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PAPER IN FOREFRONT

Chiral covalent organic framework-monolith as stationary phase for high-performance liquid chromatographic enantioseparation of selected amino acids

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Abstract

The separation of amino acid (AA) enantiomers shows significance for chemistry, food, and biology, but remains challenging due to their similar properties. A promising nanoporous chiral covalent organic framework (COF) as a stationary phase for high-performance liquid chromatography (HPLC) suffers from the irregularity and widely distributed particle size of the chiral COF. Herein, we show the facile preparation of a chiral COF-monolith as a stationary phase for HPLC enantiomeric separation of AAs via orthogonal experiments. The CTzDa-monolith is prepared by the incorporation of the model chiral COF named CTzDa into the porous poly(ethylene dimethacrylate-co-methacrylate) monolith and reveals great permeability and mechanical stability. The corresponding CTzDa-monolithic column gives better chiral HPLC separation of AAs than the commercial Poroshell 120 chiral-T column. Thermal dynamic analysis and molecular docking calculations imply the involvement of stereoscopic hydrogen, π-π, and van der Waals interactions between the CTzDa and AAs during HPLC enantioseparation. The facile incorporation of the chiral COF into the porous monolith will promote the potential of a chiral COF as a stationary phase for HPLC.

Keywords Chiral covalent organic framework · Monolithic column · Stationary phase · Amino acids · High-performance liquid chromatography

Introduction

The chiral amino acids (AAs) have been well known as basic units of protein and also widely applied in chemistry, food, and biology [1–3]. Although the AA enantiomers possess similar physicochemical properties, their biological functions and applications are different. The levorotatory AAs (L-AAs) are known for their crucial role in many biochemical activities for multicellular organisms including recognition, metabolism, and replication, while the dextrorotatory AAs (D-AAs) are largely relevant to some pathological states such as Alzheimer, cancer, and kidney dysfunction [4]. Moreover, the content of free D-AAs in food allows indicating food quality, detecting food adulteration, and distinguishing transgenic and wild materials [5–7]. Hence, the separation and quantification of AA enantiomers reveal to be significant for many areas, but remain a challenge due to their similar properties.

High-performance liquid chromatography (HPLC) based on chiral stationary phase (CSP) has been developed as a strong technique for both the enantiomeric separation of racemic mixture and preparation of pure enantiomer [8, 9]. The CSP as the core of chiral HPLC shows a dominant effect on the separation efficiency. The preparation of novel CSP is the main direction for the development of chiral HPLC. A variety of CSP including brush-type CSP [10, 11], cyclodextrin [12, 13], microporous organic networks [14], and metal-organic frameworks [15, 16] has already shown their outstanding capacity. The development of novel CSP still draws great concern.

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Covalent organic framework (COF) linked with the organic unit via covalent bond is an emerging type of crystalline organic nanoporous material with stable, well-ordered, predesignable structure. Chiral COF is prepared via the introduction of chiral groups into the COF structure [17, 18] and shows a wide application in catalysis, sensing, and separation [19–21]. The large surface areas, well-ordered structure, and tunable pore size of chiral COF make it more promising than amorphous porous materials in separation. Recently, the application of chiral COF and chiral COF/silica composites in gas chromatography and HPLC verifies their potential in chiral separation [22–24]. However, the irregularity and widely distributed particle size of COF result in the high pressure and low efficiency of HPLC column with only COF as stationary phase and limit the development of COF in HPLC [25]. The continuous monolith is prepared via in situ polymerization of organic or inorganic monomers in chromatographic column and is well known for its high permeability. Integrating the permeable porous monolith with the COF (COF-monolith) as stationary phase expects to be an efficient way to address the above-mentioned limitation of COF in HPLC, but the preparation of chiral COF-monolith as stationary phase for chiral HPLC separation has been rarely explored so far.

