Effective Detection of Phenylalanine Using Pyridine Based Sensor

Vijayakumar Sathya

Madurai Kamaraj University

Venkatesan Srinivasadesikan

Vignan's Foundation for Science Technology and Research

Shyi-Long Lee

National Chung Cheng University

Vediappen Padmini (✉ padmini.chem@mkuniversity.org)

Madurai Kamaraj University <https://orcid.org/0000-0002-6103-6432>

Research Article

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Abstract

Pyridine based organic fluorophore has been synthesized for the detection of phenylalanine (PA). The quenching has been observed between fluorophore and PA through ICT system as noticed by colorimetric and fluorimetric techniques. Under optimized conditions, the fluorophore detects PA selectively in the presence of other biomolecules. The practical application of the fluorophore has been successfully applied in human blood serum and urine.

Introduction:

Amino acid plays a significant role in protein synthesis, pharmaceutical industry, food industry and health product industry. ^[1] Natural amino acids are classified into three types as acidic, basic and neutral amino acids. ^[2] Phenylalanine (PA) is one of the essential, neutral aromatic amino acid. It exists in zwitterionic form. PA is being gathered from diet and endogenous proteolysis. ^[3] PA is found in mother's milk and numerous vegetables (carrot, onion, palm, potato, radish and beets etc.), fruits (acai palm, apple, banana, jack fruit, kiwifruit, mango etc.), meat (chicken, beef, fish and goat), milk, lentils, peanuts, cheese and sesame seeds. ^[4]

PA is a precursor of tyrosine. Tyrosine is a non-essential amino acid. It plays a vital role for the production of several neurotransmitters such as dopamine, melanin, epinephrine and norepinephrine. ^[6] Phenyl hydroxylase enzyme (PAH) is present in liver which used as a catalyst for the conversion of PA into tyrosine. Absence or inactivation of PAH leads to the inhibit of conversion of PA into tyrosine. The tyrosine level always depends on PA in human body. The normal and abnormal level of PA in human biofluids are $30 \pm 60 \mu\text{mol/L}$ and $>30 \pm 60 \mu\text{mol/L}$, respectively. Abnormal level of PA causes phenylalaninemia disease. ^[7-8] From this discussion one can understand that the essential of PA and its importance. So, to detect the PA at various places is an important. The available and predictable sensor platform are electrochemical sensors, modified carbon electrode and electrode surface techniques, ^[2, 21, 18] cyclodextrin based electrode methods, ^[19, 24, 14, 5] polymer based sensors, ^[9, 10] liquid crystal sensing, ^[11] photo luminescent sensor, ^[12] bioluminescent sensor. ^[13] However, very limited work has been reported in the literature for the detection of PA. Particularly, detect the PA at lower concentration is the challenging.

Fluorimetric method is one of the potent analytical aid to detect various toxic biomolecules and metal ions. Fluorescent organic compound has very high sensitivity, operational simplicity less time consuming, cost effective and wide range detection to the biomarker. In general, disease causing biomolecules and other microorganism containing primary amines, secondary amines and amides functional groups can interact with -OH group of blood glucose unit in our body through intermolecular hydrogen bonding. ^[14]

In this work, we have synthesized pyridine based fluorescent organic compound which selectively detects PA in the presence of other interfering biomolecules such as, leucine, asparagine, urea, creatinine, ascorbic acid, cysteine, tryptophan, alanine, glutamine and albumin. Literally, the fluorescent organic probe has been exhibit the low detection limit of 0.32 nM towards PA.

Materials And Methods

Diaminopyridine, aldehyde, glacial acetic acid, absolute ethanol, leucine, asparagine, urea, creatinine, ascorbic acid, cysteine, tryptophan, alanine, glutamine and albumin are purchased from Sigma Aldrich and Alfa Aesar. ^1H and ^{13}C NMR studies have been done with CDCl_3 as solvent containing tetramethylsilane (TMS) as internal standard in Bruker-300 MHz spectrometer at 25°C and Mass spectral has been recorder by MS-LCMS mass spectrometer respectively. Absorption and Emission spectral measurements are recorded using SHIMADZU single beam UV-vis spectrophotometer and Cary Eclipse spectrophotometer with 450W xenon lamp, respectively.^[15-18]

Experimental Section:

The probe was synthesized by a previously reported method.^[19] 2,6-diamino pyridine (1.0 mmol) was stirred in hot absolute ethanol and 4-hydroxybenzaldehyde (1.0 mmol) was added. The reaction mixture was allowed to reflux for 1h. Upon cooling, the orange colour precipitate was filtered, washed with cold ethanol and dried. (Scheme.1)^[20]

Results And Discussion:

4.1. Photo physical properties of probe

The probe has phenolic -OH which binds with the acid group in the phenylalanine as electron donor by hydrogen bond through ICT (Internal Charge Transfer) mechanism selectively. Initially the probe shows bright yellow color and with the addition of phenylalanine complete quenching has been observed.^[21] (Scheme.1)

The absorption spectrum of probe shows peaks at 411nm and 438nm in $1 \times 10^{-6}\text{M}$ ACN/PBS (v/v, 1:9) at neutral pH and the emission spectrum exhibits peak at 548nm, which is mainly characteristics of $n \rightarrow p^*$ transition (Figure.1, (i) & (ii)).

