A cyclometalated palladium–azo complex as a differential chromogenic probe for amino acids in aqueous solution†

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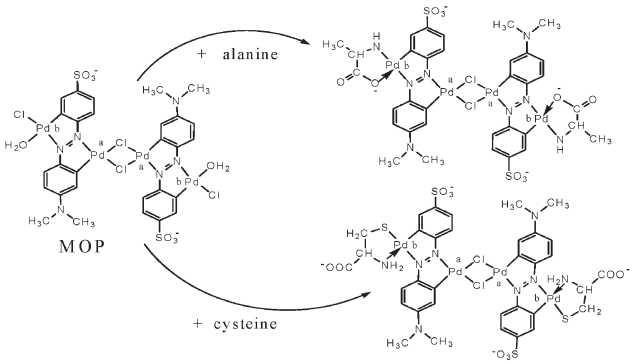
Received (in Cambridge, UK) 16th September 2004, Accepted 22nd October 2004
First published as an Advance Article on the web 2nd December 2004
DOI: 10.1039/b414131h

Solutions of a cyclometalated palladium–azo complex exhibited differential UV-Vis absorption spectra in the presence of α-amino acids with different side chain groups.

Optical sensing of amino acids is an important task in biochemistry, with a special regard to determination which requires both temporal and spatial resolution.[1] The ninhydrin reaction protocols have been widely explored for colorimetric detection of α-amino acids. However, like those based on other broad-band reactants,[2] they lack further differentiation between different α-amino acids. As known, amino acids as constituents of proteins are small molecules with various functional side chain groups, which result in different roles of amino acids in physiological processes. For examples, deficiency of cysteine is involved in slow growth, hair depigmentation, edema, lethargy, liver damage, muscle and fat loss, skin lesions, and weakness.[3] The pathogenesis of Alzheimer's disease is strongly associated with the reactions of the methionine residue in the C-terminal domain of α-amyloid peptide in the brain.[4] Therefore, the interest in developing optical chemosensors specifically recognizing a target amino acid has grown continuously.[5] Up to date, no example has been reported on differential colorimetric molecular sensing systems where different species of amino acids were differentiated by optical responses. In our opinion, differential colorimetric chemosensors for amino acids are useful not only for detection of amino acids, but also for potential applications in microscopy tracking of protein structures in metabolic processes. Herein, we describe the design of a differential chromogenic chemosensor for amino acids in aqueous solution based on a palladium–azo complex.

We chose a palladium–azo complex as the colorimetric reporting molecule because of the known strong preference in complexation of palladium or platinum with amino acids. As previously reported, palladium forms stable complexes with the N-, O- and S-donor atoms commonly present in amino acids.[6] Even the simplest α-amino acid, glycine, has the potential to bind a metal ion and form a five-membered N,O-chelating ring. In addition, amino acids often have a side chain with a metal binding group, such as imidazole, carboxyl, phenol, indole, amino and thiol. This makes the metal–amino acid chemistry in proteins more complicated and also makes the differential recognition of amino acids based on metal complexes become possible.[7] In our research, a palladium–azo complex, denoted MOP (Scheme 1), was synthesized by the reaction of methyl orange with potassium tetrachloropalladate(II) in a 1:2 molar ratio in a dioxane–water (1:1) mixture.[8] In MOP, the azo dye, methyl orange, served as a cyclometalated ligand. Such cyclometalated complexes of palladium(II) or platinum(II) have been reported to readily exhibit differential optical responses towards different incoming chelating species.[9] Thus, MOP was expected to act as a differential probe for amino acids.



Scheme 1 Reaction of MOP with different α-amino acids.