Herein, we show facile preparation of chiral COF-monolith as stationary phase for HPLC enantioseparation of AAs. The stable chiral COF named CTzDa with selective adsorption of AA enantiomers was chosen as model chiral COF to be incorporated into the permeable monolith of ethylene dimethacrylatemethyl (EDMA) and methacrylate (MAA) matrix to form the CTzDa-monolith. The effect of the essential conditions including CTzDa, porogen, and temperature on the preparation of CTzDa-monolith was investigated with an orthogonal experiment. The great permeability and mechanical stability of CTzDa-monolith as well as its high HPLC resolution for AA enantiomers were verified via systematic experimental characterization, thermal dynamic analysis, and molecular docking calculation. This work reveals the potential of chiral COF-monolith as CSP in enantiomeric HPLC separation.

Experimental

Materials

4,4',4'-(1,3,5-Triazine-2,4,6-triyl)trianiline (Tz) and 1,4-dihydroxyterephthalaldehyde (Da) were obtained from Jilin Chinese Academy of Sciences-Yanshen Technology Co., Ltd. (Jilin, China). 2,2'-Azobis(2-methylpropionitrile) (AIBN), EDMA, MAA, o-dichlorobenzene (o-DCB), AA enantiomers, 9-fluorenylmethyl chloroformate (FMOC-Cl), boric acid, and pentane were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). D-camphanic acid (D-cam) was purchased from Yuanye Biotechnology Co., Ltd. (Shanghai, China). All the organic solvents and poly(ethylene glycol) (PEG-6000) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ultrapure water was obtained from Wahaha Group Co., Ltd. (Hangzhou, China).

Apparatus

The powder X-ray diffraction spectrometry (PXRD) data were obtained with a D2 PHASER diffractometer (Bruker, German). The Fourier transform infrared spectroscopy (FTIR) spectra were measured on a Nicolet IR IS10 spectrometer (Nicolet, USA). N_2 adsorption experiments were carried on Autosorb-IQ (Quantachrome, USA). Zeta potential determination was recorded on a Malvern Nano-ZSE (Worcester shire, UK). The SU1510 (Hitachi, Japan) and JEM-2100 microscopes were used to obtain scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images, respectively. All HPLC experiments were performed on e2695 liquid chromatograph with photo-diode array detector (Waters, USA).

Orthogonal arrays for synthesis of CTzDa-monolithic column

The temperature affects the number of free radicals during polymerization. Porogen serves as a pore template and produces the pores in the prepared monolith after removal. COF as the functional part determines the performance of the obtained enantioselective material [26]. Hence, the essential synthetic factors containing chiral COF (CTzDa), porogen (PEG-6000), and temperature for the synthesis of CTzDa-monolithic column were studied in three levels. The factors and their corresponding levels were assigned in a $L_9(3^3)$ orthogonal array to investigate the optimal conditions for the CTzDa-monolithic column.

Synthesis of CTzDa-monolithic column

Typically, 16 mg CTzDa was dispersed in 1.2 mL *N*,*N*-dimethylformamide to sonicate for 1 h. Four hundred milligrams of PEG-6000, 35 μL MAA, and 400 μL EDMA were added to the above mixture to sonicate for another 2 h. Then, 10 mg AIBN was further added. The final solution was transferred to a stainless-steel column (5 cm long × 0.46 cm i.d.). After being sealed, the column was reacted at 70 °C for 24 h and finally washed with acetonitrile (ACN) to remove the unreacted monomer. The control poly(EDMA-co-MAA) monolith was prepared in the same conditions without the addition of CTzDa.

Molecular docking calculation

The molecular docking with Auto Dock4 (LaJolla, USA) was applied to produce the optimal configuration of AA enantiomers interacted CTzDa (CTzDa-AAs) [27]. One-unit cell of CTzDa was used as the model structure of COF for the molecular docking calculation. The geometry structure of isolated CTzDa and AA enantiomers was optimized before docking, respectively. An 80 × 80 × 20 Å box with a grid spacing of 0.375 Å was employed to cover all the atoms of the model CTzDa structure. The Lamarckian genetic algorithm (LGA) was applied to find the appropriate binding conformations of the AA enantiomers and CTzDa. All other parameters were set in default.

