J|A|C|S
COMMUNICATIONS

Published on Web 12/05/2002

Designing the Selectivity of the Fluorescent Detection of Amino Acids: A Chemosensing Ensemble for Histidine

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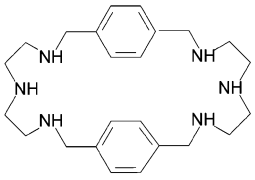
Fluorescent sensing of amino acids is a very important task in biochemistry and molecular biology, with a special regard to determinations which require both temporal and spatial resolution.[1] However, only a few fluorescent chemosensors for amino acids have been described thus far. They include a heteroditopic system, containing an NO_5 crown fragment and a guanidium subunit, suitable for linear recognition of analytes of formula $NH_3^+-(CH_2)_n-COO^-$, which displays selective behavior for $n = 3$,[2] and signals the occurrence of the recognition through a useful off/on switching of fluorescence, and a dizinc(II) cryptate, which recognizes histidine through the formation of an imidazolate bridge between the two Zn^{II} centers,[3] an event indicated by a less valuable on/off fluorescent response. In both cases, the fluorophore (a 1,9-anthracenyl fragment) acts as a spacer separating the two recognizing functionalities.

We describe now a novel type of off/on fluorescent chemosensor for amino acids, suitable for selective detection of histidine, which does not require the establishing of any covalent linking between the fluorophore and the receptor but utilizes the fluorophore and the receptor as such. According to this approach, called the "chemosensing ensemble",[4] the fluorescent indicator is bound through noncovalent interactions to the receptor, which quenches its emission; then, the added analyte displaces the indicator, which when released to the solution displays its full fluorescence. Thus, analyte recognition is signaled by the sharp appearance of the fluorescence of the indicator. Moreover, we intend to demonstrate how decisive is the choice of the indicator to achieve selectivity in recognition and sensing.



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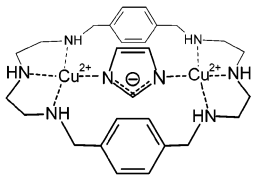
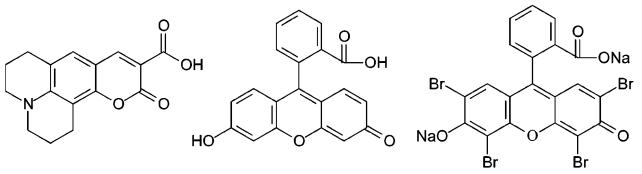


2

Selective binding of histidine requires a receptor capable of interacting with the imidazole residue rather than with the carboxylate group, which is common to all the amino acids and which cannot, therefore, induce any selectivity. The extremely weak acid imidazole ($pK_A = 14.5$), in the presence of two Cu^{II} ions, prepositioned at the right distance within an appropriate ligand, deprotonates and bridges the two metal centers.[5] As an example, an imidazolate moiety bridges the two Cu^{II} centers of the dimetallic complex of the bisdien macrocycle **1**. In particular, each Cu^{II} center in the ternary complex **2** becomes four-coordinate, according to a square geometry.[6] Thus, we decided to use the $[Cu^{II}_2(\mathbf{1})]^{4+}$ complex as a receptor for the recognition of histidine.

Then, we chose a set of fluorescent indicators including: coumarine 343 (**3**), fluorescein (**4**), and eosine Y (**5**), which share high quantum yield, excitation, and emission wavelengths in the visible region, and the presence of a carboxylate group in the molecular structure. Titration of each indicator with the $[Cu^{II}_2(\mathbf{1})]^{4+}$ receptor complex at pH = 7 resulted in a complete quenching of the emission, while nonlinear least-squares fitting of the titration profiles (fluorescence intensity, I_F, vs equiv of $[Cu^{II}_2(\mathbf{1})]^{4+}$)[7] indicated formation of 1:1 adducts, whose association constants are, in log units: coumarine 4.5, fluorescein 5.9, eosine Y 7.2.



3 **4** **5**

Formation of a 1:2 adduct had to be ruled out, due to the very poor fitting of titration data. It is suggested that, in the receptor/indicator 1:1 adduct, the two oxygen atoms of the carboxylate group of each dye bridge the two Cu^{II} ions, which quench the proximate fluorophore through either an electron- or energy-transfer process. The capability of the $-COO-$ group to encompass the two metal centers of the macrocyclic complexes has been confirmed through molecular modeling. Thus, the nonfluorescent chemosensing ensemble was generated by dissolving the $[Cu^{II}_2(\mathbf{1})]^{4+}$ receptor and the indicator in a solution buffered at pH = 7 (HEPES 0.05 M). The concentration of the indicator was 10^{-6} M, and that of the receptor was high enough to ensure quenching of the fluorescent probe. At this stage, each receptor/indicator pair was titrated with some representative L-amino acids, which included: His, Ala, Phe, Leu, Pro, plus Gly. In some cases, the amino acid was able to displace the indicator from the receptor, an event signaled by full fluorescence revival. In other cases, however, the amino acid was not able to dislodge the indicator, with no restoration of fluorescence.

