Near-Infrared Luminescence Sensing of Glutamic Acid, Aspartic Acid, and Their Dipeptides with Tris(β-diketonato)lanthanide Probes[1])

by **Hiroshi Tsukube***, **Keiko Yano**, and **Satoshi Shinoda**

Department of Chemistry, Graduate School of Science, Osaka City University, Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan (phone/fax: +81-6-66052560; e-mail: tsukube@sci.osaka-cu.ac.jp)

Dedicated to Professor *Jean-Claude Bünzli* on the occasion of his 65th birthday

A series of tris(β-diketonato)lanthanides with Yb^{3+}, Eu^{3+}, and Nd^{3+} centers were characterized as luminescent sensing probes specific to glutamic acid, aspartic acid, and their dipeptides, which are important substrates involved in nervous systems, taste receptors, and other biological systems. In particular, tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyloctane-3,5-dionato)ytterbium(III) exhibited a near-infrared emission around 980 nm in response to these biological substrates. Near-infrared-emissive complexes have several advantages over common luminescent probes; therefore, the proposed lanthanide complexes have potential analytical applications in proteomics, metabolics, food science, astrobiology, and related technologies.

Introduction. – Amino acids are key intermediates in the primary metabolism of all biological cells and are, therefore, effective biomarkers. In addition to well-established HPLC, MS, and NMR analytical methods [1b], advanced microchips, portable instrumentation, and other rapid sensing systems are required for progress in proteomics, metabolics, brain science, astrobiology, food industry, and related technologies [2]. In particular, aspartic acid (Asp), glutamic acid (Glu), and their derivatives serve as major excitatory neurotransmitters in the central nervous system of vertebrates and also play important roles in normal and diseased physiology [3]. These compounds are also recognized as taste stimuli, and a new series of their specific chemosensors could have wide applications in basic science and applied technology. Although various colorimetric and fluorescent reagents have been employed in the chemical derivatization of specific amino acids [4], some metallo-receptors have been recently reported to exhibit selective sensing properties based on their characteristic coordination profiles [5]. *Xu* and co-workers reported a cyclometalated palladium complex as a differential chromogenic probe for amino acids in aqueous solution [5a]. *Fujita* and co-workers designed a caged palladium complex to include the specific amino acid residues of oligopeptide substrates [5b]. Several lanthanide complexes have provided further applications of amino acid recognition based on their unique coordination chemistry [6][7]. *Dickins* and co-workers developed a luminescent sensing system in which a ternary complex was formed between the lanthanide center, a

[1]) Preliminary results have been reported at the XIIIth International Symposium on Luminescence Spectrometry [1a].

heptadentate cyclen-derived ligand, and an amino acid substrate (cyclen = 1,4,7,10-tetraazacyclododecane) [7a]. *Aime* and co-workers reported a magnetic-resonance-imaging (MRI) system, in which amino acids were selectively bound to lanthanide complexes with a heptadentate cyclen-type ligand [7b]. *Tamiaki* and co-workers applied porphyrinatolanthanides as circular-dichroism (CD) probes for the chiral detection of amino acids [7c].

Some luminescent tris(β-diketonato)lanthanides are reported that can be used to effectively sense Asp and Glu contained in neutral aqueous samples. Among the examined complexes, the Yb^{3+} complex **1** exhibited the highest responsivity. A series of tris(β-diketonato)lanthanides has been reported to exhibit long-lived, line-shaped, and fixed-position luminescence signals and to respond to specific external substrates upon highly coordinated complexation (*Fig. 1*) [8]. These probes have *i*) effective β-diketonato chromophores for lanthanide excitation, *ii*) two or more unoccupied coordination sites at the lanthanide center for external guests, and *iii*) high thermodynamic stability to function in the presence of H_2O. Typically, the Eu^{3+} complex **3** has been examined as a CD and an ion-selective-electrode (ISE) probe. In this article, focus was made on the near-infrared (nIR) luminescent Yb^{3+} and Nd^{3+} complexes **1**, **2**, and **4**, because nIR emission has high permeability through biotissues and no competing fluorescence from biomatrices. These complexes responded well to Asp and Glu, so that a new class of amino acid probes is expected to be developed by means of the nIR-emissive tris(β-diketonato)lanthanides [9].