Results and discussion

Design and preparation of the CTzDa-monolithic column

The unsuccessful preparation of CTzDa column via a direct package of CTzDa into stainless-steel column (5 cm long × 0.46 cm i.d.) resulted from the high back pressure due to the irregularity and widely distributed particle size of CTzDa, indicating the inaccessibility of direct application of COF as stationary phase in HPLC. Integrating the permeable porous monolith with the COF as COF-monolith is expected to be an efficient way to address the above-mentioned problem, which has rarely been explored in the chiral COF. Herein, we show facile preparation of chiral COF-monolith by incorporating chiral COF into porous organic monolith to serve as novel CSP for HPLC separation of AA enantiomers (Fig. 1). The highly permeable poly(EDMA-co-MMA) monolith matrix consisting of EDMA and MAA makes sure the great permeability of the COF-monolithic column [28]. The selective adsorption of CTzDa for AA enantiomers indicates its high potential as CSP for HPLC separation of AAs [29]. So the CTzDa was chosen as the model chiral COF to bring the poly(EDMA-co-MMA) monolith matrix with chiral separation property for HPLC separation of AA enantiomers.

The orthogonal experimental design is to test multiple independent variables in a single experiment based on the orthogonal array. The quantitative investigation of variates in different levels renders the orthogonal experiment more efficient to collect and analyze data with fewer experiments than traditional one-at-a-time processes [30, 31]. The effects of critical synthetic factors containing CTzDa, PEG-6000, and temperature in three levels on the column permeability (B_0) and efficiency (N) of CTzDa-monolithic column were conducted through an orthogonal table $L_9(3^3)$ (see Supplementary Information (ESM) Tables S1 and S2). The largest range value (R) of CTzDa among the three factors indicates that the porosity and functionality of CTzDa show the highest effect on both column permeability and efficiency (Table 1). As a pore template, porogen gave higher influence on column permeability than temperature ($R_{\text{PEG-6000}} > R_{\text{temperature}}$ for column permeability). On the contrary, the temperature gave larger effect on the column efficiency than PEG-6000 ($R_{\text{temperature}} > R_{\text{PEG-6000}}$ for column permeability).

The variance analysis of orthogonal experiment results was further applied to determine factor level for preparation of CTzDa-monolith (Table 2). The results show that the effect of CTzDa on both column permeability and efficiency is significant. Sixteen milligrams CTzDa led to the best experiment results (K_2) for both column permeability and efficiency, indicating the most suitable COF amount (16 mg) for the incorporation. The porogen shows a significant effect on column permeability rather than column efficiency. Hence, level 1 of PEG-6000 (400 mg) gave the best permeability (K_1) and was employed for subsequent preparation of CTzDa-monolithic column. The reaction temperature gives no significant influence on both column permeability and efficiency. The CTzDa-monolith was determined to prepare at 70 °C according to the obtained highest column efficiency (K_2).

Characterization of CTzDa and CTzDa-monolith

The diffraction peaks in powder X-ray diffraction (PXRD) patterns of prepared COF at 2.8°, 4.8°, 5.7°, and 7.5° matched well with that of the reported CTzDa [29] (ESM Fig. S1). Compared with the control monolith with no PXRD peaks, the CTzDa-monolith gave an obvious characteristic peak of CTzDa at 2.8° in its PXRD pattern (Fig. 2a). The peaks of the C=O band for carboxyl (1802 cm^{-1}) and ester (1746 cm^{-1}) in the Fourier transform infrared (FTIR) spectra of prepared chiral COF confirmed the preparation of CTzDa (ESM Fig. S2). The characteristic peaks of both control monolith and CTzDa

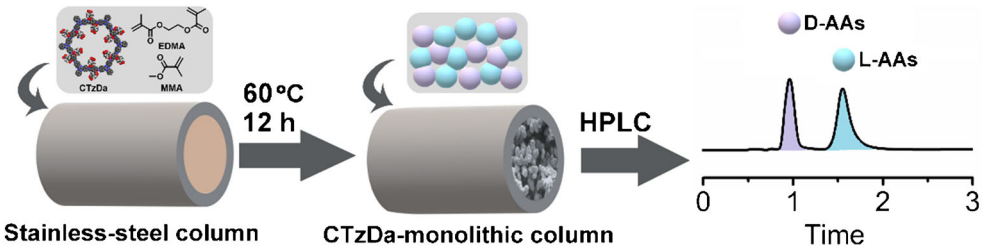

Fig. 1 Scheme for the fabrication of CTzDa-monolith as CSP for enantiomeric HPLC separation of AAs

