Chiral Fluorescence Recognition by Anthracene Fluorescent Dyes $\subset$ Water-Soluble Pillar[5]arene Containing Phosphonic Acid Group (PWP[5])

Yuliang Chen

Nanjing Tech University

Kaijie Lai

Nanjing Tech University

Jinmi Cai

Nanjing Tech University

Yicheng Li

Nanjing Tech University

Haibo Wang (✉ wanghaibo@njut.edu.cn)

Nanjing Tech University <https://orcid.org/0000-0002-4682-5580>

Research Article

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Abstract

Chirality plays a pivotal role in drugs, agrochemicals and food additives et al. The enantiomers of a chiral molecule often show huge difference in bioactivity, metabolism, and toxicity et al. thereby, the recognition of chiral molecules shows an increasingly important priority, and has become an important focus and frontier in medicine, biochemistry and other fields. In this paper, a novel method for chiral fluorescence recognition based on anthracene fluorescent dyes(AD) $\subset$ water-soluble pillar[5] arene containing phosphonic acid group(PWP[5]) is developed. The AD as guest molecule can complex with PWP[5] to form 1:1 AD $\subset$ PWP[5] assembly, and this assembly can be further used as a fluorescent probe to identify D/L-phenylalanine and D/L-phenylalaninol by fluorescent titration. The fluorescence intensity of the assembly was significantly reduced for D-phenylalanine and D-phenylalaninol, while L-phenylalanine or L-phenylalaninol was added to AD $\subset$ PWP[5] assembly, the fluorescence intensity of the assembly almost unchanged. Hence, the chiral recognition based on assembly between the achiral fused ring fluorescent dye and achiral PWP[5] was developed.

Introduction

Chirality means that an object cannot coincide with its mirror image, such as the relationship between our left and right hands [1]. Chiral molecules not only play an important role in medicine, biochemistry and agrochemistry et al.[2], they are also widely applied in asymmetric organocatalysis, molecular recognition, and material functionalization. A pair of enantiomers have different pharmacological activities[3]. R-thalidomide can alleviate pregnancy reaction, thus, S-thalidomide not only has antiemetic effect, but also has severe teratogenic effect[4]. D-penicillamine has strong toxicity and potential carcinogenicity, and L-penicillamine has antiviral activity. L-phenylalanine (Fig. 1) is an essential amino acid for humans and animals, and has broad physiological activity. As raw material it can be used in synthesis of antiviral and anticancer drugs and sweeteners. D-phenylalanine can enhance human immunity and shows an excellent analgesic effect.

Pillar(n)arenes as the 5th generation macrocyclic host after crown ethers[5], cyclodextrins[6], calixarene[7] and Cucurbituril[8] were first reported by Ogoshi[9] in 2008, and synthesized by condensation of hydroquinone ethers and paraformaldehyde with Lewis acid as a catalyst.

Compared with other macrocyclic hosts, they have higher symmetry and rigidity, and high binding selectivity to the guest. Modification of pillar(n)arenes at two ports with different substituents can provide assembling diversity, and adjust lipophilic/hydrophilic properties to realize assembling in organic solvents or water.

At present, the traditional method of chiral recognition include HPLC [10], CE [11], GC [12], CD [13] et al. However, these methods have some shortcomings and cannot meet the requirements of real-time, in-situ detection and high-quality screening in drug development.

So far, there are few reports about fluorescence chiral recognition based on assembly. Previously, our group has studied the cis/trans and chiral fluorescence recognition by NA $\subset$ CB[7] assembly [14-15]. On this basis, in order to enhance stability of binary assembly and the π - π interaction in recognizing process, PWP[5] as a substitute of CB[7] and anthracene dye as a substitute of naphthalimide dye—we further studied characteristics of the achiral AD $\subset$ achiral PWP[5] to hope to achieve better selectivity and sensitivity in fluorescence recognition of chiral molecules. In this paper, the assembly of AD $\subset$ PWP[5] was constructed and characterized by fluorescence titration and ^1H NMR. The results showed that AD can be included by PWP[5] with 1:1 stoichiometry and fluorescence intensity decreased 10 times. When D-phenylalanine or D-phenylalaninol was added to the AD $\subset$ PWP[5], the fluorescence intensity of the assembly further reduced 60% and 65%, while L-phenylalanine or L-phenylalaninol was added to AD $\subset$ PWP[5], the fluorescence intensity of the AD $\subset$ PWP[5] assembly almost unchanged. Thereby, a new method to recognize chiral substances based on AD $\subset$ PWP[5] was developed.

