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Computational studies for the elucidation of the enantiomer elution order of amino acids in chiral ligand-exchange chromatography

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ABSTRACT

Owing to the exceptional sophistication of chiral ligand-exchange chromatography (CLEC) systems operating in the presence of chiral mobile phase (CMP) additives, only few studies dealing with mechanistic investigations have been presented so far. Nevertheless, dedicated computational protocols applied to simplified models, can furnish valuable information on the factors that mainly affect the overall enantiorecognition event. Accordingly, the extraordinary accordance observed between quantum mechanical (QM) calculations and crystallographic data led us to use optimized ternary complexes carrying the chiral selector O-benzyl-(*S*)-serine [(*S*)-OBS], as starting structures to build up a computational model enabling to explain the enantiomer elution order of amino acids with this enantioresolving agent. As a result of the calculation of 113 three-dimensional descriptors on the mixed complexes, and the generation of a decision tree, the delta-Energy of solvation (delta-E_{sol}) was found to correctly classify all the compounds of the training set (20 species) according to the relative chromatographic behaviour. Thus, as a rule of thumb, the diastereomeric couples having a delta-E_{sol} value lower than 5.321 kcal/mol (splitting node) experienced a "canonical" enantiomer elution order while an opposite situation occurred for all the others (reversed elution profile). The profitable predictive power of the developed model was assessed on the selected test set (5 species).

1. Introduction

The enantiorecognition mechanism in chiral mobile phase (CMP) systems is widely recognized as a very complex matter to treat. The problem is particularly intricate as far as chiral ligand-exchange chromatography (CLEC) environments are concerned. The occurrence of a vast assortment of complexation equilibria involving the central ion and one or more chelating species (*viz* the chiral selector and/or the analyte enantiomer) [1–6] can be invoked to account for this difficulty of rationalization. Additionally, in the case of chiral selectors endowed with a hydrophobic portion, their dynamic adsorption (coating) onto the alkyl chains of the commonly employed reversed-phase (RP) packings, needs also to be taken into account when mechanistic investigations are pursued.

In connection with the crescent interest towards the "chiral HPLC" approach both at the analytical and semi-preparative-scale [7,8], to rely upon reliable computational protocols able to predict the enantiomer elution order can be of aid when pure enantiomeric forms are not available. In all these cases, basing on the elution order of structurally related species could lead to the misassignment of the absolute configuration. The actual risk of an incorrect attribution is particularly amplified in CMP-CLEC systems where even slight modifications of the physico-chemical character of the analyte or the mobile phase composition can turn into a completely different chromatographic behaviour.

In spite of the relevant contributions dealing with the computational prediction of enantiomeric selectivity in chromatography [9–11], a scanty appeal was instead exerted by such settings. The assumption that the enantioselective retention is sensitively ruled by the relative affinities of the two ternary complexes for the stationary phase when moderately high concentrations of chiral selector in the eluent are used, led us to elaborate a theoretical model enabling the rationalization of the enantiomer elution order in the presence of the N,N-dimethyl-(*S*)-phenylalanine [(*S*)-DMP] as the CMP discriminating agent [12]. With the awareness of the significant simplification made on the considered system, we however found for a small set of amino acid enantiomeric couples, an interesting correlation between the enantiomer elution order and the different water coordination capability on copper ion in the formation of the mixed ternary complexes.

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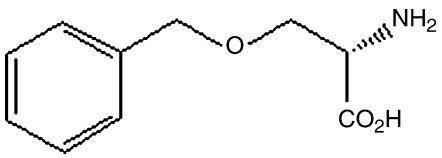

Fig. 1. Structure of O-benzyl-(*S*)-serine ((*S*)-OBS).

