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The effect of urea moiety in amino acid binding by β-cyclodextrin derivatives: A 1000-fold increase in efficacy comparing to native β-cyclodextrin

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ABSTRACT

Water soluble amphiphilic anion receptors based on urea-substituted β-cyclodextrin were synthesized via a copper(I) mediated azide-alkyne coupling reaction. The synthetic route was designed to minimize the number of operations of cyclodextrins. Stable products were obtained in 90% yield. They were successfully tested as amino acid receptors, showing excellent affinity constants (10^3–10^4 M^{-1}) in a highly competitive environment (pH 8 phosphate-buffered water solution). Isothermal titration calorimetry indicated that complex formation strongly depends on the hydrophobic nature of the guest and that the urea moiety of the receptor is necessary to efficiently bind amino acids.

1. Introduction

Molecular recognition of biological molecules is a broadly studied and important area of scientific interest. In particular, investigating selective interactions of amino acids or peptides is very important in order to understand and mimic the mechanisms of biological processes (Schneider, 2009). The utilization of hydrogen bond donors in the field of molecular recognition is a commonly developed area of scientific interest (Amendola, Fabbrizzi, & Mosca, 2010; Busschaert, Caltagirone, Van Rossom, & Gale, 2015; Choi & Hamilton, 2003). Such donors provide short-contact, relatively strong and spatially oriented interactions with negatively charged or polarized guests, allowing for selective and structurally-related binding. However, this approach is often limited by the competing environment, in which the role of the solvent cannot be neglected (Castronuovo, Elia, Pierro, & Velleca, 1999). As such, the recognition employing hydrogen bond donating groups is executed mainly in mixed solvents, where water's hydrogen bond network is shared with other polar cosolvents (e.g. acetone, acetonitrile, DMSO) (Caltagirone et al., 2008; Cametti & Rissanen, 2009; Dutta, Bose, & Ghosh, 2013).

In this study we sought to provide a water soluble compartment in which hydrogen bond donors are isolated from the solvent, so their interactions with hydrophobic L-amino acids (as model amphiphilic carboxylates) should be enhanced. For this purpose we chose β-cyclodextrin (β-CD) as a well-defined, water-soluble, amphiphilic vessel, that could be selectively modified. β-CD has a concave structure, presenting its polar hydroxy groups to the outside, while the cavity remains hydrophobic. Although several types of similar cavities have been previously tested (Dziemidowicz, Witt, & Rachoń, 2008; Mutihac, Lee, Kim, & Vicens, 2011; Richard et al., 2008; Stone, Franz, & Lebrilla, 2002) only cucurbiturils gave satisfactory (»1000 M^{-1}) affinities with amino acids (Logsdon, Schardon, Ramalingam, Kwee, & Urbach, 2011; Rajgariah & Urbach, 2008). On the other hand, underivatized CDs bind amino acids rather poorly, with K_a's below 100 M^{-1} (Cooper & MacNicol, 1978; Matsuyama, El-Gizawy, & Perrin, 1987; Rekharsky, Schwarz, Tewari, & Goldberg, 1994). Therefore, we decided to incorporate additional hydrogen bond donors in the proximity of the cavity in order to form an isolated amphiphilic environment for the recognition of amino acids. We anticipated that a urea group, having two points of interaction, would serve as a binding site efficient enough that the modification

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of just one of the 21 hydroxy groups of β-CD would be necessary to provide well defined receptors.

We hypothesized that a decorating β-CD with a side-chain having both hydrophilic (urea) and lipophilic (phenyl and isobutyl) moieties will give an appropriate environment for efficient binding of amino acids, providing a receptor far more effective than underivatized β-CD.

Having such a tool in hand the recognition or separation of amino acids would not have to be based on repetitive processes (as in chromatography or electrophoresis) but a single interaction would be efficient enough for molecular discrimination of particular pairs of substances.