Table 1 Range analysis of orthogonal experiment for the synthesis of CTzDa-monolithic column

Factors	B_0 (× 10^{-15} m^2)			N (× 10^3 plates m^{-1})		
	Temperature	PEG-6000	CTzDa	Temperature	PEG-6000	CTzDa
$K1$[a]	16.15	16.95	18.20	14.84	17.17	19.10
$K2$[a]	16.26	15.22	19.24	17.31	15.12	20.71
$K3$[a]	15.23	15.47	10.21	16.99	16.85	9.32
R[b]	1.03	1.73	9.03	2.48	2.05	11.39

[a] The K was the average experiment results of each factor level

[b] $R = K_{maximum} - K_{minimum}$

in the FTIR spectra of CTzDa-monolith demonstrated the successful incorporation of CTzDa into the monolith (Fig. 2b). The incorporation of CTzDa with large BET surface areas (523 m^2 g^{-1}) and pore volume (0.75 cm^3 g^{-1}) caused that the BET surface areas and pore volume of the porous monolith increased from 192 m^2 g^{-1}, 0.19 cm^3 g^{-1} to 252 m^2 g^{-1}, 0.21 cm^3 g^{-1} respectively (Fig. 2c). The pore size of CTzDa-monolith (2.16 nm) remained little change compared with that of the control monolith (2.19 nm), indicating no effect of the incorporated CTzDa on the pore of the monolith (Fig. 2d). The morphology of the monolith evidently changed after the incorporation of CTzDa (ESM Fig. S3). All the results verified the successful incorporation of CTzDa into the monolith.

The HPLC column efficiency is affected by morphology and particle size of the stationary phase, which can be investigated with the Van Deemter curve [32, 33]. The CTzDa-monolith column displayed higher column efficiency (23053 plates m^{-1}) for thiourea than the control monolithic column (9705 plates m^{-1}) (ESM Fig. S4). The CTzDa-monolithic column gave a lower value of all the A, B and C term of Van Deemter coefficients (corresponding to eddy dispersion, longitudinal diffusion, and mass transfer resistance, respectively) than the control monolithic column (ESM Table S3), indicating that CTzDa-monolith becomes more uniform and faster mass transfer than the control monolith. The good linearity (0.9983–0.9998) between the flow rate and column pressure with ACN, ACN/H_2O (50/50, v/v), and methanol as mobile phase in the studied flow rate range (0.5–5 mL min^{-1}) suggests the good mechanical stability of the prepared CTzDa-monolithic column (ESM Fig. S5).

HPLC separation of AA enantiomers

The AA enantiomers with weak UV absorbance were derivatized with FMOC-Cl, and then applied to investigate the performance of CTzDa-monolithic column in chiral HPLC separation. Baseline separation of FMOC-Cl derivatized AAs (FMOC-AAs) including histidine (His), tryptophan (Trp), cysteine (Cys), and serine (Ser) was achieved on the CTzDa-monolithic column within 3 min (Fig. 3 and ESM Fig. S6) according to the larger resolution (r) value (1.70–4.41) than 1.5 (ESM Table S4). All the studied L-AAs gave longer retention on the CTzDa-monolith than D-AAs. The efficiency of CTzDa-monolithic column can reach 20690 plate m^{-1} for D-His. The selectivity factor (α) of His, Trp, Cys, Ser, and Asp ranged from 1.08 to 2.38, indicating the different interactions between the stationary phase and AA enantiomers. The better chiral separation

Table 2 Variance analysis of orthogonal experiment for synthesis of CTzDa-monolithic column

Parameter	B_0			N		
	Temperature	PEG-6000	CTzDa	Temperature	PEG-6000	CTzDa
SS[a] (× 10^{-30})	2.0	5.0	147.8	10.9	7.3	227.9
df[b]	2	2	2	2	2	2
MS[c] (× 10^{-30})	1.0	2.5	73.9	5.4	3.7	113.9
F value	8.2	20.3	599.7	2.1	1.4	44.1
Significance	/	*	**	/	/	*

