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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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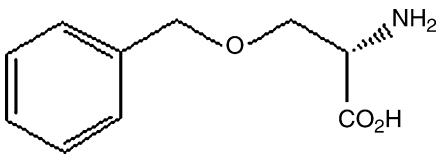

Fig. 1. Structure of O-benzyl-(*S*)-serine ((*S*)-OBS).

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