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Octaproline, a conformationally flexible chiral selector in liquid chromatographic enantioseparation

Arnau Novell[a,b], Cristina Minguillón[a,b,*]

[a] Food and Nutrition Torribera Campus, University of Barcelona, Prat de la Riba 171, E-08921-Santa Coloma de Gramenet, Barcelona, Spain
[b] Pharmaceutical Chemistry Laboratory, Faculty of Pharmacy, University of Barcelona, E-08028 Barcelona, Spain

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ABSTRACT

A proline octapeptide-derived chiral selector (CS) end-capped using a pivaloyl group was covalently linked to a silica gel chromatographic matrix by the C-terminal group. The chromatographic behaviour of the resulting chiral stationary phase (CSP) using different conditions was compared to those containing 3,5-dimethylphenylcarbamate residues on the proline units. An enantioseparation ability highly dependent on the mobile phase used is observed for these CSPs. When mixtures of alkane/alcohol or alkane/ether are used as mobile phase a similar enantioselectivity is obtained. Nevertheless, in the presence of chlorinated solvents, and without a hydrogen bonding donor in the mobile phase, enantioselectivity is extremely reduced. The reversibility of this phenomenon, attributed to a conformational change in the CS, is examined.

1. Introduction

One of the most striking and recent novelties in liquid chromatographic enantioseparation is the recognition of the outstanding and, in some way, unexpected enantioselectivity provided by oligopolyproline-derived chiral stationary phases (CSPs). Oligoproline-derived CSPs, which were firstly introduced by Li and co-workers [1], share some features in common with brush-type CSPs. The most remarkable is the relatively low-molecular weight of the chiral selector (CS). However, some chemical differences, namely low functionality and lack of aromatic rings in the CS, together with a broader selectivity [2,3], seems to point to the fact that we are facing a new class of peptide-based CSPs, as it is recognized by some authors [4].

In our previous studies on oligoproline we have compared CSPs containing as CS a single proline moiety, having a 3,5-dimethylphenylcarbamoyloxy group in the pyrrolidine ring, with those containing an oligoproline analogously derivatized [5,6]. Derivatization was introduced with the aim to provide the CS with more interaction sites able to act in the recognition of solutes, and also to introduce some analogy with the polysaccharide derivatives most used for enantioseparation purposes (3,5-dimethylphenylcarbamates of amylose and cellulose). However, the enantioseparation of aromatic-ring-containing racemates clearly demonstrated that the derivatized oligoproline CS was able to resolve compounds containing aromatic rings with the same electronic character (π-donor) than those on the CS, thus questioning the role of the derivatization. Nevertheless, it was made evident the broader application domain of oligoproline derivatives *vs* single proline derived CSPs, as well as an interesting higher loading capacity. Considering the similar density of proline units on the studied mono/oligoproline CSPs, these observations could only be explained by the presence of polyproline helical structures able to interact several at the same time with the analyte in the recognition process.

The former substituted oligoproline derived CSPs also showed a particular behaviour depending on the solvent used in the mobile phase. Concretely, enantioselectivity was reversibly lost when chloroform 100% or mixtures of this solvent with heptane, were used as mobile phase. These changes in enantioselectivity could be attributed to the known conformational change from PPII [7] (PolyProline II has all amide groups in *trans* configuration) to PPI [8] (PolyProline I has all amide groups in *cis* configuration) helical structures or any intermediate situations. It is known that PPII helices dominate in most solvent systems while PPI are

* Corresponding author at: Food and Nutrition Torribera Campus, University of Barcelona, Verdaguer Building, Office No. 12, Prat de la Riba 171, E-08921-Santa Coloma de Gramenet, Barcelona, Spain. Tel.: +34 93 403 37 87.
E-mail addresses: cminguillon@ub.edu, cristina.minguillon@gmail.com (C. Minguillón).

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only favoured in some solvents [9]. The reversible conformational change from one conformation to the other takes in the order of hours to be completed, much longer than conventional conditioning of the chromatographic column in normal phase conditions.

The goal of this work is to study this phenomenon, its consequences on chromatographic enantioselectivity, and its extensibility to non-substituted oligoproline-CS. With this aim, an octaproline peptide was synthetized and bonded to a chromatographic matrix. The chromatographic behaviour of the resulting non-substituted oligoproline-derived CSP was compared to that of a CSP containing 3,5-dimethylphenylcarbamate residues on proline units (Fig. 1) using different chromatographic conditions. The enantioseparation ability resulted to be highly dependent on the mobile phase in the two cases. This phenomenon is proved to be reversible and consistent with a conformational change of the CS.

2. Experimental

2.1. Abbreviations

ACN, acetonitrile; ClTrR-Cl, 2-chlorotrityl chloride resin; DCM, dichloromethane; DEA, diethylamine; DIC, diisopropylcarbodiimide; DIEA, N,N-diisopropylethylamine; DMF, N,N-dimethylformamide; EEDQ, 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline; EtOH, ethanol; L-Fmoc-Pro-OH, 9-fluorenylmethoxycarbonyl-L-proline; HBTU, O-benzotriazole-N,N,N′,N′-tetramethyl-uronium-hexafluoro-phosphate; HMDS, hexamethyldisilazane; HOBt, 1-hydroxybenzotriazole; MeOH, methanol; MTBE, methyl t-butyl ether; TFA, trifluoroacetic acid; Tma, trimethylacetic acid.

2.2. General supplies and equipment

ClTrR-Cl (100–200 mesh, 1% DVB, 1.3 mmol/g, NovaBiochem, Läufeldingen, Switzerland) was used as a supporting resin in solid phase synthesis. Chemicals and solvents were purchased from Aldrich (Steinheim, Germany), NovaBiochem (Darmstadt, Germany) and Panreac (Barcelona, Spain).

