Organic & Biomolecular Chemistry

RSC Publishing

COMMUNICATION

View Article Online
View Journal | View Issue

Coumarin–DPA–Cu(II) as a chemosensing ensemble towards histidine determination in urine and serum†

Cite this: *Org. Biomol. Chem.*, 2013, **11**, 717

Received 8th October 2012,
Accepted 3rd December 2012

DOI: 10.1039/c2ob26955d

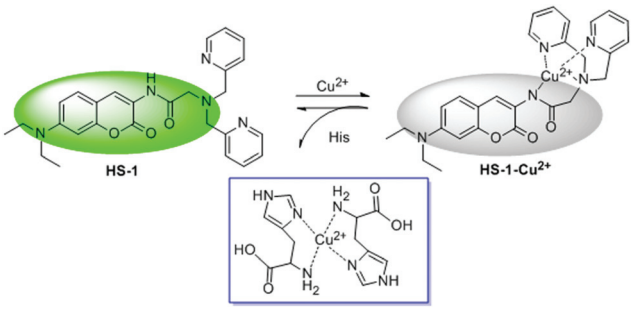
www.rsc.org/obc

Ji-Ting Hou, Kun Li,* Kang-Kang Yu, Ming-Yu Wu and Xiao-Qi Yu*

An ensemble-based fluorescent sensor HS-1 + Cu^{2+} for detection of histidine is reported. Complex HS-1 + Cu^{2+} sensitively senses histidine at pH 7.4 in aqueous media. The quantitative determination of histidine in urine and fetal calf serum is also conducted.

As the key constituents of proteins, amino acids are biologically important species.[1] Among them, histidine (His) is reported to be very essential for human growth.[2] It can play important roles in biological systems such as controlling the transmission of metal elements in biological bases owing to the high reactivity of its imidazole group.[3] Also, it is found that the deficiency of histidine may result in the impaired nutritional state of patients with chronic kidney disease.[4] Moreover, an abnormal level of histidine-rich proteins could indicate a variety of diseases such as asthma[5] and advanced liver cirrhosis.[6] Accordingly, determination of histidine in biological samples is of great importance in biochemical analysis and a number of methods have been developed for this purpose.[7,8] In these studies, fluorimetric detection has recently attracted considerable attention for its high sensitivity and temporal and spatial resolution.[9] Most of the molecular fluorescent sensors for histidine were prepared as metal–indicator complexes, namely ensembles.[7a–f,8]

In a chemosensing ensemble, the receptor is noncovalently attached to the indicator and the binding between the receptor and the analyte results in either formation of a new complex or displacement of the indicator, both of which will cause fluorescence changes.[10] There are several advantages to this method of signaling: (1) the water solubility of an ensemble can be largely improved, especially containing a metal center; (2) different species sensing can be achieved just by simply changing the receptor (metal ion) or the binding site of the indicator at will; (3) an ensemble usually does not require complicated synthesis procedures, which of course provides more practical and convenient applications. To this end, numerous chemosensing ensembles have been applied in the detection of biological species such as cations,[11] anions[12] and thiols.[13] Although several ensembles were reported for His detection, most of them could not get rid of the interference of Cys and few of them could be applied in urine and serum. Herein, we would like to report a coumarin–DPA–Cu(II) ensemble-based chemosensor **HS-1** + Cu^{2+} to selectively detect histidine in aqueous solution and biological fluids (see Scheme 1).



Scheme 1

7-Diethylamino coumarin is chosen as the fluorophore due to its good photostability, large Stokes shift and high quantum yield.[14] DPA (di(2-picolyl)amine) acts as the binding unit for Cu^{2+} for its strong binding ability with divalent heavy metal ions,[15] which may improve the selectivity of the ensemble. **HS-1** was prepared *via* a simple process and characterized by ^{1}H, ^{13}C NMR and HRMS (see ESI†).

