Investigation of Mixed Chiral Selectors of Different Metal Ion-*L*-Alanine Complex and *β*-Cyclodextrin on the Chiral Separation of Dansyl Amino Acids with Capillary Electrophoresis

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Chiral separation of dansyl amino acids by capillary electrophoresis using mixed selectors of Mn(II)-*L*-alanine complex and *β*-cyclodextrin (*β*-CD) was studied. Resolution was considerably superior to that obtained by using either Mn(II)-*L*-alanine complex or *β*-CD alone. The effects of separation parameters, such as pH value of buffer solution, capillary temperature, the concentration of Mn(II)-*L*-alanine complex, the types of CD and ligand on the migration times and resolutions were investigated. Six different transition metal complexes, Cu(II), Zn(II), Co(II), Ni(II), Hg(II) and Cd(II)-*L*-alanine complexes have been employed and compared with Mn(II) complex. Differences in retention and selectivity were found. The substitution of Cu(II), Zn(II), Co(II) and Ni(II) for Mn(II) resulted in a better chiral resolution while Hg(II) and Cd(II) showed poorer resolution abilities. The chiral separation mechanism was also discussed briefly.

Keywords chiral separation, ligand-exchange capillary electrophoresis, dansyl amino acids, *β*-cyclodextrin

Introduction

The separation of enantiomers is of great importance in biology, pharmaceutics, agriculture and environment.[1] The different separation modes and many chiral selectors available make capillary electrophoresis (CE) technique a powerful tool for chiral analysis.[2] The first application of CE in chiral separation was based on the principle of metal-complex ligand-exchange (LE) reported by Zare' group.[3,4] Using Cu(II) complexes of *L*-histidine or aspartame as chiral selectors, 14 dansyl amino acids (Dns-AAs) were resolved. So far, a variety of Cu(II)-amino acid complexes have been successfully used as chiral selectors for enantiomeric separation of amino acids,[5] hydroxy acids,[6] amino alcohols,[7] diamines and small peptides,[8] such as *L*-proline and its derivatives,[9-12] *L*-arginine.[13] It has been well known that the combination of chiral selectors is an effective approach to enhancing the selectivity and resolution in chiral CE. However, up to now, only a few papers have been published on the application of a combination of ligand-exchange and other chiral resolving mechanism.[14-16] Cyclodextrins (CDs) and their derivatives are the most commonly used chiral selectors in CE separation. The first study on the combination of CDs in LE-CE seems to be that reported by Fanali *et al*.[17] in 1989 when 15 mmol·L^{-1} *β*-CD was used in combination with sodium *L*-(+)-tartrate buffer in order to resolve the enantiomers of Co(III) complexes. Horimai[18] recently reported the separation of new quinoline drugs enantiomers using dual chiral selector systems of *γ*-CD and Zn(II)-*L*- or *D*-phenylalanine complex.

Herein, we report the chiral resolution of Dns-AAs by using mixed selectors of Mn(II)-*L*-alanine complex and *β*-CD. Six different transition metal ions, Cu(II), Zn(II), Co(II), Ni(II), Hg(II) and Cd(II) have been explored as central ions and compared with Mn(II). A possible chiral recognition mechanism was also proposed. To the best of our knowledge, no report has been published yet on the LE-CE using Mn(II) as central ion as described here.

Experimental

Chemicals

L-Alanine (*L*-Ala) was of biochemical reagent grade from the Institute of Shanghai Zhengxiang Chemical Reagents (Shanghai, China). Dns-*DL*-valine (Dns-Val), Dns-*DL*-norvaline (Dns-Nval) and Dns-*DL*-threonine (Dns-Thr) were purchased from Sigma Co. (St. Louis, MO, USA). *α*-Cyclodextrin (*α*-CD) and *β*-CD were from Tokyo Kasei (Tokyo, Japan) and copper(II) sulfate pentahydrate from Kanto Chemical (Tokyo, Japan). Other chemicals were all of analytical reagent grade obtained from Beijing Chemical Work (Beijing, China). The running electrolyte was composed of 20 mmol·L^{-1} ammonium acetate (NH_4OAc) and selectors. Water used to prepare the solution was freshly deionized and distilled with an EASYpre LF water purifier (Barnstead, USA).

