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PAPER

2-(2-Pyridyl) benzimidazole based Co(II) complex as an efficient fluorescent probe for trace level determination of aspartic and glutamic acid in aqueous solution: A displacement approach†

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A weakly fluorescent cobalt(II) complex is synthesized using 2-(2-pyridyl)-benzimidazole (PBI) as a chelating fluorescent ligand and characterized by single crystal X-ray structure. This complex serves as an efficient fluorescent probe for trace level determination of aspartic acid (AspA) and glutamic acid (GluA) in aqueous solution. Rest of the naturally occurring amino acids did not interfere. Both aspartic acid and glutamic acid replaces PBI from the coordination sphere of Co(II)-PBI complex resulting appearance of strong fluorescence signal for the released free PBI. The signal response is very fast and the interaction of both the AspA and GluA with the Co(II) is strong enough as evident from their displacement equilibrium constant values, *viz.* 4357.8 M^{-1} and 8333.33 M^{-1} respectively.

Introduction

Aspartate and glutamate are major excitatory amino acids (EAAs) that activate N-methyl-D-aspartate (NMDA) receptors in the mammalian central nervous system (CNS). Their presence at more than half of all CNS synapses play an important role in learning, memory, movement disorders, drug addiction as well as many other normal or abnormal physiological processes and behaviors. They play a key role in brain function because the level of neuronal excitability depends on the relative balance of Asp A and Glu A. Any alterations in such a balance may cause several neurological or psychiatric disorders.[1–6] Therefore, Asp A and Glu A levels are commonly monitored when studying brain chemistry for investigating the mechanisms of neurological disorders or for developing novel neuro-pharmacological agents.

Massive efflux of Asp A and Glu A was observed in different neuro-pathological models of brain injury[7–9] which caused an uncontrolled excitotoxic stimulation of postsynaptic (mainly NMDA) receptors, membrane depolarization and energy depletion resulting neuronal cell death.[10] Hence, analysis of EAAs in biological samples was very relevant in biochemistry and clinical chemistry[11–13] to know the extent of neuronal damage due to the diseases like epilepsy, Parkinson's disease[14–16] and ischemic brain injuries.[17,18]

Recently, several researchers have focused to correlate the altered levels of EAAs in humans and some pathologies, including diabetes and cancer.[19] High level Glu A content in plasma will lead to acute ischemic stroke.[20] Glaucoma, one of the major causes of blindness, was characterized by the death of retinal ganglion neurons and optic nerve damage.[21,22] Pathological release of EAAs (particularly Glu and Asp) into the extracellular fluid was supposed to cause the deterioration of retinal ganglion cells after traumatic or ischemic damage to the CNS.[23–25] Therefore, the determination of EAAs in retina samples was important to know the pathological changes that occur in retinal ganglion cells in glaucoma.

It was also well known that NMDA receptor play vital roles in descending pain modulation.[26,27] Increased release of GluA and AspA are intimately associated with both somatic and visceral nociception.[28,29] While Asp A can facilitate tricarboxylic acid cycle, potassium salt of Asp A is used to cure heart, liver disease and diabetes.[30,31]

Most previous studies have used liquid or gas chromatography with fluorescence, UV or electrochemical detection[32–35] for determination of free amino acids. But chromatography methods suffered from drawbacks like lengthy cleanup and derivatization steps while electrochemical methods needed complex treatment of electrodes.

Chemosensor[36,37] is ideal for recognition of amino acids[38] owing to their simplicity, high selectivity and sensitivity. Selective detection of a specific amino acid without interference from other amino acids is a challenging task. Recently, utilizing the unique nucleophilicity of the thiol groups, optical sensors for cysteine (Cys) and homocysteine (Hcy), have been developed.[39] Until now only a very few sensors are available for the detection of histidine (His),[40] arginine (Arg)[40c,d] phenylalanine (Phe)[41] and lysine.[42,43]

Cu(II) complex of quinacridone ligand was used as a fluorescent sensor[44] for amino acids with a protocol where Cu(II) served as the amino acid binding site and Cu(II) coordinating fluorophore (*i.e.*

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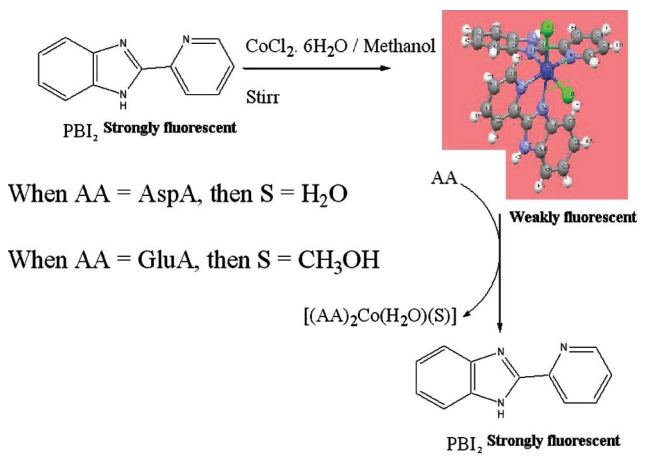
quinacridone ligand) served as the reporter molecule.[45] This concept was well known in competitive ELISA using antibodies[46,47] and also applied in designing small molecule chemosensors.[48]

