Determination of Glycine Based on Fluorimetric Enhancement of Eu(III)-TTA Complex

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Introduction

Glycine, one of the free amino acids, is widely distributed in the nervous system, playing an important role as neurotransmitters.[1-5] It is also used as a dietary supplement in pharmaceutical formulation.[6] Therefore, reliable measurements of glycine in trace amounts are an important tool. Determination of glycine has been performed by several methods, including precolumn derivatization[7] and high performance liquid chromatography[8-12] and the fluorimetric method.[13-15] They usually require multi-step preparation of extraction, clean-up and concentrating before quantitative evaluation. In some cases the methods also require chemical derivation. Some of these methods are long and tedious. In present paper, a simple, sensitive and selective determination method of glycine is presented based on the fluorescence enhancement of Eu(III)-TTA complex added to glycine.

Experimental Section

Apparatus and reagents. All fluorescence measurements were done with a Shimatzu RF-5301PC Spectrofluorophotometer, using 1 cm quartz cell. The band passes were at 10 nm for excitation and emission monochromaters. The light source was a 150 W Xenon lamp. All pHs were measured with a Mettler Toledo MP220 pH meter.

Europium oxide (99.95) was obtained from Aldrich Co. Stock solution of the europium ion was prepared by dissolving a known amount of the europium oxide in hydrochloric acid. Standard solutions were obtained by further dilution with water. Stock solutions of TTA and glycine were prepared in water. Hexamethylenetetramine-HCl[13] (pH 6.5) was used as a buffer solution. Analytical grade chemicals and distilled water were used throughout.

Procedure. To a 50 mL volumetric flask, 5 mL of pH 6.5 buffer solution, 2.5 mL of 1.0×10^{-4} M TTA, 1.0 mL of 1.0×10^{-4} M Eu(III), 10 mL of methanol and 2.5 mL of 1.0×10^{-4} M glycine were added and diluted to the mark. The fluorescence intensity of the solution was measured at 613 nm with a excitation wavelengh at 310 nm. All fluorescence intensity was corrected with blank solution and all experiments were conducted at room temperature (25 °C).

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Results and Discussion

Excitation and emission spectra. The excitation and emission spectra of the Eu(III)-TTA-glycine system are shown in Figure 1. In Figure 1, a and a' curves are the excitation spectra of Eu(III)-TTA and Eu(III)-TTA-glycine and b and b' curves are the emission spectra of the same, respectively.

The maximum excitation and emission wavelength were 305 nm and 610 nm, respectively. The presence of glycine resulted in an increase of the absorbance and emission intensity but no change in maximum wavelength.

The pH effect on the relative fluorescence intensity of Eu(III)-TTA-glycine complex was studied in the range of pH 3-9 (Figure 2). Maximum fluorescence intensity occurred at pH 5-7 of hexamethylenetetramine-HCl buffer. This pH range improved the stability of the complex. The influence of several solvents on the fluorescence intensity of the above system was examined. As shown in Figure 3, 20% methanol gave maximum intensity, therefore, determination of glycine was performed in 20% methanol solvent. This effect seemed due to the solubility of the complex. The concentration effect of TTA on the fluorescence intensity of this complex was also examined (Figure 4). The fluorescence intensity increased with the increase in TTA concentration to

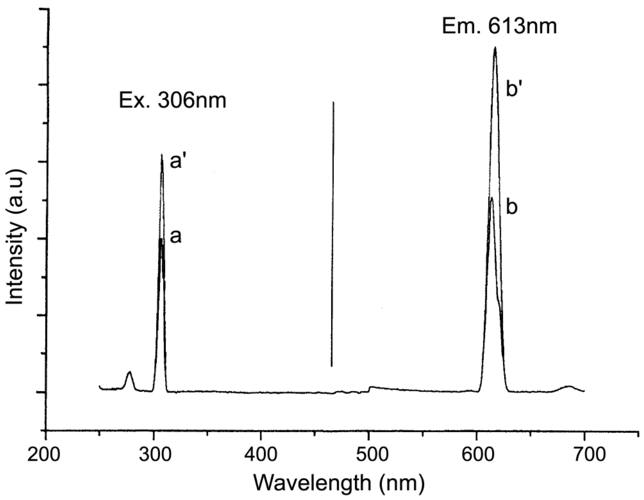

Figure 1. Excitation (a,a') and Emission (b,b') spectra of Eu^{3+}-TTA in the absence (a,b) and presence (a',b') of glycine. Eu(III) : 2×10^{-6} M, TTA : 5×10^{-6} M, Glycine : 1×10^{-6} M, pH 6.5.