Experiment

Materials and Methods

The absorption spectra were determined by Agilent Cary 60 UV-spectrophotometer. The fluorescence titration spectra were measured on Perkin Elmer Is-55 fluorescence spectrophotometer. The excitation and emission slits were set at 3 nm and 5 nm. ^1H NMR spectra were recorded on Bruker 400M spectrometer. Tetramethyl silane (TMS) was used as internal reference.

D/L-phenylalanine and D/L-phenylalaninol used in the experiment were purchased from Aladdin Biochemical Technology Co., Ltd.

PWP[5] was synthesized according to the method provided in the literature[16], AD was synthesized from anthracene. and the other reagents could be purchased and used directly.

Synthesis of 9-anthracene formaldehyde: (Fig.2) Anthracene (4 g, 22.4 mmol) was dissolved in a mixture of 4.4 mL of DMF and 5 mL of 1,2-dichlorobenzene, to the mixture POCl₃ (6 mL, 65.3 mmol) was added under stirring. The solution was then heated at 100°C for 2 h, and cooled down to 0°C, saturated sodium acetate solution was added until pH 5-6, and stirred at r.t. for 10 minutes, extracted with DCM. The organic phase was evaporated under reduced pressure and then triturated with hexane, a yellow solid was obtained (1.5 g, 32%). ¹H NMR (400 MHz, CDCl₃-d₆): δ=10.00(s, 1H), 8.91-8.86 (d, 2H), 8.83-8.74 (d, 1H), 8.10-7.99 (d, 2H), 7.69-7.52 (d, 4H).

The synthesis method steps of AD: (Fig. 2) To mixture of 9-anthracene aldehyde (5.0 g, 24.20 mmol) in ethanol (10 mL), hexamethylene diamine 12.13 mL (121.00 mmol) was added slowly at r.t.. After stirring overnight, sodium borohydride 7.0 g, 185 mmol was added and heated to 50°C for 2 h. Cooled, water (100 mL) was added and extracted with dichloromethane (100 mL), dried, and concentrated under reduced pressure to give AD. (yellow solid, 2.07g, 93%). ¹H NMR (400 MHz, DMSO-d₆): δ=8.54(s, 1H), 8.42-8.40 (d, 2H), 8.09-8.07 (d, 2H), 7.56-7.50 (q, 4H), 4.64 (s, 1H), 2.78-2.73 (q, 4H), 1.49 (s, 1H), 1.29-1.24 (d, 2H).

¹³CNMR (101 MHz, DMSO-d₆): δ=131.33, 131.15, 129.49, 127.49, 125.00, 40.58, 40.37, 40.16, 39.95, 39.74, 39.53, 39.32, 38.94, 27.15, 26.12, 25.90, 25.79

Synthesis of 1a: (Fig.5) 1,4-Dibromoethoxybenzene (2.0g, 6.0 mmol) and trioxymethylene (0.55g, 6.0 mmol) were dissolved in dichloromethane (200 mL) and stirred at 25°C for 30 min. Then Boron trifluoride etherate (0.9 g, 6.0 mmol) was added to the solution and the mixture was stirred at 25°C for 72 h. Hydrochloric acid solution (1.0 mol/L, 20 mL) was added to quenching the reaction, then the mixture was washed with water (2×50 mL) and dried over Na₂SO₄. Concentration provided a crude product, purification by column chromatography, using petroleum ether/dichloromethane (1:1, v/v) as eluent afforded 1a (1.0g, 50%). ¹H NMR (400 MHz, CDCl₃-d₆): δ=6.90 (s, 10H), 4.22 (t, q, 20H), 3.84 (s, 10H), 3.62 (t, q, 20H).

Synthesis of 1b: (Fig. 5) The mixture of 1a (0.8 g, 0.5 mmol) and triethyl phosphite (8.24g, 50 mmol) were stirred at 165°C for 24 h under nitrogen atmosphere. Then, the crude product was subjected to silica gel chromatography using methanol/ethyl acetate (1:2, v/v) as eluent, and the target 1b was obtained as a light yellow oil (1 g, 0.41 mmol, 89%). ¹H NMR (400 MHz, CDCl₃-d₆): δ=6.86 (s, 10H), 4.22-4.09 (m, 20H), 3.50-3.93 (m, 70H), 2.33-2.44 (m, 20H).

Synthesis of 1c: (Fig.5) TMSBr (4.75g, 31.03 mmol) was added to a solution of 1b (1.0 g, 0.45 mmol) in dichloromethane (15 mL) at 0°C under nitrogen atmosphere. The mixture was then allowed to warm to 25°C and stirred for 24 h. The solvent was removed under reduced pressure, and water (10 mL) was added and stirred for 30 min. Then the water was removed under reduced pressure, The residue was titrated with acetone to afford 1c as a white solid (0.6g, 1 mmol, 78%).