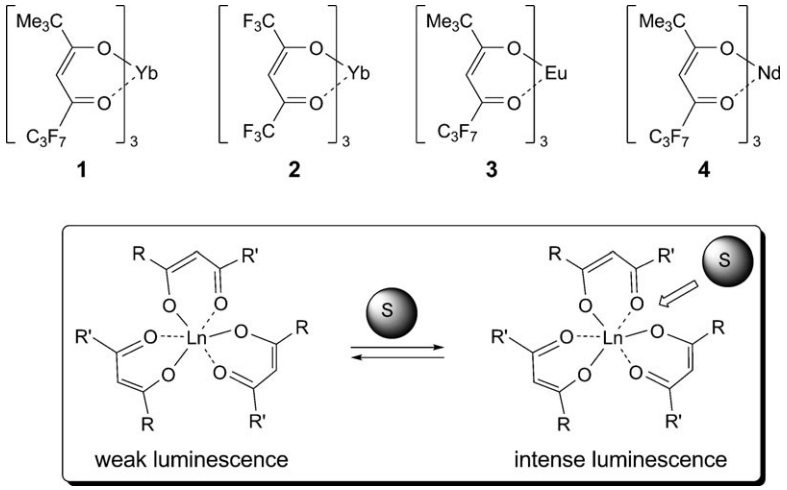

Fig. 1. *Employed tris(β-diketonato)lanthanides* **1**–**4** *and their ternary complexation.* S = external substrate.

Results and Discussion. – *Luminescence Spectral Changes upon Addition of Amino Acids.* Tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyloctane-3,5-dionato)ytterbium(III) (**1**) exhibited characteristic nIR luminescence signals in H_2O/MeCN 1:99 (pH 7.2) upon excitation of the β-diketonato chromophore at 293 nm, while the corresponding Eu^{3+} complex **3** and Nd^{3+} complex **4** had emissions centered at 612 nm and at 1062 nm,

respectively (*Fig. 2*). An aqueous solution of Glu ($2.70 \cdot 10^{-3}$ M, 0.050 ml) was mixed with a MeCN solution containing complex **1** ($3.00 \cdot 10^{-4}$ M, 0.50 ml). After dilution with MeCN to a total volume of 5.0 ml, the enhanced luminescence peak was observed around 980 nm. The peak area was enhanced 8.5 times, compared with that in the absence of Glu. *Fig. 2*,*a* also illustrates that alanine (Ala) had little influence on the luminescence intensity of complex **1** under the same conditions, indicating that complex **1** exhibited Glu-selective enhancement of the nIR luminescence. The corresponding Eu^{3+} and Nd^{3+} complexes **3** and **4** also exhibited similar Glu preference over Ala (*Fig. 2*,*b* and *c*; enhancement of 6.1 times for the complex **3** system and 4.6 times for the complex **4** system).

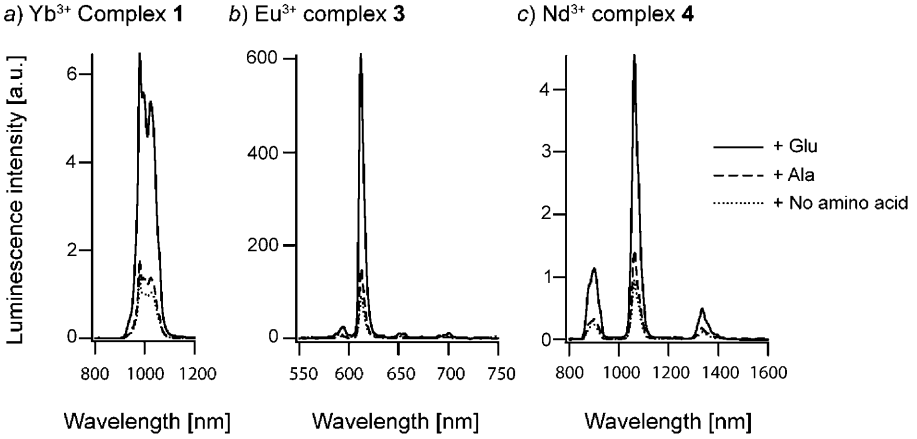

Fig. 2. *Luminescence spectral changes of lanthanide complexes* **1**, **3**, *and* **4** *upon addition of alanine and glutamic acid.* Conditions: [**1**], [**3**], or [**4**] = $3.0 \cdot 10^{-5}$ M, [Ala] or [Glu] = $2.7 \cdot 10^{-5}$ M; solvent H_2O/MeCN 1:99.