This comparative experiments have been performed to discriminate the phenylalanine by the probe in the presence of other interference competitors (Figure. 2). The selective detection of phenylalanine at $500\mu\text{M}$ concentration has been clearly revealed. Upon the addition of different concentrations of phenylalanine (20nM-500 μM) to the probe, decrease in the absorbance has been observed (Figure. 3). Thus, the Figure.4 shows the linearly decreasing absorbance of the complex.^[22]

The probe and analyte exhibit complete fluorescence quenching (TURN- OFF response) selectively compared with other competitors (Figure. 5). Further addition of phenylalanine (20nM -500mM) to probe, gradually decreases the fluorescent intensity with hypsochromic shift. (Figure. 6) The corresponding bar diagram shows selectivity towards phenylalanine, in which no spectral change has been observed with other competitors. (Figure. 7) It clearly reveals the high selectivity to phenylalanine by optical experimental method.^[23]

The competitive experiments for the probe and analyte with interfering other biomolecules such as leucine, asparagine, urea, creatinine, ascorbic acid, cysteine, tryptophan, alanine, glutamine and albumin are carried out. These experiments indicate that phenylalanine is binding with probe. Other biomolecules do not interfere during the phenylalanine binding with probe, as represented in the bar diagram. (Figure. 8) ^[24]

The job's plot plotted between mole fraction (same concentration of probe and analyte) vs fluorescence intensity. Binding stoichiometric ratio of probe with phenylalanine has been arrived by fractional addition method. From the jobs plot, the binding ratio of probe: phenylalanine is found to be 1:2 (Figure. 9) ^[25].

The pH dependance has been studied using Phosphate buffer solution (PBS). The different solutions were prepared at various pH level (pH 2-12). At basic medium (pH 8-12), deprotonation of the probe leads to less interaction with phenylalanine. At the neutral pH, high fluorescent intensity is observed while adding phenylalanine with complete quenching. Hence, all other experiments have been performed at neutral pH condition (Figure.S5).^[26]

Colorimetric and Fluorimetric Biosensor for Phenylalanine

Initially the probe is dissolved in acetonitrile (ACN) solution. Then, the addition of phenylalanine to the probe leads to the colour changes from yellow to dark brown observed by naked eye. Under the UV lamp, the colour changes observed from bright yellow fluorescence. But, there is no colour change observed for the other competitors with probe (Figure.12). From the colorimetric and fluorimetric techniques, it is confirmed that there is a selective interaction between the probe and phenylalanine at nanomolar concentration at neutral pH. ^[27, 28]

Test Strip Method:

Test strip method is a confirmation method to selective detection of phenylalanine. Filter papers were soaked overnight into the probe solution (a), probe with analyte (b) and probe with other competitors (c). The strips (a) and (c) shows yellow color and colourless, respectively. No color change has been observed with the other competitors (figure c) under UV lamp. It can be concluded that the pyridine derivative selectively detect the phenylalanine. ^[29]

5. Phenylalanine sensitivity in human biofluids

The numerical assessment for PA has been employed in human biological fluids such as urine and blood serum (Fig. 12 and 13) by standard addition method. Spiking the known concentration of PA (50 nM, 100 nM, 200 nM, 300 nM, 400 nM, 500 nM, 50 μ M, 100 μ M, 200 μ M, 300 μ M, 400 μ M and 500 μ M) into the blood and urine samples and incubate for 15-20 mins. From spectral measurement, the development of biosensor towards the detection of PA in human blood and urine sample is thus achieved. (Fig. 8 and 9) ^[30, 31]

Literature report for phenylalanine sensor:

Sl.No	Types of sensor platform	Techniques	LOD	References
1	Electrochemical sensor by using polyaniline modified carbon electrode based on β -cyclodextrin incorporated multiwall carbon nano tube and imprinted sol-gel film	Electrochemical Sensor	1.0×10^{-9} mol L ⁻¹	[32]
2	Electrochemical sensor by molecular imprinted techniques	Electrochemical sensor	1.0×10^{-9} M	[33]
3	Fluorescence quenching by using combination of cucurbit[7]uril (CB[7]) with palmatine hydrochloride.	Fluorescence quenching method	1.27×10^{-8} mol/L	[34]
4	Electrochemical enantioselectivity sensor by using Graphene-ferrocene functionalized cyclodextrin modified electrode	Electrochemical sensor	27 nM and 52 nM	[35]
5	A novel colorimetric and fluorescent multifunctional chemo-sensor by using 4-bromo-2-hydroxyben Rhodamine B hydrazide (RHBr).	colorimetric and fluorescent chemo sensor	3.0×10^{-7} M	[36]
6	potentiometric chiral sensor based on crosslinked polymethylacrylic acid–polycarbazole hybrid molecularly imprinted polymer.	Potentiometric chiral sensor	1.37 μ M	[37]
7	Spectro fluorimetric method based on fluorescence Enhancement of europium ion immobilized with sol–gel	Spectrofluorimetric Method	5.2×10^{-6} molL ⁻¹	[38]
8	Electrochemical enantioselectivity biosensor based on β -Cyclodextrin immobilized on reduced graphene Oxide	Electrochemical biosensor	0.10 μ M and 0.15 μ M	[39]
9	Biosensor by using fluorescence protein	Biosensor	3.7 μ M	[40]
10	Electrochemical biosensor by Gold Electrode Modified with Graphene Oxide Nanosheets and Chitosan	Electrochemical biosensor	416 mM	[41]
11	Optical biosensor by using fluorescent organic compound	Optical biosensor	0.32 nM	Present work