[a] squares of deviation, [b] degree of freedom, [c] mean squared deviation

* $0.01 < p \leq 0.05$, ** $p \leq 0.01$, / $p > 0.05$

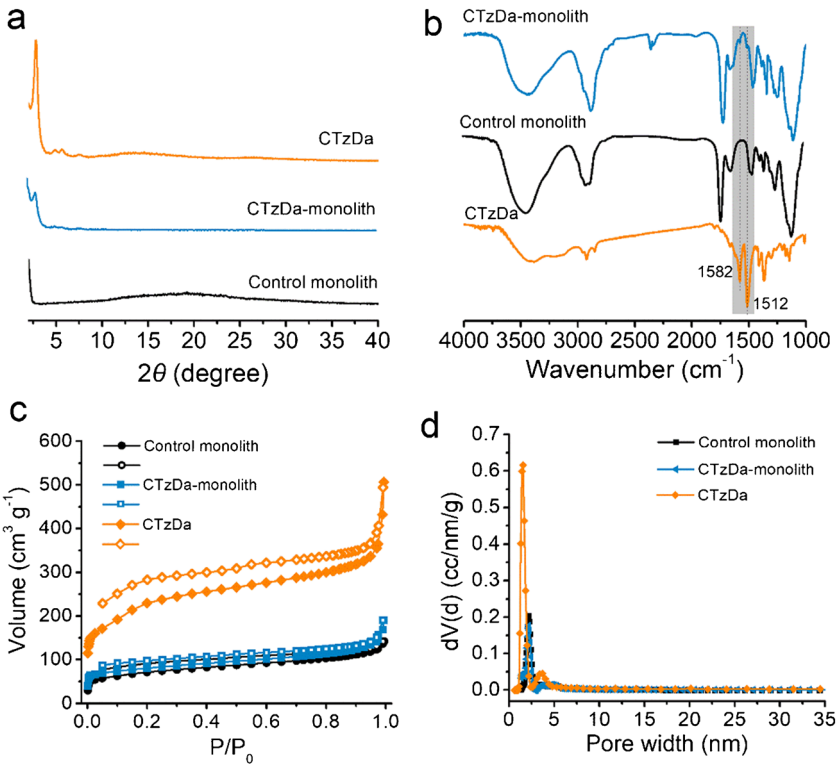

Fig. 2 **a** PXRD patterns, **b** FTIR spectra, **c** N_2 adsorption–desorption isotherms and **d** pore size distributions of CTzDa, control monolith and CTzDa-monolith

of Trp, Ser, and Asp on CTzDa-monolithic column than the commercial chiral HPLC column (Agilent Poroshell 120 chiral-T) indicates the superb potential of the chiral COF-monolithic column (ESM Fig. S7 and Table S5). The relative standard deviations of run-to-run (n = 10), day-to-day (n = 5), and column-to-column (n = 3) retention time for Trp were 1.0–1.2%, 1.1–1.2%, and 2.0–3.7%, respectively, revealing the great repeatability and reproducibility of the CTzDa-monolithic column (ESM Fig. S8).

Enantioseparation mechanism

The smaller molecular size of all the studied AAs with flexible chains than the pore of CTzDa shows the AAs can interact with the active sites in the pore of the chiral COF (ESM Fig. S9). The fact that no chiral separation occurred on the TzDa-monolithic column (which lacks chiral centers) proved the effect of the chiral microenvironment offered by CTzDa on the chiral separation (ESM Fig. S10). The retention of all the AAs on the CTzDa-monolithic column decreased evidently

