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Amino Acid Ionic Liquids as Chiral Ligands in Ligand-Exchange Chiral Separations

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Abstract: Recently, amino acid ionic liquids (AAILs) have attracted much research interest. In this paper, we present the first application of AAILs in chiral separation based on the chiral ligand exchange principle. By using 1-alkyl-3-methylimidazolium L-proline (L-Pro) as a chiral ligand coordinated with copper(II), four pairs of underivatized amino acid enantiomers—DL-phenylalanine (DL-Phe), DL-histidine (DL-His), DL-tryptophane (DL-Trp), and DL-tyrosine (DL-Tyr)—were successfully separated in two major chiral separation techniques, HPLC and capillary electrophoresis (CE), with higher enantioselectivity than conventionally used amino acid ligands (resolution (R_s) = 3.26–10.81 for HPLC; R_s = 1.34–4.27 for CE). Interestingly, increasing the alkyl chain length of the AAIL cation remarkably enhanced the enantioselectivity. It was inferred that the alkylmethylimidazolium cations and L-Pro form ion pairs on the surface of the stationary phase or on the inner surface of the capillary. The ternary copper complexes with L-Pro are consequently attached to the support surface, thus inducing an ion-exchange type of retention for the DL-enantiomers. Therefore, the AAIL cation plays an essential role in the separation. This work demonstrates that AAILs are good alternatives to conventional amino acid ligands for ligand-exchange-based chiral separation. It also reveals the tremendous application potential of this new type of task-specific ILs.

Keywords: amino acids · chirality · electrophoresis · ionic liquids · ligand design

Introduction

New discoveries in chemistry, physics, biology, and material science may provide new tools for chemical measurements. A good example is the increasing use of ionic liquids (ILs) in modern analytical chemistry.[1] ILs are a class of organic salts with a melting point close to or below room temperature. They have unique chemical and physical properties, including being air- and moisture-stable, exceedingly low vapor pressure, tunable miscibility with water, a wide electrochemical window, high conductivity, and high heat capacity.[2–4] Because of these properties, ILs have shown tremendous potential in analytical applications. Several reviews have summarized the recent application of ILs in separation and chromatography, spectroscopy, and electrochemistry.[5–13]

In high-performance liquid chromatography (HPLC), ILs have played a variety of roles in facilitating the chromatographic separation either as mobile-phase additives or as stationary phases. The IL mobile-phase additives can dynamically coat the stationary phase and more effectively mask the surface silanols than conventionally used amines. The reason is thought to be the combination of the electrostatic interaction between IL cations and surface silanols and the hydrophobic interaction between IL cations and alkyl chains of the stationary support.[14,15] This feature makes ILs useful for reducing peak tailing and enhancing the resolution of polar analytes.[16,17] Furthermore, ILs can serve as ion-pairing agents for ionized analytes[18] or as ion-exchange-based stationary phases when being covalently linked to the stationary support.[19] These applications have also been expanded to capillary electrophoresis (CE). ILs have manifested some fascinating strengths when being used as background electrolyte (BGE) additives or capillary-wall modifiers in a variety of CE applications, such as improving resolution, reducing wall adsorption, and controlling electroosmotic flow (EOF).[20–26] ILs have also been used to en-

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hance chiral separation with β-cyclodextrin (CD).[27] Conclusively, ILs provide a new approach for the optimization of HPLC and CE separations.

Current research interest in ILs includes the design of ILs with controllable chemical and physical properties or even specific functions (i.e., so-called task-specific ILs).[28,29] One interesting example is the design and synthesis of chiral ILs and their use as chiral solvents for optical resolution and asymmetric synthesis.[30–34] Chiral ILs have also been synthesized and used as chiral selectors in CE.[35–37] Unfortunately, we have found no reports on the application of chiral ILs in HPLC. On the other hand, despite their potential, chiral ILs are not commercially available and their synthesis often requires expensive reagents and complicated synthetic schemes.[30–33] These limitations severely hinder their applications.

Another interesting possibility with task-specific ILs is to introduce natural amino acids into the cations or anions of ILs.[38–43] Amino acids have many advantages including low cost, biocompatibility, and ease of chemically modification. In most cases, amino acid based ILs are also chiral ILs. Amino acids provide a valuable approach for functional design of ILs. Specifically, the term "amino acid ionic liquid", or "AAIL", was coined for the ILs with amino acids as anions; they were first synthesized by the Ohno group using an anion-exchange method.[44,45] Because the amino acids have various functional groups and keep their nature in AAILs,[46] it is possible to design AAILs for a wide range of tasks. For instance, Chen et al. have used AAILs as a support for metal scavenging and heterogeneous catalysis.[47] However, the study of AAILs is still in its infancy, and the current research mainly focuses on their synthesis and characterization.[48–51] To our knowledge, there have been no reports of using AAILs for chromatographic separation or chiral separation.