6. DFT studies

The experimental finding of pyridine based organic probe detect the PA at lower concentration were further explored using DFT atomic level simulation. All the calculations have been carried out using Gaussian 09 ^[42] suite of program. The probe and complex were optimized at B3LYP/6-31g(d,p)^[43] level of theory in the gas phase. The optimized structure of probe and complex were further confirmed with no imaginary frequencies using frequency calculation. The optimized structure of probe and complex were placed in Figure 10 (see Fig 10(a), 10(b)). The single point energy calculation of frontier molecular orbital and electrostatic potential on the optimized structure of pyridine based organic probe and PA complex were employed at B3LYP/6-31g(d,p) level in the gas phase. The electrostatic potential of probe was placed in Figure 10 (c). It can be observed from Figure 10(c) that the red region represents the electrophilic nature and blue region represents the electrophobic nature. The frontier molecular orbital (FMO) structure of probe and complex was placed in the Figure 11 (a) and 11(b), respectively. The oscillator strength at HOMO-1 to LUMO+1 observed to be 0.519 for the organic probe and the molecular orbital gap was calculated as 4.52 eV. The computed wave length for the probe was 300.88 nm. Moreover, the oscillator strength at HOMO to LUMO+2 observed to be 0.769 for the complex and the molecular orbital gap was calculated to be 3.82 eV. The computed wave length for the complex was 369.43 nm. The observed lower band gap in the complex was confirmed the biomolecule interaction with organic probe and complex formation. In probe, at HOMO-1 the electron cloud was observed at both the phenolic moiety and at LUMO+1 was observed at pyridine moiety. However, at HOMO and LUMO+2 levels the electron cloud in the complex was observed at the phenolic and pyridine moiety.

Conclusion

Pyridine derivative can able to detect analyte at neutral pH condition (pH-7.2). The probe is non-toxic in high concentration to the biological system. The probe selectively detects phenylalanine in the presence of other competitors through internal charge transfer (ICT) mechanism. The binding stoichiometric ratio is 1:2 with the excellent detection limit and the binding constant towards phenylalanine is 0.32 nM and $8.1 \times 10^7 \text{ M}^{-1}$, respectively.

Declarations

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Code availability: Not applicable

Author's contributions: **Vijayakumar Sathya:** Conceptualization, writing, original draft. **Venkatesan Srinivasadesikan:** Software resources. **Shyi-Long Lee:** Software resources. **Vediappen Padmini:** Supervision, Writing-review.

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Scheme 1

Please see the Supplementary Files for the Scheme 1.

Figures

Figure 1

(i). UV-Visible and (ii). Emission spectra of probe [1×10^{-6} M, at pH 7.2, PBS]

Figure 2

Absorption spectrum for selective detection of phenylalanine by pyridine based probe.

Figure 3

Absorption changes of probe with the 20nM to 500μm concentration of analyte in PBS: ACN (v/v, 9:1, pH7.2).

Figure 4

Linear plot of for gradually decreasing of absorbance with the phenylalanine to probe.

Figure 5

Emission spectrum for selective detection of phenylalanine by probe with other competitors.

Figure 6

Emission spectral changes of probe with the 20nM to 500 μ M concentration of analyte in PBS: ACN (v/v, 9:1, pH7.2).

Figure 7

Bar diagram for selectivity towards phenylalanine (500 μ M) with probe and other competitors.[1- probe+PA, 2- probe, 3-leucine, 4-asparagine, 5- urea, 6-creatinine, 7-ascorbic acid, 8-cysteine, 9-tryptophan, 10-alanine, 11- glutamine, 12- albumin]

Figure 8

Selective spectrum for the determination of PA in human blood serum.

Figure 9

Selective spectrum for the determination of PA in human urine sample.

Figure 10

Optimized at B3LYP/6-31g(d,p) in gas phase (a). Probe, (b). Complex & ESP at B3LYP/6-31g(d,p)//B3LYP/6-31g(d,p) in gas phase. Scale: -6.901e-2 to 6.901e-2.

Figure 11

Frontier Molecular Orbital diagram obtained at B3LYP/6-31g(d,p)//B3LYP/6-31g(d,p) in gas phase (a). Probe, (b). Complex.

Supplementary Files

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