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Mannoside and 1,2-mannobioside β-cyclodextrin-scaffolded NO-photodonors for targeting antibiotic resistant bacteria

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ABSTRACT

Two β-cyclodextrin derivatives randomly appended on the primary face with both the nitric oxide (NO) photodonor 4-nitro-3-(trifluoromethyl)aniline and a mannose or α(1→2)mannobioside residue are reported to construct targeted NO photoreleasing nanocarriers. 2D ROESY and PGSE NMR suggested supramolecular homodimerization in water by inclusion of the nitroaniline group into the facing macrocycle cavities. Isothermal titration calorimetry on their concanavalin A lectin binding showed an exothermic binding event to the lectin and an endothermic process during the dilution of the conjugates. Both α(1→2)mannobioside and the nitroaniline moieties significantly enhanced the binding to the lectin. These effects might arise from a better fit within the carbohydrate-recognition site in the former case and a multivalent effect caused by homodimerization in the latter. Direct detection of NO by amperometric technique shows that both β-cyclodextrin derivatives release this radical upon excitation with visible light with higher efficiency than the unfunctionalized NO photodonor.

1. Introduction

The emergence of pathogenic bacteria that show resistance to diverse antimicrobial active ingredients is a growing global health threat (Magiorakos et al., 2012). Commonly used antimicrobial therapies, such as the administration of antibiotic drugs, fail in providing an efficient treatment due to the ability of bacteria to develop resistance mechanisms to the action of such drugs (Woodford, 2003). Thus, biomedicine faces the demanding challenge of developing new treatment strategies against antibiotic Multi Drug Resistance (MDR) (Cohen, 2000; Laxminarayan et al., 2013; Taubes, 2008). In this regard, a very promising approach is based on the use of nitric oxide (NO) as a very efficient non-conventional antimicrobial and antioxidant. As well, this inorganic free radical has a key therapeutic role in a number of cancer and cardiovascular diseases (Carpenter & Schoenfisch, 2012; Halpenny & Mascharak, 2010). The NO radical presents a mechanism of action that avoids MDR problems, as it is considered a multitarget cytotoxic agent that is able to attack a wide range of biological targets (Szakács, Paterson, Ludwig, Booth-Genthe, & Gottesman, 2006). However, delivering gaseous NO to selected targets is a difficult challenge that has fostered the development of a range of molecular NO donors (Wang et al., 2002, 2005; Riccio & Schoenfish, 2012; Seabra & Durán, 2010). An interesting approach involves the use of biocompatible scaffolds as suitable vehicles able to release NO under light stimuli, namely NO photodonors (NOPDs). These compounds offer the great advantage to deliver NO with high spatiotemporal control, thus favoring reducing side-effects and improving therapeutic outputs (Fraix, Marino, & Sortino, 2016).

Cyclodextrins are cyclomaltooligosaccharides well known for their applications in many different fields, but particularly in the supramolecular and pharmaceutical fields. Such molecules are able to enhance the water solubility, stability, bioavailability and organoleptic properties of a large number of drugs. The doughnut-shaped structures formed by six (α-CD), seven (β-CD) and eight (γ-CD) linked glucoses define an inner cavity of a relatively hydrophobic nature, inside of which a wide range of organic molecules of similar polarity and suitable size and shape can be hosted in aqueous media. Numerous studies carried out in humans and animals have shown that CDs can be very useful to improve drug delivery of therapeutic substances (Cutrone, Casas-Solvas, & Vargas-Berenguel, 2017; Duchêne & Bochot, 2016; Popielec & Loftsson, 2017). The drug delivery strategy can be extended to NO therapy by the construction of photoactivatable CD-NOPD conjugates, either in

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covalent or non-covalent fashion, as delivery systems for the NO radical (Mazzaglia, Sciortino, Kandoth, & Sortino, 2012). Among others, 4-nitro-3-(trifluoromethyl)aniline derivatives are efficient NOPDs and suitable for bio-applications as they show dark stability and adequate absorption coefficient in the visible region, and produce NO radicals without generating toxic or light-absorbing byproducts, or altering environmental pH, temperature or ionic strength (Caruso, Petralia, Conoci, Giuffrida, & Sortino, 2007; Conoci, Petralina, & Sortino, 2006; Di Bari et al., 2016). The chromophore can enter within the cavity of β-CD, where the scarce contact with water molecules dramatically modifies both the light absorption efficiency and the mechanism of photochemical deactivation (Sortino, Giuffrida et al., 2001; Sortino, Marconi, & Condorelli, 2001). Indeed, within the hydrophobic micro-environment of a micelle, this NOPD has shown a remarkable enhancement of NO release efficiency (Di Bari et al., 2016).