In the present study dealing with the use of the O-benzyl-(*S*)-serine [(*S*)-OBS] (Fig. 1) as an alternative CMP selector [13] for the separation and resolution of racemic amino acids, focused efforts were spent to shed light on the network of interactions and perturbations that can play a relevant role in the enantioselective retention with this CLEC system. Accordingly, through the selection of two sets of amino acids (namely a training and a test set of respectively 20 and 5 enantiomer couples) (Table 1) and the adoption of a computational protocol never employed in the CLEC domain, intriguing results able to furnish a deeper insight into the molecular basis of the enantiomer elution order with the (*S*)-OBS turned out.

2. Experimental

2.1. Chemicals

The enantiomer couples of 3a,5,6,6a-tetrahydro-4H-pyrrolo[3,4-d]isoxazole-3,4-dicarboxylic acid (CIP-A) and 3a,5,6,6a-tetrahydro-4H-pyrrolo[3,4-d]isoxazole-3,6-dicarboxylic acid (CIP-B) [14] were kindly provided by Prof. M. De Amici. The enantiomers of the remaining compounds [that is, 1-aminoindane-1,5-dicarboxylic acid (AIDA) [15], 2-(5′-carboxy-thien-2′-yl)glycine (ATIDA), 2-(5-carboxy-3-methyl-2-thienyl)glycine (3-MATIDA) [16], 2-(5-carboxy-4-methyl-2-thienyl)glycine (4-MATIDA), the two 2-(2′-carboxy-3′-phenyl)cyclopropylglycine (PCCG) pairs [17] and the two 2-(2′-tetrahydrofuranyl)glycines (THFGs) pairs [18] were synthesized in our laboratories. All the remaining compounds were purchased from Sigma–Aldrich (Milano, Italy). HPLC grade water was obtained from a tandem Milli-Ro/Milli-Q apparatus (Millipore, Bedford, MA, USA). Analytes were prepared in approximate concentrations between 0.1 and 0.5 mg/mL in filtered mobile phase components and sonicated until completely dissolved.

Table 1
Investigated amino acid and relative experimental enantiomer elution order obtained with (*S*)-OBS as the chiral selector. The membership set is also specified.

Amino acid	Elution order		Membership set	
	Canonical	Reversed	Training	Test
3-MATIDA	•		•	
4-MATIDA	•		•	
AlloIle	•		•	
a-THFG	•		•	
b-THFG	•		•	
CIP-A	•		•	
Ile	•		•	
Met	•		•	
NorLeu	•		•	
NorVal	•		•	
PCCG-13/15	•		•	
Phe	•		•	
Phg	•		•	
Pro	•		•	
Tyr	•		•	
Val	•		•	
AlloThr		•	•	
His		•	•	
PCCG-2/4		•	•	
Thr		•	•	
ATIDA	•			•
CIP-B	•			•
Leu	•			•
AIDA		•		•
Cys		•		•

2.2. Mobile phase preparation and instrumentation

The mobile phase as well as the instrumentation employed for the analytical runs was the same as in Ref. [19].