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Instrumentation

All CE separations were carried out with a Beckman P/ACE MDQ system (Fullerton, CA, USA) equipped with a photodiode array detection. An uncoated fused-silica capillary (50 μm I.D., 375 μm O.D.) with a length of 57 cm (50 cm to detector) was used throughout (Yongnian Optical Fiber Work, Hebei, China). Data were acquired and processed using P/ACE station software (version 4.0, Beckman Instruments, Fullerton, CA, USA) on an IBM computer. The capillary temperature was maintained by the cooling system of the CE instrument. New capillaries were initially conditioned with methanol for 5 min, followed by H_2O (2 min), 0.1 mol·L^{-1} HCl (5 min), 0.1 mol·L^{-1} NaOH (5 min), H_2O (2 min). Before each separation, the capillary was rinsed with 0.1 mol·L^{-1} NaOH, H_2O and running buffer each for 5 min. The applied voltage is 20 kV. Samples were injected with pressure at 0.5 p.s.i. for 5 s. and the eluent was monitored at 254 nm. Acetone was used to mark the electroosmotic flow (EOF).

Samples were prepared by dissolving the dansyl amino acids in 20 mmol·L^{-1} NH_4OAc solution (pH 4.0) adjusted by acetic acid (HAc) at the concentration range of 0.1—1 mmol·L^{-1}.

Results and discussion

Chiral separation of dansyl amino acids

Chiral separations of Dns-Val, Dns-Nval and Dns-Thr enantiomers using either Mn(II)-*L*-alanine complex or β-CD alone as selector were not satisfactory. As shown in Fig. 1a, no optical resolution of the Dns-AAs was observed in a buffer containing only Mn(II)-*L*-alanine complex. Also, as shown in Fig. 1b, using running electrolyte containing 5 mmol·L^{-1} β-CD and without metal complex, only Dns-*DL*-Nval got partly resolved and the separation of the other two pairs of Dns-AAs could not be carried out. However, when Mn(II)-*L*-alanine complex and β-CD were simultaneously present in electrolyte solution, the chiral separation resolution was greatly improved (Fig. 1c). The combination of Mn(II) complex and β-CD resulted in better selectivity than when using either of them alone. This shows clearly that Mn(II)-*L*-Ala complex and β-CD are cooperated in chiral separation. For any Dns-AAs, the *D*-enantiomers invariably migrated faster than the corresponding *L*-enantiomers. Under our pH condition, the velocity of free Dns-AAs was slower than that of the electroosmosis using acetone as a marker. This suggests that the free Dns-AAs are negatively charged. The formed enantiomer-metal-ligand ternary complexes and the ternary complexes-CD inclusion complex of *L*- or *D*-AAs are neutral and will move faster than the free Dns-AAs. This indicates that the higher the stability of ternary complexes or the stronger interaction of those with the β-CD, the higher the migration velocity of the enantiomer. Therefore, the complex of the *D*-enantiomer will be more stable than *L*-enantiomer; thus, the *D*-enantiomer is eluted faster ($L < D$). The reversal of the migration order of the enantiomers was observed when *D*-Ala was used instead of the *L*-isomer. In the present system, the migration order of Dns-AA enantiomers is thought to be dependent on the stability of the inclusion complex formed between the ternary complexes and β-CD. Changing the chirality of the ligand from *L*-Ala to *D*-Ala changes the stability of the inclusion complex, which leads to an inversion in the migration order of the enantiomers ($D < L$).

Effects of separation conditions

The pH value of the running electrolyte is one of the most important factors in chiral recognition since it directly influences the electroosmotic flow (EOF), the effective charge and the stability of the complex. The effect of pH value on the resolution was investigated over the range of 3.0—6.0 with a running electrolyte containing 2.5 mmol·L^{-1} Mn(II), 5 mmol·L^{-1} *L*-Ala and 5 mmol·L^{-1} β-CD. The pH value of the electrolyte was adjusted by adding 1 mol·L^{-1} acetic acid or ammonia and leads to a great change in the resolution. The optimized pH value for the separation varies for different Dns-AAs. The resolutions of Nval increased with the increasing of pH ranging from 3.0 to 4.0, then apparently decreased above pH = 4.0. The optimum separation condition for these two pair enantiomers was at pH = 4.0. On the other hand, the optimum pH valne for resolution of Val was found to be 4.5. While at this pH value, the other two pairs of enantiomers were somewhat more poorly resolved. pH value of 4.0 was thus considered to be optimum for the separation.