Herein, we report the trace level selective sensing of aspartic acid and glutamic acid with a new crystallographically characterized weak fluorescent Co(II) complex with 2-(2-pyridyl)benzimidazole using displacement protocol.[44]

Results and discussion

Synthesis, structural and spectral characteristics

Scheme 1 shows the facile one step synthesis of the Co(II)-PBI complex. A distorted octahedral geometry around Co(II) is found from the single crystal X-ray structure analysis (Fig. 1) of the complex. Two Cl atoms are in *cis*-position providing a suitable geometry for substitution with chelating AspA and GluA. Different structural parameters of the complex are presented in Table 1 whereas its detail bond lengths and bond angles are presented in Table 2. Mass spectra of the products obtained from the reaction of Co(II)-PBI complex with aspartic or glutamic acid are presented in Figure S1 and Figure S2 respectively.† Both the mass spectra indicate the replacement of PBI moieties from the coordination sphere by AspA and GluA. FTIR spectra of the Co(II)-PBI complex and its adducts with AspA and GluA are presented in Figure S3, Figure S4 and Figure S5 respectively.† Distinctively different band positions and shape of those spectra indicate formation of new adducts. Absorption spectral changes of the Co(II)-PBI complex upon gradual addition of AspA and GluA are presented in Figure S6 and Figure S7 respectively.† A gradual increase of the absorption peak at 320 nm is observed for both the systems. Effect of all the naturally occurring 20 amino acids on the fluorescence spectra of Co(II)-PBI complex is shown in Fig. 2 which clearly indicates that a significant increase of the emission intensity is observed upon addition of AspA and GluA while rest of the amino acid have no effect. However, GluA induced higher emission intensity than that of AspA. Fig. 3 shows the changes in the emission spectra (λ_{Em} = 370 nm, λ_{Ex} = 280 nm) of Co(II)-PBI complex as a function of externally added AspA. After addition of 5 times AspA (5×10^{-5} M), the emission intensities of a weakly and wide fluorescence band centered at 470 nm has been increased



Scheme 1 Synthesis of the Co(II)-PBI complex and its use for the fluorescence assay of aspartic acid and glutamic acid.

Table 1 Crystal parameters of the Co(II)PBI complex

Emperical formula	C_{24} H_{18} Cl_2 Co N_6, $2H_2O$
F.W	556.31
T/K	93(2)K
Crystal color	Red
Crystal description	Prism
Crystal dimension/mm	0.35 × 0.30 × 0.08
Crystal system	Monoclinic
Space group	*C*2/*c*
Z	8
a/Å	24.215(4)
b/Å	14.850(2)
c/Å	16.053(2)
α (°)	90.00
β (°)	124.684(3)
γ (°)	90.00
V/Å^3	4746.7(13)
$2\theta_{max}$ (°)	54.79
$2\theta_{min}$/deg	6.17
No. of rflcns measured	15672
No. of rflcns used	6740
P (Calcd.) (g cm^{-3})	1.546
μ (M_0K_α) (cm^{-1})	0.984
F(000)	2248
Goodness of fit	1.044
R_1, wR_2[a] $I > 2\sigma(I)$	0.0539, 0.1613
All data	0.0862

[a] $R1 = \sum||F_0| - |F_c||/\Sigma|F_0|$; $wR2 = |\Sigma w(F_0^2 - F_c^2)^2/\Sigma w(F_0^2)^2|^{1/2}$

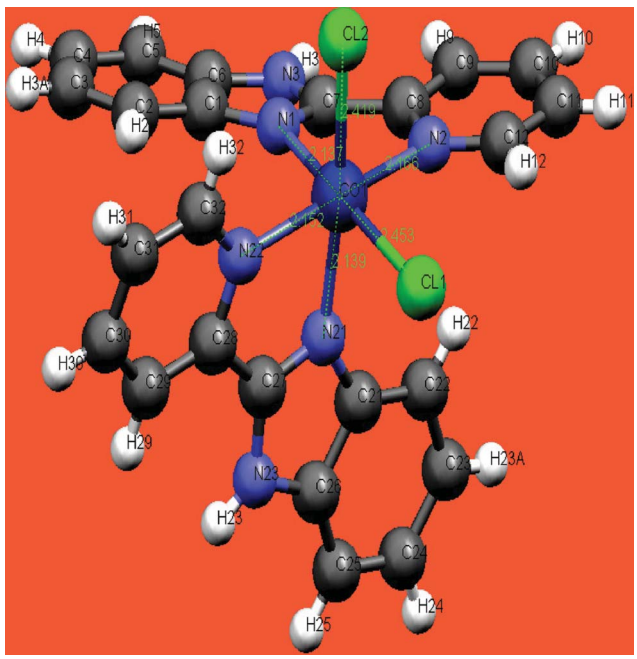


Fig. 1 Single crystal X-ray structure of the Co(II)-PBI complex.

significantly. Similar trend is observed in the fluorescence titration of Co(II)-PBI complex (λ_{Ex} = 280 nm, λ_{Em} = 370 nm) with GluA (Fig. 4). Only difference that has been observed between the two fluorescence titration is the ratio of emission intensities at 370 nm and 470 nm which is higher in former.