In this paper, we demonstrate for the first time the application of AAILs in chiral separation. Four underivatized amino acids were used as model analytes. Because the amino acids maintain their nature in AAILs,[46] we performed the separations based on the ligand-exchange principle.[52–54] The L-proline ILs coupled with Cu^{II} were used as chiral selectors because L-proline has been proven to be an outstanding ligand in organocatalysis and organometallic catalysis.[55] Chiral recognition is achieved based on the formation of ternary mixed-metal complexes between the AAIL ligand and the analyte ligand. The different complex stability constants of the mixed complexes with D and L enantiomers result in the enantioseparation of racemic amino acids. The method was validated by means of two major chiral separation techniques, HPLC and CE. In both techniques, AAILs show a significant superiority over the conventionally used amino acid ligands. Specifically, we have made an elaborate comparison between the HPLC and CE results. On the basis of the comparison, the detailed separation mechanisms, including the function of AAIL cation, are discussed in this paper.

Results

Enantioseparations by HPLC with AAILs as chiral ligands: In our HPLC study, the AAILs coupled with Cu^{II} were used as chiral mobile-phase additives. The influencing factors were studied with DL-Phe as a model analyte. Herein, AAILs are used instead of conventionally used amino acid ligands. Our first concern was whether or not the amino acids can still serve as chiral ligands when being made into AAILs and what the function is of the AAIL cation in the separation. Thus, we first performed the separation using AAILs with different alkyl chain lengths in the cation. As shown in Figure 1, good baseline separations of DL-Phe enantiomers are obtained with all the four L-Pro ILs (separation factor (α) and resolution (R_s) > 2.0), thereby indicating that the AAILs are indeed effective chiral ligands. For comparison, we also performed the separation with L-Pro as a chiral ligand under the same conditions. Although the baseline separation is also obtained with L-Pro ($\alpha = 1.82$ and $R_s = 2.70$), its enantioselectivity is evidently poorer than that of L-Pro ILs. Another interesting control experiment is the separation using L-Pro and a common IL, 1-butyl-3-methylimidazolium bromide, [C_4mim][Br], as a binary mobile-phase additive. Surprisingly, the obtained resolution ($R_s = 1.46$) is even lower than that of pure L-Pro. Therefore, just the presence of the [$C_n$mim] cation in the mobile phase does not cause the better separation, and it is essential to combine the [$C_n$mim] cation and L-Pro into AAILs. Notably, by increasing the alkyl chain length of [$C_n$mim] from C_2 to C_8, the enantioselectivity is significantly enhanced (i.e., R_s in-

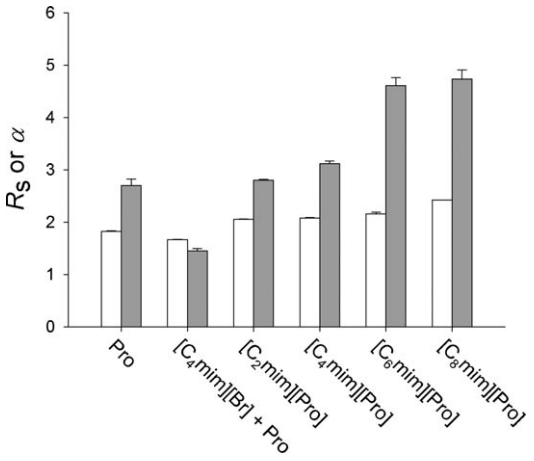


Figure 1. Separation factor (α; shown in white) and resolution (R_s; shown in gray) of DL-Phe for different chiral selectors in HPLC. Pure L-Pro, L-Pro+[C_4mim][Br], and four Pro ILs with different alkyl chain lengths coupled with Cu^{II} were used as chiral mobile-phase additives. Mobile phase: 1 mM chiral mobile-phase additive, 0.5 mM $Cu(Ac)_2$, and MeOH (15% v/v) in water; column: Ultimate XB C18 (150×4.6 mm i.d.); flow rate: 1 mL min^{-1}; temperature: 25 °C; fluorescence detection: $\lambda_{ex}/\lambda_{em}$ = 215 nm/295 nm.

creases from 2.80 to 4.73). The above phenomena indicate that the AAIL cation plays an important role in the separation mechanism. Furthermore, the retention time of analytes also increases with the alkyl chain length. Considering that the enantioselectivity for [C_6mim][Pro] is comparable to that of [C_8mim][Pro] and that it is more difficult to synthesize AAILs with longer alkyl chain lengths because of the larger viscosity, we used [C_6mim][Pro] as a model AAIL in the following studies.

