Switching of Terbium(III)-sensitized Luminescence by Ligand Exchange Reaction: Determination of Catecholamines

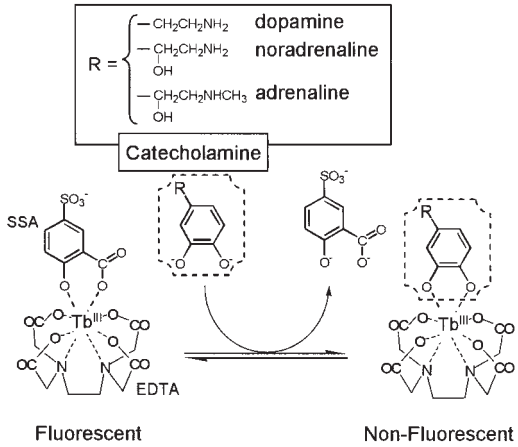
Yukiko Takahashi, David A. Pacheco Tanaka, Hideyuki Matsunaga, and Toshishige M. Suzuki*
AIST-Tohoku, National Institute of Advanced Industrial Science and Technology (AIST), 4-2-1 Nigatake, Miyagino-ku, Sendai 983-8551

(Received March 22, 2002; CL-020262)

Ligand exchange reaction between catecholamines and sulfosalicylic acid (SSA) in the ternary complex, $[Tb(SSA)EDTA]^{4-}$ leads to switch the luminescence signal, providing a simple detection system of catecholamines available in an aqueous solution.

Ternary complexes consisting of Tb(III)-EDTA (EDTA=ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid) and some aromatic bidentate ligands exhibit a characteristic sharp emission band in an aqueous solution.[1] This phenomenon has been applied to the fluorometric determination of terbium[2] and fluorometric immunoassay.[3] Among the aromatic bidentate ligands sulfosalicylic acid (SSA), 2,3-dihydroxynaphthalene, *o*-hydroxybezoic acid and 1,2-dihyroxy-3,5-benzenedisulfonic acid (Tiron) typically show excellent sensitizing effect for the energy transfer luminescence upon ternary complex formation with Tb(III)-EDTA.[1,2] The sensitizing pathway in the Tb(III) complexes has been interpreted by the excitation of aromatic probe molecule from its singlet state to the triplet state (T_1) followed by the energy transfer to $^5D_4$ level of Tb(III) and then, the transition of $^5D_4$-$^7F_5$ gives a characteristic sharp emission peak.[1,3,4] Since the proper matching of ligand excited state (T_1) with $^5D_4$ level of Tb(III) is essentially required for the energy transfer process, effective sensitizing ligands are rather limited. Therefore substitution of the sensitizing ligand with inactive ligand (*vice versa*) is an interesting approach to switch the luminescence signal.

We found that the emission signal intensity of $[Tb(SSA)EDTA]^{4-}$ decreased upon addition of catecholamines (dopamine, adrenaline and noradrenaline) due to ligand substitution with SSA (Scheme 1). Thus, in the present study we attempted to apply the above ternary complex system as the signal probe for the direct fluorometric analysis of catecholamines via ligand substitution. Despite a variety of methods including titrimetric, spectrophotometric chromatographic and polarographic techniques[5] have been reported for the determination of catecholamines in pharmaceutical drags and physiological samples, most of these methods require derivatization or oxidation of catecholamines prior to instrumental analysis. Simple and rapid detection methods of catecholamines by direct sample introduction are scarce.



Scheme 1. Ligand exchange reaction of Tb-EDTA-SSA complex with catecholamine.

Tb(III)-EDTA complex contains three coordinated waters, which are labile and can easily be replaced by more strongly binding ligands.[6,7] Such an ability has led to the use of Ln(III)-EDTA (Ln = lanthanoid) as aqueous NMR shift reagents[8] and Zr(IV)-EDTA as an anion receptor.[9] Among the several candidates of ligands, which can sensitize the energy transfer luminescence, we have chosen SSA because of its superior chemical stability to other aromatic dihydroxy derivatives. A mixture of $Na[Tb(H_2O)_3EDTA]$ (2×10^{-5} M, M = mol dm^{-3}) and SSA (8×10^{-5} M) in an aqueous solution at pH 12, gave a sharp emission band at 548 nm ($\Delta\lambda_{1/2} < 10$ nm) upon excitation at 258 nm.