In order to study the effect of capillary temperature on the chiral separation, the electrophoretic experiments were performed in the temperature range of 15 to 35 ℃. As shown in Fig. 2, in the 15 ℃ separation, *D*- and *L*-Thr were nearly resolved, while *L*-Val and *D*-Nval migrated together. Increasing the temperature to 20 ℃ induced a splitting of the *D*-Val and *L*-Nval coelution peak. Further increasing of the temperature to 25 ℃ resulted in a baseline resolution of *D*-Val and *L*-Nval. Although a further increase in the temperature brought about a decrease in the migration time of Dns-AAs, the resolutions decreased apparently and even disappeared when the temperature was increased up to 30 ℃ and then to 35 ℃. A decreased buffer viscosity with increase in separation temperature was the main contributor to the decreased migration times of the analytes. Taking into consideration of resolution and migration time simultaneously, the operation temperature was kept constant at 25 ℃ for further investigation in this work.

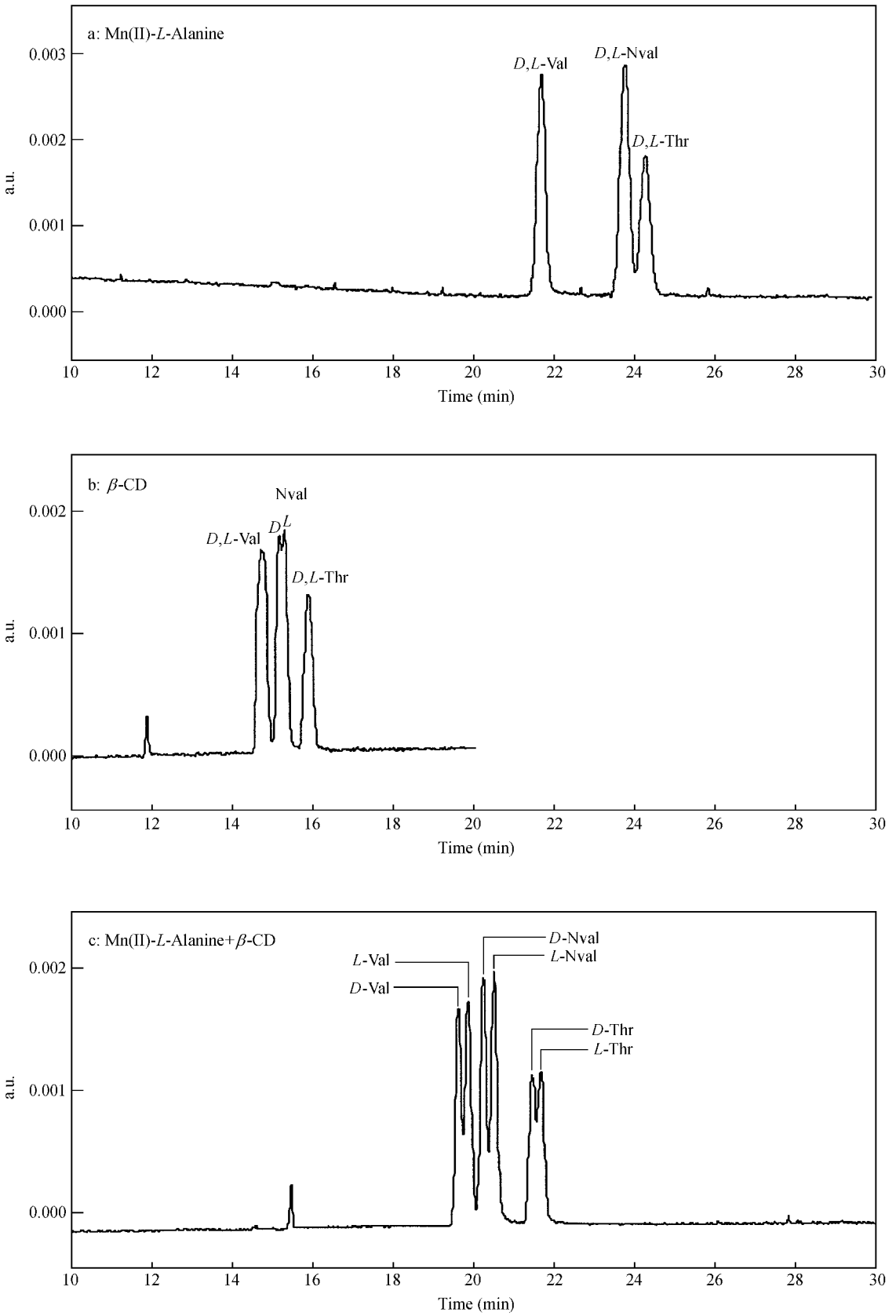

Fig. 1 Chiral separation of Dns-Val, Nval and Thr in the presence of (a) 2.5 mmol·L^{-1} $MnSO_4$, 5 mmol·L^{-1} *L*-alanine; (b) 5 mmol·L^{-1} β-CD; (c) 2.5 mmol·L^{-1} $MnSO_4$, 5 mmol·L^{-1} *L*-alanine and 5 mmol·L^{-1} β-CD. Buffer, 20 mmol·L^{-1} NH_4OAc at pH = 4.0; injection, 0.5 p.s.i. × 5 s; separation voltage, 20 kV (350 V/cm); capillary, 57 cm × 50 μm (I.D.); capillary temperature, 25 ℃.

