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Short communication

Evaluation of the enantiomeric selectivity in the chiral ligand-exchange chromatography of amino acids by a computational model

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Abstract

The chromatographic resolution of enantiomeric amino acids is accomplished on a reversed phase column using aqueous mobile phase containing the chiral reagent *N*,*N*-dimethyl-*S*-phenylalanine-Cu(II). The separation is a result of the whole interaction between the diastereomeric complex surface and the mixed stationary phase realized by the dynamic coating of the RP-18 carbon chains layer. The elution order seems to be related to the different water coordination capability on copper ion in the formation of the mixed ternary complexes.

Keywords: Enantiomer separation; Computational model; Solvent accessible area; Elution order; Amino acids

1. Introduction

Chiral ligand-exchange chromatography (CLEC) [1,2] is a well established method for resolving a wide variety of racemic mixtures, including free and derivatized amino acids, hydroxy acids, amino alcohols and peptides. Chiral discrimination is achieved by interaction of each enantiomer with a chiral metal complex [usually copper(II)], leading to the formation of diastereomeric complexes of different stability and/or different affinity for the column [3]. We have recently described the useful application of the chiral mobile phase (CMP) CLEC procedure to the analytical resolution of racemic 1-aminoindane-1,5-dicarboxylic acid (AIDA) followed by the preparative separation of its enantiomers [4]. In this case, the practical exploitation we have chosen is due to the early observation that in most instances, ligand exchange in free solution takes place at higher rates compared to the situation in which the chiral selector is chemically bound to the sorbent surface [5], and a considerably improved mass transfer capability in the stationary phase of the CMP approach, with respect to the employment of a chiral stationary phase (CSP) [6], is realized as well. It is worth noting that the elution order observed with AIDA is $k_S < k_R$: this sequence differs from previous reported findings [6] claiming that with the CMP-CLEC procedure and the *N*,*N*-dimethyl-*S*-phenylalanine as the chiral selector, the elution order in a series of amino acids is $k_R < k_S$ in most cases where the aminoacid enantiomers act as bidentate ligands. An exception occurs when an additional coordinative bond from the amino acid molecule is possible (histidine): in this case, the analyte acts as a tridentate ligand and $k_S < k_R$ was observed [6].

As a continuation of our work, we have investigated possible improvements of this important analytical technique focusing our attention, in particular, on the possibility to develop theoretical models able to predict the elution order of diastereomeric complexes formed with racemic amino acids. Certainly, the prediction of the elution order for a given pair of enantiomers on a specific chromatographic system can be a useful complementary method to assess the absolute configuration of new compounds. Studies on chiral recognition of enantiomers in CLEC have been initially formulated by Davankov et al. [7–9], then pursued by many other researchers [10–13] and more recently, reported in two detailed reviews [14,15] describing the theoretical background and recent achievements in CLEC, respectively. Although early observations pointed out on the relative stability of the diastereomeric complexes formed in the mobile phase in order to support the elution order observed [16], more recently it has been claimed that the selective retention is determined

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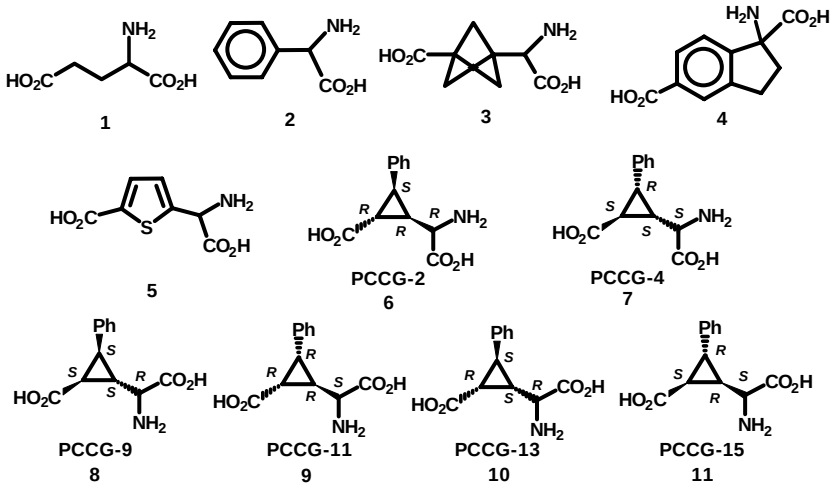

