Colorimetric Determination of Amino Acids using Fluorescent Copper Nanoparticle

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Abstract*: Among the 20 amino acids, cysteine plays a major role in communication of the cells, especially towards immune system and thus developing sensor for cysteine is very important to understand the status of the human health. Copper nanoparticles decorated with Rhodamine B (RBCN) have the potential to detect the biologically important species such as amino acids, especially cysteine. RBCN has been previously has demonstrated for the sensing of host molecules such as cucurbituril based on the relative binding potential of rhodamine B on the surface of copper nanoparticles. Based on that concept, now we have developed the sensor for amino acids, especially for the cysteine

Keywords: Copper nanoparticles, Amino acids, Cysteine sensor, colorimetric sensor

I. INTRODUCTION

The essential component of all creatures on earth is protein which is made up of amino acids [1], There are 20 different amino acids with different functional groups, which have been actively involved in several of the cell processing such as Amino acid are key cell signaling, synthesis of hormones, gene expression regulator and phosphorylation cascade [2]. All the amino acids have been encoded by the DNA and amino acid synthesis is believed controlled by the DNA and RNA codes. The concentrations of amino acids in the cell are very essential in cell protection and functioning the metabolites are more important. For example, excess amino acids may cause serious cardiovascular disease, oxidative stress and neurological disorders [1]. On the other hand amino acids deficiency may cause irritability, hormonal imbalance, fatigue and at the extreme scarcity will cease the synthesis of protein. Several methods have been explored to detect amino acid that include high performance liquid chromatography [3], UV-vis detection [4], fluorescent detection [5], [6], capillary electrophoris [7], Fourier Transform Infrared spectroscopy [8], mass spectrophotometer [9], etc. However, these methods require extensive preparation and laborious and for that purpose, we have developed naked eye detection method for the amino acids. Among the analytical methods, fluorescence method has gained much attention due to its high sensitivity and furthermore, visual detection of amino acid could very useful for various applications [2], [10], [11]. Due to the high selectivity, sensitivity and ease of functionalization of copper nanoparticle, it gained much attention for the development of fluorescent sensor for several analytes [12], [13], [14]. Until now, gold and silver nanoparticles have been explored for the for the detection of amino acids such as arginine, lysine and histidine and many others [15]. Recently, selective detection of arginine has been reported using bis-rhodamine-thiourea/Al^{3+} [16]. On the other hand, cysteine modified silver nanoparticles were explored for the detection of histidine [17]. In recent years, many of the biosensing applications have been developed using copper, gold and silver nanoparticles based on their unique optical properties and facile derivatization [12]. Development of "turn-on" fluorescence sensor for the detection of amino acid in water will provide great pathway in the field of biosensor [18], [19]. We recently reported, a rhodamine B decorated copper nanoparticle for detection of CB7 with a simple fluorescence turn-on method. In the reported method, the rhodamine B tethered copper nanoparticle released the rhodamine B based on the higher binding affinity of CB7 towards the rhodamine B to act as a sensor for CB7 and that also displayed good selectivity towards the CB7 compared to the other homologues such as CB6 and others [20]. Similarly, we also have published another sensor for CB7 using the graphene oxide bound rhodamine B [21].

In a similar manner, we have developed a new sensor for amino acids using rhodamine decorated copper nanoparticles. Rhodamine is a fluorescent molecule, which turn off the fluorescence upon binding with copper nanoparticles. Here, we reported a sensor for amino acids employing RBCN nanoparticle as fluorescence probe in aqueous medium, in which cysteine showed better sensitivity among other amino acids. The amino acids such as alanine, glutamine, leucine, phenylalanine, tryphtophan, tyrosine and cysteine were examined with RBCN nanoparticle in aqueous media. Interestingly, the nanoparticle showed turn-on fluorescence with amino acids under neutral condition in aqueous medium, especially with cysteine it displayed good fluorescence, which apparently showed a marked difference from other amino acids. We presume that the thiol group present in cysteine could be responsible for this huge difference in fluorescence response of cysteine in comparison to other amino

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acids, because thiol compounds have been used as a capping agent to stabilize the nanoparticles and known to have higher affinity with the metal nanoparticles [22]. Furthermore, the selectivity of the cysteine sensor has been proved by the naked eye detection method. Overall, RBCN has been utilized to sense the amino acids, especially for cysteine. Among the amino acids, cysteine has the thiol group, which has been taken advantage to demonstrate the selectivity for cysteine. RBCN can be used to identify the cysteine qualitatively by the naked eye detection method. Therefore, the present method provides a very facile way to identify the biologically important, cysteine amino acid more selectively.