We then investigated the effects of AAIL concentration and eluent pH on the separation. As shown in Figure 2A, both α and R_s increase with an increase of the AAIL concentration. It should be noted that the baseline separation can be achieved even at a very low concentration of AAIL (e.g., 0.25 mM), thus indicating the strong enantioseparation capability of AAILs. Generally, it is desirable to use a low concentration of mobile-phase additive to control the background noise and to minimize the cost. Since the enantioselectivity with 1 mM AAIL is sufficiently high ($\alpha > 2$ and $R_s > 4$), we used 1 mM AAIL in the following studies. Figure 2B depicts the dependence of enantioselectivity on the eluent pH. By increasing the pH from 3.9 to 7.9, both α and R_s increase. For R_s especially, it increases significantly from 0.76 at pH 3.9 to 8.11 at pH 7.9. This phenomenon is similar to that in a previous report.[56] When increasing the pH over 7.9, however, both α and R_s begin to decline. A further, higher pH will yield precipitates in the mobile phase. On the other hand, increasing the pH greatly prolongs the retention time of analytes; for example, at pH 7.9, the retention time (t_R) of L-Phe exceeds 30 min. The peak shape also gets worse at higher pH. Taking the high resolution, short analysis time, and good peak shape into account, we used pH 5.8 to perform the separation.

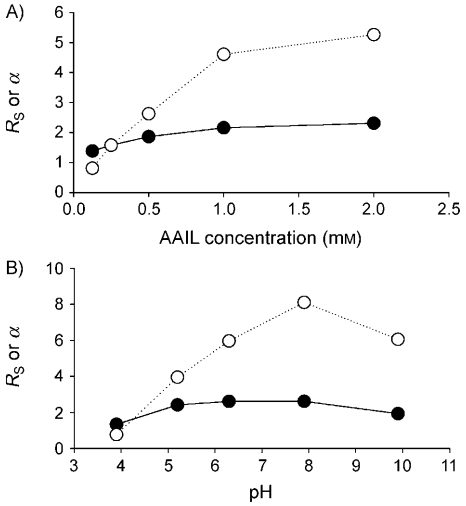


Figure 2. A) Effects of AAIL concentration and B) mobile phase pH on the enantioseparation of DL-Phe in HPLC (●=α, ○=R_s). For A), the concentration of $Cu(Ac)_2$ in the mobile phase was kept at half of AAIL concentration. For B), the mobile phase was 1 mM [C_6mim][Pro], 0.5 mM $Cu(Ac)_2$, and MeOH (15% v/v) in water. The pH was adjusted by an aqueous solution of HCl or NaOH. Other conditions were the same as indicated in Figure 1.

We have also investigated other influencing factors including the concentration of Cu^{II} and organic solvent in the mobile phase. The best molar ratio of AAIL to Cu^{II} was found to be 2:1, which is consistent with the expected value based on the ligand-exchange principle that the tetracoordinated Cu^{II} ions combine with bifunctional solute molecules and bidentate chiral matrix fixed ligands.[52–54] The addition of MeOH does not exert a significant impact on the enantioselectivity. However, it was added into the mobile phase to adjust the retention of analytes on the column. In this study, 15% v/v MeOH was used to tailor conditions for a suitable retention time. Incidentally, we also found that adding 7.5% v/v acetonitrile in the mobile phase can play a similar role.

Figure 3 shows the chromatograms for the enantioseparations of four pairs of amino acid enantiomers in HPLC. The corresponding separation data are listed in Table 1. Undoubtedly, this AAIL-based method shows excellent enantioselectivity for the tested racemic amino acids: α ranges from 1.27 to 2.16, and R_s ranges from 3.26 to 10.81. It should

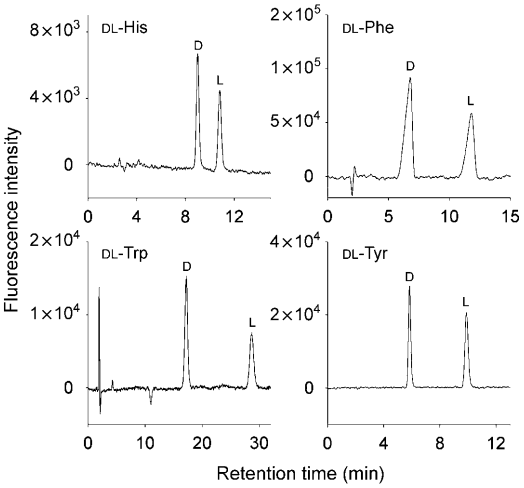


Figure 3. Enantioseparations of DL-His, DL-Phe, DL-Trp, and DL-Tyr by HPLC. Mobile phase: 1 mM [C_6mim][Pro], 0.5 mM $Cu(Ac)_2$, and MeOH (15% v/v) in water (pH 5.8); fluorescence detection: $\lambda_{ex}/\lambda_{em}$=280 nm/348 nm for DL-His, 215 nm/295 nm for DL-Phe, 270 nm/304 nm for DL-Trp, and 216 nm/295 nm for DL-Tyr. Other conditions were the same as indicated in Figure 1.