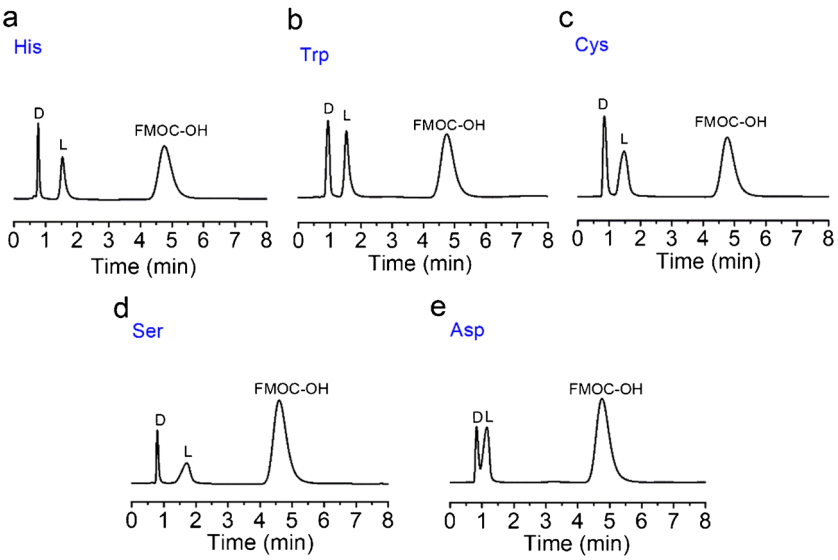

Fig. 3 HPLC enantioseparation chromatograms of FMOC-AAs **a** His, **b** Trp, **c** Cys, **d** Ser, and **e** Asp on CTzDa-monolithic column (5 cm long × 0.46 cm i.d.). Mobile phase: ACN/H_2O (40/60, v/v) at 0.8 mL min^{-1}, UV detection wavelength: 254 nm

with the increase of ACN in the mobile phase, revealing the involvement of hydrophobic interaction in the chiral separation (ESM Fig. S11). Furthermore, the reduction of retention time with the increase of temperature indicates the exothermic interaction of AAs and CTzDa-monolith (ESM Fig. S12).

Thermal dynamic analysis shows the energy change during the chiral separation and was conducted to understand the retention and chiral separation of AAs on CTzDa-monolithic column (ESM method, ESM Fig. S13 and ESM Table S6). The negative of enthalpy change (ΔH) and entropy change (ΔS) confirmed the retention of AAs on the CTzDa-monolith was exothermic and driven by enthalpy. The negative chiral part of enthalpy change ($\Delta\Delta H$) and positive chiral part of entropy change ($\Delta\Delta S$) reveal that both enthalpy and entropy drive the chiral separation of His, Trp, and Asp on CTzDa-monolith. The negative $\Delta\Delta H$ and $\Delta\Delta S$ of Cys and Ser indicate the enthalpy drives their chiral separation. Moreover, the value of $\Delta\Delta H$ was observed between −0.4 and −4.2 kJ mol^{-1}, indicating the contribution of weak π-π and hydrogen interaction for the chiral separation of AAs on the CTzDa-monolith in addition of chiral steric hindrance [34].

Molecular docking based on one-unit cell of CTzDa was performed to further investigate the interaction of CTzDa-monolith and AAs. The molecular docking calculation can provide the probable binding configurations of the AA enantiomers and CTzDa (CTzDa-AAs) with different binding energy (BE). The configuration with the lowest BE means the highest feasibility of the binding configuration for CTzDa-AAs [35, 36] and was chosen as the representative of CTzDa-AAs for further analysis. The BE of CTzDa-L-AAs for His, Trp, Cys, Ser, and Asp (−4.17, −4.88, −3.70, −4.06, −3.09 kcal mol^{-1}) appeared more negative than that of CTzDa-D-AAs (−3.60, −4.39, −3.37, −3.50, −2.90 kcal mol^{-1}), respectively (Table 3). The lower BE verified the higher binding strength of L-AAs with CTzDa than D-AAs, which caused the faster elution of D-AAs from the CTzDa-monolith. All the representative of CTzDa-AAs showed the AA enantiomers tended to bind around the chiral moiety of CTzDa (Fig. 4, ESM Figs. S14 and S15). Moreover, the interaction between CTzDa and AAs involved hydrogen, π-π, and van der Waals interactions according to the atom distance. Further

