A water-soluble pillar[5]arene-based chemosensor for highly selective and sensitive fluorescence detection of L-methionine†

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L-Methionine (L-Met) is an essential amino acid for the human body. The detection of L-Met in water solution is very important. Herein, we report an efficient approach for the fluorescent detection of L-Met with high selectivity and sensitivity in water using a cationic pillar[5]arene-based chemosensor (AWP5). When various L-amino acids are added to the AWP5 water solution, only L-Met can induce fluorescence in AWP5 at λ_{em} = 520 nm, showing dramatic enhancement; thus, other amino acid do not interfere in the L-Met recognition process. AWP5 also shows high sensitivity for L-Met in water, with the lowest detection limit of AWP5 for L-Met being 5.46 × 10^{-7} mol L^{-1}.

1. Introduction

L-Amino acids play a significant role in chemistry and biology. In particular, L-Met is one of the most essential amino acids for protein synthesis in the human body, which can promote the growth of hair and nails, enhance muscle activity etc. L-Methionine acts as a methyl group donor in many methyltransferase reactions including biosynthesis of phospholipids and bioelectrical activity. Loss of L-methionine in brain cells of aged animals is linked with the loss of dopaminergic β-adrenergic binding sites and changes in cell membrane composition. Therefore, the selective detection of L-Met is very important. To date, there are various useful strategies or methods such as artificial chemosensors, high-performance liquid chromatography, and capillary zone electrophoresis that have been applied for the detection of amino acids. However, these methods suffer from a number of problems. First, the instrument detection methods often employ expensive equipment. Second, owing to the low water solubility of organic groups, most synthesized chemosensors do not work well in water solution. However, most biological or environmental procedures are carried out in water systems. Therefore, it is still a big challenge and an important task to develop a water soluble chemosensor for the detection of L-Met in water.

Recently, pillararene chemistry has undergone rapid development and pillararene derivatives have been widely used in the field of molecular recognition, drug delivery, molecular devices and so on. Note that two kinds of important water-soluble pillararene derivatives have been developed to date: (1) cationic pillararene and (2) anionic pillararene. Due to their nice water solubility, inclusion and host–guest properties, these kinds of pillararene derivatives provide a great opportunity for the design of water-soluble chemosensors. In view of this and based on our research interest in molecular recognition, herein, we report a novel and efficient way for the detection of L-Met in water via a cationic pillar[5]arene-based chemosensor (AWP5). As shown in Scheme 1, the

Scheme 1. The chemical structure of AWP5 and possible selective detection mechanism of the sensor AWP5 for L-Met.
chemosensor AW5 possesses high water solubility and can enable fluorescence detection of L-Met in water with high selectivity and sensitivity through a host–guest mechanism.

2. Results and discussion

Cationic pillar[5]arene (AW5) was synthesized according to Scheme S1†. To investigate L-amino acid recognition abilities of the sensor AW5 in water, we carried out a series of host–guest recognition experiments. The recognition profiles of the sensor AW5 for various L-amino acids, including L-α-Ala, L-Arg, L-Cys, L-Gly, L-Lys, L-Pro, L-Met, L-Thr, L-Ser, L-His, L-Val, L-Tyr, L-Asn, L-Glu, L-Trp, L-Gln, L-Ile, L-Phe, L-Asp, and L-Leu, were primarily investigated using fluorescence spectroscopy in water.

As shown in Fig. 1, when 10 equiv. of L-Met was added to the water solution of sensor AW5, a blue fluorescence emission appeared. Moreover, in the corresponding fluorescence spectrum, the maximum emission peak appeared at 617 nm. However, other L-amino acids such as L-α-Ala, L-Arg, L-Cys, L-Gly, L-Lys, L-Pro, L-Thr, L-Ser, L-His, L-Val, L-Tyr, L-Asn, L-Glu, L-Trp, L-Gln, L-Ile, L-Phe, L-Asp, and L-Leu did not induce similar fluorescence changes (Fig. 2 and S10†). Therefore, in water solution, AW5 showed specific fluorescence selectivity for L-Met.