Table 1. Separation data of HPLC and CE for racemic amino acids.

	HPLC[a]			CE[b]		
	k'_D	α	R_s	t_{mD}[c] [min]	α[d]	R_s
DL-Phe	3.72	2.16	4.61	15.95	1.037	1.48
DL-Trp	9.11	1.74	8.84	16.06	1.080	3.52
DL-Tyr	2.44	1.97	10.81	19.52	1.092	4.27
DL-His	3.97	1.27	3.26	12.39	1.023	1.34

[a] Experimental conditions for HPLC are the same as indicated in Figure 3. [b] Experimental conditions for CE are the same as indicated in Figure 7. [c] t_{mD}=migration time of the D enantiomer. [d] The separation factor in CE separation is defined as the ratio of migration time of L and D enantiomers (i.e., $\alpha = t_{mL}/t_{mD}$).

also be noted that the chromatographic conditions are optimized based on using DL-Phe as the model enantiomers. As the aim of this work is to explore the mechanism of AAIL-based enantioseparation, we did not perform further optimization with respect to each individual racemic amino acid. In spite of that, the obtained enantioselectivities for the other tested racemic amino acids are also satisfactory. To our knowledge, this should be one of the best achievements for enantioseparation of underivatized amino acids by chiral ligand exchange chromatography.[57,58]

Enantioseparations by CE with AAILs as chiral ligands: As another important chiral separation technique, CE is always regarded as complementary to HPLC due to its distinct separation mechanism from HPLC. Thus, we think, it should be interesting to study the same chiral selective system, AAILs–Cu^{II}, in both HPLC and CE. In CE, the AAILs coupled with Cu^{II} were used as both chiral selectors and background electrolytes (BGEs), and thus needed no additional BGEs. This is the first advantage brought by these bifunctional ILs in CE. We first investigated the effect of alkyl chain length of the AAIL cation on the separation. As shown in Figure 4, the R_s is significantly enhanced by the

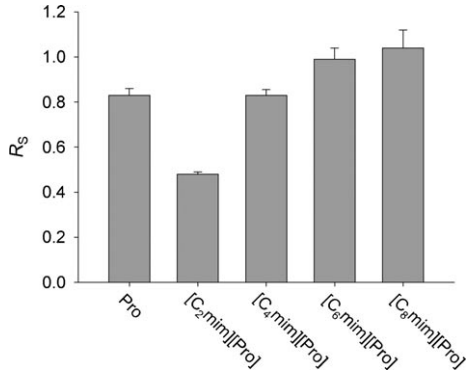


Figure 4. Effect of alkyl chain length of AAIL cation on resolution of DL-Phe in CE. Buffer: 30 mM AAIL, 15 mM $Cu(Ac)_2$, and MeOH (30% v/v) in water; capillary: fused silica of 50 cm total length (40 cm to detector) × 50 μm i.d.; injection: 3.4 kPa for 5 s; detection: 200 nm; applied voltage: 30 kV; temperature: 25 °C.

long alkyl chain in the AAIL cation. This phenomenon is consistent with the result obtained in HPLC, thereby demonstrating again that the AAIL cation plays an important role in the separation mechanism. However, it should be noted that the resolution in CE is lower than that in HPLC. For comparison, L-Pro was also tested as a chiral ligand, and its performance was approximately the same as that of [C_4mim][Pro] and inferior to [C_6mim][Pro] and [C_8mim]-[Pro]. While using L-Pro together with [$C_n$mim][Br] in CE, the electroosmotic flow (EOF) was suppressed thoroughly (ca. $4.3\times10^{-6}\,cm^{-2}V^{-1}s^{-1}$ estimated by using the Williams and Vigh method[59]) and no analytes were detected within 30 min. This distinct phenomenon of L-Pro from L-Pro ILs indicates again that the use of AAILs is not simply equal to the combined use of amino acids and IL cations. As a convenient comparison with HPLC, [C_6mim][Pro] was also selected as a suitable AAIL in the following studies.