The enhanced luminescence profiles of complex **1** were significantly dependent on the pH of the amino acid solutions. *Fig. 3*,*a*, shows the effect of pH on the luminescence intensity of **1** in the presence of Glu and Ala. The observed pH dependency corresponded to the ionization of the two amino acids. Ala has a zwitterionic form at neutral pH, in which the NH_3^+ and CO_2^- moieties are highly hydrated and electrostatically interactive. The favored Glu substrate has an additional CO_2^- moiety in the side chain at neutral pH, which provides effective coordination with the Yb^{3+} center. *Fig. 3*,*b* and *c*, compare the titration curves for Glu and Ala at pH 7.2 and 10.0. At neutral pH, Glu formed a 1:1 complex with complex **1** and exhibited enhanced luminescence, but Ala did not appear to interact with complex **1**. In contrast, Ala enhanced the luminescence signals at pH 10.0, with two molecules of Ala required to bind one of complex **1**. The titration curve at pH 10.0 indicates that Glu forms a 1:2 complex (Glu/**1**); therefore, both amino acids exhibit different coordination modes at high pH. ^{13}C-NMR Experiments were carried out with ^{13}C-labeled Ala and Glu. When 15 mol-% of complex **1** were added to each amino acid solution (D_2O/CD_3CN 1:99, pD 7), somewhat different spectral changes were displayed upon interaction with the Yb^{3+} center. The signals for CH(2) and CO_2^- of Ala broadened, while those for Glu, the

CO_2^- group neighboring the NH_3^+ group and $CH_2(4)$ connected with the other CO_2^- group were shifted slightly (*ca.* 1 ppm). Therefore, the two amino acids interact with the Yb^{3+} center in a different fashion at neutral pH.

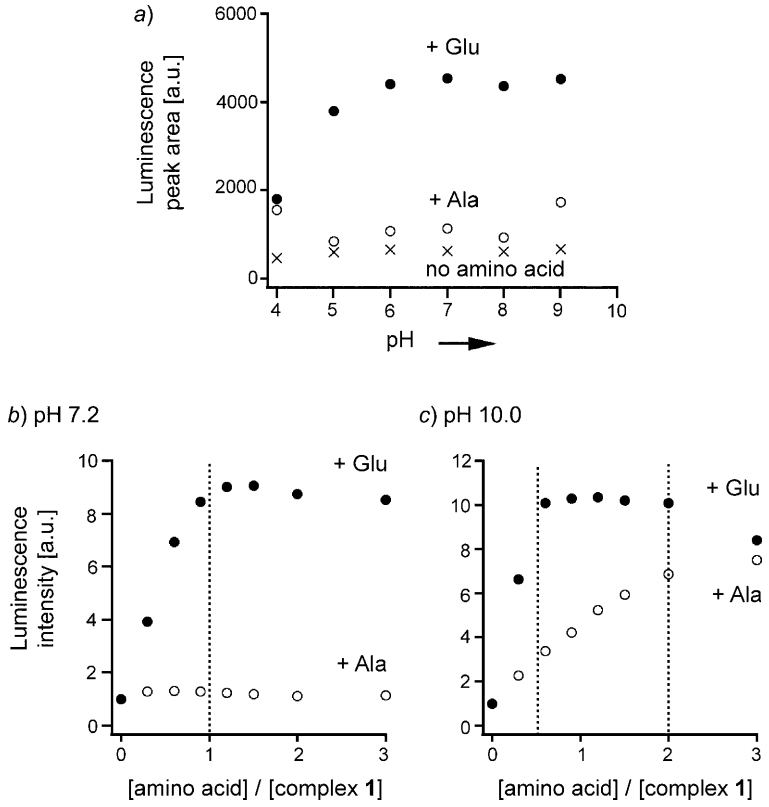

Fig. 3. *pH Dependency of ytterbium luminescence of complex* **1** *upon addition of Ala and Glu.* Conditions: $[\mathbf{1}] = 3.0 \cdot 10^{-5}$ M; solvent H_2O/MeCN 1:99; for *a*) [Ala] or [Glu] $= 2.7 \cdot 10^{-5}$ M.