Some typical titration profiles are reported in Figure 1. The $[Cu^{II}_2(\mathbf{1})]^{4+}$/coumarine ensemble (Figure 1a) does not discriminate His and Gly. In fact, both His and Gly displace the indicator and restore its full emission. The situation is more favorable with the fluorescein-containing ensemble (Figure 1b), which satisfactorily discriminates His (full recovery of fluorescence) from Gly, whose I_F profile is distinctly less steep. However, the highest sensing selectivity is observed with the $[Cu^{II}_2(\mathbf{1})]^{4+}$/eosine Y ensemble (Figure 1c), which sufficiently discriminates His from Gly and other investigated amino acids. Nonlinear least-squares treatment of competitive titration profiles shown in Figure 1 allowed the determination of the equilibrium constants for the interaction of $[Cu^{II}_2(\mathbf{1})]^{4+}$ with each amino acid,[9] at pH = 7. Corresponding values are reported in the bar diagram in Figure 2; the highest binding constant is shown by His, which offers to the two Cu^{II} centers the strongly donating imidazolate bridging group. Binding by the

10.1021/ja027110l CCC: $25.00

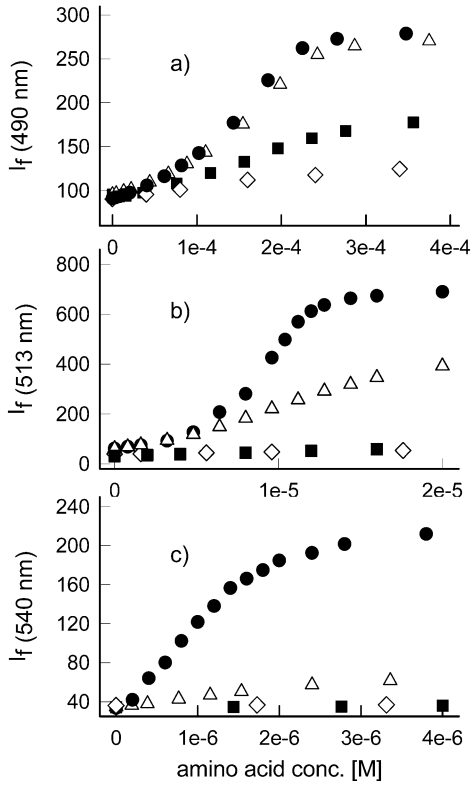


Figure 1. Titration of the chemosensing ensemble $[Cu^{II}_2(\mathbf{1})]^{4+}$/a, b, c with amino acids: (a) coumarine 343: 10^{-6} m, $[Cu^{II}_2(\mathbf{1})]^{4+} = 2.5 \times 10^{-4}$ m; (b) fluorescein: 10^{-6} m, $[Cu^{II}_2(\mathbf{1})]^{4+} = 1.6 \times 10^{-5}$ m; (c) eosine Y: 10^{-6} m, $[Cu^{II}_2(\mathbf{1})]^{4+} = 2.4 \times 10^{-6}$ m. Circles, histidine; triangles, glycine; squares, alanine; diamonds, phenylalanine.

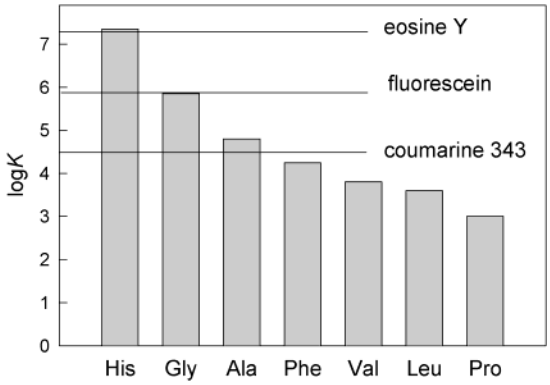


Figure 2. Equilibrium constants for the interaction of the receptor $[Cu^{II}_2(\mathbf{1})]^{4+}$ with selected natural amino acids (bars) and fluorescent indicators (horizontal solid lines). The position of the horizontal line with respect to the bars determines the selectivity of the chemosensing ensemble $[Cu^{II}_2(\mathbf{1})]^{4+}$/indicator toward the chosen amino acid.

imidazolate residue of His has been confirmed by the fact that plain imidazole shows the same log *K* value as His. Then, other amino acids come, which all interact with the metals of the receptor with the less donating bridging carboxylate group of each NH_3^+−CH(**R**)-COO− zwitterion. The observed trend of stability (Gly > Ala > Phe > Val > Leu > Pro) seems to be related to the increasing steric repulsive effects exerted by the **R** substituent.