As given in Figure 1, the emission signal intensity at 548 nm decreased upon addition of catecholamine. The change in signal intensity apparently depends on the concentration of catecholamine added. The 1 : 1 complex formation between Tb(III)-EDTA and SSA was confirmed by molar ratio plots of the emission intensity by changing the concentration of SSA under the fixed concentration of $[Tb(H_2O)_3EDTA]^-$. Similar molar ratio plots by use of absorption intensity at 344 nm also indicated 1 : 1 complex

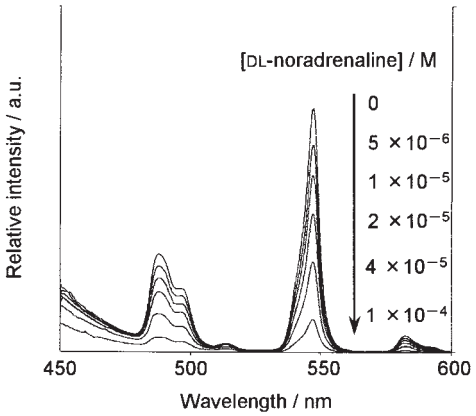


Figure 1. Luminescence spectral change of Tb-EDTA-SSA solution upon addition of DL-noradrenaline in water. $[Tb(H_2O)_3EDTA] = 2 \times 10^{-5}$ M; $[SSA] = 8 \times 10^{-5}$ M; [NaOH] = 0.01 M, room temperature.

formation between Tb(III)-EDTA and catecholamine. However unlike $[Tb(SSA)EDTA]^{4-}$, the characteristic emission band was not observed in this case presumably due to improper matching of energy levels between catecholamine and Tb(III). Therefore the observed quenching of signal intensity can be interpreted by the replacement of SSA by catecholamine (Cat) as the competing ligand (eq 1).

$$[Tb(SSA)EDTA]^{4-} + Cat^{2-} = [Tb(Cat)EDTA]^{3-} + SSA^{3-} \quad (1)$$

The change of signal intensity was appreciably rapid and terminates within 30 sec, indicating the facile ligand exchange reaction. Since addition of any types of catecholamines, i.e., DL-adorenaline, DL-noradorenaline and DL-dopamine, commonly decreased the emission signal intensity, coordination through two oxygens of catechol moiety is most likely to take place as shown in Scheme 1.

The emission signal intensities at 548 nm were measured as a function of pH in the absence and presence of catecholamine (2×10^{-5} M) under the fixed concentrations of $[Tb(H_2O)_3EDTA]^-$ and SSA at 2×10^{-5} M and 8×10^{-5} M, respectively. Complexation of SSA begins to occur at pH above 10 and the maximum Tb(III) emission was observed in the pH range 11.5–12.5. This seems to relate to the high pK_a value of the phenol group of SSA (p$K_{a1} = 2.5$, p$K_{a2} = 11.7$).[10] The signal intensity sharply decreased beyond pH 12.5, which can be attributed to the breakdown of the ternary complex by the increasing concentration of hydroxide ion. The emission intensity of the present system at around pH 12 did not change on standing for 1 h.

Concentration dependency of the signal intensity was examined at optimum pH (pH 12) for the DL-noradrenaline and the result is given in Figure 2. The detection range of DL-noradrenaline is from 1×10^{-6} to 5×10^{-4} M. A similar trend in concentration dependency was commonly observed for other catecholamines. Presence of common anions including Cl^-, NO_3^-, phosphate and acetate did not interfere significantly the signal intensity up to 50 times to that of DL-noradrenaline (2×10^{-5} M). Typical biomolecules such as glycine and citrate did not interfere the emission at the concentrations up to 1×10^{-3} M and 1×10^{-5} M, respectively. Metal ions including Fe(III), Cu(II) and Zn(II) which may be contained more or less in biological sample appreciably interfered due to competed complexation with catecholamine. However, addition of 1 mM EDTA effectively masked Fe(III), Cu(II) and Zn(II) at the concentrations below 5×10^{-5} M, 1×10^{-5} M and 1.4×10^{-4} M, respectively.