L-Amino acids with different side chain groups were tested in buffered aqueous solutions of MOP. Fig. 1 shows the color changes of the MOP solution in the presence of histidine (His), cysteine (Cys), alanine (Ala), lysine (Lys) and tyrosine (Tyr), respectively. This phenomenon was not affected by the coexistence of ethanol, acetic acid or glucose. When the analog of MOP, synthesized by the reaction of methyl orange with K_2PdCl_4 in 1:1 ratio,[8a] was used as an alternative probe in this experiment, no color change occurred. This indicated that the Pd(b) atoms in MOP, but not Pd(a) atoms, acted as the reacting sites for amino acids in the chromogenic reactions. For further investigation on the selectivity of the reaction of MOP with α-amino acids, other organic acids such as hydroxyacetic acid, 4-aminobenzoic acid and

Fig. 1 Color changes of aqueous solutions of MOP (blank, 2.50×10^{-5} M; buffered at pH 7.4 with 0.02 M phosphate buffer) with various L-amino acids (5.00×10^{-4} M).

† Electronic supplementary information (ESI) available: Experimental section. See http://www.rsc.org/suppdata/cc/b4/b414131h/

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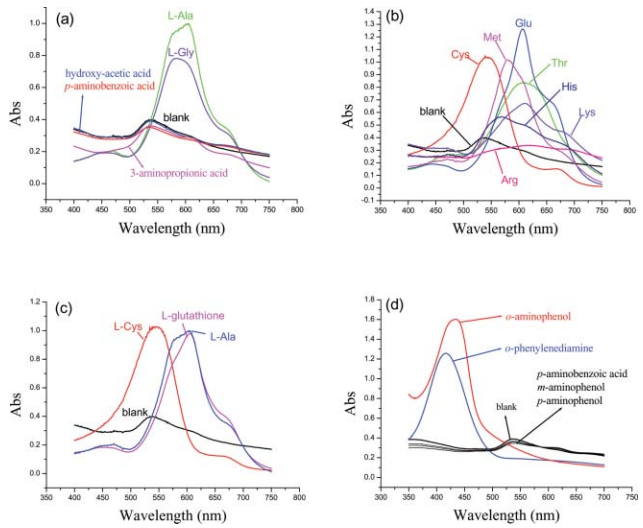

Fig. 2 Absorption spectra of MOP solutions (2.50×10^{-5} M; buffered at pH 7.4 with 0.02 M phosphate buffer) in the presence of different analytes (4.00×10^{-4} M): (a) α-amino acids and other organic acids; (b) different L-α-amino acids; (c) cysteine and glutathione; (d) different derivatives of aniline. Absorption spectra were recorded after 2 h incubation in a 50 °C water-bath.

3-aminopropionic acid were also tested. As shown in Fig. 2(a), only the α-amino acids, glycine (Gly) and alanine, induced the MOP sensing solutions to give a remarkable color shift from light purple to deep blue. Therefore, MOP is determined to be a suitable colorimetric probe for α-amino acids.

The donor atom preferences in complexes of palladium with amino acids and related molecules has been reviewed previously.[6] It was concluded that, when an amino acid was in the presence of a palladium complex containing easily displaced ligands, the thermodynamically preferred product would be that in which a five-membered N,O-chelating ring was formed. This results in the selective response of MOP to α-amino acids. Other N-, O- or S-donors commonly present in amino acids are also involved in palladium–amino acid chemistry, with a thermodynamic preference for S- and N- donors over O-donors. Therefore, the differential spectral response of MOP to amino acids can be explained and the sensing mechanism of amino acids is illustrated in Scheme 1. This mechanism was further confirmed by following experiments. As shown in Fig. 2(c), addition of L-glutathione into the MOP sensing solution induced an absorption spectrum similar to that of alanine but not cysteine. Therefore, the five-membered N,S-chelating ring rather than the –SH donor group only, is thought to be responsible for the large color shift to pink induced by cysteine. This Pd–N chelating mode was also successfully applied to the colorimetric recognition of *o*-aminophenol from *p*- and *m*-aminophenol (Fig. 2(d)). Among the tested aniline derivatives, those containing a α-donor group favoring the formation of a five-membered chelating ring, such as *o*-aminophenol and o-phenylenediamine selectively reacted with MOP in a chromogenic manner.

