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## COMMUNICATION

## A facile enantioseparation for amino acids enantiomers using $\beta$ -cyclodextrins functionalized Fe<sub>3</sub>O<sub>4</sub> nanospheres<sup>†</sup>

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Herein is presented a strategy for the enantioseparation of amino acids enantiomers using  $\beta$ -CD functionalized Fe<sub>3</sub>O<sub>4</sub> nanospheres, in which  $\beta$ -CD provides the ability to chirally discriminate amino acids enantiomers, while the Fe<sub>3</sub>O<sub>4</sub> nanoparticles serve as magnetic separators.

The importance of amino acids (AAs) in a variety of bioscience areas and food analysis is undeniable. AAs can be present as L- or D-isomers. Although the D-amino acids (D-AAs) and L-amino acids (L-AAs) have similar physical characteristics and chemical properties in nonstereo environments, they can have very different pharmacological properties and bioactive effects in stereo environments, specifically in biological environments. Thus, the enantioseparation of amino acids enantiomers has become vitally significant in pharmaceutical and biochemical fields.<sup>1-3</sup> Currently, crystallization<sup>4,5</sup> is the simplest and most widely used process for large-scale enantiomeric resolution. However, this process requires large amounts of mother liquor and leads to high losses of valuable products. Apart from crystallisation, a number of complicated approaches have been used for the chiral separation of organic compounds, including gas chromatography (GC),<sup>6</sup> high-performance liquid chromatography (HPLC),7 capillary electrophoresis (CE)8,9 and the use of chiral reagents in chromatography.<sup>10</sup> Most of these techniques are based on chromatography or electrophoresis, which are usually limited to the analytical scale, only allow a small amount of materials to be separated per run. Hence scale-up is uneconomical.<sup>11,12</sup> Furthermore, membrane based chiral separation,<sup>13,14</sup> and methods using molecularly imprinted polymers (MIPs)<sup>15</sup> and surface modified nanoparticles<sup>16–18</sup> have also been started to be used to separate various mixtures of amino acids in recent years. Nanoscale magnetite (Fe<sub>3</sub>O<sub>4</sub>) as one important phase of iron oxide has been extensively used in many magnetic application areas, such as electrochemical analysis,<sup>19</sup> separation of proteins<sup>20</sup> and so on. In this communication,

we focus on a more efficient and economical enantioseparation of amino acids enantiomers by synthesizing  $\beta$ -cyclodextrin ( $\beta$ -CD) modified Fe<sub>3</sub>O<sub>4</sub> nanospheres as a chiral selecting system. Furthermore, photo-controlled inclusion and exclusion reactions of azobenzene derivatives with  $\beta$ -CD were used to induce the release of the absorbed amino acids with specific chirality from the nanoparticles and lead to their repetitious recycle application in enantioseparation.

The magnetite nanoparticles and azobenzene derivatives (AzoTAB) were synthesized in analogy to the previous procedures, respectively.<sup>21,22</sup> The morphology and structure of the prepared Fe<sub>3</sub>O<sub>4</sub> nanoparticles modified with or without β-CD had been characterized by TEM. XRD and IR analysis (as shown in Fig. S1-S3, ESI<sup>†</sup>), which confirmed the successful preparation of β-CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles. The excellent magnetically controllable aggregation behavior of β-CD functionalized Fe<sub>3</sub>O<sub>4</sub> nanoparticles was verified by placing a magnet near the vessels containing the aqueous dispersion of the nanoparticles (as shown in Fig. S1c, ESI<sup>+</sup>), which provides the basis for our system to be further applied in magnetic separation. Thus, the chiral recognition ability of amino acids enantiomers provided by  $\beta$ -CD, and the magnetic separation ability provided by Fe<sub>3</sub>O<sub>4</sub> nanoparticles, gives the combined system an effective ability to separate amino acids enantiomers. The detailed preparation procedures and enantioselectivity mechanism of β-CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles for amino acids isomers are shown in Scheme 1. To verify the enantioseparation capacity of β-CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles for



Scheme 1 Preparation procedures and separation mechanism of  $\beta$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles for amino acids isomers.

