ChemComm

The restoration of pyrene fluorescence of a Cu^{II}–β-cyclodextrin–pyrene complex

Swadeshmukul Santra, Peng Zhang and Weihong Tan*

Department of Chemistry and UF Brain Institute, The University of Florida, Gainesville, FL 32611-7200, USA. E-mail: tan@chem.ufl.edu

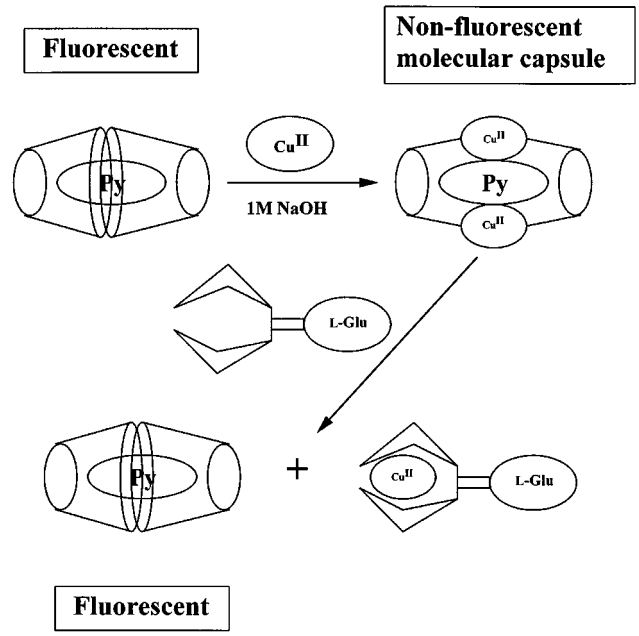
Received (in Corvallis, OR, USA) 23rd April 1999, Accepted 3rd June 1999

A new photochemical mechanism has been developed to restore the fluorescence of an interesting complex of copper ion–pyrene–cyclodextrins upon interaction with glutamate.

We describe a new photochemical mechanism to restore the fluorescence of an interesting complex of copper(II) ion–cyclodextrin–pyrene upon interaction with glutamate. This process will enable us to understand cyclodextrin (CD) based chemistry and to develop novel techniques for amino acid analysis. Cyclodextrins have been widely used in many areas including chemistry, material science and biotechnology.[1,2] They are sugar molecules having the structure of a hollow truncated cone with a hydrophobic cavity. Cyclodextrins (host) are capable of encapsulating appropriately sized fluorophores (guest) in their hydrophobic cavity by forming inclusion complexes.[1,2] These complexes can be formed based on four possible intermolecular interactions.[2] The inclusion of pyrene molecules inside the cavity of cyclodextrins has been well-studied.[3,4] Upon inclusion of a fluorophore, CDs offer a more protective microenvironment and generally enhance the luminescence of the guest molecule by shielding the excited species from quenching and non-radiative decay processes that occur in bulk solution. In general, the guest molecule loses its solvation sphere upon entering the cyclodextrin cavity, and solvent molecules are simultaneously expelled out from the cavity. On the other hand, copper(II) (Cu^{2+}) ions can (i) quench the fluorescence *via* a photo-induced electron transfer process which involves a transfer of electron from the fluorophore in the excited state to the metal ion;[5] (ii) form complexes with CDs in alkaline solutions;[6] and (iii) bind with L-glutamate,[7] a tridentate ligand. Cu^{2+}–CD complexes are capable of binding organic molecules in the cavity,[8] and Cu^{2+} can bind strongly with EDTA in alkaline media leaving free CDs in solution.[9]

Here we report a new mechanism for fluorescence enhancement based on the above-described chemical reactions taking place in aqueous media. A new Cu^{2+}–β-CD–pyrene ternary complex is prepared in which the pyrene fluorescence is quenched by the Cu^{2+} ion, as shown in Scheme 1. Cu^{2+} is weakly bound to the deprotonated secondary hydroxy groups of β-CD.[6] It is expected that strong chelating agents, such as amino acids, will bind to the Cu^{II} complex, leaving free β-CD–pyrene and resulting in fluorescence restoration. We have chosen glutamate, a major excitatory neurotransmitter in the central nervous system,[10] as an example to demonstrate the principle that the ternary complex has strong capability of binding amino acids. The restoration of the pyrene fluorescence in this study is accomplished upon the addition of glutamate.