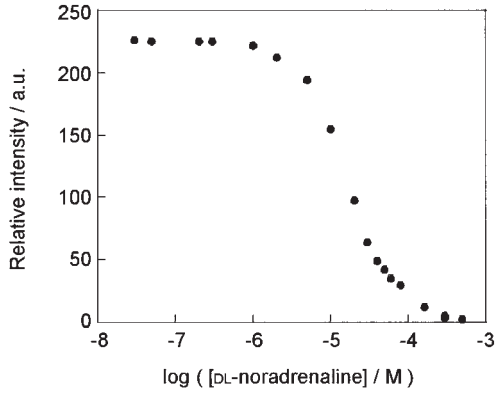


Figure 2. Change in luminescence signal intensity at 548 nm as a function of DL-noradrenaline concentration. $[Tb(H_2O)_3EDTA] = 2 \times 10^{-5}$ M; $[SSA] = 8 \times 10^{-5}$ M; [NaOH] = 0.01 M.

In conclusion, the sharp emission signal due to the energy transfer process in the ternary complex, $[Tb(SSA)EDTA]^{4-}$, can be switched by substitution of SSA with catecholamines leading to provide a direct detection system of catecholamines in an aqueous solution.

Y. T. wishes to acknowledge the New Energy and Industrial Technology Development Organization for the fellowship support.

References

1 I. Hemmila, *Anal. Chem.*, **57**, 1676 (1985); N. Arnaud and J. Georges, *Analyst*, **125**, 1487 (2000).
2 S. J. Lyle and N. A. Za'tar, *Anal. Chem. Acta*, **162**, 447 (1984); S. J. Lyle and N. A. Za'tar, *Anal. Chem. Acta*, **162**, 305 (1984); S. J. Lyle and N. A. Za'tar, *Anal. Chem. Acta*, **153**, 229 (1983).
3 E. P. Diamondis and T. K. Christopoulos, *Anal. Chem.*, **62**, 1149A (1990); T. K. Christ and E. P. Diamondis, *Anal. Chem.*, **64**, 342 (1992).
4 H. Hohsino, S. Utsumi, and T. Yotsuyanagi, *Talanta*, **41**, 93 (1994).
5 W. I. Mohamed and F. B. Salem, *Anal. Lett.*, **17**, 191 (1984); P. Nagaraja, K. C. Srinvasa, K. S. Rangappa, and N. M. Made Gowda, *Talanta*, **46**, 39 (1998); J. J. B. Nevaro, J. M. L. Gallego, and P. B. Laguna, *Anal. Chem. Acta*, **300**, 293 (1995); F. B. Salem, *Anal. Lett.*, **26**, 1959 (1993); C. S. Prakasa Sastry, K. E. Rao, and U. V. Prasad, *Talanta*, **29**, 917 (1982); M. Ufer-Weiss, H. Jork, and H. E. Kelleer, *Fresenius' Z. Anal. Chem.*, **318**, 273 (1984).
6 W. D. Horrocks, Jr. and D. R. Sudnick, *J. Am. Chem. Soc.*, **101**, 334 (1979).
7 L. Spaulding and H. G. Brittain, *Inorg. Chem.*, **24**, 369 (1985).
8 G. A. Elgavish and J. Reuben, *J. Am. Chem. Soc.*, **98**, 4755 (1976); A. Dean Sherry, C. A. Stark, J. R. Ascenso, and C. F. G. C. Geraldes, *J. Chem. Soc., Dalton Trans.*, **1981**, 2078; K. Kabuto and Y. Sasaki, *Chem. Commun.*, **1984**, 316.
9 T. M. Suzuki, D. A. P. Tanaka, M. A. L. Tanco, M. Kanesato, and T. Yokoyama, *J. Envir. Monit.*, **2**, 555 (2000); Y. Takahashi, D. A. P. Tanaka, H. Matsunaga, and T. M. Suzuki, *J. Chem. Soc., Perkin Trans. 2*, **2002**, 759 (2002).
10 A. E. Martell and R. M. Smith, "Crytical Stability Constants," Plenyum Press, New York and London (1985), Vol. 6, p 368.