¹H NMR (400 MHz, DMSO-d₆): δ=6.88 (s, 10H), 4.10-4.05 (t, 20H), 3.68 (s, 10H), 2.27-2.22 (t, 20H) (Fig.6), ³¹P NMR (162 MHz, DMSO-d₆): δ=22 (Fig.7)

¹³CNMR (101 MHz, DMSO-d₆): δ=149.32, 127.93, 114.28, 63.14, 29.76.

Synthesis of (PWP[5]): (Fig.5) 1c (100 mg, 0.059 mmol) and NaOH (23.6 mg, 0.59 mmol) were added to H₂O (10 mL) and stirred at 25°C for 12 h. Water was then removed, and the PWP[5] was obtained as a light yellow solid (112.7 mg, 0.059 mmol, 100%).

Results And Discussion

Formation of AD ⊂ PWP[5] Assembly

The binding behaviors of AD ⊂ PWP[5] was characterized by fluorescence and UV-Vis spectroscopy. AD (concentration is 6×10⁻⁵ mol/L) was titrated by different concentration of PWP[5] solution (0~9×10⁻⁵ mol/L), and its fluorescence curves (Fig.9a) and inclusion constant (Fig.10) were obtained, respectively. With the increase of PWP[5], the emission wavelength changes from 498 nm to 495 nm, and the fluorescence intensity decreased about 6 times. From Fig.9b, red shift of the absorption wavelength from 375 to 395 nm appeared, this phenomenon indicated an interaction between PWP[5] and AD. The fluorescence emission mechanism of anthracene dye AD belongs to intramolecular charge transfer (ICT), that is, the charge is transferred from the aminohexyl chain to the anthracene ring in the molecule. When NA was titrated with PWP[5], the aminohexyl group of AD was encapsulated by the cavity of PWP[5], which blocked electron transfer from amino chain to anthracene ring, led to fluorescence decrease. When the concentration of AD was equal to that of PWP[5], the fluorescence intensity decreased to minimum. Even if more PWP[5] was added, the fluorescence intensity almost unchanged, which indicates that AD and PWP[5] form a 1:1 complex. (Fig. 9).

Here we define ΔF by ΔF=F-F₀. Where F represented the fluorescence intensity of PWP[5] at different concentrations of PWP[5], and F₀ was the fluorescence intensity of the guest (AD) solution. Figure 10 showed the relationship between 1/ΔF and 1/PWP[5]. The ratio of the intercept of the regression line to the slope gave the inclusion constant K_{AD⊂PWP[5]} = 2.05×10⁵ L/mol, indicating that a more stable inclusion

compound is formed. (Compared with the previously studied NA ⊂ CB[7] assembly, the inclusion constant is increased about 4 times. $K_{\text{NACB}[7]} = 4.94 \times 10^4 \text{ mol/L}$ [15].)

In order to further confirmed AD ⊂ PWP[5], the ^1H NMR was performed. As shown in Figure 11, in the presence of PWP[5], the signal of the guest AD proton exhibited higher field shifts ($\Delta\delta = -0.10, -0.15, -0.12, -0.21, -0.19$, and -0.10 ppm for protons a,b,c,d,e,f).

At the same time, due to the deshielding effect, the proton peaks in anthracene shifted down field ($\Delta\delta = 0.31, 0.10, 0.12, 0.13, 0.13$ and 0.1 ppm for protons of 10,1,2,4,6 and 8, respectively). These results indicate that the aminoalkyl side chain of AD penetrates into the cavity of PWP[5].

Fluorescent Recognition of D/L-phenylalanine and D/L-phenylalaninol by AD ⊂ PWP[5]

A concentration of $6 \times 10^{-5} \text{ mol/L}$ solution of AD ⊂ PWP[5] was titrated by different concentration ($0 \sim 9 \times 10^{-5} \text{ mol/L}$) of D/L-phenylalanine, fluorescence spectra had been measured and recorded. $6 \times 10^{-5} \text{ mol/L}$ of AD ⊂ PWP[5] solution and $0 \sim 9 \times 10^{-5} \text{ mol/L}$ of D/L-phenylalanine were mixed, as shown in Fig.12b, with the addition of different concentrations of L-phenylalanine solution to the AD ⊂ PWP[5] assembly, the emission wavelength and fluorescence intensity almost unchanged. However, when different concentrations of D-phenylalanine were added to AD ⊂ PWP[5], the fluorescence intensity decreased about 2.5 times. (Fig.12a) at the same time, blue shift of emission wavelength from 504 nm to 500 nm appeared.