Figure 5 depicts the effects of the concentration of AAIL and organic solvent on the separation. It can be seen from Figure 5A that a much higher concentration of AAILs is required to obtain effective enantioseparation in CE than in HPLC. The R_s increases with AAIL concentration until the AAIL concentration reaches 30 mM. At an AAIL concentration over 30 mM, obvious band broadening is observed that compromises the selectivity, thus the R_s begins to decline. For instance, by increasing AAIL concentration from 30 to 50 mM, the efficiency of D- and L-Phe decreases from 83600 and 56000 plates m^{-1} to 75100 and 51200 plates m^{-1}, respectively. As a result, 30 mM was used as the optimal AAIL concentration. Furthermore, it is usually essential to add buffer additives such as sodium dodecylsulfate (SDS) or organic solvents to enhance the enantioselectivity in CE.[60–62] Here, considering that SDS may cause greater background noise, we chose MeOH to adjust the separation selectivity. As shown in Figure 5B, the enantioselectivity for DL-Phe is poor without MeOH in the buffer. Adding MeOH in a concentration no higher than 30% v/v into the buffer remarkably improves the selectivity. At the same time, addition of MeOH greatly prolongs the migration time. At an MeOH concentration over 30% v/v, the rather long migration time aggravates the band broadening and thereby compromises the selectivity. Thus 30% v/v was used as a proper additive concentration. It is worth noting that the organic solvent has exerted a larger effect on the enantioselectivity in CE than in HPLC.

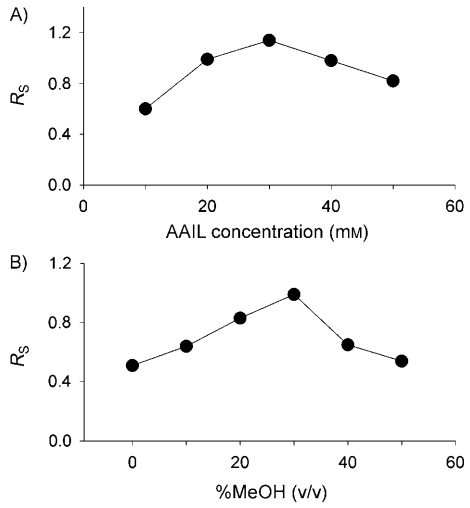


Figure 5. A) Effects of AAIL and B) MeOH concentration on resolution of DL-Phe in CE. For A), the concentration of $Cu(Ac)_2$ in buffer was kept at half of the AAIL concentration. For B), the buffer was 30 mM [C_6mim][Pro], 15 mM $Cu(Ac)_2$ with different percentages of MeOH in water. Other conditions were the same as indicated in Figure 4.

Figure 6 shows the enantioseparation of racemic Phe over a range of buffer pH. The effect of pH in CE is, surprisingly, distinct from that in HPLC: the R_s dramatically decreases as

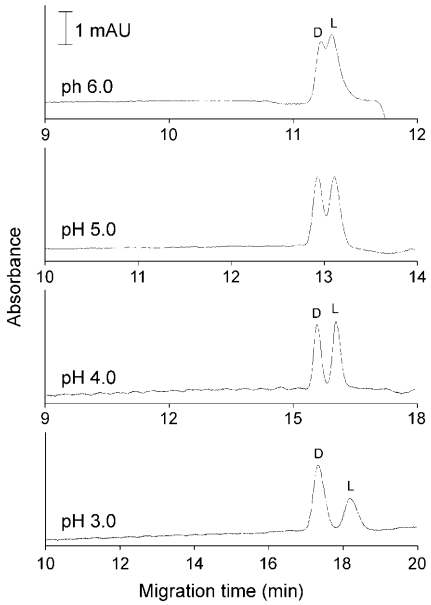


Figure 6. Effect of buffer pH on enantioseparation of DL-Phe in CE. Buffer: 20 mM [C_6mim][Pro], 10 mM $Cu(Ac)_2$, and MeOH (30% v/v) in water. The buffer pH was adjusted by an aqueous solution HCl or NaOH to the desired value. Other conditions were the same as indicated in Figure 4. Note: In the case of pH 3.0, the concentration of D and L enantiomer are 1 and 0.5 mM, respectively, thus indicating that their migration order is D before L.