First, the fluorescence response of **HS-1** (5 μM) toward Cu^{2+} was tested in HEPES (20 mM, pH = 7.4, containing 0.5% DMSO as cosolvent). Upon the addition of Cu^{2+}, the fluorescence of **HS-1** at 500 nm gradually decreased and was quenched completely when 1 equiv. Cu^{2+} was added, indicating the formation of a 1 : 1 bonding mode between **HS-1** and Cu^{2+} (Fig. S1†). Concomitantly, the quantum yield of **HS-1** decreased from 41% to 0.85% (fluorescein in 1 N NaOH as

Key Laboratory of Green Chemistry and Technology, Ministry of Education, College of Chemistry, Sichuan University, Chengdu, 610064, China.
E-mail: kli@scu.edu.cn, xqyu@scu.edu.cn; Fax: +(86)-28-85415886;
Tel: +(86)-28-85415886

†Electronic supplementary information (ESI) available: Experimental procedures, additional plots of UV, fluorescence, NMR and mass analysis data. See DOI: 10.1039/c2ob26955d

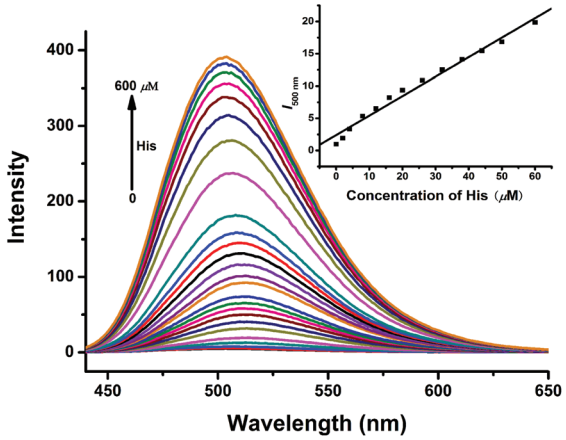


Fig. 1 The titration profile of **HS-1** + Cu^{2+} (5 μM) toward His in HEPES (20 mM, pH = 7.4, containing 0.5% DMSO as cosolvent). Inset: The linear relationship between **HS-1** + Cu^{2+} (5 μM) and His in a low concentration range from 2–60 μM (λ_{ex} = 410 nm, slits: 2 nm/2 nm).

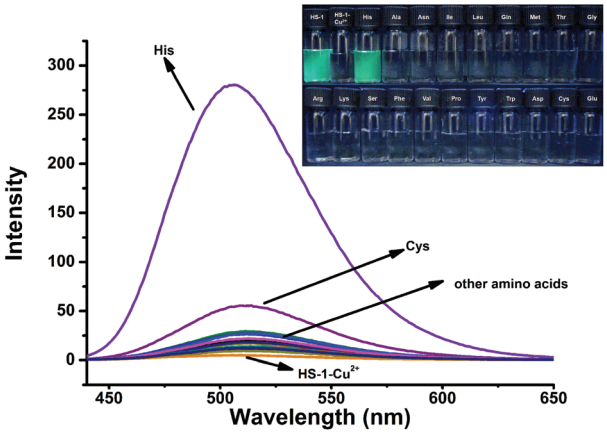


Fig. 2 The fluorescence spectra of **HS-1** + Cu^{2+} (5 μM) toward 100 equiv. amino acids (50 equiv. for His) in HEPES (20 mM, pH = 7.4, containing 0.5% DMSO as cosolvent) (λ_{ex} = 410 nm, slits: 2 nm/2 nm). Inset: Fluorescence color changes of **HS-1** + Cu^{2+} upon addition of various amino acids under a UV lamp (Ex = 365 nm). From top to bottom and left to right: **HS-1**, **HS-1** + Cu^{2+}, His, Ala, Asn, Ile, Leu, Gln, Met, Thr, Gly, Arg, Lys, Ser, Phe, Val, Pro, Tyr, Trp, Asp, Cys, Glu.

reference, Φ = 0.85).[16] A Job plot for the complexation also showed a 1 : 1 stoichiometry (Fig. S2†). Based on the 1 : 1 binding mode, the binding constant derived from the fluorescence titration data was calculated to be 1.3 × 10^6 M^{-1} (Fig. S3†).