To further investigate the efficiency of the sensor AW5 for L-Met detection, we carried out fluorescence titration experiments. As shown in Fig. 3, in the fluorescence spectrum, with an increasing amount of L-Met, emission peak at 594 nm gradually increased. Furthermore, the detection limit of the fluorescent spectrum changes calculated on the basis of 3σ/m (ref. 49) is 5.46 × 10⁻⁷ M for L-Met (Fig. S11, ESI†). Moreover, we carried out similar experiments in the presence of 10 equiv. of L-his (Fig. S12, ESI†). The L-Met detection limit was 2.07 × 10⁻⁶ M in the presence of 10 equiv. of L-His, which indicated that AW5 showed high sensitivity for L-Met in the presence of other amino acids.

To further exploit the utility of AW5 as a selective sensor for L-Met, competitive experiments were carried out in the presence of 10.0 equiv. of L-Met and 2.0 equiv. of various L-amino acids in water. The fluorescence emission spectrum of the sensor AW5 with L-Met was not influenced by the subsequent addition of other competing L-amino acids, which indicated that AW5 had specific selectivity for L-Met (Fig. 4).

The recognition mechanism of AW5 towards L-Met was investigated by ¹H-NMR titrations, 2D NOESY-NMR, ESI-mass spectrometry, and job plots. As shown in Fig. 5, in ¹H-NMR, upon the addition of L-Met to an AW5 D₂O solution, proton peaks of AW5 and L-Met show obvious shifts. The triplet peaks of H₅, H₆, H₇, and H₈ of L-Met show obvious upfield shifts (Δδ = -0.15, -0.23, -1.54, -0.10, -0.12 ppm), which indicates that L-Met is located in the cavity of AW5. Simultaneously, the proton peaks of H¹ and H² of AW5 shift upfield (Δδ = -0.25 and -0.40 ppm), which indicate electrostatic interactions between the carboxylic acid groups of L-Met and ammonium salts of AW5. The electrostatic interactions directly lead to charge transfer from the carboxylate groups of L-Met to the AW5 ammonium salt groups. By this way, these proton chemical shift changes indicated that the complexation took place between AW5 and L-Met in solution.
Furthermore, a 2D NOESY NMR study of a mixture of AWP5 (10.0 mM) and L-Met (10.0 mM) in D2O was carried out to investigate the relative spatial positions of this host–guest complex. As shown in Fig. 6, clear correlation signals were observed between proton H1 of AWP5 and proton Hα of L-Met, indicating that L-Met penetrated through the cavity of AWP5.

To confirm the binding stoichiometry between the sensor AWP5 and L-Met in H2O, a job plot was constructed. The results illustrate that L-Met forms a 1 : 1 complex with AWP5 (Fig. 7). Furthermore, mass spectrometry experiments also support the abovementioned proposed host–guest mechanism. In the ESI-MS of the sensor AWP5 and L-Met solution, [AWP5 + L-Met + 4Br]5+ a peak at m/z = 444.0376 proved the 1 : 1 complexation stoichiometry between AWP5 and L-Met.

Through the abovementioned phenomena and description, a possible mechanism was proposed (Scheme 1). On addition of L-Met, AWP5 forms a stable inclusion complex with L-Met in solution because of their multiple interactions. The methylene groups of L-Met can result in C–H⋯π interactions with the pillar[5]arene group, whereas the amino groups of L-Met can form hydrogen bonds with oxygen atoms of the pillar[5]arene group. In addition, electrostatic interactions occur between ammonium groups of AWP5 and carboxylic acid group of L-Met. These multiple interactions stabilize the resulting inclusion complex between AWP5 and L-Met.

3. Conclusions

In summary, we employed cationic pillar[5]arene (AWP5) as a water-soluble chemosensor for the recognition of amino acids. The chemosensor AWP5 could fluorescently detect L-Met in water with high selectivity and sensitivity by a host–guest mechanism. Moreover, the detection limit of the sensor AWP5 for L-Met was 5.46 × 10⁻⁷ mol L⁻¹, which indicated that the sensor AWP5 could be useful as a highly selective and sensitive sensor for detecting L-Met. The chemosensor AWP5 can thus serve as a practical and convenient fluorescence test kit to detect L-Met.

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