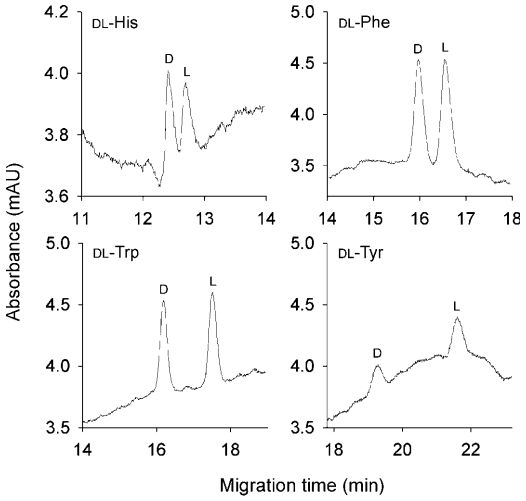


Figure 7. Enantioseparations of DL-His, DL-Phe, DL-Trp, and DL-Tyr by CE. Buffer: 30 mM [C_6mim][Pro], 15 mM $Cu(Ac)_2$, and MeOH (30% v/v) in water (pH 4.0). Other conditions were the same as indicated in Figure 4.

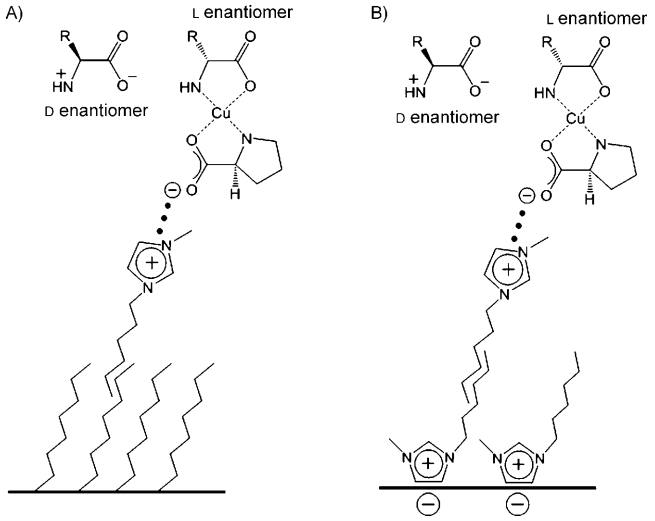
the pH increases from 3.0 to 6.0. When at pH 7.0 or higher, no effective enantioseparation was achieved. In addition, because a much higher concentration of Cu^{II} was used in CE than in HPLC (15 mM vs. 0.5 mM), the precipitates were observed at pH > 7.0 in CE buffer, whereas they were absent even at pH 9.9 in the HPLC mobile phase. On the other hand, lower pH causes longer migration times and lower efficiency, that is, by varying the pH from 5.0 to 3.0, the efficiency of D- and L-Phe decreases rapidly from 148100 and 132500 plates m^{-1} to 26200 and 21900 plates m^{-1}, respectively. To compromise between high enantioselectivity and high efficiency, pH 4.0 was used in CE. Interestingly, the same pH was also used in a previous study that used L-Pro and L-hydroxyproline as chiral ligands.[63]

We have also investigated the effects of Cu^{II} concentration. As expected, the highest enantioselectivity was obtained when the molar ratio of AAIL to Cu^{II} ions was 2:1. Changing the Cu^{II} to Co^{II} shows no enantioselectivity for the racemic Phe, probably because the complexes formed with Co^{II} are too stable.[64] When changing the Cu^{II} to Ni^{II}, the R_s is only 0.29. Zn^{II} was also tested, but it greatly suppressed the EOF[65] and no useful data were obtained. Conclusively, Cu^{II} is the best metal ion to serve as the coordination center. This result is consistent with the previous report using an amino acid–Cu^{II} system.[64,66]

Figure 7 shows the electropherograms of the tested amino acid enantiomers by CE. Their corresponding separation data are given in Table 1. As listed, R_s ranges from 1.34 to 4.27. Although this resolution is somewhat lower than that in HPLC, it is competitive with or even better than the previously reported results for enantioseparation of underivatized amino acids by ligand-exchange CE.[60,61]

Discussion

From the above results, we realize that AAILs are good alternatives as chiral ligands for coordinating with metal ions in ligand-exchange chiral separations. Interestingly, AAILs show different performances in HPLC and CE. This provides a potential approach to gain a better insight into the separation mechanism. It should be noted that the enantioselectivity in HPLC is evidently higher than that in CE, even though a much higher concentration of AAILs was used in CE. This result implies that the separation is facilitated more effectively by different retention of amino acid–Cu^{II} complexes and uncomplexed amino acids in HPLC than by their different electrophoretic mobilities in CE. Notably, the AAIL cation greatly affects the separation in HPLC (as shown in Figure 1): the longer the alkyl chain the AAIL cation has, the better the obtained resolution. Such a significant impact was not expected initially, because the AAILs should be ionized in aqueous solution and their cations should not be involved in the coordination with Cu^{II}. The potential way for AAIL cations to exert such an impact is for the alkylimidazolium cations and L-Pro to form ion pairs on the surface of the stationary support (as illustrated in Scheme 1A). The ternary copper complexes are attached onto the stationary-phase surface by means of the ion-pair formation, thus yielding an ion-exchange type of retention for the DL enantiomers. The enantiomer that has a larger complex stability constant will have a stronger retention on the column. This retention can be enhanced by increasing the alkyl chain length of [$C_n$mim] because of the enhance-