The vast majority of viruses, bacteria and cells present on their surfaces a family of proteins called lectins that are able to specifically bind carbohydrates. This fact has allowed the development of drug delivery strategies based on the use of carbohydrates as selective biological vectors (Casas-Solvas & Vargas-Berenguel, 2016; Vargas-Berenguel, Ortega-Caballero, & Casas-Solvas, 2007). Thus, by conjugating a molecular carrier to carefully selected carbohydrates it is possible to transport therapeutics to those cells presenting on their surfaces the complementary saccharide receptor. In particular, oligosaccharidic mannosyl structures, such as mannobioside (α-D-Man-(1→2)-D-Man) and mannotrioside (α-D-Man-(1→2)-α-D-Man-(1→2)-D-Man), are intimately related with the pathogenicity of a good number of viruses and bacteria (Schuster, Vijayakrishnan, & Davis, 2015). For example, mannose-binding lectins (MBLs) such as FimH are displayed on the surface of many bacteria. Furthermore, cells of the immune system present a series of MBLs on their surfaces, such as macrophage-mannose receptors (MMR) on the macrophages and Dendritic Cell-Specific Intercellular Adhesion Molecule-3 (ICAM-3)-Grabbing Non-integrin (DC-SIGN) on the dendritic cells (Figdor, van Kooyk, & Adema, 2002). Thus, mannoside-CD conjugates able to produce antibacterial effects or to encapsulate antibacterial drugs could be used as target-specific antimicrobial delivery systems as a way to fight antibiotic resistance. However, this strategy is hindered by the intrinsic weakness of the saccharide-protein interaction, which can be overcome through a multivalent presentation of the carbohydrate moieties to achieve a global binding potency toward the lectin higher than that for the sum of the monovalent entities (Baussanne et al., 2000; Benito et al., 2004; Casas-Solvas & Vargas-Berenguel, 2016; Vargas-Berenguel et al., 2007).

In this context, we describe herein the construction of photo-responsive β-CD derivatives having the CD scaffold covalently linked to the NOPD 3-(trifluoromethyl)-4-nitrobenzenamine and mannose or α(1→2)mannobioside as targeting functionalities, these latter having specific avidity for pathogens showing mannose-receptor lectins on their surface. We hypothesized that such structures would undergo a supramolecular self-aggregation process in aqueous solution through the inclusion of the NOPD within the β-CD. Such arrangement would result in a multivalent display of the carbohydrate moieties, as well as enhancing the NO-release efficiency due to the NO donor confinement inside the CD cavity. The formation of self-inclusion complexes was investigated by 2D ROESY and pulse-gradient stimulated echo (PGSE) NMR experiments, while amperometric detection allowed us to estimate the light-stimulated release of the NO radical. Isothermal titration calorimetry (ITC) served to measure the binding efficiency of these new conjugates towards Concanvalin A (ConA) lectin as a mannose-specific model protein.

2. Experimental part

2.1. Materials and methods

Merck silica gel 60 F254 aluminum sheets were used for Thin Layer Chromatography (TLC). Plates were developed by UV–vis light and stained with 5% v/v sulfuric acid in ethanol. Merck silica gel (230–400 mesh, ASTM) was used as stationary phase for flash column chromatography. Merck Celite 545 (0.002-0.1 mm) was used for filtration when stated. Uncorrected melting points measurements were taken with a Büchi B-450 melting point equipment. Optical rotations ($[\alpha]_D$ values given in 10^{-1} deg cm^{-1} g^{-1}) were measured on a Jasco P-1030 polarimeter at room temperature. Attenuated Total Reflectance (ATR) infrared spectra were measured on a Bruker Alpha FTIR equipment. MALDI-TOF mass spectra using 2,5-dihydroxybenzoic acid (DHB) as matrix were recorded on a 4800 Plus AB SCIEX spectrometer, while an Agilent LC/MSD-TOF spectrometer was used to measure ESI-TOF mass spectra. Dialysis was performed using Biotech CE Tubing MWCO: 100–500 D. Absorbance UV–vis spectra were obtained using a Jasco V 650 spectrophotometer.