The insets show plot of fluorescence intensities *vs.* externally added AspA and GluA. They indicate that after a certain amount of externally added AspA and GluA, there is no further change in the emission intensity of the systems. Up to 20 times (2×10^{-4} M) of

Table 2 Bond lengths and angles of the Co(II)-PBI complex

Bond Length	(Å)	Bond Length	(Å)
O_2 O_2	1.332(14)	C_6 C_5	1.406(6)
Co_1 N_1	2.137(3)	C_6 C_1	1.416(6)
Co_1 N_{21}	2.139(3)	C_{32} C_{31}	1.377(5)
Co_1 N_{22}	2.152(3)	C_{32} H_{32}	0.9500
Co_1 N_2	2.166(3)	C_1 C_2	1.401(6)
Co_1 Cl_2	2.4193(11)	C_{31} C_{30}	1.384(6)
Co_1 Cl_1	2.4526(13)	C_{31} H_{31}	0.9500
N_2 $C1_2$	1.334(5)	C_2 C_3	1.387(6)
N_2 C_8	1.346(5)	C_2 H_2	0.9500
N_{22} C_{32}	1.348(5)	C_{30} H_{30}	0.9500
N_{22} C_{28}	1.350(5)	C_{25} C_{24}	1.361(6)
N_1 C_7	1.327(5)	C_{25} H_{25}	0.9500
N_1 C_1	1.390(5)	C_{22} C_{23}	1.379(6)
C_{27} N_{21}	1.323(5)	C_{22} C_{21}	1.395(5)
C_{27} N_{23}	1.354(5)	C_{22} H_{22}	0.9500
C_{27} C_{28}	1.464(5)	C_{11} C_{10}	1.368(7)
N_{21} C_{21}	1.394(5)	C_{11} C_{12}	1.385(6)
C_{26} C_{25}	1.378(6)	C_{11} H_{11}	0.9500
C_{26} N_{23}	1.391(5)	C_5 C_4	1.395(6)
C_{26} C_{21}	1.410(5)	C_5 H_5	0.9500
C_8 C_9	1.379(5)	C_{23} C_{24}	1.399(6)
C_8 C_7	1.477(5)	C_{23} H_{23A}	0.9500
N_{23} H_{23}	0.8800	C_{12} H_{12}	0.9500
C_{29} C_{28}	1.383(5)	C_9 C_{10}	1.391(6)
C_{29} C_{30}	1.387(6)	C_9 H_9	0.9500
C_{29} H_{29}	0.9500	C_3 C_4	1.385(7)
N_3 C_7	1.364(5)	C_3 H_{3A}	0.9500
N_3 C_6	1.368(5)	C_{24} H_{24}	0.9500
N_3 H_3	0.8800	C_{10} H_{10}	0.9500
		C_4 H_4	0.9500

Bond angles of the Co(II)-PBI complex

Bond Angle	(°)	Bond Angle	(°)
N_1 Co_1 N_{21}	87.47(12)	N_3 C_6 C_5	132.0(4)
N_1 Co_1 N_{22}	94.65(12)	N_3 C_6 C_1	106.0(4)
N_{21} Co_1 N_{22}	76.96(12)	C_5 C_6 C_1	122.0(4)
N_1 Co_1 N_2	77.44(12)	N_{22} C_{32} C_{31}	122.8(4)
N_{21} Co_1 N_2	95.06(12)	N_{22} C_{32} H_{32}	118.6
N_{22} Co_1 N_2	169.13(12)	C_{31} C_{32} H_{32}	118.6
N_1 Co_1 Cl_2	92.22(9)	N_1 C_1 C_2	130.6(4)
N_{21} Co_1 Cl_2	172.69(9)	N_1 C_1 C_6	109.0(3)
N_{22} Co_1 Cl_2	95.79(9)	C_2 C_1 C_6	120.3(4)
N_2 Co_1 Cl_2	92.00(9)	C_{32} C_{31} C_{30}	118.9(4)
N_1 Co_1 Cl_1	171.62(9)	C_{32} C_{31} H_{31}	120.5
N_{21} Co_1 Cl_1	87.17(10)	C_{30} C_{31} H_{31}	120.5
N_{22} Co_1 Cl_1	90.41(9)	C_3 C_2 C_1	117.3(4)
N_2 Co_1 Cl_1	96.63(10)	C_3 C_2 H_2	121.4
Cl_2 Co_1 Cl_1	93.91(4)	C_1 C_2 H_2	121.4
C_{12} N_2 C_8	118.0(3)	C_{31} C_{30} C_{29}	119.3(3)
C_{12} N_2 Co_1	127.0(3)	C_{31} C_{30} H_{30}	120.4
C_8 N_2 Co_1	114.9(2)	C_{29} C_{30} H_{30}	120.4
C_{32} N_{22} C_{28}	117.5(3)	C_{24} C_{25} C_{26}	116.5(4)
C_{32} N_{22} Co_1	126.6(2)	C_{24} C_{25} H_{25}	121.8
C_{28} N_{22} Co_1	115.8(2)	C_{26} C_{25} H_{25}	121.8
C_7 N_1 C_1	105.0(3)	C_{23} C_{22} C_{21}	117.0(4)
C_7 N_1 Co_1	111.8(3)	C_{23} C_{22} H_{22}	121.5
C_1 N_1 Co_1	141.7(3)	C_{21} C_{22} H_{22}	121.5
N_{21} C_{27} N_{23}	113.0(3)	C_{10} C_{11} C_{12}	119.3(4)
N_{21} C_{27} C_{28}	120.7(3)	C_{10} C_{11} H_{11}	120.3
N_{23} C_{27} C_{28}	126.2(3)	C_{12} C_{11} H_{11}	120.3
C_{27} N_{21} C_{21}	105.4(3)	N_{22} C_{28} C_{29}	123.1(4)
C_{27} N_{21} Co_1	113.2(2)	N_{22} C_{28} C_{27}	112.9(3)
C_{21} N_{21} Co_1	141.2(2)	C_{29} C_{28} C_{27}	123.9(3)
C_{25} C_{26} N_{23}	132.1(4)	$C_{4\,C5}$ C_6	116.0(4)
C_{25} C_{26} C_{21}	122.7(4)	C_4 C_5 H_5	122.0
N_{23} C_{26} C_{21}	105.2(3)	C_6 C_5 H_5	122.0
N_2 C_8 C_9	122.9(4)	N_{21} C_{21} C_{22}	131.0(4)
N_2 C_8 C_7	113.2(3)	N_{21} C_{21} C_{26}	109.1(3)
C_9 C_8 C_7	123.8(4)	C_{22} C_{21} C_{26}	119.9(4)
C_{27} N_{23} C_{26}	107.2(3)	C_{22} C_{23} C_{24}	121.6(4)
C_{27} N_{23} H_{23}	126.4	C_{22} C_{23} H_{23A}	119.2