II. RESULT AND DISCUSSION

The fluorescence sensor probe, RBCN was synthesized according to the reported procedure [20] Briefly, Rhodamine B and copper sulfate (1 mM) were prepared in 200 mL of water. To this, hydrazine hydrate (30%, 100 µL) was added dropwise and allowed to stir for 24 hrs. After stored it for 3 days in dark, the product (RBCN) has been isolated by centrifugation and it was further washed with DI water followed by acetone to remove the unbound rhodamine B. This was further diluted to 10 mL volume and used as stock solution.

Detailed isolation and purification of RBCN has been published [20], in which the residual removal of rhodamine B was the key success, which has been achieved by the multiple washings of acetone and water and that allowed to turn off the fluorescence. Upon addition of amino acids, the fluorescence was turn-on depends on the competitive binding affinity between rhodamine B and the respective amino acids. For example, 850 µL of amino acid (10^{-2} M) was as added to the vial containing 150 µL of RBCN, which allowed to release the rhodamine B quantitatively depending on the relative binding affinity of amino acids in competition with the bound rhodamine B. The fluorescence release of rhodamine B was tested with various amino acids that include Alanine (Ala), Glutamine (Glu), Leucine (Leu) , Phenylalanine (Phe), Tryptophan (Try), Tyrosine (Tyr) and Cysteine (Cys). Fluorescence sensing of these amino acids has been summarized in fig.1 and 2. As shown in fig.2, the intensities of various amino acids against the RBCN has been displayed in a bar diagram. Among them, cysteine displayed the best out of all the amino acids that has been tested. Tryptophan displayed the second best among the other amino acids. Phenylalanine is the third best among the amino acids that have been tested. There is a correlation between the tryptophan and phenylalanine as both having the aromatic ring and that displayed the better affinity toward the copper nanoparticles. The difference between phenylalanine and tyrosine is only a hydroxyl group at the para position of the phenyl part and that created the hydrophilic environment and the reason why it failed to get attracted towards either nanoparticles or rhodamine B. All the other amino acids displayed no marked difference between them. Collectively, thiol groups have good affinity towards the metal nanoparticles, which is the key success for attaining the selectivity and sensitivity of cysteine. In the literature, it has proved often the affinity between the copper and thiols [22]. Thus thiols get attracted towards the copper nanoparticles, in which rhodamine B has been attached non-covalently. Basically, thiols could compete with rhodamine B for the surface of the nanoparticles, which resulted in release of rhodamine B quantitatively and subsequently, release of rhodamine B indirectly provides the opportunity to develop sensor for cysteine. To confirm the relation between the concentration and the quantity of release of rhodamine B, we have performed the experiments with different concentrations of cysteine. As shown in Fig. 3, when the cysteine concentration was raised from 10^{-3} to 10^{-2} M, there was marked raise in the intensity of the rhodamine B fluorescence release, which has been proved that the cysteine concentration can be indirectly measured from the release of rhodamine. For example, 1 and 10 mM solutions of cysteine were prepared in a phosphate buffer (pH 7.0) and measured the fluorescence intensity after the addition of 300 µL of RBCN in each vials containing cysteine. The excitation wavelength for rhodamine B was 554 nm and the emission wavelength spectra were observed at 577 nm.

Another advantage of the present method is that we can also visualize the cysteine qualitatively. As shown in fig. 4. amino acids Alanine (Ala), Glutamine (Glu), Leucine (Leu) , Phenylalanine (Phe), Tryptophan (Try), Tyrosine (Tyr) and Cysteine (Cys) can be visualized under the UV light (365 nm) by mixing the respective amino acids (150 µL, 10-2 M) with RBCN (850 µL) in a vial. As shown in fig. 4, except cysteine, all the other amino acids displayed no fluorescence. This indicates that RBCN, can be used to detect by naked eye without any laborious protocol and thus, our method could be

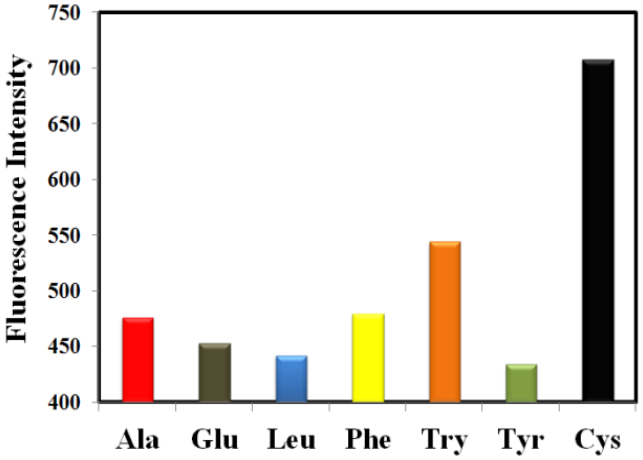

Figure 1: Emission spectral changes of RBCN upon addition of amino acids (1 x 10^{-2} M) Ala, Cys, Glu, Leu, Phe, Try and Tyr respectively (λ_{max}= 577 nm).