Fig. 1. Structures of the investigated amino acids.

by the relative affinities of the two complexes for the stationary phase [11]. This observation prompted us in the elaboration of a computational model for the two diastereomeric complexes formed with *N*,*N*-dimethyl-*S*-phenylalanine as the chiral selector with the aim to determine the degree of water coordination by calculating the solvent accessible surface area of the coordination centre. According to this theoretical model, the first eluted complex should be the one in which the coordination centre possesses the higher solvent accessible surface area so that two water molecules better accommodate in the formation of an hexadentate assembly thus giving rise to an energetically more stable complex. As a result, the enantio-recognition of the enantiomers will come through the difference in the interaction of the two diastereomeric complexes with the hydrophobic surface of the column packing.

A small set of enantiomeric couples of amino acids (Fig. 1) endowed with different steric and/or electronic properties was then explored in order to test our hypothesis. Table 1 shows the different HPLC factors for glutamic acid (**1**), phenylglycine (**2**), 2-(3′-carboxy-[1.1.1]bicyclopentyl)glycine (CBPG, **3**) [17], AIDA (**4**), and 2-(5′-carboxy-thien-2-yl)glycine (ATIDA, **5**) [18], while Table 2 relates to the three 2-(2′-carboxy-3′-phenyl)cyclopropylglycines pairs [PCCG-2 (**6**) and PCCG-4 (**7**), PCCG-9 (**8**) and PCCG-11 (**9**), PCCG-13 (**10**) and PCCG-15 (**11**) [19] which required the addition of an organic modifier (MeOH) for their elution.

2. Experimental

All reagents were of analytical grade and purchased from Sigma–Aldrich (Milano, Italy). HPLC grade water was obtained from a tandem Milli-Ro/Milli-Q apparatus (Millipore, Bedford, MA, USA).

Table 1
Amino acid (AA) HPLC retention and separation factors obtained with different pH values

AA	pH 3.5				pH 4.0				pH 4.5			
	k_R	k_S	R	α	k_R	k_S	R	α	k_R	k_S	R	α
Glu	0.48	0.59		1.23	1.61	2.39		1.49	2.08	3.51		1.68
Phg	2.11	4.15	7.70	1.97	5.15	16.13	15.38	3.13	8.68	24.12	17.36	2.78
CBPG	0.59	0.87	2.09	1.46	1.67	2.98	5.43	1.78	1.87	4.49	9.39	2.41
AIDA	3.27	2.43	3.79	1.34	12.33	7.76	5.94	1.59	17.17	13.17	4.28	1.35
ATIDA	1.54	1.02	3.64	1.51	6.43	3.65	7.63	1.76	8.63	4.46	7.82	1.94

Table 2
Amino acid (AA) HPLC retention and separation factors obtained with the addition of MeOH

AA	pH 3.5 (5% MeOH)				pH 3.5 (10% MeOH)			
	k_R	k_S	R	α	k_R	k_S	R	α
PCCG (**2, 4**)	54.04	30.5	21.72	1.77	18.4	14.89	10.07	1.23
PCCG (**9, 11**)	5.07	5.69	1.90	1.12	3.44	3.79	1.25	1.10
PCCG (**13, 15**)	36.52	44.43	3.19	1.21	18.57	19.98	1.76	1.07

k_R, k_S: capacity factors; R: resolution factor; α: separation factor.

2.1. Instrumentation

The analytical HPLC measurements were made on a Shimadzu (Kyoto, Japan) LC-Workstation Class LC-10 equipped with a CBM-10A system controller, two LC-10AD high pressure binary gradient delivery systems, a SPD-10A variable-wavelength UV-Vis detector and a Rheodyne 7725i injector (Rheodyne Inc., Cotati, CA, USA) with a 20 μl stainless steel loop.