Table 2 (*Contd.*)

Bond Angle	(°)	Bond Angle	(°)
C_{26} N_{23} H_{23}	126.4	C_{24} C_{23} H_{23A}	119.2
C_{28} C_{29} C_{30}	118.3(4)	N_1 C_7 N_3	113.0(3)
C_{28} C_{29} H_{29}	120.9	N_1 C_7 C_8	120.6(4)
C_{30} C_{29} H_{29}	120.9	N_3 C_7 C_8	126.2(4)
C_7 N_3 C_6	107.0(3)	N_2 C_{12} C_{11}	122.5(4)
C_7 N_3 H_3	126.5	N_2 C_{12} H_{12}	118.8
C_6 N_3 H_3	126.5	C_{11} C_{12} H_{12}	118.8
		C_8 C_9 C_{10}	118.3(4)
		C_8 C_9 H_9	120.9
		C_{10} C_9 H_9	120.9
		C_4 C_3 C_2	122.2(4)
		C_4 C_3 H_{3A}	118.9
		C_2 C_3 H_{3A}	118.9
		C_{25} C_{24} C_{23}	122.3(4)
		C_{25} C_{24} H_{24}	118.9
		C_{23} C_{24} H_{24}	118.9
		C_{11} C_{10} C_9	119.1(4)
		C_{11} C_{10} H_{10}	120.5
		C_9 C_{10} H_{10}	120.5
		C_3 C_4 C_5	122.3(4)
		$C_3C_4H_4$	118.9
		C_5 C_4 H_4	118.9

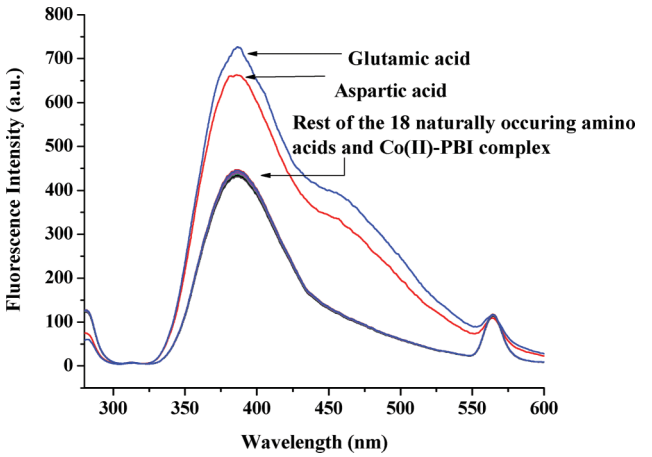


Fig. 2 Effect of different amino acids on the fluorescence spectra of Co(II)-PBI complex([complex] = 10^{-5}M,[AA] = 10^{-4}M).

the externally added AspA and GluA, we observed linearity. Thus, by making use of this linear relationship, one can easily find out the concentration of any unknown AspA and GluA. Stern–Volmer type plots (Figure S8 and S9†), revealed the linear relationship between the changes of fluorescence intensity of the system with the externally added AspA and GluA which would be very useful to determine the unknown concentrations of the two amino acids.

Fluorescence quantum yields (Φ) for Co(II)-PBI complex, Co(II)-PBI complex +AspA and Co(II)-PBI complex +GluA molecular systems are 0.024, 0.104 and 0.106 respectively.