As frequently reported [6], tris(β-diketonato)lanthanides are directly bound to two or three H_2O molecules at neutral pH in the absence of coordinative solvents and substrates. When a coordinative amino acid is added to a solution of the lanthanide complex, some of the bound H_2O molecules are thought to be replaced. This process was easily followed by comparison of the luminescence behavior between the H_2O/MeCN 1:99 and D_2O/MeCN 1:99 solution systems (*Fig. 4*). When 1 equiv. of Ala was added to a D_2O/MeCN solution containing complex **1**, the luminescence signal was 4 times stronger than in H_2O/MeCN, indicating that the H_2O molecules still occupied the vacant sites of complex **1**, even in the presence of Ala. Similar D_2O-enhanced luminescence was not observed for the addition of Glu. The Glu guest gave almost the same luminescence intensities in H_2O/MeCN and D_2O/MeCN solutions, indicating that Glu effectively replaced the H_2O molecules at the Yb^{3+} center.

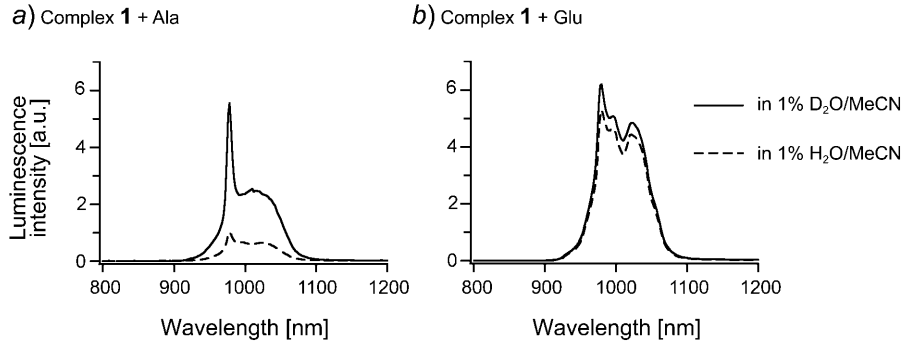

Fig. 4. *Comparison of luminescence spectra in H_2O/MeCN and D_2O/MeCN* 1:99 *of* a) **1**/*Ala and* b) **1**/*Glu.* Conditions: $[\mathbf{1}] = 3.0 \cdot 10^{-5}$ M, [Ala] or [Glu] $= 2.7 \cdot 10^{-5}$ M.

Luminescence Sensing of Amino Acids. The luminescence-sensing properties of lanthanide complexes **1**–**4** were examined for a series of amino acids and dipeptides at pH 7.2, as shown in *Fig. 5*, *a* and *b*. When nIR-emissive complex **1** was employed, Asp offered a comparable enhancement (8.1 times) to Glu, but γ-aminobutyric acid (GABA) exhibited modest luminescence (4.9 times). For glycine (Gly), Ala, serine (Ser), and other amino acids (His, Lys, and Gln), only slight responses were observed (< 1.5 times), whereas complex **1** favored Asp and Glu with lower isoelectric points (p*I*): Asp (p$I = 2.79$), Glu (p$I = 3.22$) $\ll$ Gln (p$I = 5.65$), Ser (p$I = 5.68$), Gly (p$I =$ 5.97), Ala (p$I = 6.00$) $\ll$ His (p$I = 7.59$) $\ll$ Lys (p$I = 9.74$). Competitive experiments were conducted with Yb^{3+} complex **1**, in which two kinds of equimolar amino acids were added: [Yb^{3+} complex **1**] = [amino acid A] = [amino acid B] $= 3.0 \cdot 10^{-5}$ M. Combinations of Glu/His, Glu/Gly, and Glu/Gln were examined. Each combination of amino acids exhibited almost the same luminescence intensity at 980 nm as that observed in the presence of Glu alone, which confirmed that Glu interacts more strongly with the Yb^{3+} center than His, Gly, and Gln (*Fig. 6*). The other tris(β-diketonato)lanthanide complexes **2**, **3**, and **4** exhibited similar luminescence responsivities for a series of amino acids; however, the selectivity toward Glu and Asp was modest (see *Fig. 5*, *a*). Among these complexes, Yb^{3+} complex **2** exhibited the lowest responsivity, even toward Glu and Asp, which indicates that an appropriate selection of the fluorinated β-diketonato ligand is required for effective luminescent sensing of amino acids. Yb^{3+} Complex **1** was also applicable for the sensing of Glu-containing dipeptides (*Fig. 5*, *b*). The Glu-Glu, Glu-Ala, and Ala-Glu substrates examined all exhibited enhanced luminescence intensities at similar levels to that for Glu, while Ala-Ala gave only slightly enhanced ytterbium luminescence. This complex also responded more effectively to Glu residues of the dipeptide substrates than complexes **2**, **3**, and **4**.