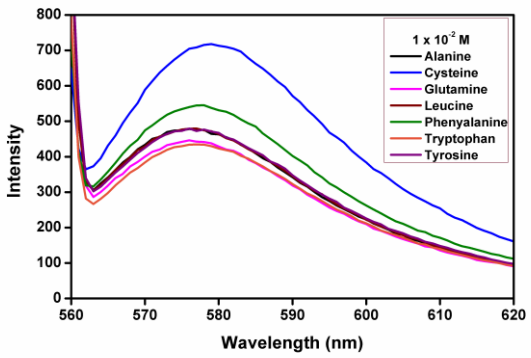

Figure 2: Fluorescence intensity comparison upon addition of various amino acids Ala, Cys. Leu, .Phe, Try, Tyr and Cys with RBCN respectively.

A complimentary to the existing method of detection for amino acids and the ease synthesis of RBCN will be an added advantage.

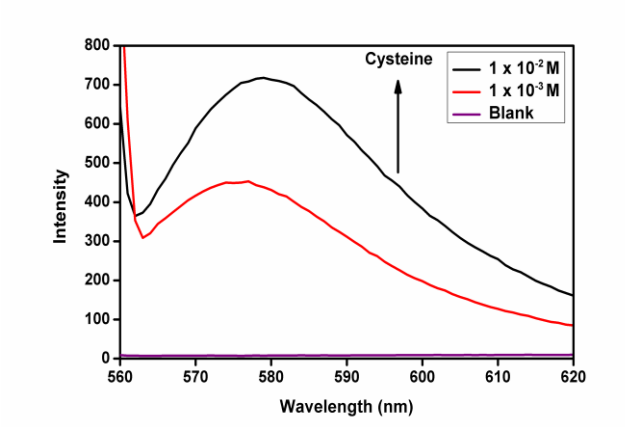

Fig. 3. Fluorescence spectra of RBCN upon the addition of increasing concentration of cysteine in water at pH 7 (λmax= 577 nm, excitation wavelength is 554 nm).

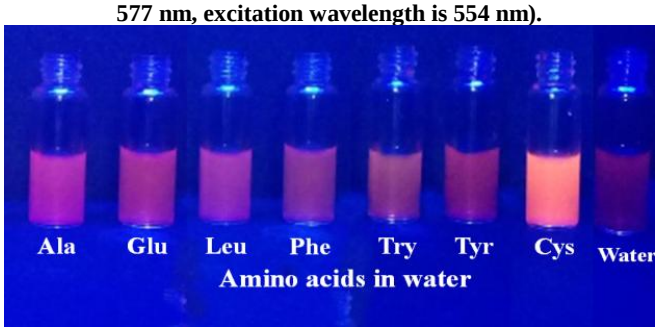

Fig. 4: Naked eye detection of amino acids with RBCN- under the UV light (365 nm) (To the 150 µL of RBCN in water, 850 µL of 10^{-2} M amino acids were added)

III. CONCLUSION

In summary, we developed an economical and selective amino acid sensor using inexpensive copper nanoparticle, RBCN The nanoparticle displayed good selectivity for cysteine, when it was examined with various amino acids such as Alanine, Glutamine, Leucine, Phenylalanine, Tryphtophan, Tyrosine and Cysteine in water. Based on the higher affinity of cysteine towards the copper nanoparticles, cysteine displayed remarkable turn on fluorescence with RBCN. Therefore RBCN can be complementary to the other existing amino acid sensors, especially for cysteine