Calculation of displacement equilibrium constant

The interaction between the AspA or GluA with the Co(II)-PBI complex is a replacement reaction as evident from the mass spectra of the isolated products. The displacement equilibrium constants are calculated by the equation, $(F_{lim} - F_0)/(F_x - F_0) = 1 + 1/K[Q]$, where F_0 is the fluorescence intensity of the Co(II)-PBI complex, F_{lim} is the fluorescence intensity of the system at complete

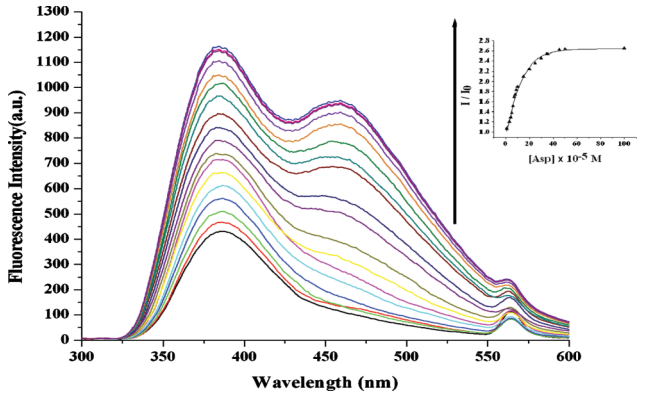


Fig. 3 Changes in the fluorescence spectra of Co(II)-PBI complex (10^{-5} M) as a function of externally added AspA. (From bottom to up, [AspA] = 10, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 450, 500, 1000 μM. Inset: Plot of fluorescence intensity of Co(II)-PBI complex (10^{-5} M) as a function of externally added [AspA] (solvent = water, λ_{ex} = 280 nm, λ_{em} = 370 nm).

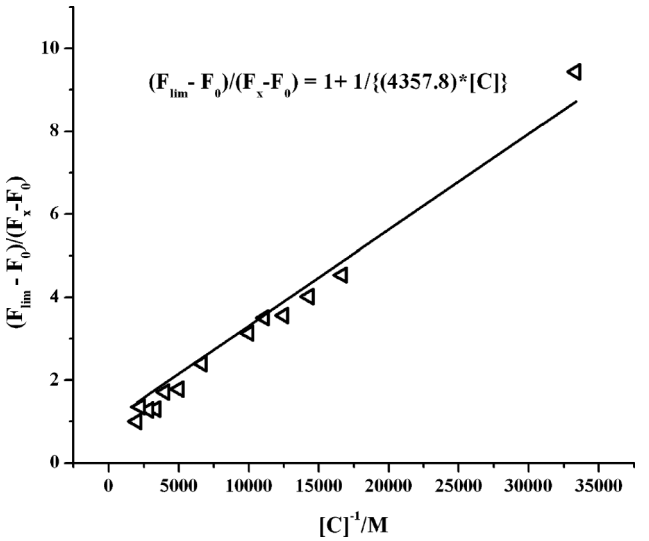


Fig. 5 Displacement equilibrium constant of AspA with the Co(II)-PBI complex. Where F_0 is the emission intensity of Co(II)-PBI complex, F_x is the emission intensity of Co(II)- AspA system at any intermediate concentration of AspA, F_{lim} is the emission intensity of Co(II)- AspA system at complete interaction (no further change in emission intensity occurs) with AspA, C is the concentration of AspA.

interaction *i.e.* when no further change in the emission intensity is observed. F_x is the fluorescence intensity of the system at any intermediate concentration of AspA or GluA, Q is either AspA or GluA, and K is the displacement equilibrium constant, the values of which are, 4357.8 M^{-1} for AspA and 8333.33 M^{-1} for GluA (Fig. 5 and Fig. 6) indicating a significant molecular level interaction of these two amino acids with the Co(II)-PBI complex.

Selectivity

The selectivity behavior is obviously one of the most important characteristics of an amino acid selective chemosensor, which is the relative optode response for the amino acid over others present in the solution. Thus, the influence of a number of common naturally occurring amino acids on the fluorescence intensity of Co(II)-PBI complex is investigated. Fig. 7 and Fig. 8 (bar diagrams) show the effect of other common accompanying amino acids on the fluorescence intensity of Co(II)-PBI+AspA and Co(II)-PBI+GluA molecular assembly in a ternary mixture (Co(II)-PBI complex + AspA/GluA + AA, where AA is an amino acid other than AspA and GluA). Rest of the 20 naturally occurring amino acids have no significant interference on the fluorescence spectra of Co(II)-PBI+AspA/GluA molecular systems.

Thermal studies

To study the thermal stablities of the Co(II)-PBI complex, Co(II)-AspA and Co(II)-GluA molecular systems, we performed the TGA studies which are presented in Figure S10, S11 and S12 respectively.† While the Co(II)-PBI complex is stable up to 86 °C whereas Co(II)-AspA and Co(II)-GluA molecular systems are