The above result that the fluorescence of **HS-1** can be completely quenched by Cu^{2+}, and the fact that the Cu^{2+}-complex can selectively discriminate His from other amino acids,[7a–c,8b] allowed us to speculate that the **HS-1** + Cu^{2+} ensemble is promising as a turn-on fluorescent sensor for histidine. To confirm our assumption, **HS-1** was preincubated with Cu^{2+}, and the titration of His to the resulting ensemble was conducted. As Fig. 1 and Fig. S4† display, the fluorescence of **HS-1** + Cu^{2+} increased significantly upon the addition of histidine and up to an ~80-fold enhancement was observed when 120 equiv. His was added finally. Furthermore, the fluorescence intensity of the ensemble at 500 nm increased linearly with the treatment of His in a low concentration range from 2–60 μM (R^2 = 0.983), and the detection limit (S/N = 3) of His from the titration profile was detected to be 3.1 μM,[12c] which is sensitive enough for its practical applications in determination of His in biological fluids.

To examine the selectivity, changes in the fluorescence intensity of **HS-1** + Cu^{2+} promoted by addition of excess amounts of various amino acids were measured in HEPES (20 mM, pH = 7.4, containing 0.5% DMSO as cosolvent). As shown in Fig. 2, the addition of 100 equiv. (0.5 mM) of other naturally occurring amino acids including tryptophan (Trp), asparagine (Asn), lysine (Lys), leucine (Leu), isoleucine (Ile), methionine (Met), threonine (Thr), tyrosine (Tyr), valine (Val), aspartic acid (Asp), alanine (Ala), serine (Ser), glutamine (Gln), glutamic acid (Glu), glycine (Gly), phenylalanine (Phe), cysteine (Cys), proline (Pro) and arginine (Arg) did not cause any obvious fluorescence enhancement except that the addition of 100 equiv. Cys could lead to an approximate 10-fold reinforcement. A fluorescence titration experiment of **HS-1** toward Cys was also conducted, and only an ~11-fold enhancement was observed even though more Cys was added (Fig. S5†). By sharp contrast, the fluorescence of **HS-1** + Cu^{2+} at 500 nm was dramatically amplified with up to an ~60-fold enhancement while 50 equiv. of His was added into the solution, which is profound enough to distinguish His from Cys. Usually, a Cu^{2+}/Ni^{2+}-ensemble sensing His can distinctly respond to Cys.[7a–c,8] The spectroscopic studies suggest that the ensemble exhibits a high selectivity over other amino acids including Cys, indicating the strong binding capacity of the imidazole group of His to the copper ion. Since the formation constants of His with Cu^{2+} were reported to be 10.16 (log β_1) and 18.11 (log β_2),[17] which were far larger than those of **HS-1** and Cu^{2+}, a displacement approach might be reasonable to explain the optical changes above.

To further investigate the interaction between His and **HS-1** + Cu^{2+}, the ESI spectra were obtained (Fig. S6†). Before the treatment of His, an intense peak at m/z 533.1511 was found to correspond to the [**HS-1** + Cu^{2+} − H].[12c] Upon addition of 4 equiv. and 40 equiv. His respectively, the peak at m/z ~ 533.15 gradually weakened and the peak at m/z ~ 472.23 related to **HS-1** was observed, indicating that the binding between His and Cu^{2+} led to the release of **HS-1**. Thus, the selective His detection of the **HS-1** + Cu^{2+} ensemble is based on a displacement approach. In addition, the appearance of a peak at 373.0664 evidenced the formation of the complex [2His + Cu^{2+}].