The effect of concentration of Mn(II) and *L*-Ala on the separation was investigated by altering concentration of Mn(II) and *L*-Ala with a fixed concentration ratio of chelating amino acid to manganese of 2∶1. Fig. 3 shows the electropherogram of the optical resolution of three pairs of Dns-*DL*-AAs using a running solution containing 10 mmol·L^{-1} $MnSO_4$, 20 mmol·L^{-1} *L*-Ala and 5 mmol·L^{-1} β-CD. Near and complete baseline resolutions of the enantiomers were obtained. As expected, chiral separation gradually becomes better with increasing the concentration of Mn(II) complex, however, which caused a prolonged separation time. The migration velocity of Dns-AAs decreased significantly with increase of Mn(II) complex concentration. A general improvement in the resolution and increase of migration time by increasing the amount of Mn(II) complex in the buffer may be due to the stronger complexation between analyte and the complex. The decrease of EOF resulting from the absorbtion of positively charged Mn(II) ion on the inner wall of the capillary may be another reason.

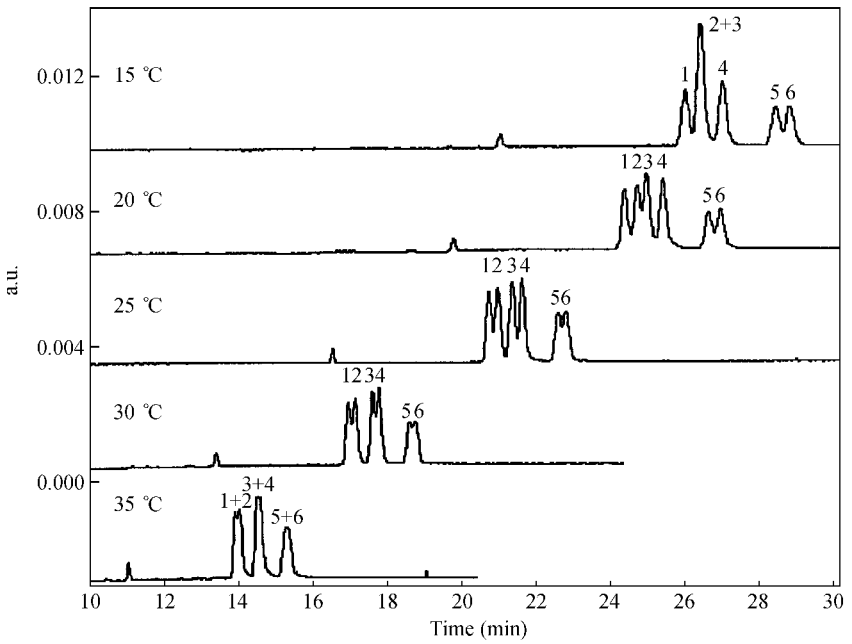

Fig. 2 Effect of capillary temperature on the enantioseparation of three pairs of Dns-amino acids. Running electrolyte solution, 2.5 mmol·L^{-1} $MnSO_4$, 5 mmol·L^{-1} *L*-alanine and 5 mmol·L^{-1} β-CD; capillary temperature, 15—35 ℃; other conditions are the same as in Fig. 1. Peaks: 1, *D*-Val; 2, *L*-Val; 3, *D*-Nval; 4, *L*-Nval; 5, *D*-Thr; 6, *L*-Thr.