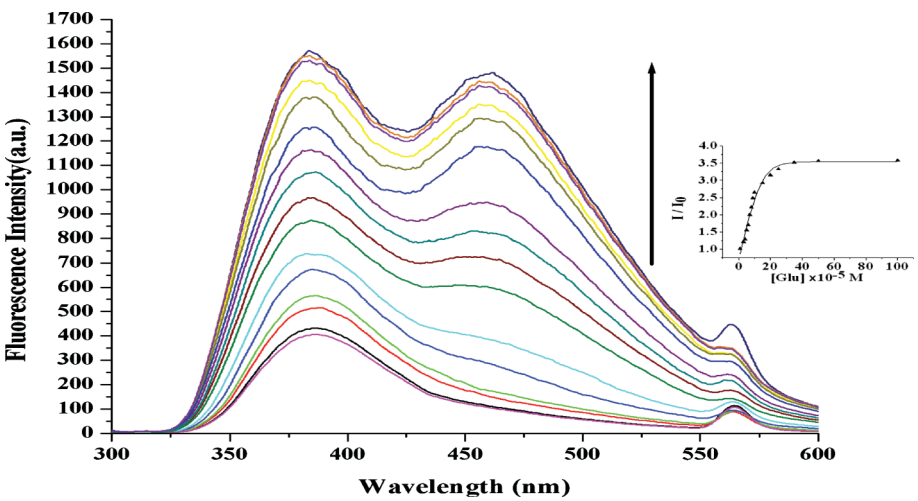


Fig. 4 Changes in the fluorescence spectra of Co(II)-PBI complex (10^{-5} M) as a function of externally added GluA. (From bottom to up, [GluA] = 10, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 450, 500, 1000 μM. Inset: Plot of fluorescence intensity of Co(II)-PBI complex (10^{-5} mol L^{-1}) as a function of externally added GluA] (solvent = water, λ_{ex} = 280 nm, λ_{em} = 370 nm).

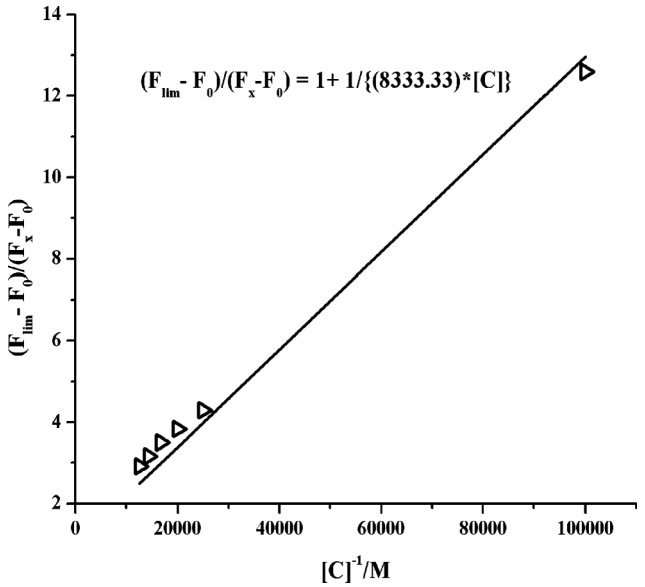


Fig. 6 Displacement equilibrium constant of GluA with the Co(II)-PBI complex.

relatively more stable, *viz.* 187 °C and 178 °C respectively, probably, due to the hydrogen bonding interaction.

Molecular level interaction

A stereoscopic view of the energy minimized structures (B3LYP/6-31G(d,p) basis set) of Co(II)-PBI complex, Co(II)-AspA and Co(II)-GluA systems, are presented in Fig. 9a, 9b and 9c respectively. Fig. 10 a, b and c show the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of Co(II)-PBI complex, Co(II)-AspA and Co(II)-GluA systems respectively. Distinct differential nature of their HOMOs and LUMOs indicate a molecular level interaction between the Co(II)-PBI complex and AspA as well as GluA. Table 3 presents the theoretical bond lengths and bond angles obtained from the optimized geometrical structures of the complexes.

Table 3 Theoretical bond lengths and bond angles obtained from B3LYP/6-31G(d,p) - optimized geometrical structures of complexes

Bond	Bond length(Å)	Bond angle	Angle(°)
$[(PBI)_2CoCl_2]^0$(*cis*)			
Cl_1–Co_{46}	2.311	N_{22}–Co_{46}–N_{25}	76.43
Cl_{51}–Co_{46}	2.576	Cl_1–Co_{46}–N_{25}	85.58
N_{22}–Co_{46}	1.979	N_{48}–Co_{46}–N_{25}	96.20
N_{25}–Co_{46}	2.383	N_{47}–Co_{46}–N_{25}	88.36
N_{48}–Co_{46}	1.968	Cl_{51}–Co_{46}–N_{22}	100.47
N_{47}–Co_{46}	1.982	Cl_{51}–Co_{46}–N_{47}	82.32
		Cl_{51}–Co_{46}–N_{48}	86.24
		Cl_{51}–Co_{46}–Cl_1	103.88
[(Aspartate)$_2$ Co$(H_2O)_2$]			
N_{17}–Co_{16}	1.955	O_{15}–Co_{16}–O_{32}	71.19
O_{31}–Co_{16}	1.904	N_{17}–Co_{16}–O_{32}	78.93
O_{35}–Co_{16}	2.463	O_{31}–Co_{16}–O_{32}	108.22
N_1–Co_{16}	1.961	N_1–Co_{16}–O_{32}	103.65
O_{15}–Co_{16}	1.914	O_{35}–Co_{16}–O_{31}	72.24
O_{32}–Co_{16}	2.498	O_{35}–Co_{16}–N_{17}	84.69
		O_{35}–Co_{16}–N_1	92.70
		O_{35}–Co_{16}–O_{15}	108.61
[(Glutamate)$_2$ Co$(CH_3OH)(H_2O)]^0$			
O_{23}–Co_{12}	1.904	O_{23}–Co_{12}–O_{26}	72.56
N_{13}–Co_{12}	1.948	N_{13}–Co_{12}–O_{26}	81.81
O_{24}–Co_{12}	2.506	O_{11}–Co_{12}–O_{26}	107.64
O_{11}–Co_{12}	1.902	N_1–Co_{12}–O_{26}	99.67
N_1–Co_{12}	1.951	O_{24}–Co_{12}–N_{13}	97.45
O_{25}–Co_{12}	2.468	O_{24}–Co_{12}–O_{23}	107.76
		O_{24}–Co_{12}–O_{11}	72.02
		O_{24}–Co_{12}–N_1	81.05

Experimental

Materials and methods

2-(2-Pyridyl) benzimidazole (PBI) and L-amino acids have been purchased from Sigma-Aldrich® Inc. (St. Louis, USA) and SRL (Mumbai, India) respectively. $CoCl_2$. $6H_2O$ has been purchased from Merck, India. Solvents used are of spectroscopic grade. De-ionized water from Milli–Q Millipore® 18.2 MΩ cm^{-1} conductivity system (Bedford, MA, USA) is used.