Thus, it can be suggested that all the other natural amino acids not investigated here, which present more hindering substituents, should have a log *K* lower than Gly and could be discriminated when using **4** and **5**.

In Figure 2, the equilibrium constants for the $[Cu^{II}_2(\mathbf{1})]^{4+}$−indicator interaction are also reported as horizontal solid lines. Noticeably, the relative position of each line with respect to the bars accounts for the more or less selective behavior illustrated in Figure 1. In particular, discrimination would require that the binding constant of the indicator (horizontal line) is distinctly lower than that of the envisaged amino acid and considerably higher than that of the interfering amino acid. This is not the case of coumarine, which cannot discriminate His and Gly (both exhibiting much higher association constants) and show competitive behavior with Ala and Phe. On the other hand, the binding constant of fluorescein is significantly lower than that of His, but very close to that of Gly, which shows, therefore, competitive behavior. The more favorable situation, but perhaps not the ideal one, is observed with eosine Y, whose log *K* is adequately higher than that of any possible interferent, and is lower, even though slightly, than that of the analyte of interest.

A Zn^{II}-containing colorimetric chemosensing ensemble had been previously used for the detection of aspartate in a 1:1 water−methanol mixture;[10] amino acid recognition resulted from both metal−ligand interactions and hydrogen bonding involving a carboxylate and a guanidinium group appended to the terpyridine ligand. In the present study, the use of a receptor containing two genuine transition metal ions, Cu^{II}, prepositioned at the correct distance within a hexamine macrocycle, provides (i) selective recognition of the ambidentate imidazole residue of histidine over the carboxylate group of natural amino acids and (ii) metal-induced quenching of the bound fluorescent probe. Moreover, it has been demonstrated that careful choice of a fluorescent indicator with tuned affinity toward the receptor (lower than that of the envisaged analyte, higher than that of the interferent) can provide discrimination in sensing of a desired substrate.

Acknowledgment. This work was supported by CNR (Progetto Finalizzato "Biotecnologie"). N.M. is grateful to UE for a "Marie Curie" fellowship (HPMF-CT-2000-01030).

Supporting Information Available: Experimental details for the synthesis of receptor and fluorescent titrations (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Czarnik, A. W. *Chem. Biol.* **1995**, *2*, 423.

(2) de Silva, A. P.; Gunaratne, H. Q. N.; McVeigh, C.; Maguire, G. E. M.; Maxwell, P. R. S.; O'Hanlon, E. *Chem. Commun.* **1996**, 2191.

(3) Fabbrizzi, L.; Francese, G.; Licchelli, M.; Perotti, A.; Taglietti, A. *Chem. Commun.* **1997**, 581.

(4) Wiskur, S. L.; Aït-Haddou, H.; Lavigne, J. J.; Anslyn, E. V. *Acc. Chem. Res.* **2001**, *34*, 963.

(5) Coughlin, P. K.; Martin, A. E.; Dewan, J. C.; Watanabe, E.-I.; Bulkowski, J. E.; Lehn, J.-M.; Lippard, S. J. *Inorg. Chem.*, **1984**, *23*, 1004.

(6) Zhu, H.-L.; Hang, Q.-W.; Zhao, J.; Duan, C.-Y.; Tang, W.-X. *Transition Met. Chem.* **1999**, *24*, 131.

(7) Gans, P.; Sabatini, A.; Vacca, A. *Talanta* **1996**, *43*, 1739.

(8) Connors, K. A. *Binding Constants, the Measurements of Molecular Complex Stability*; Wiley: New York, 1987.

(9) In competition assays, titrations were carried out until a constant value of I_{max} was reached. For amino acids with a low affinity towards the receptor, saturation of fluorescence intensity could not be reached even at high substrate concentration; thus, I_{max} was evaluated from data fitting. However, the I_{max} value calculated from curve fitting was found coincident (within the experimental error) with the I_{max} value determined for the same indicator in an experiment in which displacement was complete (e.g., with His).

(10) Aït-Haddou, H.; Wiskur, S. L.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123*, 11296.

JA027110L