Fig. 7, *a*, illustrates the enhanced luminescence spectra of complex **1** by the stepwise addition of Glu, *i.e.*, 0.05 ml of aqueous samples containing $0.09 - 2.70 \cdot 10^{-3}$ M Glu and a MeCN solution of complex **1** ($3.00 \cdot 10^{-4}$ M) were mixed to prepare the H_2O/MeCN 1:99 solution to a total volume of 5.00 ml. *Fig. 7*, *b*, shows the observed luminescence intensity *vs.* the Glu concentration in the original aqueous sample. These results confirm that the method enabled detection of Glu in the original aqueous solution at a level of $0.15 \cdot 10^{-3}$ M. Even when Cl^- anions (30 equiv., $4.5 \cdot 10^{-3}$ M) was included in the

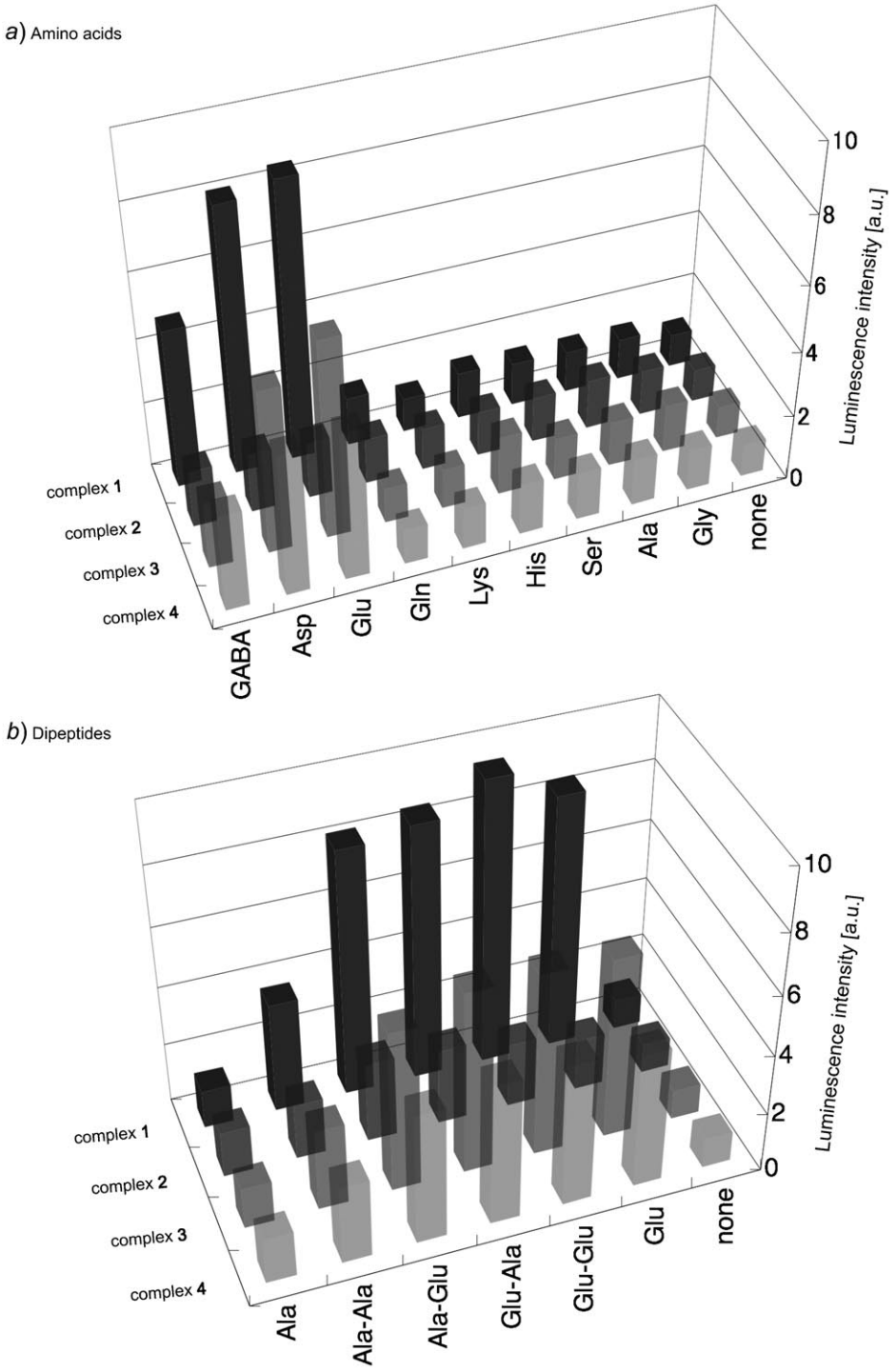