The competition experiments were conducted to investigate the further utility of **HS-1** + Cu^{2+} (5 μM) in the presence of other amino acids in HEPES (20 mM, pH = 7.4, containing 0.5% DMSO as cosolvent). As shown in Fig. 3 and Fig. S7,† the addition of 100 equiv. other amino acids to the solution of **HS-1** + Cu^{2+} containing 50 equiv. His did not induce any considerable changes. Hence, **HS-1** + Cu^{2+} seems to be useful for

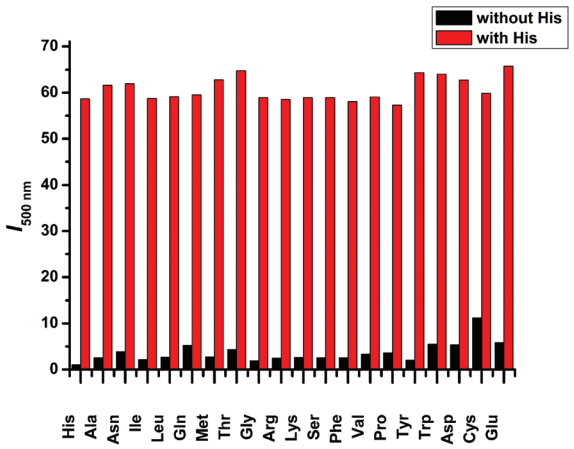


Fig. 3 Fluorescence response of **HS-1** + Cu^{2+} (5 μM) toward amino acids (100 equiv.) in the absence (black bars) or presence (red bars) of His (50 equiv.) in HEPES (20 mM, pH = 7.4, containing 0.5% DMSO as cosolvent) (λ_{ex} = 410 nm, slits: 2 nm/2 nm).

selectively sensing His even in the presence of other relevant species.

As an essential bioactive amino acid component of many proteins, the detection of histidine in biological fluids such as urine and serum is in urgent demand. With previous results in hand, we would like to explore the quantitative determination of histidine in urine and serum samples using **HS-1** + Cu^{2+}. We first studied the fluorescence response of **HS-1** + Cu^{2+} to His in the presence of some biological species, including K^+, Na^+, Mg^{2+}, Ca^{2+}, Fe^{3+}, glucose, ascorbic acid and urea (Fig. S8†). No obvious fluorescence changes of **HS-1** + Cu^{2+} in the presence of His were observed when these biological species were added, indicating the potential utility of **HS-1** + Cu^{2+} for the determination of His in biological fluids. Then, urine was diluted before use and His was spiked into the urine as an internal standard without further treatment. To the spiked urine samples was added **HS-1** + Cu^{2+} (5 μM) and then the fluorescence of the mixtures was measured. The average His concentrations in two urine samples were determined to be 510 and 990 μM (the urine was totally diluted 150 times before measurement), respectively, which is in good agreement with its normal levels in human urine (normal level: 130–2100 μM in urine).[18]

Next, the His determination in fetal calf serum was conducted. Fetal calf serum was deproteinized by methanol before use and various amounts of the supernatant were added to the HEPES solution containing **HS-1** + Cu^{2+} (5 μM). An excellent linear relationship between the amount of deproteinized fetal calf serum and the fluorescence intensity of **HS-1** + Cu^{2+} at 500 nm was obtained (Fig. S9†). Inspired by this result, we subsequently measured the concentration of His in fetal calf serum by spiking His into serum as an internal standard. A good linear correlation was observed as well and the His concentration in fetal calf serum was measured to be 495 μM (Fig. 4).

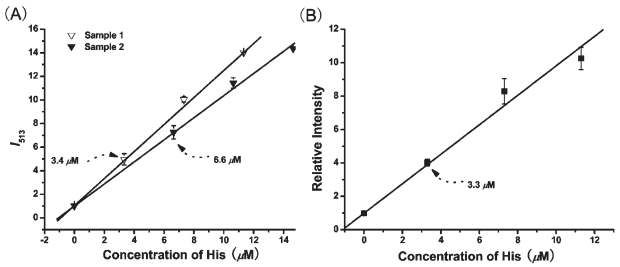


Fig. 4 Determination of His concentration in spiked (A) human urine and (B) fetal calf serum using His as an internal standard. His concentrations measured were 0, X, X + 4, X + 8 μM. The data represent the average of three independent experiments.