2.3. Computational methods

A dataset of 25 amino acidic enantiomer couples was collected and splitted into two classes on the basis of their chromatographic behaviour. More specifically, while class 1 comprised all amino acids showing the "canonical" elution order, class 2 included those experiencing the reversal elution profile. Ternary complexes encompassing the amino acid enantiomer, the central copper(II) and the chiral discriminating agent (*S*)-OBS were designed using Maestro 9.0 [20] and geometrically optimized in gas-phase using Macromodel 9.7 [21] and the OPLS-2005 force field [22]. In order to get a more accurate assessment of the final energy and geometry, each resulting complex was further optimized in gas-phase using quantum mechanical (QM) calculations with Jaguar 7.6 [23], the DFT-B3LYP level of theory and the 6-31G** basis-set. The approximation of the self-consistent field (SCF) was set at the ultra-fine accuracy level. During all these calculations, a formal charge of +2 and a spin multiplicity of 2 were assigned to each complex. The resulting QM optimized conformation was instrumental for the calculation of 112 3D-descriptors included in the MOE software version 2008.10 [24]. In particular, the default all-atom MMFF94x force field [25] was used for the computation of the potential energy descriptors. With the aim of statistically selecting consistent training and test set compounds belonging to both class 1 and class 2, a principal components analysis (PCA) [26] was carried out on the above collection of 3D descriptors plus QM energy values. Finally, a decision tree was developed to classify compounds of the training set according to their elution order. In the decision tree, data are organized into nodes along branches. Nodes are questions that are posed incrementally on independent variables to split the training set into its classes of target property. In this study, the independent variables were defined as the difference between the value of each 3D descriptor calculated on the complex carrying the (*S*)-enantiomer and that of the ternary assembly containing the (*R*)-enantiomer. The resulting decision tree was then used to classify compounds of the test set in order to statistically validate the predicting power of the model. While the "quest" method was selected to construct the decision tree, a maximum number of split that could yield a terminal node (maximum tree depth) was set to a value of 5, the significance level to a value of 5% and the number of intervals to a value of 10. A cross-validation analysis was carried out to assess the robustness of the model. This analysis consisted in a leave-one-out procedure on the training set and the ensuing construction of 20 additional decision trees. All the statistical analyses were carried out using the statistical package XLSTAT2010.4 [27].

3. Results and discussion

In two previous works [13,19] we demonstrated the (*S*)-OBS (Fig. 1) performing as a profitable CMP-CLEC selector for the enantioseparation of physico-chemically different natural and unnatural amino acids. Moreover, it was there remarked its contemporary presence in both chromatographic phases of the usually employed reversed-phase (RP) environments, being ascribed to the co-existence, in the molecule, of an aromatic lipophilic side-chain

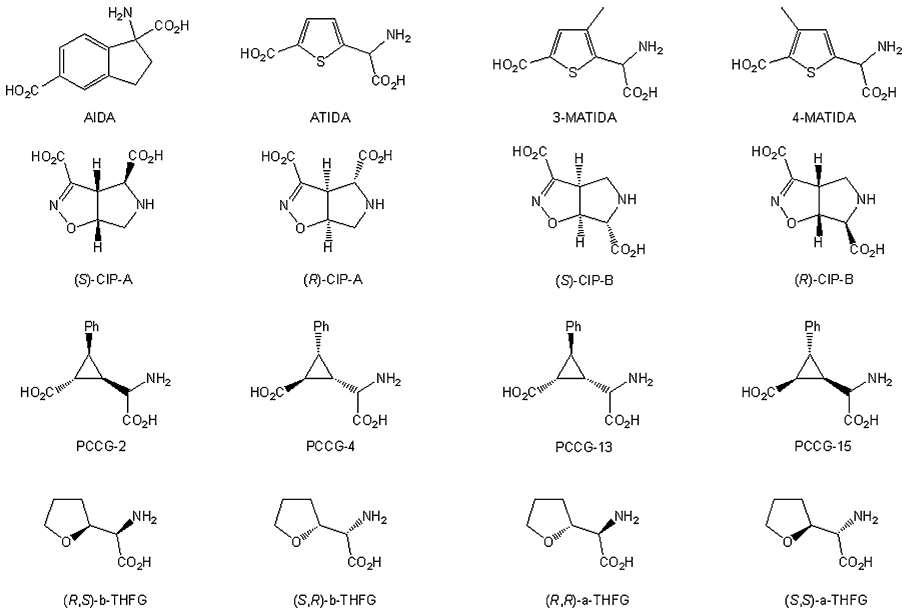

Fig. 2. Structures of the synthesized investigated amino acid analytes.

residual (namely the benzyl portion) and a hydrophilic underivatized amino acidic moiety connected through an ether spacer. As a result of the network of complexation equilibria that takes place in both chromatographic phases, a comprehensive identification of all the forces that drive the enantiomer elution order comes to be particularly challenging when such a type of CMP setting is concerned.