2. Experimental

2.1. General

All solvents were used as received, unless stated otherwise. Purification of products was performed using chromatography on silica gel (Merck Kieselgel 60, 230–400 mesh) with mixtures of hexane/ethyl acetate, methanol/dichloromethane, acetonitrile/water/ammonia or using gel filtration on styrene resin (Diaion® HP-20) with water/methanol gradient. Thin-layer chromatography (TLC) was performed on silica gel plates (Merck Kieselgel $60F_{254}$). Visualization of the developed chromatogram was accomplished using UV light or ninhydrin and cerium molybdate stains. Reported NMR spectra were recorded in $CDCl_3$ or $(CD_3)_2SO$ using a Varian Unity Plus 200 MHz and Agilent 300 MHz and 500 MHz spectrometers. Chemical shifts of ^1H NMR and ^{13}C NMR are reported as δ values relative to TMS (δ = 0.00) and $CDCl_3$ (δ = 77.0) or $(CD_3)_2SO$ (δ = 39.5), respectively. The following abbreviations are used to indicate the multiplicity: s – singlet; d – doublet; t – triplet; q – quartet; m – multiplet; dm – doublet of multiplets. Mass spectra were measured on a Shimadzu LCMS-IT-TOF using ESI technique.

Distilled water (>18 MΩ/cm grade) was supplied by Mili-Q water system. Phosphate buffers were prepared by mixing specified amounts of $NaH_2PO_4{\cdot}2H_2O$ and $Na_2HPO_4{\cdot}H_2O$ and subsequent titration with NaOH, to desired pH, using Elmetron CP-401 pH-meter.

Dialysis was performed using Spectrum Labs Biotech Grade Cellulose Ester Dialysis Membrane, MWCO: 100–500 Da, preserved with 0.05% sodium azide.

2.2. Synthesis

2.2.1. Mono-2-propargyl-β-CD (*1*)

To a solution of dry β-CD (10 g, 10 mmol) in DMSO (100 mL) LiH (1 equiv.) was added and stirred overnight, until the solution became clear. Next, propargyl bromide (1 equiv.) and LiI (a few mg) were added and the mixture was stirred at 55 °C for 5 h, avoiding light exposure. The solvent was evaporated under reduced pressure, resulting slurry was dissolved in water (20 mL) and precipitated with acetone (500 mL). Solid residue was filtered, air-dried and then the resulting powder was dissolved in water and passed through styrene resin (Diaion® HP-20) using gradient of water/methanol (0–10%) as solvent. Prior to use, the resin was conditioned in methanol for 1 h and rinsed with water. Fractions containing pure product were evaporated under reduced pressure giving 4.1 g of **1** (3.5 mmol, 35%). COSY, TOCSY, HSQC and HMBC NMR spectra of **1** are in Appendix A.

^{1}HNMR (500 MHz, DMSO) δ 5.80 (br s, 11H), 4.99 (d, *J* = 3.7 Hz, 1H), 4.82 (br s, 6H), 4.47 (dd, *J* = 15.8 Hz, *J* = 2.4 Hz, 1H), 4.46 (br s, 9H), 4.39 (dd, *J* = 15.8 Hz, *J* = 2.4 Hz, 1H), 3.79 (t, *J* = 9.2 Hz, 1H), 3.65–3.55 (m, 27H), 3.52 (t, *J* = 2.4 Hz, 1H), 3.43–3.40 (m, 2H), 3.37–3.27 (m).

^{13}C NMR (75 MHz, DMSO): δ 102.0–101.0, 100.1, 82.2–81.5, 79.9, 79.1, 77.8, 73.2–71.7, 72.6, 60.0–59.7, 58.7.

HRMS ESI+ *m*/*z*: 1195.3756 (MNa^+, $C_{45}H_{72}N_{35}O_3Na^+$ requires 1195.3752).