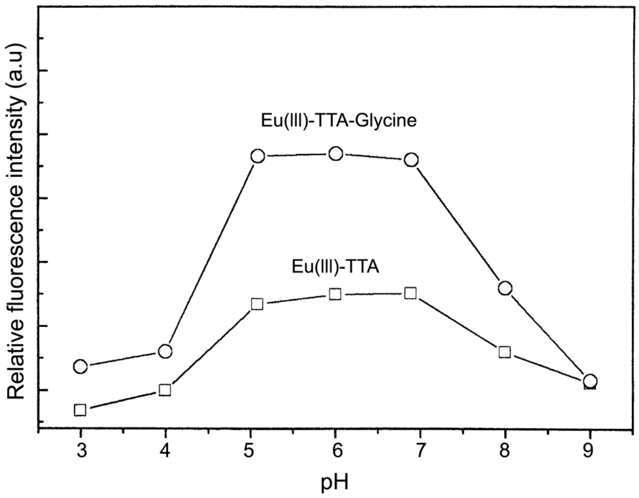

Figure 2. pH effect on the fluorescence intensity of Eu(III)-TTA and Eu(III)-TTA-glycine. Eu(III) : 2×10^{-6} M, TTA : 5×10^{-6} M, Glycine : 1×10^{-6} M.

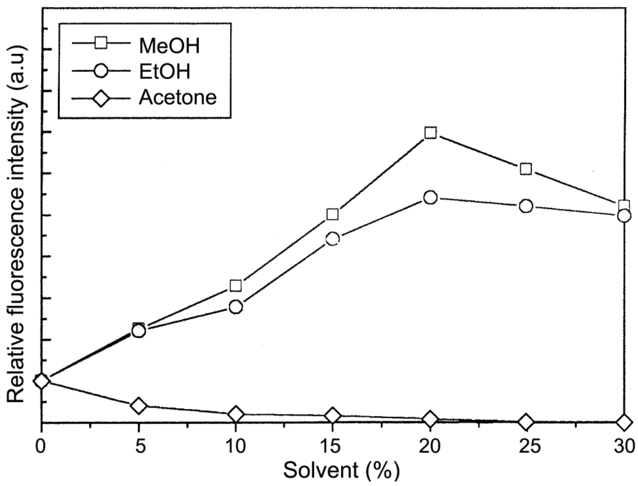

Figure 3. Solvent effect on the fluorescence intensity of Eu(III)-TTA-glycine. Eu(III) : 2×10^{-6} M, TTA : 5×10^{-6} M, Glycine : 1×10^{-6} M, pH 6.5.

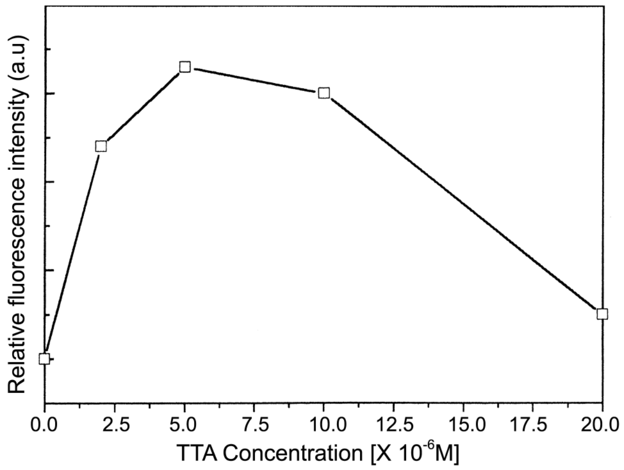

Figure 4. TTA concentration effect on the fluorescence intensity of Eu(III)-TTA-glycine. Eu(III) : 2×10^{-6} M, Glycine : 5×10^{-6} M, pH 6.5.

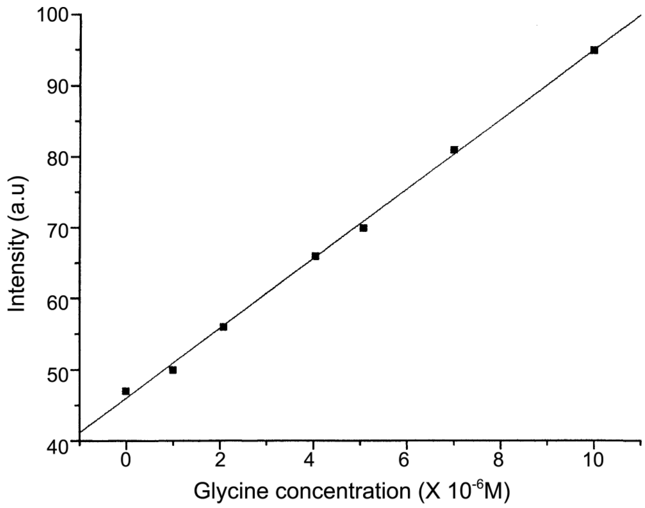

Figure 5. Calibration curve of glycine. Eu(III) : 2×10^{-6} M, TTA : 5×10^{-6} M, pH 6.5.