A LiChrospher 100 RP-18 (Merck, 250 mm × 4.0 mm i.d., 5 μm, 100 Å) analytical column was conditioned by recycling the selected mobile phase for at least 24 h. The mobile phase was prepared by dissolving copper(II)acetate (1 mM) and *N*,*N*-dimethyl-*S*-phenylalanine (2 mM) separately in HPLC-grade water; the latter solution was roughly adjusted to the desired pH with AcOH or NaOH. The solutions were then mixed, filtered through a 0.45 μm Millipore filter (Bedford, MA, USA) and degassed with 10 min sonication; finally, the pH was adjusted and the desired amount of organic modifier added. Analytes were prepared in approximate concentrations between 0.1 and 0.5 mg/ml in filtered mobile phase components and sonicated until completely dissolved. The UV detection wavelengths were set at 254 and 210 nm, and the flow rate was 1.0 ml/min. Injection peak was used for a completely unretained marker in all analyses.

2.2. Molecular modeling studies

The eight diastereomeric couples of copper complexes with *N*,*N*-dimethyl-*S*-phenylalanine and the enantiomeric amino acid were built using the sketch module of Cerius-2 (Accelrys, San Diego, CA). The copper ion was connected to the oxygens and nitrogens of the alpha aminoacidic groups along the direction of their lone pairs so that five atoms (O and N of the selector, Cu, O and N of the analyte) defined a plane of coordination. Two water molecules were axially inserted above and below the plane of coordination (V and VI position of copper coordination) by bonding their oxygens to the copper ion with the only exception of *R*- and *S*-histidine where only one water molecule was inserted. Indeed, in both isomers of histidine the 3-nitrogen of the imidazolic ring was bonded along its lone pair to the copper atom [16]. Atomic charges of the complexes were calculated using the charge-equilibration method [20]. Each complex was minimized using the Universal Force-Field v.1.2 [21] and the smart minimizer protocol of the open force field module (OFF) until the overall atomic root mean square displacement and the energy difference were below 0.003 Å and 0.001 kcal/mol, respectively. In all the studied complexes the conformation of the chiral selector was set in such a way to maximize the hydrophobic surface on one side of the complex, thus representing the putative side of interaction with the stationary phase. Before proceeding to the calculation of the atomic solvent accessible surfaces, the water molecules were deleted from the optimized complexes, the atoms belonging to the plane of coordination were constrained to their respective atomic coordinates to preserve the obtained geometry of coordination, and the complexes minimized again as described above. During this latter minimization protocol, the solvent was implicitly considered by setting a dielectric constant of 80. Where possible, the conformational space of isomers connected to the complex was explored using the grid search module of Cerius-2. Consequently, the global minimum conformation obtained for each complex was stored and minimized again using a dielectric constant of 80. Atomic solvent accessible surfaces were calculated using the Connolly methodology. In particular, the computation was performed by rolling a sphere of radius 1.4 Å around each complex and calculating the copper ion area exposed to the solvent (Table 3).

Table 3
Solvent accessible surface area of the diastereomeric complexes with *N*,*N*-dimethyl-*S*-phenylalanine

	Geom.	Accessible surface area		Surface area (VdW)	
		Cu (Å^2)	Total (Å^2)	Polar	Apolar
R-Glu	*t*	6.252	328.783	213.2	398.3
S-Glu	*c*	5.733	330.389	216.7	319.9
R-Phg	*t*	2.042	326.546	120.0	489.0
S-Phg	*c*	1.922	322.343	119.9	478.4
R-His	*t*	1.496	308.453	171.0	402.5
S-His	*c*	1.785	310.559	162.2	392.0
R-CBPG	*t*	3.263	353.013	204.4	430.7
S-CBPG	*c*	1.926	340.756	208.1	385.8
R-AIDA	*t*	2.487	377.346	213.2	398.3
S-AIDA	*c*	5.559	379.771	195.8	491.3
R-ATIDA	*c*	1.923	351.230	225.9	406.0
S-ATIDA	*t*	4.094	353.306	232.5	413.4
PCCG-2	*t*	5.470	404.982	177.3	538.2
PCCG-4	*c*	7.206	397.732	192.2	530.5
PCCG-9	*t*	5.498	395.471	187.4	533.9
PCCG-11	*c*	4.913	393.824	182.5	536.1
PCCG-13	*t*	5.424	397.064	154.4	543.5
PCCG-15	*c*	5.170	399.047	151.5	537.7