Accordingly, for the present study dealing with the use of the (*S*)-OBS as the enantiodiscriminating agent, we performed the construction of decision tree models [10,28,29] to explain the observed enantiomer elution order of a pool of selected amino acids. The experimental dataset was formed by 25 enantiomer couples (Table 1) displaying two different chromatographic behaviours: the majority (19 out of 25) of tested enantiomer couples underwent the "canonical" $k_R < k_S$ elution order, while few pairs (6 out of 25) experienced a "reversed" $k_R > k_S$ elution profile. For 3-MATIDA, 4-MATIDA, ATIDA and Cys, the priority in the absolute configuration assignment based on the Cahn–Ingold–Prelog rules is driven by the presence of the S atom. In these cases, the (*R*)-enantiomer corresponds to the L-isomer and, for the first three compounds, the observed $k_R > k_S$ elution profile reflects the same sequence experienced by the canonically classified species. Conversely, for Cys, the observed $k_R < k_S$ elution profile corresponds to a reversed chromatographic behaviour. In Fig. 2, the synthesized compounds employed in the study are shown.

The ternary complexes were modelled and optimized in their global minimum conformation using high-level QM calculations as detailed in Section 2. In agreement with crystallographic data, the result of the QM optimizations showed in most cases a planar tetracoordination of Cu(II), in which the amino and carboxylate groups of the two chelators were equatorially arranged around the metal ion in alternate fashion ["glycine-like (gly-like)" interaction] [30]. In Fig. 3A the exemplary case of the (*R*)-Val/Cu(II)/(*S*)-OBS ternary complex is shown. Only two compounds, namely cysteine (Cys) and histidine (His), behaved as tridentate ligands (Fig. 3B and C, respectively), with a further functional group being axially linked to the metal ion.

The chelation behaviour of His was deeply investigated by several authors. As a confirmation of the reliability of the executed computational geometry optimization, QM results of the complexes carrying His residue were found to be in strict accordance with the determined X-ray "diamine- (or histamine-)" type structure [31]. In analogy to His, also the tridentate chelation ability of Cys (with the amino acidic function being located equatorially) was described for a series of metal ions [32]. The robustness of the QM computational approach led us to use the optimized complexes as starting structures to build up a computational model to explain the enantiomer elution order. Initially, we evaluated the possibility to explain the observed elution behaviour solely on the basis of the different energetic value between the complexes of diastereomeric couples. In particular, we correlated the delta QM energy, calculated as the difference between the energy of the complex bearing the (*S*)-isomer and that of the assembly containing its specular (*R*), with the experimental elution order. As a result, we did not observe such a correlation for the following species: Allo-Thr, ATIDA, Met, Ile, Leu, PCCG-2/4, Phe, b-THFG and Thr. This finding confirms that the thermodynamic stereoselectivity plays a relevant but not exclusive role in the CMP-CLEC enantiorecognition process [1,3,33]. Therefore, in order to reduce the number of outliers species, we tested a conspicuous pool (112) of 3D descriptors calculated by MOE software

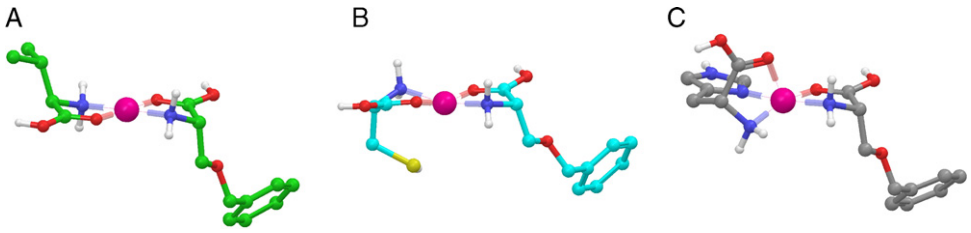

Fig. 3. 3D models for the (a) (*S*)-OBS/Cu(II)/(*R*)-Val, (b) (*S*)-OBS/Cu(II)/(*R*)-Cys, and (c) (*S*)-OBS/Cu(II)/(*R*)-His optimized complexes. The central sphere in each complex represents the cupric cation. Only polar hydrogens are visualized.