Scheme 1. Hypothetical schemes showing the retention mechanisms of [Cu(AAIL)(L-amino acid)] complexes on A) the HPLC column and B) the capillary wall. The dots between L-Pro and alkylimidazolium cations represent the formation of ion pairs. Note: D-amino acid is shown in an uncomplexed form, but actually it can also form complexes with Cu^{II} and AAIL, albeit with a smaller stability constant than L-amino acid.

ment of the hydrophobic interaction with the C18 column. Therefore, the resolution increases with the alkyl chain length of [$C_n$mim] in HPLC.

This hypothesis is also supported by two other facts: first, the retention time of the enantiomers increases with the alkyl chain length, as the retention of [$C_n$mim] is associated with the retention of the amino acid–Cu^{II} complexes by means of ion-pairing; second, the combined use of L-Pro and [$C_n$mim][Br] yields a poorer separation than [$C_n$mim]-[Pro], for the presence of $[Br]^-$ interferes the formation of ion pairs. Note that the pK_1 (−COOH) of L-Pro is 1.99. At pH above this value, the ion pairing of alkylimidazolium cations and L-Pro is feasible. Furthermore, in all the cases, the D enantiomer is eluted before the L enantiomer (as shown in Figure 3), suggesting that the stability of [Cu(L-Pro IL)(L-amino acid)] is higher than [Cu(L-Pro IL)(D-amino acid)]. This is consistent with the previously reported results upon [Cu(L-Pro)(DL-amino acid)].[67–69]

In CE, the separation is generally driven by different electrophoretic mobilities of amino acid–Cu^{II} complex and uncomplexed amino acid.[70] At pH 4.0, the tested amino acids have net positive charges, and thus they migrate faster than their neutral complexes with Cu^{II}.[63,71] Figure 7 shows that D migrate faster than L enantiomers, thereby indicating that [Cu(L-Pro IL)(L-amino acid)] has a higher stability than [Cu(L-Pro IL)(D-amino acid)], which is in agreement with the results from HPLC. On the other hand, the capillary dynamically coated with alkylimidazolium cations can be regarded as an open-tubular stationary phase.[21] Thus, the alkyl chain length of [$C_n$mim] can also affect the separation, probably by means of the formation of ion pairs of alkylimidazolium cations and L-Pro IL on the surface of the capillary (as illustrated in Scheme 1B). The longer the alkyl chain the AAIL cation has, the stronger the retention the capillary wall causes to the amino acid–Cu^{II} complex, and thus the better the resolution obtained. Similar to that in HPLC, this hypothesis is also supported by the fact that the combined use of L-Pro and [$C_n$mim][Br] does not yield a comparable separation to L-Pro ILs. However, the open-tubular stationary phase should be a small part in the CE separation mechanism. Thus, the alkyl chain length of the AAIL cation exerts a similar but less pronounced impact on the separation than that in HPLC. From the above discussion, we can realize that it is the formation of alkylimidazolium cations and L-Pro ion pairs on the surface of the support that makes the AAILs superior to conventional amino acids in chiral ligand exchange chromatography. However, the AAIL cation plays a less essential role in CE than in HPLC due to their different separation mechanisms. This also is the reason that HPLC shows better enantioselectivity than CE.

It is also interesting to discuss the distinct effects of pH in HPLC and CE. This difference can be readily interpreted based on the above separation mechanisms. In HPLC, increasing the eluent pH makes L-Pro more anionic and thus is favorable to L-Pro interaction with alkylimidazolium cations and the formation of ion pairs on the support surface. Thus, the enantioselectivity increases with the eluent pH ranging from 3.9 to 7.9. A superfluously high pH (i.e., over 7.9) decreases the enantioselectivity probably due to the hydration of Cu^{II} ions and the augmentation of the ternary complex stability.[70] In CE, the separation is mainly dependent on the difference in charge-to-mass ratio between amino acid–Cu^{II} complex and uncomplexed amino acid.[70] Under acidic conditions, the uncomplexed amino acid is more cationic than the neutral amino acid–Cu^{II} complex and thus migrates before the latter.[63,71] Increasing the buffer pH makes the amino acid less cationic and thus minimizes the difference in electrophoretic mobilities between the ternary mixed complex and the uncomplexed amino acid. Therefore, the enantioselectivity deteriorates with an increase in pH.