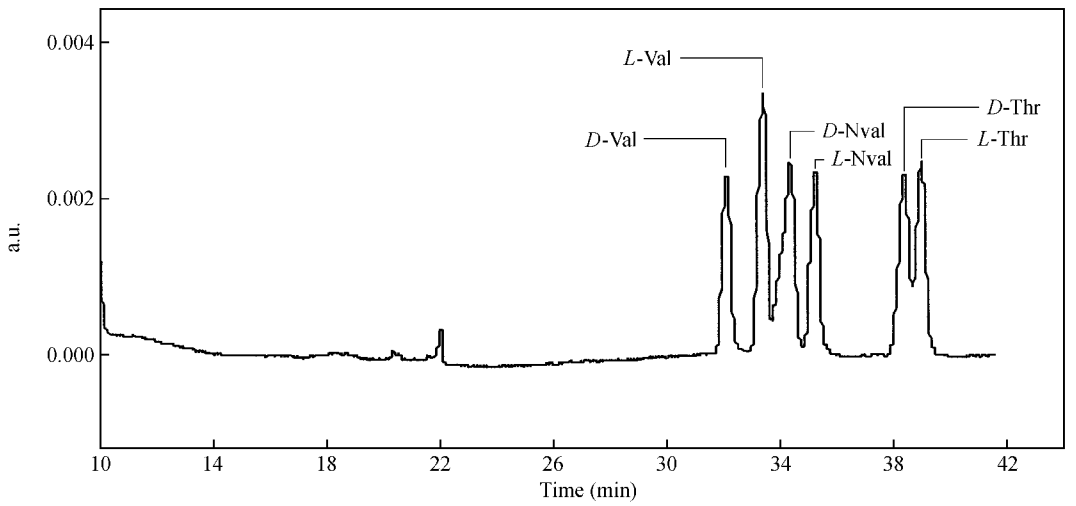

Fig. 3 Electropherogram of the chiral separation of Dns-Val, Nval and Thr in the presence of 10 mmol·L^{-1} $MnSO_4$, 20 mmol·L^{-1} *L*-alanine and 5 mmol·L^{-1} β-CD. Other conditions are the same as in Fig. 1.

Chiral separation using different metal-L-alanine complexes with β-CD

The influence of metal ion on the retention and chiral separation of the Dns-AAs was examined. In particular, Cu(II), Zn(II), Co(II), Ni(II), Hg(II) and Cd(II) were selected due to their large *L*-Ala-Metal(II) complexation constants.[19] Therefore, six different complexes of Cu(II)-*L*-Ala, Zn(II)-*L*-Ala, Co(II)-*L*-Ala, Ni(II)-*L*-Ala, Hg(II)-*L*-Ala and Cd(II)-*L*-Ala were used as the chiral selector for the separation of Dns-AAs in this paper and the results were compared with Mn(II) complex. Table 1 gives the migration times and the relative resolution values for the 3 pairs of Dns-AA enantiomers in the presence of 2.5 mmol·L^{-1} metal ion, 5 mmol·L^{-1} *L*-Ala and 5 mmol·L^{-1} β-CD in 20 mmol·L^{-1} ammonium acetate (pH = 4.0). For a clear explanation, the extent of separation of the two peaks of a racemate is represented by R', which can be defined by $R' = 100(H - H')/H$ and where H and H' are the height of the first peak and that of the valley between the two peaks, respectively.[20] In this definition, the greater R' value corresponds to a better resolution, and a baseline resolution of the two peaks is generally achieved when R' is equal to 100. As it can be seen from Table 1, efficiency was found to vary significantly with the metal ion studied. Using Cu(II), Zn(II), Co(II) or Ni(II) as central ions instead of Mn(II) resulted in a better chiral resolution and Zn(II) yielded the best efficiency and peak symmetry. When Zn(II)-*L*-alanine complex and β-CD were used simultaneously, Dns-Val, Dns-Nval and Dns-Thr could achieved a complete or almost complete baseline resolution; while no resolutions were observed when either of them was absent. Although Co(II) resolved aliphatic Dns-Val and Dns-Nval well, poor chiral recognition was found for the polar Dns-Thr. Interestingly, when Co(II) was substituted for Mn(II), the elution order for Dns-AAs was inverted. These behaviors may be inherent for Co(II). In addition, the elution time for Cu(II) was longer than any other metal ions due to its largest *L*-Ala-Cu(II) stability constant.[19] However, the resolving power of Hg(II) and Cd(II) was poorer than that of Mn(II). Obviously, the difference in retention and selectivity may arise from the properties of the metal ions such as charge to radius ratio, coordination number and structure. The order of the decreasing charge to radius ratio of decreasing metal ion acidity was found to be Ni(II) ≈ Cu(II) ≈ Co(II) > Zn(II) > Cd(II) > Hg(II). Thus, electrostatic bidentate attachment of the carboxylate anion and the sulfonamide anion arising from the induced proton loss from the acidic metal center would seem to play a role in the complexation of these metals. While the Zn(II) chelate exhibited the highest separation of all the metals for the Dns-amino acids. Clearly, the coordination number and structure of the metal play a significant role in the separation. More work is necessary to elucidate in more detail concerning these differences between metal central ions.