In protein chemistry, amino acids are usually divided into several groups according to a structure–function relationship. In our study, L-amino acids commonly found in proteins are divided into six groups based on their side-chain compositions: (a) carboxyl-containing groups; (b) basic groups; (c) S-donor-containing groups; (d) hydroxy-containing groups; (e) heterocyclic rings; (f) alkyl groups. Their absorption behaviors in MOP solutions were compared (ESI†) and it was found that MOP exhibited a good spectral differentiation towards amino acids with different functional side chain groups (Fig. 2(b)). Appropriate selection of the testing pH in practice might improve the spectral resolution between different amino acids (ESI†).

For further evaluation of the application of MOP to determinations of amino acids, the spectral evolution of MOP sensing solutions upon titration with different L-amino acids were recorded (Fig. 3). For most of the amino acids, such as Cys (Fig. 3(a)), Met (Fig. 3(b)) and Ala (Fig. 3(c)), the characteristic absorbances increased linearly with the concentrations of the amino acids titrated into the MOP sensing solutions over a concentration range 2.0×10^{-6}–10^{-5} M, without shifting the spectral shapes. However, in the case of Lys (Fig. 3(d)), λ_{max} shifted from 570 to 609 nm at relatively higher concentration. It was considered that the amino side group of Lys at high concentration was able to chelate the Pd(a) atoms through the breakage of the Cl–Pd–Cl bridges in MOP, in a reaction manner previously reported.[8a] The efficiency of the colorimetric response was also susceptible to the presence of coexisting inorganic ions. In our experiments, when the sensing solutions of MOP were prepared as aqueous mixtures of 0.10 M NaCl, 0.020 M Na_2SO_4, 0.030 M $NaHCO_3$, 2.0×10^{-3} M $NaNO_3$ and buffer phosphates, the reduction of the sensing responses were usually below 10% even at a concentration level of the tested amino acid down to 1.0×10^{-6} M. However, it was noticeable that the responding equilibrium was prolonged by this artificial disturbance to a degree dependent on the species and concentration of the tested amino acid (ESI†).

In summary, a cyclometalated palladium–azo complex, MOP, is proposed as a novel chromogenic chemosensor for α-amino acids

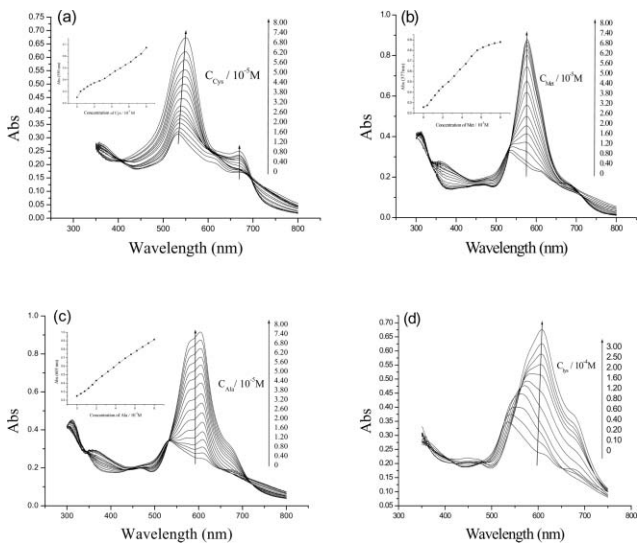

Fig. 3 Spectral evolution of MOP solutions (2.00×10^{-5} M; buffered at pH 7.4 with 0.02 M phosphate buffer) upon titration with different L-amino acids (a, Cys; b, Met; c, Ala; d, Lys). Inset: Absorbance at the maximum absorption wavelength plotted *vs.* concentration of the tested amino acid. Absorption spectra were recorded after 2 h incubation in a 50 °C water-bath.

in aqueous solution at physiological pH. Amino acids with different side chain groups were clearly distinguishable in their UV-Vis absorption spectra. This new concept of amino acid sensing facilitates the detection of a wide range of amino acids and opens the possibility of applications in microscopy tracking of protein structures. Further study along this line is currently under way.

This study was supported by the National Natural Science Foundation of China (No. 20275033).

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