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 Table 1
 The specific rotation data for the racemic mixture of amino acids isomers

	Specific rotation data		
	$[\alpha]^a$	$[\alpha]^b$	$[\alpha]^c$
Alanine (L-: D- = 1:1, 0.01 mol $L^{-1}$ )	0	11.2	-7.9
Tryptophan (L-: D- = $1:1, 0.01 \text{ mol } L^{-1}$ )	0	35.7	-34.6
Tyrosine $(L : D = 1 : 1, 0.01 \text{ mol } L^{-1})$	0	6.2	-4.5

<sup>*a*</sup> The racemic mixture of amino acids isomers. <sup>*b*</sup> For the residual solution after enantioseparation with  $\beta$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles. <sup>*c*</sup> For the residual solution after the release of the absorbed amino acids isomers with AzoTAB. The specific rotation data [ $\alpha$ ] for pure L- and D-alanine (0.01 mol L<sup>-1</sup>), L- and D-tryptophan (0.01 mol L<sup>-1</sup>), L- and D-tyrosine (0.005 mol L<sup>-1</sup>) solutions were -14.4, 14.6 -36.9, 36.9, -10.5 and 10.4, respectively.

amino acids isomers, the absorption coefficients *P* of  $\beta$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles for different amino acids racemic mixture solutions, which can indicate the binding property of the modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles to racemic mixtures, were measured. The values of *P* are 2.8 × 10<sup>-3</sup> mol g<sup>-1</sup> (alanine, L-:D- = 1:1, 0.01 mol L<sup>-1</sup>), 2.5 × 10<sup>-3</sup> mol g<sup>-1</sup> (tryptophan, L-:D- = 1:1, 0.01 mol L<sup>-1</sup>) and 2.5 × 10<sup>-3</sup> mol g<sup>-1</sup> (tyrosine, L-:D- = 1:1, 0.005 mol L<sup>-1</sup>), respectively, which were found to change in proportion to the concentration of the amino acids isomers in the range of 0.005 and 0.02 M.

As expected the specific rotation of a pure racemic mixture of amino acids isomers was zero due to the external compensation.

However, after enantioseparation with B-CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles, the specific rotation of the resulting racemic mixture of amino acids solutions was 11.2 (alanine), 35.7 (tryptophan) and 6.2 (tyrosine) (Table 1), which indicated change in the relative proportion of L- and D-type amino acids isomers in solution. D-Type amino acids isomers were the dominant residual species left in solution. Hence the β-CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles showed more efficient complexation of L-type amino acids isomers. The difference in the enantioselectivity for different D and L-amino acids isomers was attributed to the shape selectivity of the  $\beta$ -CD cavity and the different inclusion binding strength of the different amino acid enantiomer guests to  $\beta$ -CD host (for example, the stability constants  $(\log K)$  of the inclusion complex for L-tryptophan and D-tryptophan with  $\beta$ -CD should be 2.33 and 1.11, respectively).23

To explore the enantioselectivity mechanism of β-CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles for amino acids isomers, FTIR spectrum and  $H^1$  NMR analysis of the  $\beta$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles mixing with the racemic mixture of tryptophan solution, and in the absence of tryptophan was performed. As shown in Fig. 1a, an obvious shift from 1646 ( $\beta$ -CD, OH in-plane bending vibration absorption) to 1629 cm<sup>-1</sup> could be observed after mixing with tryptophan solution, which is ascribed to the inclusion reaction of  $\beta$ -CD and guest molecules. As for tryptophan, peaks at 3049 cm<sup>-1</sup> (carboxylic acid OH stretching absorption), 1665  $cm^{-1}$ , 1590  $cm^{-1}$ , and 1450  $cm^{-1}$  (benzene's structure skeleton vibration) disappeared after mixing with β-CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles. This latter result provides evidence that tryptophan enters the hydrophobic cavity of  $\beta$ -CD. The H<sup>1</sup> NMR gave further evidence of the formation of this inclusion complex for  $\beta$ -CD and tryptophan.



**Fig. 1** (a) FT-IR spectra and (b)  $H^1$  NMR of (i) tryptophan, (ii)  $\beta$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles and (iii)  $\beta$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles interacting with tryptophan.

As shown in Fig. 1b, the signals for H-2, H-3 and H-4 all shift upfield by about 0.02 ppm, while the band due to the H-5 proton has a maximum upfield shift of 0.06 ppm. These shifts suggest that the tryptophan interacts with the internal protons near the smaller opening of the cavity. L- and D-type can form different complex constructions with  $\beta$ -CD. When L-tryptophan interacts with the  $\beta$ -CD, the aromatic part of tryptophan inserts into the hydrophobic cavity of  $\beta$ -CD, however, the hydroxyl-terminated part of tryptophan would insert into the hydrophobic cavity of  $\beta$ -CD when D-tryptophan interacts with  $\beta$ -CD.<sup>24</sup> As shown in Fig. 1b, the signal for H-g is shifted upfield by about 0.015 ppm, and the signal for H-c is dramatically shifted upfield by about 0.45 ppm; both of which belong to the aromatic portion of tryptophan. All the above results indicated that  $\beta$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles mostly absorbed L-tryptophan in the racemic mixture; however, D-tryptophan still remained in the solution. Thus the use of β-CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles was highly applicable to the robust and effective separation of the amino acids isomers.