Fig. 5. *Luminescence-sensing profiles of complexes* **1**–**4** *for amino acids and dipeptides.* Conditions: [**1**], [**2**], [**3**], or [**4**] = $3.0 \cdot 10^{-5}$ M, [amino acid] or [dipeptide] = $2.7 \cdot 10^{-5}$ M; solvent H_2O/MeCN 1:99.

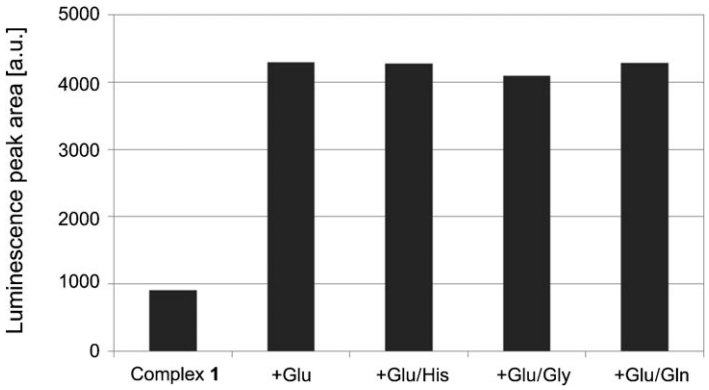

Fig. 6. *Competitive binding experiments with complex* **1** *and Glu and other amino acids.* Conditions: [**1**] = $3.0 \cdot 10^{-5}$ M, [Glu] = $3.0 \cdot 10^{-5}$ M, [amino acid] = $3.0 \cdot 10^{-5}$ M; solvent H_2O/MeCN 1:99.

aqueous sample, similar enhancements of the ytterbium luminescence from the presence of Glu were seen (*Fig. 8*). Therefore, it is evident that complex **1** could be possibly applied in bioanalytical processes.

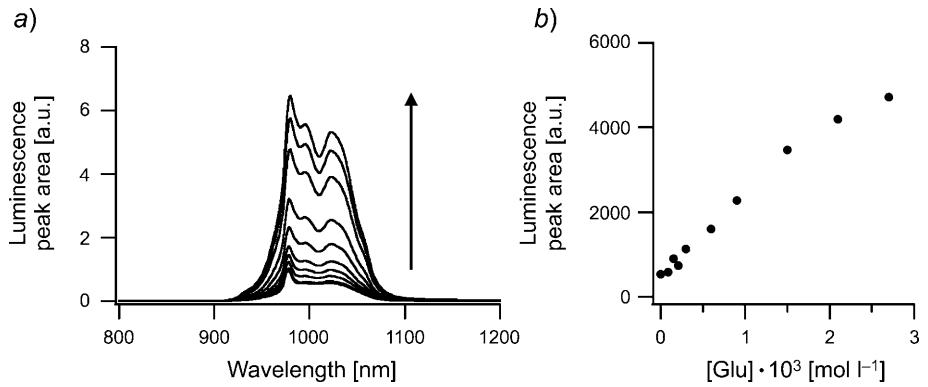

Fig. 7. a) *Enhanced luminescence spectra and* b) *peak areas of complex* **1** *upon addition of Glu*