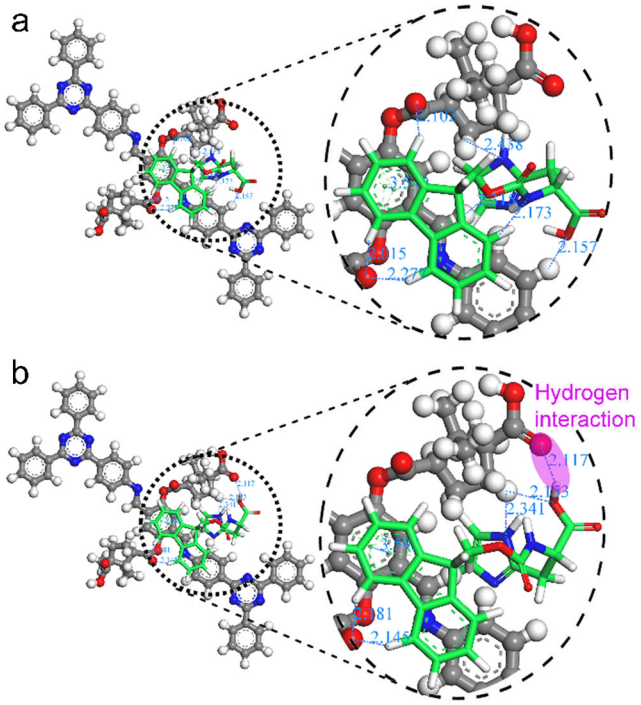

Fig. 4 Representative binding configuration of CTzDa and **a** D-His and **b** L-His with lowest BE produced with molecular docking (distance Å). The COF was shown in the ball-and-stick model (C grey, N blue, O red H white), while the amino acid enantiomer was displayed in the stick model (C bule, N blue, O red, S yellow, H white)

comparison of the CTzDa-AAs concluded that the difference of stereoscopic hydrogen interactions mainly resulted in chiral separation of the AAs on CTzDa-monolith. The L-AAs for His, Cys, and Ser can form hydrogen bonds with CTzDa. In contrast, the hydrogen interaction absented in their corresponding CTzDa-D-AAs due to the increase of molecular distance (Fig. 4, ESM Fig. S14c and d, Fig. S15a and b), leading to the lower affinity of D-AAs towards CTzDa. Although the interaction type of L-Trp and CTzDa was similar to that of D-Trp, the shorter distance of the interactions indicates the higher interaction strength of L-Trp and CTzDa (ESM Fig. S14a and b). The similar interaction and strength of Asp and CTzDa (ESM Fig. S15c and d) resulted in no chiral baseline separation of Asp on the CTzDa-monolith in HPLC (Fig. 3e).

Table 3 The lowest BE of CTzDa-AAs

Analyte	BE (kcal mol^{-1})	
	D-AA	L-AA
His	−3.60	−4.17
Trp	−4.59	−4.88
Cys	−3.37	−3.70
Ser	−3.50	−4.06
Asp	−2.90	−3.09

Conclusions

In summary, we have shown the facile preparation of chiral COF CTzDa-monolith by incorporating CTzDa into the porous organic polymer monolith as a stationary phase for HPLC enantioseparation of AAs. Integrating the permeable porous poly(EDMA-co-MAA) monolith and crystalline CTzDa with selective adsorption of AA enantiomers not only precluded the problem of directly applying chiral COF as the

stationary phase, but also brought the monolith with the chiral property. The prepared CTzDa-monolith with great permeability and mechanical stability well interacted with the AAs via the dominant stereoscopic hydrogen, π-π, and van der Waals interactions, rendering the CTzDa-monolithic column with high chiral HPLC resolution of some AAs. This research offered a general strategy for the application of chiral COF as stationary phase and will promote more chiral COF stationary phase for HPLC.

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Declarations

Conflict of interest The authors declare no competing interests.

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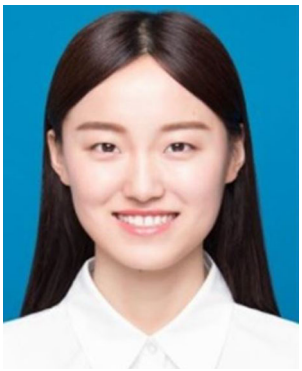

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