Therefore, AD ⊂ PWP[5] was able to recognize the enantiomers of D/L-phenylalanine. We supposed that in the process of hydrogen bond formation between the hydrogen of the amino and carboxyl groups in D-phenylalanine and phosphonic acid group in the port of PWP[5] (Fig.14), AD ⊂ PWP[5] assembly could induce the benzene ring and anthracene ring to be parallel, thereby, a stable $\pi - \pi$ interaction appeared, which led to the fluorescence decrease. However, L-phenylalanine was different from D-phenylalanine in the spatial configuration, the assembly could not induce the parallel between benzene ring and anthracene ring and a stable $\pi - \pi$ interaction could not occur, so the fluorescence almost unchanged.

The same titration was also performed to D/L-phenylalaninol solution, as shown in Fig. 13. When D-phenylalaninol was added to AD ⊂ PWP[5], the fluorescence intensity decreased by about 2.7 times, and the emission wavelength shifted from 504 nm to 498 nm (Fig. 13a). For L-phenylalanine, the emission wavelength and fluorescence intensity almost unchanged (Fig. 13b). The recognition mechanism of D/L-phenylalaninol is similar to that of D/L-phenylalanine.

Conclusions

In summary, the assembly based on AD ⊂ PWP[5] had been successfully applied to the recognition of chiral molecule.

AD ⊂ PWP[5] assembly was constructed and characterized by fluorescent titration and ^1H NMR with 1:1 ratio and $K_{\text{AD} \subset \text{PWP}[5]} = 2.05 \times 10^5 \text{ mol/L}$. The AD ⊂ PWP[5] assembly was used as fluorescent probe to recognized D/L-phenylalanine and D/L-phenylalaninol, When assembly was titrated by D-phenylalanine or D-phenylalaninol, a stable $\pi - \pi$ interaction occurred which led to the fluorescence decrease, and for L-phenylalanine or L-phenylalaninol, a stable $\pi - \pi$ interaction could not occur, so fluorescence intensity almost not changed.

In a words, we have successfully recognized D/L-phenylalanine, D/L-phenylalanine by AD ⊂ PWP[5]. Thus we have provided a new method to recognize chiral molecule.

Declarations

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Authors' Contribution YC, KL, YL, and JC completed all experimental work. YC analyzed the results and wrote papers, HB proposed concepts, designed assemble model ideas, and provided supervision and completed review.

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Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest There is no conflict to declare.

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Figures

Figure 1

Structures of D/L-phenylalanine, D/L-phenylalaninol, PWP[5] and AD

Figure 2

Synthesis of AD

Figure 3

¹H NMR spectrum of AD

Figure 4

^{13}C NMR spectrum of AD

Figure 5

Synthesis of PWP[5]

Figure 6

¹H NMR spectrum of 1c

Figure 7

³¹P NMR spectrum of 1c

Figure 8

¹³C NMR spectrum of 1c

Figure 9

Fluorescence (a) and absorption(b) spectra of AD (6×10⁻⁵mol/L) titrated by different concentration of PWP5(×10⁻⁵mol/L):(1) 0; (2) 1; (3) 2; (4) 3; (5) 4; (6)5; (7) 6; (8) 7; (9) 8; (10) 9 in aqueous solution

Figure 10

Inclusion constant of AD $\subset$ PWP[5]

Figure 11

^1H NMR spectrum of AD before and upon addition of Pillar[5] arene: (A) PWP[5], (B) AD $\subset$ PWP[5], (C) AD

Figure 12

Fluorescence spectra of AD $\subset$ PWP[5] (6×10^{-5} mol/L) titrated by different concentration of D-phenylalanine (a) and L-phenylalanine (b) ($\times 10^{-5}$ mol/L): (0) 0; (1) 1; (2) 2; (3) 3; (4) 4; (5) 5; (6) 6; (7) 7; (8) 8; (9) 9

Figure 13

Fluorescence spectra of AD $\subset$ PWP[5] (6×10^{-5} mol/L) titrated by different concentration of D-phenylalaninol (a) and L-phenylalaninol (b) ($\times 10^{-5}$ mol/L): (0) 0; (1) 1; (2) 2; (3) 3; (4) 4; (5) 5; (6) 6; (7) 7; (8) 8; (9) 9

Figure 14

Interacting mechanism between AD-C-PWP[5] and D/L-phenylalanine