In conclusion, an ensemble **HS-1** + Cu^{2+} is reported to selectively detect His in aqueous solution. No considerable interference is observed in the presence of the other amino acids and common biological species. Notably, as a common interferent for chemosensing ensembles of His, Cys causes negligible impact on the emission intensity of the **HS-1** + Cu^{2+} ensemble, which enables more practical applications of **HS-1** + Cu^{2+}. Ultimately, this ensemble was successfully applied for the parallel measurement of His concentration in human urine and fetal calf serum.

This work was financially supported by the National Program on Key Basic Research Project of China (973 Program, 2012CB720603, 2013CB328900) and the National Science Foundation of China (Nos. 20732004 and 21001077). We also thank the Analytical & Testing Center of Sichuan University for NMR analysis.

Notes and references

1 S. Shahrokhian, *Anal. Chem.*, 2001, **73**, 5972–5978.
2 (*a*) J. D. Kopple and M. E. Swendseid, *J. Clin. Invest.*, 1975, **55**, 881–891; (*b*) S. E. Snyderman, A. Boyer, E. Roitman, L. E. Holt Jr. and P. H. Prose, *Pediatrics*, 1963, **31**, 786–801.
3 G. N. Chen, X. P. Wu, J. P. Duan and H. Q. Chen, *Talanta*, 1999, **49**, 319–330.
4 M. Watanabe, M. E. Suliman, A. R. Qureshi, E. Garcia-Lopez, P. Bárány, O. Heimbürger, P. Stenvinkel and B. Lindholm, *Am. J. Clin. Nutr.*, 2008, **87**, 1860–1866.
5 A. L. Jones, M. D. Hulett and C. R. Parish, *Immunol. Cell Biol.*, 2005, **83**, 106–118.
6 (*a*) H. Saito, L. T. Goodnough, J. M. Boyle and N. Heimburger, *Am. J. Med.*, 1982, **73**, 179–182; (*b*) F. W. Leebeek, C. Kluft, E. A. Knot and M. P. De Matt, *J. Lab. Clin. Med.*, 1989, **113**, 493–497.
7 (*a*) M. A. Hortalá, L. Fabbrizzi, N. Marcotte, F. Stomeo and A. Taglietti, *J. Am. Chem. Soc.*, 2003, **125**, 20–21; (*b*) Y.-Y. Fu, H.-X. Li and W.-P. Hu, *Sens. Actuators, B*, 2008, **131**, 167–173; (*c*) Z. Huang, J. Du, J. Zhang, X. Q. Yu and L. Pu, *Chem. Commun.*, 2012, **48**, 3412–3414; (*d*) Y. Zhang, R. H. Yang, F. Liu and K. A. Li, *Anal. Chem.*, 2004, **76**, 7336–7345; (*e*) L. Fabbrizzi, G. Francese, M. Licchelli,

A. Perotti and A. Taglietti, *Chem. Commun.*, 1997, 581–582; (*f*) D.-L. Ma, W.-L. Wong, W.-H. Chung, F.-Y. Chan, P.-K. So, T.-S. Lai, Z.-Y. Zhou, Y.-C. Leung and K.-Y. Wong, *Angew. Chem., Int. Ed.*, 2008, **47**, 3735–3739; (*g*) X. Li, H. Ma, L. Nie, M. Sun and S. Xiong, *Anal. Chim. Acta*, 2004, **515**, 255–260.

8 (*a*) P. Wu and X.-P. Yan, *Biosens. Bioelectron.*, 2010, **26**, 485–490; (*b*) F. Pu, Z.-Z. Huang, J.-S. Ren and X.-G. Qu, *Anal. Chem.*, 2010, **82**, 8211–8216; (*c*) S.-K. Sun, K.-X. Tu and X.-P. Yan, *Analyst*, 2012, **137**, 2124–2128.