2.2.2. (S)-O-Tosyl-2-(boc-amino)-4-methylpentanol

N-Boc-L-Leucinol (2.5 g, 11.5 mmol) was dissolved in dichloromethane (100 mL) and cooled to 0 °C. Triethylamine (1.1 equiv.) and *p*-tosyl chloride (1.05 equiv.) were added and the resulting mixture was stirred overnight (allowing the mixture to warm up to rt). It was next washed with saturated sodium bicarbonate (100 mL) and brine (20 mL). Organic layer was then dried with $MgSO_4$ and evaporated under reduced pressure. The product was purified on silica gel using a 10:90 ethyl acetate:hexane mixture resulting with 2.91 g of **2** (7.8 mmol, 68%).

Careful purification of the aminoalcohol **2** before the tosylation process is crucial, as mixed anhydride (formed prior to the reduction step) acts as an inhibitor.

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 8.5 Hz, 2H), 4.70–4.34 (m, 1H), 3.97 (dd, *J* = 14.4, 11.0 Hz, 2H), 2.43 (d, *J* = 9.6 Hz, 3H), 1.64 (s, 2H), 1.50–1.35 (m, 9H), 1.14 (dd, *J* = 8.6, 5.9 Hz, 1H), 1.00–0.70 (m, 6H).

2.2.3. (S)-2-(Boc-amino)-1-azido-4-methylpentane (*3*)

2 (2.35 g, 5.9 mmol) was dissolved in DMF (20 mL) and NaN_3 (3 equiv.) was added. The addition of more than 3 equivs of NaN_3 caused the elimination of the tosylated aminoalcohol due to the basicity of azide ions in DMF. The resulting mixture was stirred at 60 °C for 24 h. It was then poured into water (300 mL) and extracted with diethyl ether. The combined organic layers were then evaporated under reduced pressure. The oily residue was dried under vacuum until it solidified resulting with 2.26 g (4.7 mmol, 80%) of **3**.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.56–4.39 (m, 1H), 3.80 (d, *J* = 4.0 Hz, 2H), 3.54–3.10 (m, 2H), 1.71–1.51 (m, 1H), 1.49–1.40 (m, 9H), 0.92 (dd, *J* = 6.6, 2.2 Hz, 6H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 155.40, 79.75, 70.81, 55.35, 51.03, 48.69, 44.53, 41.48, 28.49, 25.25, 24.88, 23.08, 22.26.

2.2.4. Synthesis of urea 4

(*S*)-2-(Boc-amino)-1-azido-4-methylpentane (600 mg, 2.5 mmol) was dissolved in 1:1 mixture of TFA and dichloromethane (5 mL) and the resulting mixture was stirred for 1 h, after which all volatiles were evaporated. In order to neutralize residual TFA and deprotonate the amine, the solid residue was treated with triethylamine (1 mL), which excess was subsequently evaporated. Dichloromethane (20 mL) was added and the solution was treated with the respective isocyanate (1 equiv., 2.5 mmol). After 24 h of stirring at rt the mixture was washed with saturated sodium bicarbonate (50 mL) and brine (50 mL). The organic layer was dried with $MgSO_4$ and the product was purified on silica gel using a 3:97 mixture of methanol:dichloromethane, yielding, after evaporation and vacuum drying, urea **4**.

2.2.5. (S)-1-(1-Azido-4-methylpentan-2-yl)-3-phenylurea (*4a*)

Yield: 590 mg (2.25 mmol, 90%)

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.49–7.19 (m, 5H), 7.06 (d, *J* = 6.9 Hz, 2H), 4.15–3.93 (m, 1H), 3.42 (ddd, *J* = 37.1, 12.1, 4.0 Hz, 2H), 2.14 (s, 2H), 1.64 (dt, *J* = 13.0, 7.5 Hz, 1H), 0.93 (d, *J* = 6.7 Hz, 6H).

^{13}C NMR (50 MHz, $CDCl_3$) δ 187.87, 138.38, 129.51, 124.06, 121.33, 55.67, 48.48, 41.51, 25.03, 22.35.

HRMS ESI+ *m*/*z*: 262.1609 (MH^+, $C_{13}H_{19}N_5OH^+$ requires 262.1662).