A series of tris(β-diketonato)lanthanide complexes were characterized as luminescent-sensing probes for amino acids and dipeptides. Of these complexes, tris(β-diketonato)ytterbium **1** exhibited the highest selectivity for nIR emission in response to Glu, Asp, and their derivatives. Although several lanthanide complexes have been attached to the targeted proteins and DNA in luminescent-labeling experiments [10], further design of nIR-emissive lanthanide complexes will provide more selective and rapid screening of specific amino acid targets.

The authors wish to thank Prof. *Jean-Claude G. Bünzli* of Ecole Polytechnique Federate de Lausanne, Switzerland, for his encouragement of their sensing research. This work was financially supported by *Grants-in-Aid of Science Research* from the *Ministry of Education, Culture, Sports, Science, and Technology*, Japan (Nos. 20900138 and 20245014).

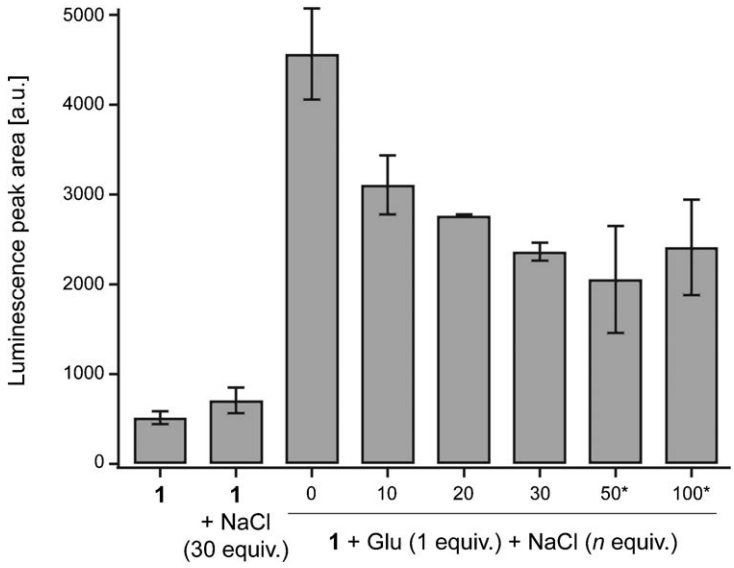

Fig. 8. *Effect of Cl^- anion on Glu-enhanced luminescence of complex* **1**. Conditions: $[\mathbf{1}] = 3.0 \cdot 10^{-5}$ M; solvent H_2O/MeCN 1:99. A small amount of precipitate was formed at $n = 50$ and 100 (indicated by an asterisk).

Experimental Part

General. Luminescence spectra: luminescence spectrophotometer (*Perkin-Elmer LS-50B*) and spectrofluorometer (*Horiba Fluorolog-3*) with a liquid-N_2-cooled InGaAs detector.

Materials. Tris(*β*-diketonato)lanthanides complexes **1**–**4** were obtained from *Sigma-Aldrich*, Japan, and the amino acids were purchased from *Wako Pure Chemical Ind. Ltd.*, Japan (Gly and GABA) and *Nacalai Tesque Inc.*, Japan (Glu, Lys, Gln, Ser, His, Asp, and Ala). Dipeptides, received from *Kokusan Chemical Company, Ltd.*, Japan, were used without further purification. Fluorescence-grade MeCN was also used as received.

Luminescence and UV Experiments. Most of the luminescence experiments were carried out in H_2O/MeCN 1:99 upon excitation of the diketonato ligands (293 nm). The concentrations of tris(*β*-diketonato)lanthanides and external guests are given in each figure. Because the amino acid complexation offered only slight changes in the UV absorbance, the ligand-excitation luminescence spectra were recorded with the complex solns. at constant concentration. The pH values of the sample solns. were usually adjusted to *ca.* 7.2 by the addition of NaOH. The relative luminescence-signal areas indicated in several figures were normalized for each complex system based on the observed area without amino acid.