3. Results and discussion

The chromatographic profile of the amino acid examined is reported in Tables 1 and 2. Mobile phase recycling (2 l) provides for an effective dynamic coating of the C-18 column surface, thus giving rise to a mixed stationary phase in which lipophilic aromatic moieties besides the aliphatic carbon chains have to be considered in the mechanism of retention. The copper(II) diastereomeric complexes formed in solution with the aminoacidic groups in a *trans* disposition on the plane, normally show an elution order in which the heterochiral complex elutes before the homochiral one: this behaviour reflects the possibility for the complex formed with a chiral *S*-selector to better accommodate the two axial water molecules in the transoid

arrangement (*S*, *R*-complex) than in the cisoid disposition (*S*, *S*-complex). Hence, the resulting heterochiral complex is more polar and elutes first. In the presence of a tridentate ligand (histidine) [6] the reversal of the elution order ($k_S < k_R$) comes through a more polar cisoid, homochiral complex.

Among the analyzed racemic mixtures: glutamic acid (**1**); phenylglycine (**2**), CBPG (**3**), PCCG-9 and PCCG-11 (**8** and **9**), PCCG-13 and PCCG-15 (**10** and **11**) confirmed the normal elution order ($k_R < k_S$) (Tables 1 and 2). Instead, an opposite elution order ($k_S < k_R$) has been observed with AIDA (**4**), ATIDA (**5**), and PCCG-2 and PCCG-4 (**6** and **7**). With ATIDA (**5**), the non-conventional elution order is only apparent: the priority in the absolute configuration assignment is driven by the presence of the sulfur atom. In this case, the *R*-enantiomer corresponds to the L-isomer and the observed elution order ($k_S < k_R$) reflects the same sequence experienced by common amino acids with the same spatial arrangements of the substituents around the aminoacidic centre where $k_R < k_S$ takes place.

A real inversion in the elution order is present with AIDA (**4**) and the couple PCCG-2 and PCCG-4 (**6** and **7**). In order to explain this unusual behaviour, we have hypothesized a crucial role played by the solvent accessible area on copper ion reflecting its ability of coordinating water molecules in the two axial positions. Accordingly, we have calculated both total and partial accessible surface area with the Connolly methodology, and both polar and apolar Van der Waals surface area for each diastereomeric complex. Table 3 reports the results of this computation along with the geometry of the complex: transoid (*t*) or heterochiral and cisoid (*c*) or homochiral. The analysis of these surface area values suggests that the polarity of the diastereomeric complex correlates well only with the accessible surface area on copper ion. Since the insertion of water in the formation of the hexadentate complex clearly increases the polarity of the analyte then the accessible surface area for the axial water coordination on copper ion will contribute to the control of the overall hydrophobic–hydrophilic balance, and in particular, to the interaction of one side of the diastereomeric complex with the hydrophobic surface of the stationary phase. Moreover, the presence of a couple of methyl groups on the aminoacidic nitrogen of the selector in addition to its phenyl moiety strongly affects the interaction with the stationary phase. In fact, when the fixed orientation of both methyl groups and phenyl ring imposed by the chirality of the selector joins the alternate handedness of the analyte, an extensionally different hydrophobic surface is realized in the two resulting diastereomeric complexes formed by the planar coupling of the four aminoacidic functions with the copper ion. The resulting final molecular shape, realized with the axial addition of water, gives rise to different interactions with the above described mixed surface area of the column resulting in a different retention time for the two complexes.

In the specific case of AIDA (**4**), PCCG-2 and PCCG-4 (**6** and **7**), the cisoid, homochiral complex displays a more accessible area on copper ion then the transoid one. Hence, two water molecules are allowed to better coordinate the copper ion thus giving rise to a more hydrophilic complex which elutes first.

4. Conclusion

With the CMP-CLEC procedure and the *N*,*N*-dimethyl-*S*-phenylalanine as the chiral selector we succeeded in resolving difficult amino acid racemates such as AIDA (**4**) and ATIDA (**5**). The functional groups displayed by the chiral selector strongly affect the mechanism of chiral recognition in the CMP mode. The elution order seems to be related to the different water coordination capability on copper ion in the formation of the mixed ternary complexes. In fact, it is independent from both the amount of total water accessible surface area (Connolly surface) and Van der Waals total surface area, while seems to be strictly correlated with the solvent accessible area on copper ion. Further investigations into the enantio-separation of these and other amino acids are underway in our laboratory and will be reported in due course.

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