Conclusion

In this paper, we show for the first time the application of AAILs in chiral separation by HPLC and CE. AAILs coupled with Cu^{II} were used as chiral selectors for the enantioseparation of racemic amino acids based on the chiral ligand exchange principle. In both of the two techniques, AAILs have shown higher enantioselectivity than conventionally used amino acid ligands. This superiority is thought to be related to the ion pairing of alkylimidazolium cations and L-Pro on the surface of the stationary support or capillary wall. The AAIL cation plays a more essential role in HPLC than in CE due to the different separation mechanisms of these two techniques. This study not only shows that AAILs are good ligands for ligand-exchange chiral separation techniques, but also reveals the tremendous application potential of AAILs.

Experimental Section

Chemicals: AAIL 1-alkyl-3-methylimidazolium L-proline salts ([$C_n$mim][Pro]; $n=2$, 4, 6, and 8) were synthesized according to the procedure reported in the literature.[44] Their structure and purity were examined by NMR spectroscopy and mass spectrometry.[46] The detailed synthesis procedure and NMR spectroscopy and mass spectrometry results are given in the Supporting Information. L-Proline (L-Pro), DL-phenylalanine (DL-Phe), DL-histidine (DL-His), DL-tryptophane (DL-Trp), DL-tyrosine (DL-Tyr), and other raw materials for the synthesis of AAILs including *N*-methylimidazole, 1-bromoethane, 1-bromobutane, 1-bromohexane, and 1-bromooctane were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Copper acetate monohydrate ($Cu(Ac)_2{\cdot}H_2O$) was obtained from Damao Reagent (Tianjin, China). HPLC-grade methanol (MeOH) and acetonitrile were purchased from Merck (Darmstadt, Germany). Ultrapure water from a Synergy UV system (Millipore SAS, Molsheim, France) was used throughout. All reagents were of analytical grade unless otherwise noted.

Apparatus: The HPLC experiments were performed using an HPLC system (Shimadzu, Kyoto, Japan) consisting of two LC-20AT pumps, a CTO-10AS VP column oven, and an RF-10A XL fluorescence detector. The system was integrated using a communication bus module (Model CBM-20A) and controlled by a Shimadzu LCSolution workstation. HPLC analysis was performed using an Ultimate XB-C18 column (5 μm, 4.6×150 mm) (Welch Materials, Inc., Ellicott, USA).

The CE experiments were performed using a Beckman–Coulter P/ACE MDQ CE system (Fullerton, CA, USA) with a UV detector working at 200 nm. The data acquisition rate was 8 Hz. Untreated fused-silica capillaries (Reafine Chromatography Ltd., Hebei, China) with a total length of 50 cm (40 cm to the detector), an inner diameter of 50 μm, and an outer diameter of 375 μm were used. All the rinse processes were conducted under a high pressure of 138 kPa. The capillaries were maintained at (25±0.1) °C using a thermostat.

Enantioseparation of racemic amino acids by HPLC: An aqueous solution containing AAILs and $Cu(Ac)_2$ (molar ratio 2:1) was used as an isocratic elution solvent. MeOH (15% v/v) was added into the mobile phase to adjust the retention time of analytes unless otherwise noted. The mobile phase was filtered through a 0.45 μm membrane and degassed by ultrasonication before use. The racemic amino acid samples were prepared in MeOH at 0.1 mg mL^{-1}. Because the tested aromatic amino acids have native fluorescence, fluorescence detection was adopted to reduce the background noise. The $\lambda_{ex}/\lambda_{em}$ for DL-Phe, DL-Trp, DL-Tyr, and DL-His were set at 215/295, 270/304, 216/295, and 280 nm/348 nm, respectively. The enantioselectivity was evaluated by observing the separation factor (α) and resolution (R_s).

Enantioseparation of racemic amino acids by CE: The new capillary was treated with MeOH, 1 M HCl, 1 M NaOH, and buffer in sequence. Between runs, if the buffer was changed, the capillary was refreshed with 1 M NaOH and buffer; otherwise, the capillary was rinsed with buffer for 5 min. The racemic amino acid samples were prepared in water at 1 mM unless otherwise noted. The samples were injected from the anode end of the capillary at 3.4 kPa for 5 s, and then were separated under a constant voltage of 30 kV. The buffer reservoirs were replenished after every three runs. The UV detection was adopted for CE because there was no suitable fluorescence detector for CE that matched the native fluorescence wavelengths of the tested amino acids. The enantioselectivity in CE was evaluated by resolution (R_s).

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