Explanation of the effect of mixed selectors for the chiral separation

Using a β-CD-metal(II)-amino acid solution as the running electrolyte, the enantioseparation of Dns-AAs was achieved in this work. No or very bad resolution was observed when either the metal-ligand complex or β-CD was absent in the separation system. The chiral resolution mechanism is considered to involve the ligand-exchange interaction of Dns-AAs with metal-*L*-alanine complexes in combination of a host-guest inclusion complexation of those with β-CD. The separation mechanism of LE is based on the enantioselective complexation between both enantiomers and the metal-ligand complex to form a ternary diastereomer of the enantiomer-metal-ligand complex.[2] This is given by the equations as follows:

$$\mathrm{Mn}(L\text{-S})_2 + D\text{-A} \rightleftharpoons \mathrm{Mn}(L\text{-S})(D\text{-A}) + L\text{-S} \quad (1)$$

$$\mathrm{Mn}(L\text{-S})_2 + L\text{-A} \rightleftharpoons \mathrm{Mn}(L\text{-S})(L\text{-A}) + L\text{-S} \quad (2)$$

Here, S is selector (alanine), and A is analyte (Dns-AAs). According to this mechanism, an enantioseparation occurs only when there is the required difference in stabilities of the enantiomer-metal-ligand complexes for both enantiomers. In the present work, the Dns-AAs enantiomers could not be resolved when using metal-*L*-alanine

Table 1 Chiral separation of Dns-amino acids using different metal-*L*-alanine complex and β-CD as selectors

Metal ion	Dns-Val			Dns-Nval			Dns-Thr		
	t_L (min)	t_D (min)	R'	t_L (min)	t_D (min)	R'	t_L (min)	t_D (min)	R'
Cu^{2+}	26.4	26.8	77.3	29.6	30.1	96.5	32.6	33.0	58.4
Zn^{2+}	24.4	24.8	93.6	25.7	26.4	100	30.0	30.5	82.6
Co^{2+}	23.5	23.9	89.1	21.9	22.4	97.7	20.7	21.0	29.8
Ni^{2+}	20.5	20.8	72.4	22.7	23.3	100	26.8	27.1	60.6
Mn^{2+}	18.3	18.5	50.2	21.3	21.7	85.9	24.8	25.2	40.4
Hg^{2+}	14.3	14.4	23.6	16.9	17.2	84.7	19.6	19.8	5.1
Cd^{2+}	17.0	17.2	24.8	19.1	19.4	74.7	20.7	20.7	0

Running electrolyte: 2.5 mmol·L^{-1} metal ion, 5 mmol·L^{-1} *L*-Ala and 5 mmol·L^{-1} β-CD in 20 mmol·L^{-1} ammonium acetate, pH = 4.0; other conditions are the same as in Fig. 1. R' is relative resolution; t_L and t_D are the migration times of the *L*- and *D*-enantiomers, respectively.

complexes alone as selector. This suggests that these metal cations form kinetically unstable adducts with *L*-alanine ligand[21] and the differences in stabilities of ternary complexes for each enantiomer are very small, which could not be achieved in the absence of β-CD.