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REFERENCES

1. G. Wu, Amino acids, "Amino acids: metabolism, functions, and nutrition" Springer, vol. 37, pp. 1-17, May 2009
2. A. M. Pettiwala and P. K. Singh, "Recent developments in designing optical sensors for detection of basic amino acids," Indian J Chem, vol. 57B, pp. 293-300, Feb 2018.
3. Y. V. Tcherkas and A. D. Denisenko, "Simultaneous determination of several amino acids, including homocysteine, cysteine and glutamic acid, in human plasma by isocratic reversed-phase high-performance liquid chromatography with fluorimetric detection," J Chromatogr A Elseveir, vol. 913, pp. 309-313, 2001.
4. K. Amarnath, V. Amarnath, K. Amarnath, H. L. Valentine, W. M. Valentine, "A specific HPLC-UV method for the deermination of cysteine and related aminothiols in biological samples," Talenta Elsevier, vol. 60, pp. 1229-1238, Mar 2003.
5. X. Shang, J. Yu, X. Wei, X. Li, Y. Feng, and X. Xu, "A Highly selective Colorimetric sensor for cysteine in waater solution and bovine serum albumin," J Sens Hindawi, vol. 2016, Article ID 5862929, Nov 2015.
6. H. Wang, W. Wang, H. Zhang, "Spectrofluorimetric determination of cysteine based on the fluorescence inhibition of Cd(II)-8-hydroxyquinoline-5-sulphonic acid complex by cyseine," Talenta Elsevier, vol. 53, pp. 1015-1019, Jan 2001.
7. T. Inoue and J. R. Kirchhoff, "Electrochemical detection of thiols with a coenzyme pyrroloquinoline quinone modified electrode," Anal. Chem. ACS, vol. 72, pp. 5755-5760, Oct 2000.
8. K. Kargosha, S. H. Ahmadi, M. Zeeb, S. R. Moeinossadat, "Vapour phase fourier transform infrared spectrometric determination of L-Cysteine and L-Cystine," Talanta Elsevier, vol. 74, pp. 753-759, Jul 2007.
9. N. Burford, M. D. Eelman, D. E. Mahony and M. Morash, "Definitive identification of cysteine and glutathione complexes of bismuth by mass specrometry: assessing the biochemical fate of bismuth pharmaceutical agents," Chem Comm RSC, pp. 146-147, Jan 2003.
10. Y. Sun, L. Zhang and H. Li, "Chiral colorimeric recognition of amino acids based on silver nanoparticle clusters,"New J Chem RSC, vol. 36, pp. 1442-1444, May 2012.
11. J. F. Folmer-Andersen, V. M. Lynch, and E. V. Anslyn, "Colorimetric enantiodiscrimination of Alpha-Amino acids in protic media," JACS, vol. 127(22), pp. 7986-7987, Mar 2005.
12. Y. Guo, F. Cao, X. Lei, L. Mang, S. Cheng, and J. Song, "Fluorescent copper nanoparticles: recent advances in synthesis and applicaions for sensing metal ions," Nanoscale RSC, vol. 8, pp. 4852-4863, Jan 2016.
13. G. Doria, J. Conde, B. Veigas, L. Giestas, C. Almeida, M. Assuncao, J. Rosa and P. V. Baptista, "Noble metal nanoparticles for biosensing applications," Sensors, vol. 12, pp.1657-1687, Feb 2012.
14. J. Cao, L Ding, W. Hu, X. Chen, and Y. Fang, "A ernary sysem based onn fluorophore-surfactant assemblies-Cu^{2+} for highly sensiive and selective detection of argenine in aqueous solution," Langmuir ACS, vol. 30, pp. 15364-15372, Dec 2014.
15. A. M. Pettiwala and P. K. Singh, "Recent developments in designing optical sensors for detection of basic amino acids," Indian J Chem, vol. 57B, pp. 293-300, Feb 2018.
16. L. He, V. L. L. So, J. H. Xin, "A new rhodamine-thiourea/Al^{3+} complex sensor for the fast visual detection of arginine in aqueous media," Sens Actuators B Elsevier, vol. 192, pp. 496-502, Nov 2013.
17. H. Li, and Y. Bian, "Selecive colorimetric sensing of hisidine in aqueous solution using cysteine modified silver nanoparticles in the presence of Hg^{+}," Nanotechnology IOP publishing, vol. 20, Mar 2009.
18. T. Minami, N. A. Esipenko, B. Zhang, L. Isaacs and P. Anzenbacher, "Turn-on fluorescent sensor array for bsic amino acids in water," ChemComm RSC, vol. 50, pp. 61-63, 2014.
19. M. Bnizzoni, L. Fabbrizzi, G. Piovani and A. Taglietti, "Fluorescent detection of glutamate with a dicopper (II) polyamine cage," Terahedron Elsevier, vol. 60, pp. 11159-11162, Oct 2004.
20. R. Prakash, G. Usha, L. Piramuthu and N. Selvapalam, "Facile detection of cucurbit[7]uril by rhodamine decorated nanoparticle," Chem Lett Chem Soc Jpn, vol. 46, pp. 1300-1303, 2017.
21. R. Prakash, G. Usha, P. Sivaranjana, K. Karpagalakshmi, L. Piramuthu and N. Selvapalam, "Graphene oxide based fluorescence sensor for cucurbit[7]uril," New J Chem RSC, vol. 42, pp. 13038-13043, Jun 2018.
22. A. Rigo, A. Corazza, M. L. Paolo, M. Rossetto, R. Ugolini, M. Scarpa, "Interaction of copper with cysteine: stability of cuprous complexes and catalytic role of cupric ions in anaerobic tthiol oxidation," J Inorg Biochem Elsevier, vol. 98, pp. 1495-1501, Jul 2004.
23. R. A. Soomro, A. Nafady, Sirajuddin, N. Memon, T. H. Sherazi, N. H. Kalwar, "L-Cysteine protected copper nanoparticles as colorimetric sensor for mercuric ions," Talanta Elsevier, vol.130, pp. 415-422, Jul 2014

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