D-(+)-Mannose (≥99%, Acros), acetic anhydride (purum, Panreac), $BF_3{\cdot}Et_2O$ (≥46.5% BF_3 basis, Aldrich), sodium (purum, Panreac), benzoyl chloride (99%, Aldrich), trichloroacetonitrile (98%, Aldrich), potassium carbonate (purum, Panreac), benzoyl cyanide (98%, Aldrich), trimethyl orthoacetate (99%, Aldrich), D/L-10-camphorsulfonic acid (98%, Acros), benzoic anhydride (≥95%, Aldrich), 4-dimethylaminopyridine (99%, Fluka), trimethylsilyl triflate (≥98%, Fluka), and copper(I) iodide (98%, Aldrich) were purchased from commercial sources and used without further purification otherwise indicated. β-CD (CycloLab) was dried at 50 °C in vacuum in the presence of P_2O_5 until constant weight was achieved. 7% HCl solution in MeOH was prepared by diluting 37% aqueous HCl solution (Panreac) in distilled MeOH. Triethylenamine (≥99%, Sigma-Aldrich), pyridine (purum, Panreac), propargyl alcohol (99%, Acros), 2-aminoethanol (≥98%, Sigma-Aldrich), and organic solvents were dried according to literature procedures (Perrin & Armarego, 1989). Dry DMF (99.8%, over molecular sieves, AcroSeal) was purchased from Acros. 4 Å molecular sieves (VWR Chemicals) were heated at 200 °C under high vacuum for 12 h for activation. 6^I-Deoxy-6^I-azido-6^X-deoxy-6^X-*N*-{3-[*N*′-(4′-nitro-3′-trifluoromethylphen-1′-yl)amino]propylamino}cyclomaltoheptaose **1** was synthesized as a mixture of regioisomers as previously reported by Benkovics et al. (2017) (see supplementary data for details). 6^I-Azido-6^I-deoxy-β-cyclodextrin **2** was purchased from CycloLab. Propargyl α-D-mannose **3** was prepared as described in literature (Poláková, Beláňová, Mikušová, Lattová, & Perreault, 2011; Zhao, Liu, Park, Boggs, & Basu, 2012) with small modifications. Specifically, purifications of both compounds after deacetylation were carried out by column chromatography using EtOAc-MeOH 6:1 as eluent. NMR data for these compounds in D_2O completely agreed those described by Erdmann and Wennemers (2010) and van der Peet et al. (2006), respectively. *O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl) trichloroacetamidate **8** was prepared as described by Hartmann et al. (2012).

2.2. NMR measurements

^1H, ^{13}C and 2D NMR spectra were recorded on a Bruker Avance III HD 600 MHz spectrometer equipped with a QCI $^1H/^{13}C/^{15}N/^{31}P$ proton-optimized quadrupole inverse cryoprobe with ^1H and ^{13}C cryochannels, a Bruker Avance III HD 500 MHz spectrometer equipped with an inverse TBI $^1H/^{31}P$/BB probe, or a Bruker Nanobay Avance III HD 300 MHz spectrometer using a QNP $^1H/^{13}C/^{19}F/^{31}P$ probe, depending on the sample. Chemical shifts (δ) are given in parts per million (ppm) and *J* values are expressed in hertz (Hz). Residual solvent signals (δ_H 7.26 and δ_C 77.16 ppm for $CDCl_3$; δ_H 4.79 ppm for D_2O) were used as internal references. Water signal was suppressed in two-dimensional phase-sensitive gROESY experiments using WATERGATE 3-9-19 pulse sequence with gradients. Stimulated echo diffusion measurements (Pregosin, Kumar, & Fernández, 2005) were performed on 10 mM samples in 500 μL of D_2O on the Bruker Avance 500 without spinning using rectangular gradient pulses of variable strength, which was