9 (*a*) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515–1566; (*b*) L. Prodi, F. Bolletta, M. Montalti and N. Zaccheroni, *Coord. Chem. Rev.*, 2000, **205**, 59–83; (*c*) C. Bargossi, M. C. Fiorini, M. Montalti, L. Prodi and N. Zaccheroni, *Coord. Chem. Rev.*, 2000, **208**, 17–32.

10 S. L. Wiskur, H. Ait-Haddou, J. J. Lavigne and E. V. Anslyn, *Acc. Chem. Res.*, 2001, **34**, 963–972.

11 (*a*) L. Xue, Q. Liu and H. Jiang, *Org. Lett.*, 2009, **11**, 3454–3457; (*b*) Z. Huang, J. Du, J. Zhang, X. Q. Yu and L. Pu, *Anal. Methods*, 2012, **48**, 3412–3414.

12 (*a*) Y. L. Liu, X. Lv, Y. Zhao, J. Liu, Y. Q. Sun, P. Wang and W. Guo, *J. Mater. Chem.*, 2012, **22**, 1747–1750; (*b*) B. L. Ma, F. Zeng, F. Y. Zheng and S. Z. Wu, *Chem.–Eur. J.*, 2011, **17**, 14844–14850; (*c*) X. Cao, W. Lin and L. He, *Org. Lett.*, 2011, **13**, 4716–4719.

13 (*a*) H. S. Jung, J. H. Han, Y. Habata, C. Kang and J. S. Kim, *Chem. Commun.*, 2011, **47**, 5142–5144; (*b*) X.-F. Yang, P. Liu, L. Wang and M. Zhao, *J. Fluoresc.*, 2008, **18**, 453–459; (*c*) Y.-K. Yang, S. Shim and J. Tae, *Chem. Commun.*, 2010, **46**, 7766–7768.

14 (*a*) A. Helal, M. H. Or Rashid, C.-H. Choi and H.-S. Kim, *Tetrahedron*, 2011, **67**, 2794–2802; (*b*) L. Yuan, W. Lin, J. Song and Y. Yang, *Chem. Commun.*, 2011, **47**, 12691–12693.

15 (*a*) G. K. Walkup, S. C. Burdette, S. J. Lippard and R. Y. Tsien, *J. Am. Chem. Soc.*, 2000, **122**, 5644–5645; (*b*) S. A. de Silva, A. Zavaleta, D. E. Baron, O. Allam, E. V. Isidor, N. Kashimura and J. M. Percarpio, *Tetrahedron Lett.*, 1997, **38**, 2237–2240; (*c*) J. Y. Kwon, Y. J. Yoon, Y. J. Lee, K. M. Kim, M. S. Seo, W. Nam and J. Yoon, *J. Am. Chem. Soc.*, 2005, **127**, 10107–10111; (*d*) C. Lu, Z. Xu, J. Cui, R. Zhang and X. Qian, *J. Org. Chem.*, 2007, **72**, 3554–3557; (*e*) Z. Liu, C. Zhan, W. He, Z. Yang, X. Gao and Z. Guo, *Chem. Commun.*, 2010, **46**, 6138–6140; (*f*) H. H. Wang, L. Xue, Z. J. Fang, G. P. Li and H. Jiang, *New J. Chem.*, 2010, **34**, 1239–1242.

16 T. Miura, Y. Urano, K. Tanaka, T. Nagano, K. Ohkubo and S. Fukuzumi, *J. Am. Chem. Soc.*, 2003, **125**, 8666–8671.

17 L. D. Pettit, *Pure Appl. Chem.*, 1984, **56**, 247–292.

18 P. M. Kovach and M. E. Meyerhoff, *Anal. Chem.*, 1982, **54**, 217–220.