5.0×10^{-6} M and decreased at the higher concentration. The fluorescence intensity of Eu(III)-TTA-glycine complex was influenced by the concentration of Eu(III) and TTA, so these concentrations must be retained constantly to determine glycine. The composition of the Eu(III)-TTA-glycine complex was investigated. The mole ratio between Eu(III) and TTA was 1 : 3 and the mole ratio between Eu(III) and glycine was 1 : 2. Therefore, the composition of the complex was $Eu(III)(TTA)_3(glycine)_2$.

Possible enhancement mechanism. The fluorescence of the europium ion was weak due to the low oscillatory strength of its absorption.[17] A fluorescence increase for Eu^{3+} can be achieved by energy transfer from the TTA ligand to the Eu^{3+} in the complex. The composition of Eu^{3+}-TTA complex in water is $Eu^{3+}(TTA)_3(H_2O)_2$.[18] In the presence of glycine, the structure of the complex included two glycine molecules instead of two water molecules and the glycine prevented quenching by water molecule.[19] Therefore, the fluorescence intensity of the Eu^{3+}-TTA-glycine complex was enhanced with the addition of glycine.

Calibration curve of glycine. The increase in the fluorescence intensity of the Eu(III)-TTA-glycine complex was a linear function of glycine concentration. The linear range was 1×10^{-6}-10×10^{-6} M glycine concentration when the concentration of TTA and Eu(III) was 5×10^{-6} and 2×10^{-6} M, respectively and pH was 6.5 (r = 0.998) (Figure 5). The detection limit was 1×10^{-7} M glycine (S/N=3), which was comparable with limits obtained by other methods.[7,10] The present method was simpler and sensitive than the other methods. The linear range can be changed according to the concentration of TTA and Eu(III).

Interference. The effects of some metallic ions and amino acids on the fluorescence intensity of Eu(III)-TTA-glycne complex were investigated in the prescence of 5×10^{-6} M glycine (Table 1). Some metallic ions, such as Pr(III), V(IV),

Table 1. Fluorescent characteristics of Eu(III)-TTA-glycine in the different metal ions and amino acids Eu(III) : 2×10^{-6} M, TTA : 5×10^{-6} M, Glycine : 5×10^{-6} M

	Concentration (M)	Flu. intensity	RFI[a]
None		124	1.00
Pr(III)	5×10^{-5}	17	0.14
U(IV)	5×10^{-5}	65	0.52
La(III)	5×10^{-5}	157	1.27
Gd(III)	5×10^{-5}	150	1.21
Tb(III)	5×10^{-5}	111	0.90
V(IV)	5×10^{-5}	12	0.10
Pt(IV)	5×10^{-5}	50	0.40
Threonine	5×10^{-5}	130	1.04
Histidine	5×10^{-5}	121	0.97
Cystine	5×10^{-5}	103	0.83
Arginine	5×10^{-5}	157	1.26
Lysine	5×10^{-5}	137	1.08
Aspartic acid	5×10^{-5}	135	1.08
Serin	5×10^{-5}	126	1.01
Valine	5×10^{-5}	120	0.96

[a]Relative fluorescence intensity

Pt(IV) and cystine acid, decreased the fluorescence intensity of the above complex, but other amino acids had negligible effect in a concentration of 5×10^{-5} M.

Application. To test the validity of this method, two synthetic samples were prepared and the glycine content was determined by this method and reversed-phase high-performance liquid chromatograpy.[10] The analytical data obtained by the standard addition method of this method was compared with the results of chromatographic method. In two cases good agreement was obtained as seen in Table 2.

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Table 2. Composition of synthetic samples and analytical results

	Concentration	Found		
		Present method	RSD[a] (%)	HPLC[b]
No.1	1×10^{-5} M of threonine, histidine, cystine, argine and aspartic acid in 1.00×10^{-6} M glycine	1.05×10^{-6} M	3.0	non detectable
No.2	1×10^{-5} M of threonine, histidine, cystine, argine and aspartic acid in 5.00×10^{-6} M glycine	5.06×10^{-6} M	3.0	5.0×10^{-6} M

[a]Relative standard deviation (n = 5). [b]LC 10 AD. C_{18} Resil HL column