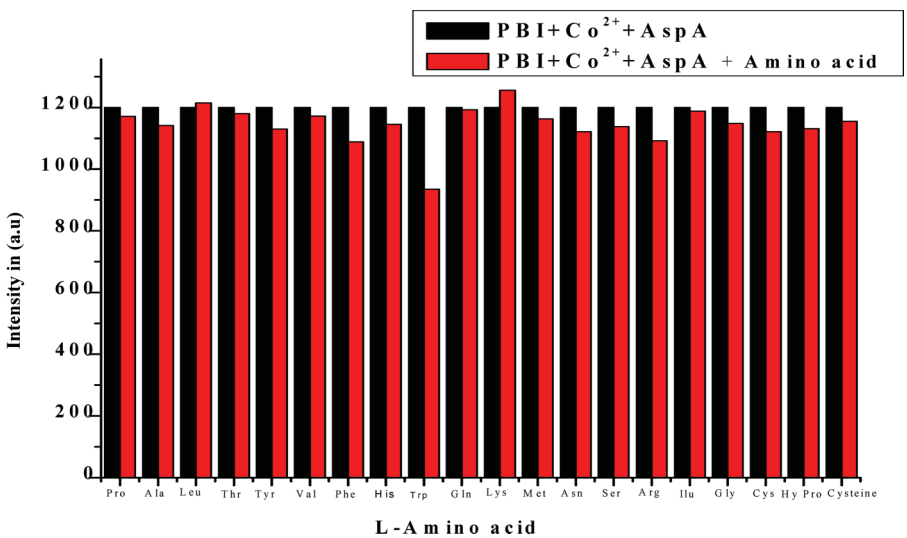


Fig. 7 Interferences of other amino acids (20 μM) on the emission intensities of Co(II)- AspA system (10 μM) in aqueous solution.

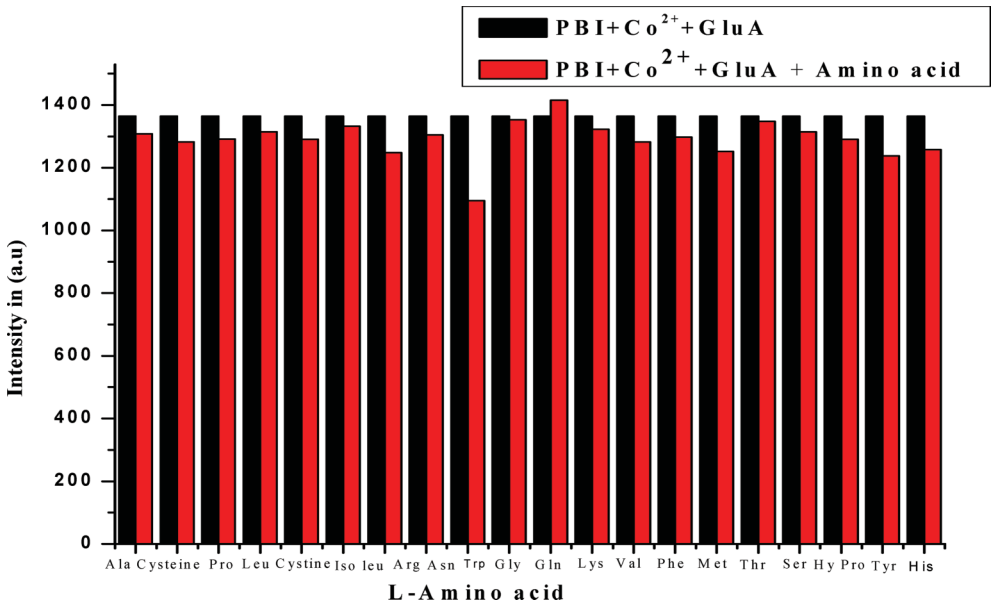


Fig. 8 Interferences of other amino acids (20 μM) on the emission intensities of Co(II)- GluA system (10 μM) in aqueous solution.

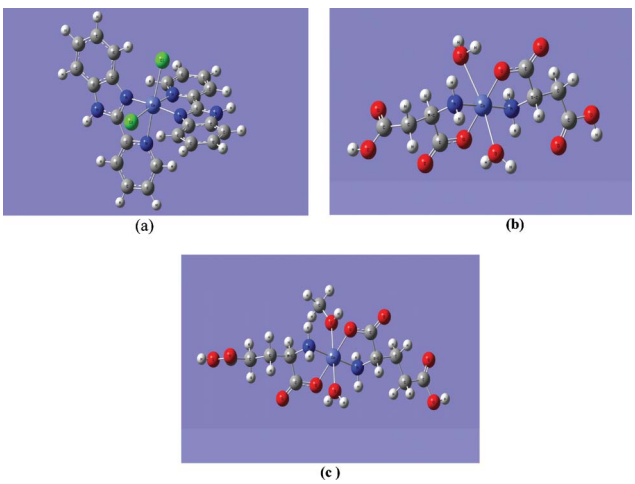


Fig. 9 B3LYP/6-31G(d,p)-optimized geometrical structures of complexes: (a) $(PBI)_2CoCl_2$]; (b) [$(Aspartate)_2Co(H_2O)_2$] and (c) [$(Glutamate)_2Co(CH_3OH)(H_2O)$].