REFERENCES

[1] a) H. Tsukube, K. Yano, S. Shinoda, *Luminescence* **2008**, *23*, 274. b) I. Molnár-Perl, *J. Chromatogr., A* **1997**, *763*, 1; S. Wagner, K. Scholz, M. Donegan, L. Burton, J. Wingate, W. Völkel, *Anal. Chem.* **2006**, *78*, 1296; N. Shanaiah, M. A. Desilva, G. A. N. Gowda, M. A. Raftery, B. E. Hainline, D. Raftery, *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 11540.

[2] J. L. Bada, *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 797; A. M. Skelley, J. R. Scherer, A. D. Aubrey, W. H. Grover, R. H. C. Ivester, P. Ehrenfreund, F. J. Grunthaner, J. L. Bada, R. A. Mathies, *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 1041.

[3] X. Li, L. Staszewski, H. Xu, K. Durick, M. Zoller, E. Adler, *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4692; S. Okumoto, L. L. Looger, K. D. Micheva, R. J. Reimer, S. J. Smith, W. B. Frommer, *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 8740.

[4] O. Rusin, N. N. St. Luce, R. A. Agbaria, J. O. Escobedo, S. Jiang, I. M. Warner, F. B. Dawan, K. Lian, R. M. Strongin, *J. Am. Chem. Soc.* **2004**, *126*, 438; F. Tanaka, N. Mase, C. F. Barbas III, *Chem. Commun.* **2004**, 1762.

[5] a) S.-H. Li, C.-W. Yu, J.-G. Xu, *Chem. Commun.* **2005**, 450; b) S. Tashiro, M. Kobayashi, M. Fujita, *J. Am. Chem. Soc.* **2006**, *128*, 9280.

[6] H. Tsukube, S. Shinoda, *Chem. Rev.* **2002**, *102*, 2389; S. Shinoda, H. Miyake, H. Tsukube, in ' Handbook on the Physics and Chemistry of Rare Earths', Eds. K. A. Gschneider Jr., J.-C. G. Bünzli, V. K. Pecharsky, Elsevier, Amsterdam, 2005, Vol. 35, p. 273.

[7] a) R. S. Dickins, A. S. Batsanov, J. A. K. Howard, D. Parker, H. Puschmann, S. Salamano, *Dalton Trans.* **2004**, 70; b) S. Aime, E. Terreno, M. Botta, J. I. Bruce, D. Parker, V. Mainero, *Chem. Commun.* **2001**, 115; c) H. Tamiaki, S. Unno, E. Takeuchi, N. Tameshige, S. Shinoda, H. Tsukube, *Tetrahedron* **2003**, *59*, 10477.

[8] H. Tsukube, M. Hosokubo, M. Wada. S. Shinoda, H. Tamiaki, *Inorg. Chem.* **2001**, *40*, 740; R. K. Mahajan, I. Kaur, R. Kaur, S. Uchida, A. Onimaru, S. Shinoda, H. Tsukube, *Anal. Chem.* **2004**, *76*, 7354.

[9] S. Comby, J.-C. G. Bünzli, in ' Handbook on the Physics and Chemistry of Rare Earths', Eds. K. A. Gschneider Jr., J.-C. G. Bünzli, V. K. Pecharsky, Elsevier, Amsterdam – Tokyo, 2007, Vol. 37, p. 217; H. Tsukube, Y. Suzuki, D. Paul, Y. Kataoka, S. Shinoda, *Chem. Commun.* **2007**, 2533.

[10] C. Turro, P. K.-L. Fu, P. M. Bradley, in 'Metal Ions in Biological Systems', Eds. A. Sigel, H. Sigel, Dekker, New York, 2003, Vol. 40, pp. 323 – 353; N. Weibel, L. J. Charbonnière, M. Guardigli, A. Roda, R. Ziessel, *J. Am. Chem. Soc.* **2004**, *126*, 4888; B. R. Sculimbrene, B. Imperiali, *J. Am. Chem. Soc.* **2006**, *128*, 7346.

Received March 26, 2009