The stability constant  $(\log K)$  of the inclusion complex for azobenzene mesogen and  $\beta$ -CD was about 4.0, much higher than those of L-tryptophan (2.33) and D-tryptophan (1.11). Thus the formation of the inclusion complex for azobenzene derivatives with  $\beta$ -CD could be used to induce the release of the absorbed amino acids from the nanoparticles. Further, the two isomers of azobenzene mesogen, the *trans* and *cis* forms, can be reversibly switched upon photo-irradiation. When the *trans*-azobenzene is transformed to the *cis*-azobenzene, CDs cannot include the bulky *cis* form. Therefore, the photocontrolled exclusion reaction of azobenzene derivatives with  $\beta$ -CD could lead to the repetitious recycle application of  $\beta$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles in enantioseparation.

In order to demonstrate the above idea, repetitious recycle experiments in enantioseparation for  $\beta$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles were performed. The nanoparticles containing L-tryptophan were added to water-soluble azobenzene (AzoTAB,  $10^{-2}$  M) solution. After waiting for 2 hours for the release of the absorbed amino acids isomers, the nanoparticles



Fig. 2  $H^1$  NMR of (i) AzoTAB, (ii)  $\beta$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles and (iii) β-CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles interacting with AzoTAB

were removed by a magnetic separation process. The release process of the absorbed amino acids isomers was monitored by  $H^1$  NMR analysis and the optical activity characterization. As shown in Fig. 2, the signals for H-3, H-5 and H-6 were all upfield shifted dramatically by about 0.08, 0.07 and 0.1 ppm. respectively. Moreover, the signals of the aromatic protons H-a, H-b and H-c for AzoTAB also showed a dramatic upfield shift by about 0.18, 0.19 and 0.07 ppm, respectively. These chemical shifts of different protons indicate that the AzoTAB formed an inclusion complex with  $\beta$ -CD in this case, and the absorbed L-tryptophan was released into the solution. The specific rotation of the resulting solution showed -34.6, close to that of pure L-tryptophan solutions. The concentration of the released L-tryptophan was comparable to the concentration decrease of the racemic mixture tryptophan solution after the magnetic separation process, which gave further evidence of the total release of the absorbed L-tryptophan into the solution.

Then above  $\beta$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles containing AzoTAB were re-dispersed in water and irradiated with UV light for 10 min. The photo-controlled exclusion reaction of azobenzene derivatives with β-CD was monitored by UV-Vis absorbance spectra and H<sup>1</sup> NMR analysis. After 5 min UV irradiation, the trans AzoTAB had been entirely photoisomerized into the cis form (as shown in Fig. S4, ESI<sup>†</sup>), which indicated that AzoTAB had been released from the β-CD. The nanoparticles were then removed by the magnetic separation process for repetitious recycle application in enantioseparation. The typical H<sup>1</sup> NMR signals between 6 and 8 ppm for AzoTAB disappeared (as shown in Fig. S5, ESI<sup>†</sup>), which indicated that AzoTAB had been entirely removed from  $\beta$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles. It should be noted here that after a few cycles, the enantioseparation capacity of the  $\beta$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles tended to decrease. However, after 5 cycles, β-CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles still showed good enantioselectivity for L-tryptophan (as listed in Table 2), which indicated that  $\beta$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles were highly applicable to the repetitious recycle application in enantioseparation of amino acids isomers.

In summary, we have presented a simple and convenient chemical method for the preparation of water-soluble and stable  $\beta$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles using  $\beta$ -CD as a chiral selector to conduct the enantiomeric separation of

**Table 2** The repetitious recycle application of  $\beta$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles in enantioseparation for the racemic mixture solution of tryptophan (L-: D- = 1:1, 0.01 mol  $L^{-1}$ )

	Specific rotation data			
	$[\alpha]^a$	$[\alpha]^b$	$[\alpha]^c$	
lst recycle	0	35.4	-34.6	
2nd recycle	0	34.3	-33.2	
3rd recycle	0	32.6	-25.5	
4th recycle	0	33.2	-22.4	
5th recycle	0	31.1	-21.8	

<sup>a</sup> The racemic mixture of amino acids isomers. <sup>b</sup> For the residual solution after enantioseparation with β-CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles. <sup>c</sup> For the residual solution after the release of the absorbed amino acids isomers with AzoTAB.

amino acids. The method using  $\beta$ -CD modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles effectively separates the isomers of different amino acids, especially for the tryptophan. We believe that the surface architectures of magnetic nanoparticles on introducing the CD-based host-guest recognition would afford the chemical and bio-analytical separation of various molecules in aqueous media.

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