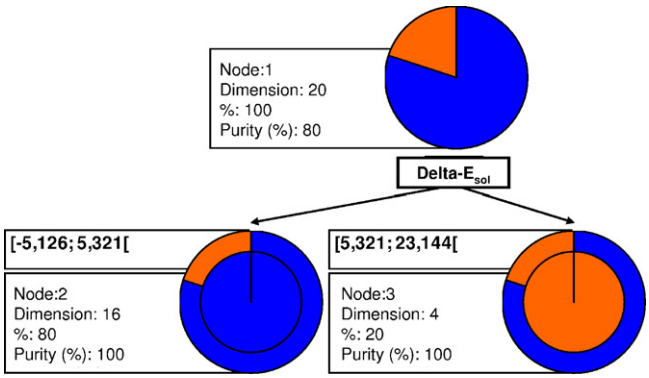

Fig. 4. Decision tree model. Classification of the training set compounds according to the relative chromatographic behaviour through the delta-Energy of solvation (delta-E_{sol}) descriptor. For each node, the cut-off value (in bold square brackets), the number of objects (Dimension, %) and the within class homogeneity (Purity), are specified.

on the optimized complexes. Computed QM energy values of the ternary complexes were also used in addition to these descriptors, leading to a total of 113 variables.

With the intent of properly selecting the training and test sets, a PCA was carried out on the 113 descriptors and the ternary complexes. The sum of the two principal components (PC1 and PC2) was able to encode for the 57% of the information that differentiates the investigated compounds. By plotting PC1 (*x*-axis) and PC2 (*y*-axis), a test set of five enantiomeric couples of amino acids was defined, sampling the four quadrants. In particular, we selected three couples displaying a canonical elution profile (ATIDA, CIP-B and Leu) and two ones following the reversed elution order (AIDA and Cys). The remaining 20 species composed the training set (Table 1). The ratio between the number of species of the training and test sets was defined in agreement with the conventional rules of statistics [34].

Assuming the concept that, for every enantiomeric pair, the elution order contemporarily refers to both diastereomeric complexes, we deemed as more productive to consider for each descriptor the difference between the values calculated on the assemblies containing the (*S*)-enantiomer and the (*R*)-enantiomer, respectively. The collection of values achieved in this way was then employed to build a decision tree [10,28] and identify the discriminating descriptors. As a result, the delta-Energy of solvation (delta-E_{sol}), which was selected by the decision tree algorithm, was found to correctly classify all the compounds of the training set according to the relative chromatographic behaviour (Fig. 4). This descriptor, in particular, defines the difference of the energy of solvation between the complexes of diastereomeric couples.

The splitting node of delta-E_{sol} was identified as equal to 5.321 kcal/mol. Thus, as a rule of thumb, the diastereomeric couples having a delta-E_{sol} value lower than the above cut-off experienced a $k_R < k_S$ enantiomer elution order while an opposite situation occurred for all the others ($k_R > k_S$). In order to evaluate the internal robustness of the model, a cross-validation protocol was performed using a leave-one-out procedure on the training set. In all of the resulting 20 runs, the descriptor selected by the decision tree was always the delta-E_{sol}, sustaining the statistical significance of this descriptor in explaining the elution order of the analytes. Likewise, the average value and standard deviation of the splitting-node were 5.495 ± 1.085 kcal/mol, being in agreement with the value of 5.321 kcal/mol found in the original model. Moreover, the inspection of the Pearson matrix of correlation among the descriptors showed a maximum r^2 value of 0.68 for the delta-E_{sol}, thus evidencing a lack of correlation with this descriptor.

Interestingly, all the models from the leave-one-out procedure showed the AIDA as the only misclassified compound of the test set. Collectively, the cross-validation analysis confirmed the results of the original decision tree.