Elemental analyses were performed by the Scientific and Technological Centres (University of Barcelona) using a Thermo Organic Elemental Analyzer, Flash 2000. MALDI-TOF Mass spectrometry was performed on a 4700 Proteomic analyzer (Applied Biosystems). The scaled-up synthesis was performed on an automatic peptide synthesizer Abi 433 A (Applied Biosystems). The CSP was packed into stainless-steel tubes (100 mm × 4.6 mm id) by the slurry method.

The chromatographic assays were carried out on a Waters HPLC system (Mildford, MA, USA) consisting of 1525 binary HPLC pump, 717plus autosampler and a 2467 dual λ UV Absorbance Detector. Retention factor (k) was calculated as $(t_r - t_0)/t_0$ being t_r the retention time and t_0 the void time. The enantioseparation factor (α) was calculated as the ratio of the retention factors for the two enantiomers. The void volume (t_0) was determined with 1,3,5-tri-*tert*-butylbenzene. Flow rate was fixed at 1 mL/min and UV detection was either 220 nm or 254 nm, depending on the analyte used. Analytes **1**–**12** and **22**–**24** were synthetized in our laboratory by conventional procedures and spectroscopically characterized. The elution order of enantiomers was determined by injecting the enantiomerically pure compound when it was available. **CSP-2** was prepared as reported in a previous study [5].

2.3. Synthesis of the octaproline CS

Analogously to what is described [1] (Fig. 2), to ClTrR-Cl (100–200 mesh, 506 mg, 1.30 mmol/g) washed and pre-swelled with DCM (5 mL × 1 min ×4) the mixture of L-Fmoc-Pro-OH (129 mg, 0.38 mmol) and DIEA (133 μL, 0.76 mmol) in 5 mL of DCM was added. Additional 399 μL (2.28 mmol) of DIEA was added after 3 min and the mixture was allowed to react for 75 min. The unreacted 2-chlorotrityl chloride groups on the resin were end-capped by the treatment with 405 μL of MeOH (30 min). Washings with DMF (5 mL ×4) and DCM (5 mL ×4) were performed. The Fmoc-protecting group was removed by treating the resin with 4 mL of 20% (v/v) piperidine in DMF for 5 min. This operation was repeated two more times (10 + 10 min). The 9-methylenefluorene produced was collected and quantified by UV absorption at 290 nm. The actual functionalization attained on the resin was determined to be 0.59 mmol/g [10].

The mixture of L-Fmoc-Pro-OH (308 mg, 0.91 mmol), HOBt (138 mg, 0.91 mmol) and DIC (140 μL, 0.91 mmol) in 3 mL of anhydrous DMF was added to the ClTrR-Pro-H resin prepared above. After stirring for 2 h, the resin was filtered and washed with DMF (5 mL ×4) and DCM (5 mL ×4). The Fmoc group was then removed with piperidine. The next six amino acids were coupled following exactly the same procedure for coupling/deprotection. The successive coupling of Pro units was periodically controlled on the basis of 9-methylenefluorene UV determination after Fmoc cleavage.

Finally, pivalic acid (93 mg, 0.91 mmol) was coupled to the ClTrR-Pro_8-H using HOBt (138 mg, 0.91 mmol) and DIC (140 μL, 0.91 mmol) as coupling agents. The free peptide was cleaved from the resin by the treatment with 3% (v/v) TFA in DCM (5 mL ×5). The liquors obtained from this treatment were collected and co-evaporated several times with DCM (25 mL). The resulting oily residue was precipitated in diethyl ether and collected by centrifugation. The solid was dissolved in ACN/water (50:50) and finally lyophilized. The purity of the resulting peptide was checked by HPLC (k: 3,1; Column: Symmetry® C18, 5 μm, 150 mm × 4.6 mm id.; mobile phase: linear gradient from 0 to 100% ACN (0.04% in TFA) in 15 min; Flow rate: 1 mL/min, λ: 214 nm) and characterized by MALDI-TOF MS (m/z: [M+Na] 901.46, [M+K] 917.43).

When the synthesis was performed in an automatic synthesizer HBTU and DIEA were used instead of DIC and HOBt as coupling agents.

2.4. Preparation of *CSP-1*

To a solution of HO-Pro_8-Tma (400 mg, 0.45 mmol) in 40 mL of DCM, EEDQ (240 mg, 0.94 mmol) and γ-aminopropylsilica gel (2 g), obtained from spherical silica gel (5 μm, 100 Å, Kromasil) following the conventional procedure [11,12] (Elemental analysis: C, 4.48%; H, 1.20%; N, 1.45%), were added. The suspension was allowed to react overnight at room temperature. The resulting bonded silica gel was collected by filtration, washed exhaustively with DCM and toluene (elemental analysis: C, 11.20%; H, 1.98%; N, 2.96%, 235 μmol CS/g CSP or 1.9 mmol Proline/g CSP, calculated on the basis of nitrogen percentage). The solid material was suspended in toluene (10 mL) and HMDS (1 mL) was added. The mixture was allowed to react for 1 h at reflux temperature. The resulting modified silica gel was collected by filtration, washed exhaustively and consecutively with toluene, DCM, EtOH and water until neutral washing liquors were obtained, EtOH, acetone and diethyl ether, and dried in vacuum at room temperature [5] (elemental analysis: C, 11.84%; H, 2.07%; N, 2.70%).

3. Results and discussion

Given their ready availability as enantiomerically pure compounds, many amino acids have been used as chiral moieties in Pirkle-type CSPs [13]. Among these, L-proline constitutes a particularity from the structural point of view. Proline is the only proteinogenic amino acid provided with a secondary amino group.