert figure: the relation between BSA concentration and intensity ratio of $I_{468\,nm}/I_{499\,nm}$ (EXC = 350 nm).

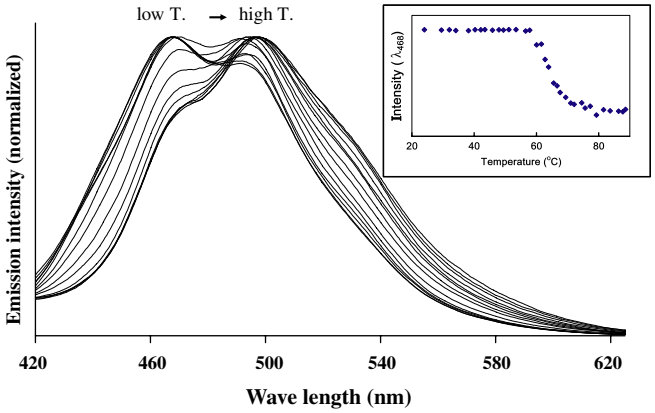


Fig. 5. The normalized emission spectra of compound 6-Cl-2-StQ-NMe_2 (2.0×10^{-5} M) with 0.04% BSA as a function of temperature in aqueous solution (EXC = 350 nm). Insert figure: the relation between temperature and emission intensity $\lambda_{468\,nm}$ (EXC = 350 nm).

shown in Fig. 5. At room temperature, 6-Cl-2-StQ-NMe_2 displays a structured fluorescence with the maximum located at 468 nm. As temperature increases, a new structured fluorescence with the maximum located at 499 nm appears. The inserted figure in Fig. 5 is the plot of the emission intensities (after normalization) at 468 nm vs. the solution temperature. This clearly indicates a dramatic change in the emission intensity occurring above 60 °C.

In a previous work, the denaturation temperature of BSA was recorded using Fourier transform infrared (FTIR) spectroscopy. The secondary structure of BSA exhibited an obvious change beyond 60 °C in the heating process [26]. Therefore, we concluded that the emission intensity change that occurred beyond 60 °C was from the denaturation of BSA. Since the emission maxima display a red-shift after denaturation, we conclude the polarity inside BSA increase after denaturation.

Globular proteins with quaternary structures dissociate before being denatured [27]. In addition to the disruption of noncovalent bonds, there is more exposure of the thiol and hydrophobic groups, which were previously buried, to the aqueous medium. This consequential exposure of the hydrophobic core of the molecule into an aqueous environment results in a polarity increase around the probe (6-Cl-2-StQ-NMe_2) molecules.

Two important results need to be mentioned in Fig. 5. First, the absorption maximum of 6-Cl-2-StQ-NMe_2 after incorporation into denatured BSA is located at 400 nm, a typical absorption of monomer molecule. Therefore, it was considered that the addition of a protein broke the assembled structure of the aggregate of 6-Cl-2-StQ-NMe_2. The emission spectrum of 6-Cl-2-StQ-NMe_2 after incorporation into denatured BSA was similar with the emission spectrum of the aggregate in an aqueous solution. Since only a monomer molecules exists inside denatured BSA, this concludes that the emission spectrum of an aggregate in an aqueous solution is from the emission of monomer species. This result confirms the occurrence of the excited-state deaggregation, as suggested in Scheme 2, and the polarity inside denatured BSA is similar with the monomer located inside the aggregate. Second, the emission spectrum of 6-Cl-2-StQ-NMe_2 after incorporation into denatured BSA also displayed a fine structure. Noncovalent protein–fluorophore interactions can occur by different physical mechanisms, including hydrophobic interactions, electrostatic interactions and hydrogen bonding [20]. Although the exact nature of these interactions is difficult to determine in our system, the existence of an interaction restricted the motion of 6-Cl-2-StQ-NMe_2, and the rigid environment around 6-Cl-2-StQ-NMe_2 produced a structured emission spectrum.