The selection of the delta-E_{sol} descriptor perfectly fits with the assumption by Davankov et al. [33] about an active (and in some cases decisive) participation of achiral molecular structures (in this case the "carpet" of C-18 chains) in the enantioselective process. These authors clearly stated that the exclusive consideration of the association energy within two diastereomeric adducts may led to consider erroneous enantioselectivity profiles if solvation and adsorbing events are ignored. The selection of the delta-E_{sol} descriptor as the splitting node highlighted an outstanding role of the conformational and configurational dependent difference of the solvation energy between diastereomers in driving the enantiorecognition event. Indeed, for each pair of diastereomers, the measure of the difference of the hydrophobic/hydrophilic balance and, in turn, of the relative solvation, determines their differential partitioning between the mixed stationary phase and the water-based eluent.

In order to assess the predictive power of this model, we then used the selected test set. The results showed promising, with the only misclassified specie being AIDA. On the basis of the above reflections, the incorrect prediction for AIDA enantiomers can be plausibly ascribed to the structural rigidity of the molecule coupled with the lack of the otherwise present amino acid α-hydrogen: in this case, these elements produce an underestimation of the delta-E_{sol} descriptor (2.467 kcal/mol) with the consequent, resulting misclassification.

The reversed elution order of His and Cys can be conceivably demanded to the possibility of an adjunctive axial coordination by the analyte side-chain, which produces a signifying difference in the physico-chemical properties of the relative ternary diastereomeric complexes.

In accordance with the literature data, both Allo-Thr and Thr were QM optimized as gly-like coordinants [35,36]. Thus, their reversed elution order can be tentatively demanded to the very intriguing polymeric structures observed for the relative Cu(II)A_2 systems [35]. Accordingly, a multimodal connection of adjacent mixed complexes was fully described for such type of aggregates which reflects into peculiar solvation profiles.

The possibility to undergo a stereochemically dependent polymerization and, in turn, unusual solvation mechanisms, can be also hypothesized for the constrained analogues of glutamic acid (Glu), PCCGs [17]. Indeed, in analogy to the OH group in Thr and Allo-Thr, the distal COOH function of PCCGs is a substituent of an asymmetric carbon. However, it can be likely supposed the controversial elution behaviour of the two PCCG couples (that is $k_R > k_S$ for PCCG 2/4 and $k_R < k_S$ for PCCG 13/15) being a consequence of the fixed geometry imposed by the cyclopropyl ring leading to a different conformation (which means different solvation) of the resulting diastereomeric ternary complexes. Moreover, the spatial flexibility of the distal, sp3-linked COOH group is a distinctive feature of PCCGs with respect to the constrained distal, sp2-linked carboxylic moieties in all the other acidic amino acids (namely, AIDA, ATIDA, CIP-A, CIP-B, 3-MATIDA and 4-MATIDA). In this connection, a multimodal coordination capability (both bidentate and tridentate) of the ternary complexes [37] carrying the PCCG enantiomers can be presumed. However, the gly-like coordination was preferred for the calculation of the 3D descriptors being the same experimentally observed for Glu/Cu(II) complexes [38] and found to be profitable for the developed predictive model.

4. Conclusions

Dedicated molecular modelling protocols have been fruitfully engaged to get new insights into the main features governing the

mechanism of enantiorecognition in CMP-CLEC settings. Here we have showed the profitable use of a decision tree model for explaining the observed enantiomer elution order of a pool of selected physico-chemically diverse amino acids in the presence of the (*S*)-OBS as the chiral dopant into the eluent. The computational model was performed after the calculation of 113 3D descriptors on quantum mechanically optimized enantiomer analyte/Cu(II)/chiral selector complexes. As a result, the delta-E_{sol} was selected as the suitable descriptor for correctly classifying the submitted species according to the relative chromatographic behaviour. This methodology can be of aid when stereochemically known enantiomeric forms are not available, thus reducing the risk of an incorrect attribution of the absolute configuration on the basis of the chromatographic behaviour of structurally related species.

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