β-CD is a neutral cyclic molecule with a hydrophobic interior cavity and a polar exterior containing many chiral centers. It can form inclusion complexes with a large number of analytes. Chiral resolution thus occurs on the basis of differences in the stability of formation of the solute-CD inclusion complex for each enantiomer. None of the Dns-AAs enantiomers could be resolved in the presence of 5 $mmol \cdot L^{-1}$ β-CD alone except Dns-Nval. When β-CD was used as chiral selector mixed with metal-*L*-alanine complexes, it can form an inclusion complex with the ternary complexes of each Dns-AAs enantiomers. The inclusion process involves inclusion of a dansyl group in the hydrophobic cavity and a sulfonylamido and/or COO^- group close contact with hydrophilic rim by forming hydrogen bond with the secondary hydroxy group located on the rim of the CD cavity (Fig. 4). Note that the non-polar dansyl portion of the molecule is found inside the cavity and that the amino group forms hydrogen bonds with the hydroxyl groups at the rim of the toroid. The remaining side chain R of the *L*- and *D*-enantiomer would be located in the proximity of and far from the secondary hydrophilic rim, respectively.[22] The approach of the side chain to the hydrophilic rim is considered to be less favourable for the inclusion because the stereo hindrances occur. Therefore, the inclusion complex of the *D*-enantiomer will be more stable than *L*-enantiomer. When included in a β-CD cavity, the ternary complex is transported towards detection window slower, because of the increase in molecular size. This, therefore, indicates that the stronger interaction with the β-CD, the slower the migration time of the inclusion-complex. Thus, the migration order of Dns-AAs should be *D*-enantiomer before the corresponding *L*-enantiomer. Our experiments clearly agree with these results.

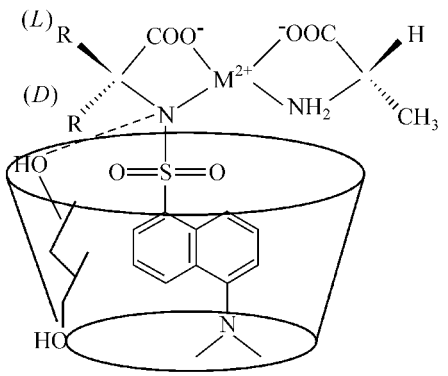

Fig. 4 Schematic diagram of the inclusion complex between β-CD and the ternary complex of Dns-*D*- or *L*-AA with metal(II)-*L*-Ala complex.

When *L*-leucine (Leu), *L*-glutamic acid (Glu) or *L*-tryptophan (Trp) was used instead of *L*-alanine as ligands, no chiral resolution of Dns-AAs was achieved. This suggests that the enantioseparation of amino acids may be related to the properties of the ternary complexes formed by different ligands. The stability of each ternary complex depends on the enantioselective interactions occuring among the chiral ligand, central metal ion and the analyte. An enantioseparation occurs only when there is the required difference in stabilities of the enantiomer-metal-ligand complexes for both enantiomers. Compared with other amino acid ligands, *L*-Ala is a relatively stable coordinator.[19] As a result of it, the ligand-exchange interaction between the analyte and the ternary Cu(*L*-Ala)$_2$ complex can be more effective. The resolution also depends on the size of the cavity of CD. Substitution of α-CD for β-CD, all the test amino acid enantiomers could not be resolved when α-CD was present. This effect was contributed to the cavity size of the CDs. Selectivity or differential complexation of Dns-AAs results from the size of the hydrophobic group with respect to the ability of the solute to penetrate the cavity. Compared with the inner cavity dimension of α-CD (0.57 nm) and β-CD (0.78 nm),[2] the Dns-AAs (0.48 nm) may form more stable inclusion complex with β-CD. The above results indicate that the two mechanisms of ligand-exchange and host-guest inclusion complexation both play crucial role in the chiral recognition process and the enantioseparation may be greatly dependent on the properties of the ternary complexes formed by different metal ions and ligands as well as the property of CD. Further work is continuing on the study of the mechanism for the resolution of Dns-AAs.

Conclusion

In this study, enantioseparation of three different dansyl-*DL*-AAs was achieved by mixture of Mn(II)-*L*-alanine complexes and β-CD; the separation was impossible or not good when either the complexes or β-CD was absent in the running electrolyte solution. The chiral separations of Dns-Val, Dns-Nval and Dns-Thr show that the two interactions of ligand-exchange and host-guest inclusion complexation both play a crucial role in the chiral recognition process. The role of metal ion on separation was examined. Four different transition metal ions, Cu(II)-, Zn(II)-, Co(II)- and Ni(II)-*L*-alanine complexes have been employed and compared with Mn(II) complex. Differences in retention and selectivity were found.

This work clearly illustrates that using metal-ligand complex in combination with β-CD would help to widen the application range of LE-CE on the separation of enantiomers, as well as understand of fine mechanisms of chiral separation better.

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