4. Conclusion

The emission maxima of compound 6-Cl-2-StQ-NMe_2, possessing ICT characters, correlate excellently with the static polarity of the environment. In an aqueous solution, 6-Cl-2-StQ-NMe_2 formed an 'H type' aggregate. Upon photoexcitation, a deaggregation reaction in the excited state was observed because of a larger charge separation occurring in the excited-state disfavored the H-aggregate. 6-Cl-2-StQ-NMe_2 is an excellent biosensor in monitoring the content of protein and the conformational change in thermal denaturation process. The detection limit of the BSA concentration was found to be 40 μg/ml, and a conformational change was observed beyond 60 °C for BSA in the thermal denaturation reaction.

Acknowledgement

Financial support from the National Science Council is acknowledged.

References

[1] C. Sun, J. Yang, L. Li, X. Wu, Y. Liu, S. Liu, J. Chromatogr. B 803 (2004) 173.
[2] A.P. Demchenko, Anal. Biochem. 343 (2005) 1.
[3] S.L. Wang, T.I. Ho, J. Photochem. Photobiol., A 135 (2000) 119.
[4] S.L. Wang, G.-Y. Gao, T.-I. Ho, L.-Y. Yang, Chem. Phys. Lett. 415 (2005) 217.
[5] S.L. Wang, T.-W. Yeh, T.-I. Ho, Chem. Phys. Lett. 418 (2005) 418, 393.
[6] R. Das, D. Guha, S. Mitra, S. Kar, S. Lahiri, S. Mukherjee, J. Phys. Chem. A 101 (1997) 4042.
[7] Y. Suzuki, K. Yokoyama, J. Am. Chem. Soc. 127 (2005) 17799.
[8] A. Sarkar, S. Chakravorti, Chem. Phys. Lett. 235 (1995) 195.
[9] X.-K. Jiang, Acc. Chem. Res. 21 (1988) 362.
[10] F.H. Quina, E.A. Lissi, Acc. Chem. Res. 37 (2004) 703.
[11] D. Horn, J. Rieger, Angew. Chem., Int. Ed. 40 (2001) 4330.
[12] Y.-B. Jiang, M.-G. Jin, L.-R. Lin, Spectrochim. Acta, Part A 54 (1998) 1927.
[13] B.-K. An, S.-K. Kwon, S.-D. Jung, S.Y. Park, J. Am. Chem. Soc. 124 (2002) 14410.
[14] S.L. Wang, T.C. Lee, T.I. Ho, J. Photochem. Photobiol., A 151 (2002) 21.
[15] M. Kasha, H.R. Rawls, M.A. El-Bayoumi, Pure Appl. Chem. 11 (1965) 371.
[16] C.A. Hunter, J.K.M. Sanders, J. Am. Chem. Soc. 112 (1990) 5525.

[17] P. Relkin, Crit. Rev. Food Sci. 36 (1996) 565.
[18] D.C. Carter, J.X. Ho, Adv. Protein Chem. 45 (1994) 153.
[19] A. Mallick, B. Haldar, N. Chattopadhyay, J. Phys. Chem. B 109 (2005) 14683.
[20] F. Welder, B. Paul, H. Nakazumi, S. Yagi, C.L. Colyer, J. Chromatogar. B 793 (2003) 93.
[21] K. Murayama, M. Tomida, Biochemistry 43 (2004) 11526.
[22] T. Kosa, T. Maruyama, M. Otagiri, Pharm. Res. 15 (1998) 449.
[23] A. Michnik, K. Michalik, Z. Drzazga, J. Therm. Anal. Calorim. 80 (2005) 399.
[24] V. Militello, V. Vetri, M. Leone, Biophys. Chem. 105 (2003) 133.
[25] C. Scharnagl, M. Reif, J. Friedrich, Biochim. Biophys. Acta 1749 (2005) 187.
[26] S.-L. Wang, S.-Y. Lin, M.-J. Li, Y.-S. Wei, T.-F. Hsieh, Biophys. Chem. 114 (2005) 205.
[27] O.B. Ptitsyn, Adv. Protein Chem. 47 (1995) 83.