To demonstrate the principle, we have used a fluorescence spectroscopic method to characterize the complexes synthesized at different stages of the chemical reactions shown in Scheme 1. All the experiments were done at room temperature using a Perkin-Elmer Luminescence Spectrometer [Model LS 50B, UK]. Fluorescence spectra of the solutions prepared at different stages of the process were recorded with the same pyrene concentration of 2×10^{-8} M. First, a stock solution of pyrene was prepared in cyclohexane. A calculated amount of the pyrene stock solution was added to a cuvette, and the cyclohexane was evaporated by dry nitrogen. A trace amount of pyrene was thus obtained for the preparation of experimental solutions for spectroscopic analysis. The solutions were stirred for 24 h and 12 h before and after, respectively, the addition of the required amount of solid β-CD to make its concentration in the solution 0.01 M. It was assumed that pyrene molecules were completely dissolved in all solutions. Fig. 1(*a*) and (*b*) show an almost two-fold fluorescence enhancement of the pyrene emission in β-CD [Fig. 1(*b*)] when compared to the free pyrene emission in 1 M NaOH solution [Fig. 1(*a*)]. Second, we added 0.01 M Cu^{2+} solution [$CuSO_4 \cdot 5H_2O$] to the above complex solution followed by stirring for 2 h. No precipitate of $Cu(OH)_2$ was observed and the resulting complex formed in the solution (deep blue in color) was non-fluorescent, as shown by Fig. 1(*c*). The enhanced fluorescence of pyrene complexed with β-CD was quenched by Cu^{2+} due to electron transfer. In the third step, glutamate was added to the same solution. The fluorescence spectrum of the resulting new solution is shown in Fig. 1(*d*). It is clear that the addition of glutamate to the complex solution resulted in complete restoration of pyrene fluorescence. This restoration is due to the removal of Cu^{2+} from the ternary complex. The pyrene fluorescence band is slightly distorted in Fig. 1(*d*), showing a larger full width at half maximum (FWHM). Although the vibrational structure of the pyrene fluorescence bands remained intact, their relative intensity ratios varied when compared to those of free pyrene, and pyrene encapsulated inside the β-CD cavity. The strongest vibrational peak of the pyrene fluorescence band at 374 nm became a shoulder after the complex interacted with glutamate. We also obtained excitation spectra for all the solutions at different stages of the process, as shown in the left side of Fig. 1. Overall,



Scheme 1 Proposed mechanism for the formation of a ternary Cu^{2+}–β-CD–pyrene molecular capsule and the restoration of pyrene fluorescence upon interaction with glutamate.

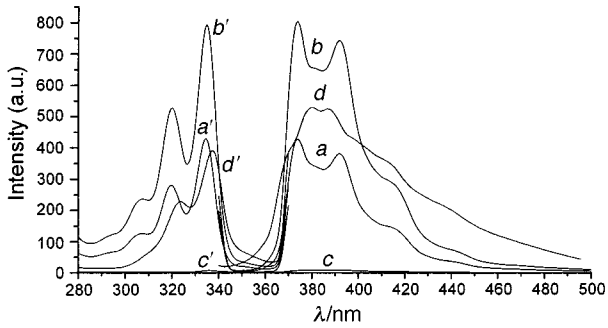


Fig. 1 Fluorescence emission spectra [λ_{exc} = 335 nm] (right) and fluorescence excitation spectra [λ_{em} = 374 nm] (left); (*a*) and (*a*′): free pyrene [*A* = 16018 au]; (*b*) and (*b*′): pyrene encapsulated in β-CD (0.01 M) cavity [*A* = 31346 au]; (*c*) and (*c*′): in the presence of 0.01 M Cu^{II} [*A* = 412 au]; (*d*) and (*d*′): after addition of 1.87 M glutamate [*A* = 30566]; [pyrene] = [2×10^{-8} M] and *A* = 392 au for the background. *A* is the uncorrected total area under the pyrene fluorescence band.

similar results to those from the fluorescence spectra were obtained. This further supports the proposed mechanisms of this series of chemical reactions. The total area under the fluorescence band for the restored emission and the emission before Cu^{2+} addition were the same. Based on the total area under each emission band as shown in Fig. 1, there is a more than 1500 times {(30566 − 392)/(412 − 392) = 1510} enhancement of fluorescence yield. This result clearly shows that there is no loss of fluorescence yield even in the solution phase, where there is a huge amount of other ionic substances. It is thus expected that an amino acid sensor, such as a glutamate sensor, developed in this manner will be highly sensitive.

One of the most important steps in this series of chemical reactions is the quenching of fluorescence by Cu^{2+}. One key question that has to be answered is how much Cu^{2+} has to be added to the complex solution in order to totally quench the pyrene fluorescence. We thus studied the effect of [Cu^{2+}] on the quenching of pyrene fluorescence. Fluorescence spectra of the complex solutions with various amounts of Cu^{2+} were taken, and the fluorescence intensity at 374 nm was used to monitor the effect of Cu^{2+} on pyrene fluorescence quenching. As shown in Fig. 2, pyrene emission decreased exponentially as the [Cu^{2+}] increased to about 0.01 M. There was no change essentially in fluorescence intensity as more Cu^{2+} was added to the solution. 0.01 M happened to be the concentration of β-CD in the complex solution. This suggests the stoichiometry of the formation of the Cu^{2+}–β-CD complex be of 1:1. Further increase of [Cu^{2+}] led to its precipitation as $Cu(OH)_2$. Based on the crystal structure of β-CD–pyrene–alcohol complex,[11] we thus propose a 2:2:1 Cu^{2+}–β-CD–pyrene molecular capsule, as shown in Scheme 1. The reaction between glutamate and the ternary complex in solution could take place in a single step (glutamate binds directly with the ternary complex) or in two steps (glutamate binds with the free Cu^{2+} ion which is in equilibrium with the ternary complex). Detailed kinetic measurements are being carried out to understand both the quenching and the restoration mechanisms.