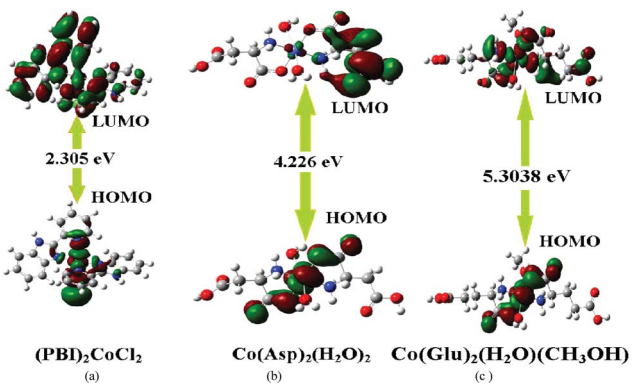


Fig. 10 HOMO and LUMO of (a) $(PBI)_2CoCl_2$]; (b) [$(Aspartate)_2Co(H_2O)_2$] and (c) [$(Glutamate)_2Co(CH_3OH)(H_2O)$].

Instrumentation

A JASCO (model V-570) UV-vis. spectrophotometer is used for measuring UV-vis. spectra. FTIR spectra are recorded on a JASCO FTIR spectrophotometer (model: FTIR-H20). Mass spectra are recorded on a QTOF Micro YA 263 mass spectrometer in ES positive mode. Thermogravimetric analyses are performed on a Perkin Elmer TG/DTA lab system I (Technology by SII). Fluorescence spectra are measured with a Hitachi F-4500 spectrofluorimeter equipped with a temperature controlled cell holder. X-ray crystal data are collected at 93 K by using a Rigaku MM007 High brilliance RA generator/confocal optics and Mercury CCD system. Intensities are corrected for Lorentz polarization and for absorption. The structure is solved by direct methods. Hydrogen atoms bound to carbon are idealized. Structural refinements are obtained with full matrix least-squares based on F2 by using the program SHELXTL[49] (SHELXTL 6.11, Bruker AXS, Madison, WI, USA, 2004). Structures of Co(II)-PBI complex and its molecular assemblies with AspA and GluA are optimized by DFT (B3LYP/6-31G(d,p) basis set) using Gaussian '03 software package.[50]

Synthesis of dichloro-bis-2-(2-pyridyl) benzimidazole Co(II) complex (Co(II)PBI)

2 g (10.24 mmol) PBI is dissolved in 10 mL methanol. To this solution, a methanol solution of $CoCl_2$. $6H_2O$ (2 g, 5.12 mmol, 10 mL) is added drop wise with continuous stirring. The mixture is refluxed at 50 °C for 4 h. The dark pink color solution is kept for slow evaporation of the solvent. After two weeks, red color crystals have been found. The structure of Co(II)PBI complex have been established by X-ray crystallography

Synthesis of Co(II)-AspA system

To characterize the end product of the reaction between Co(II)PBI complex and AspA, they are mixed in 1 : 10 mole ratio (0.5 g, 0.96 mmol of Co(II)PBI complex with 2.55 g, 19.2 mmol of AspA)

in water and stirred for 3 h. After slow evaporation of water, a straw color compound have been isolated and characterized.

Synthesis of Co(II)-GluA system

Co(II)PBI complex and AspA, are mixed in 1 : 10 mole ratio (0.5 g, 0.96 mmol of Co(II)PBI complex with 2.82 g, 19.2 mmol of GluA) in water and stirred for 3 h. After slow evaporation of water, a straw color compound have been isolated and characterized.

Preparation of solutions

Working solutions of AspA and GluA are prepared by serial dilution of a 1×10^{-2} M stock solution (0.0133 mg of AspA and 0.0147 mg GluA in10 mL water). A stock solution of Co(II)-PBI complex (1×10^{-5} M) have been prepared by dissolving its appropriate amount in water.

Measurement procedure

Solutions of AspA and GluA have been mixed separately with the solution of Co(II)PBI complex in different ratios (v/v) and their fluorescence studies are performed. The fluorescence emission intensity is measured at 370 nm while the excitation wavelength was fixed at 280 nm. 1 cm quartz cell was used for all the measurements.

Conclusion

We have developed an exceptionally simple, rapid and sensitive method for determination of AspA and GluA *via* a displacement protocol. The water soluble 2-(2-pyridyl)-benzimidazole based weakly-fluorescent Co(II)-complex serves as an efficient fluorescent sensor for AspA and GluA. These two amino acids could recover the quenched fluorescence of Co(II)PBI complex while the rest 18 amino acids failed to do so. Probably, AspA and GluA being acidic amino acids, they could produce more stable Co(II) chelate through hydrogen bonding.

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