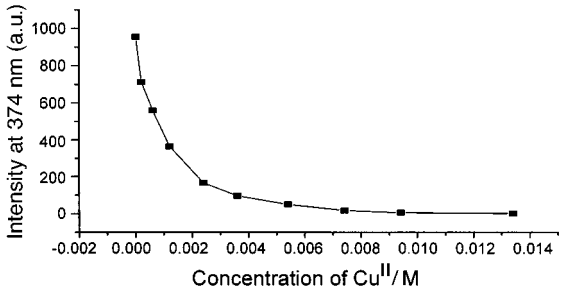


Fig. 2 Effect of copper ion concentration on the fluorescence intensity of pyrene emission in the β-CD–pyrene inclusion complex.

When different amounts of glutamate were used to restore the pyrene fluorescence, we observed a dramatic increase in fluorescence of pyrene in the complex solution. This provides an essential basis for designing a Cu^{2+}–β-CD–pyrene based bioanalysis assay for amino acids. Presently a few strategies are being pursued to take advantage of the dramatic rise of the restored fluorescence to achieve excellent detection capability. The selectivity for individual amino acid analysis could appear as a major disadvantage of this mechanism. But some of our preliminary studies have revealed that different amino acids have different binding capabilities with the copper(II) ion. We have also found that the same ternary copper(II) complex displays different sensitivity ranges for different amino acids. It is thus possible that ultrasensitive assays could be developed for glutamate and other interesting neurotransmitters. Another possible application of this photochemical mechanism could be amino acid analysis by capillary electrophoresis or HPLC using the ternary complex by either on-column or post-column reactions. We will also explore optical fiber technology to covalently bind the ternary complex for biosensor development.[12]

In conclusion, we report here for the first time the restoration of pyrene fluorescence in a Cu^{2+}–β-CD–pyrene ternary complex upon interaction with glutamate. The restoration is complete and fast. Based on Cu^{2+} concentration experiments, we have proposed a feasible molecular interaction mechanism of the complexes formed *via* a few interesting chemical reactions. The Cu^{2+}–β-CD–pyrene complex has a molecular ratio of 2:2:1. The formation of the ternary complex and the restoration of pyrene fluorescence by glutamate demonstrate the feasibility of a bioanalysis technique for the detection of glutamate and other similar ligands. The principle operates similarly to a molecular beacon (non-fluorescent probe) used for the detection of DNA sequences in the target through a hybridization process followed by the restoration of quenched fluorescence.[13] The chemistry involved in the present technique is relatively simple. Synthesis of the complex capsule can be carried out in the solution phase which should facilitate the usage of this capsule as a biosensor for *in vivo* glutamate monitoring. This inclusion system may also become a new model for multifactorial biological systems for molecular recognition and for mimicking active transport or concentration of substances.

This work was supported by US NSF Career Award CHE-9733650, by the Whitaker Foundation Biomedical Engineering Award and by US Department of Defense Office of Naval Research Young Investigator Award N00014-98-1-0621.

Notes and references

1 J. Szejtli, *Cyclodextrins and their Inclusion Complexes*, Academiai Kiado, Budapest, 1982.
2 D. B. Amabilino and J. F. Stoddart, *Chem. Rev*., 1995, **95**, 2725; A. Harada, L. Li and M. Kamachi, *Nature*, 1993, **364**, 516.
3 T. Yorozu, M. Hoshino and M. Imamura, *J. Phys. Chem.*, 1982, **86**, 4426.
4 H. Yang and C. Bohne, *J. Phys. Chem.*, 1996, **100**, 14 533.
5 T. Nakamura, A. Kira and M. Imamura, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 2033.
6 Y. Matsui and D. Suemitsu, *Bull. Chem. Soc. Jpn.*, 1985, **58**, 1658.
7 C. M. Gramaccoili and R. E. Marsh, *Acta Crystallogr*., 1966, **21**, 594.
8 O. Egyed and V. Weiszfeiler, *Vib. Spectrosc*., 1994, **7**, 73.
9 Y. Matsui and K. Kinugawa, *Bull. Chem. Soc. Jpn.*, 1985, **58**, 2981.
10 J. A. Zivin and D. W. Choi, *Sci. Am*., July 1991, **265**, 56; J. Cordek, X. Wang and W. Tan, *Anal. Chem*., 1999, **71**, 1529.
11 K. A. Udachin and J. A. Ripmeester, *J. Am. Chem. Soc*., 1998, **120**, 1080.
12 W. Tan, *Trends Anal. Chem.*, 1998, **17**, 501; J. P. Alarie and T. Vo-Dinh, *Talanta*, 1991, **38**, 529.
13 X. Fang, X. Liu, S. Schuster and W. Tan, *J. Am. Chem. Soc*., 1999, **121**, 2921.

Communication 9/03244D