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Designed positively charged cyclodextrin hosts with enhanced binding of penicillins as carriers for the delivery of antibiotics: The case of oxacillin

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Octakis[6-(2-carboxyethyl)thio-6-deoxy]-γ-CD (γPSP, Sugammadex: PubChem CID: 6918584)

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ABSTRACT

In an effort to identify the optimal cyclodextrin (CD) host for delivery of penicillins to mammalian cells that will also offer protection against β-lactamase-induced hydrolysis, nuclear magnetic resonance (NMR) spectroscopy and isothermal titration calorimetry (ITC) have been employed to study the inclusion complexes formed in aqueous solution between designed CD derivatives and two aminopenicillins, ampicillin and amoxicillin, and two antistaphylococcal penicillins, methicillin and oxacillin. Anionic and cationic thioether-substituted-β- and −γCD derivatives were thus synthesized and compared with the neutral, parent CDs for complexation with the penicillins. The synthesized derivatives were shown to present ~20% elongated cavity space in solution. Moreover, the cationic ones are >98% protonated at physiological pH. The most efficient host was the positively charged octakis[6-(2-aminoethylthio)-6-deoxy]-γ-CD (γCys) that formed the strongest complex with oxacillin ($K_b \sim 1700\ M^{-1}$) in an enthalpically and entropically favorable process ($\Delta H_b = -10.5$ kJ/mol, $T\Delta S_b = 8.0$ kJ/mol). *In vitro* biological tests demonstrated that γCys reduces 2.3-fold the rate of hydrolysis of oxacillin in the presence of oxa-1 β-lactamase while displaying cell crossing capability and efficient internalization into macrophages as well as a sufficiently safe cytotoxicity profile. Overall, γCys could be considered as a promising vehicle for protection and delivery of oxacillin.

1. Introduction

Resistance of pathogens to antibiotics is widely recognized as an imminent threat to contemporary society with dreary outlook and consequences (Spellberg et al., 2008; Fisher et al., 2005). Very recent reports by specialized authorities have proposed a series of measures to be taken among which is the recommendation to "increase the number of effective antimicrobial drugs to defeat infections that have become resistant to existing medicines"

Abbreviations: AMP, ampicillin; AMX, amoxicillin; MET, methicillin; OXA, oxacillin; βCD, β-cyclodextrin; γCD, γ-cyclodextrin; APA, 6-aminopenicillanic acid; PBS, phosphate buffered saline; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; FBS, fetal bovine serum; DMEM, Dulbecco's Modified Eagle Medium; Tris buffer, tris(hydroxymethyl)aminomethane buffer; FACS, fluorescence-activated cell sorting.

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(O'Neill, 2016). Although more than one approach can be adopted towards this goal, revitalization of the clinically available drugs using nanotechnological approaches to evade the known and well understood mechanisms of resistance is a desirable quest. The latter approach aspires to take advantage of the emerging power of nanoscience and is the motivation for the present work: nanosized cyclodextrin (CD) molecular carriers were designed to maximize their binding to penicillin antibiotics in order to protect them from the hydrolytic action of β-lactamase enzymes (a common bacterial resistance mechanism) and simultaneously to act as efficient delivery vehicles to cells.

Cyclodextrins (CDs) are well studied macrocyclic molecules comprising n glucopyranose units (n = 7: βCD, n = 8: γCD, Table 1a) connected circularly to form a hollow structure. CDs can host in their cavity hydrophobic molecules or parts of molecules thus forming inclusion complexes and supramolecular structures (Crini, 2014). Overall, CDs can improve drastically the aqueous solubility, the bioavailability and the stability of lipophilic drugs, and consequently the natural CDs and few derivatives have been approved as drug excipients (Loftsson and Brewster 1996; Davis and Brewster, 2004). Moreover, recent studies have suggested that cavity inclusion can be accompanied by external interactions among the components and by aggregation phenomena which cannot be readily quantified but are important for the delivery of drugs (Kurkov and Loftsson, 2013). Introduction of substituents at the narrow side of CDs can result in (i) cavity elongation to engulf larger guests (ii) positively charged derivatives (terminal amino groups) potentially able to cross cell membranes to deliver their cargo (Mourtzis et al., 2008), and (iii) negatively charged hosts (terminal carboxyl groups) tailored for suitably-sized cationic guests, like sugammadex (Bridion®) (γPSP, Table 1a) (Bom et al., 2002). Optimization of the CD structure through suitable chemistry can therefore enable binding of drugs with maximized strength.

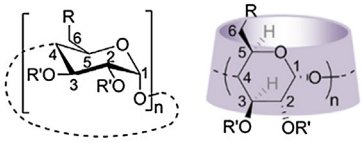
Penicillins are extensively prescribed antibiotics with a chemical structure characterized by the 6-aminopenicillanic acid core (6-APA, $-NH_2$ at position 8, Table 1b) and a side chain. The most common mechanism of resistance to penicillins developed by pathogens is the enzymatic hydrolysis of the β-lactam ring by bacterial β-lactamases (Page, 2012). Aminopenicillins (broad spectrum antibiotics, e.g. ampicillin, AMP, and amoxicillin, AMX) and antistaphylococcal penicillins (narrow spectrum, e.g. methicillin, MET, and oxacillin, OXA) are two widely used categories of semi-synthetic penicillins (Table 1b) that are zwitterionic and anionic, respectively, at neutral pH. Their side chains as well as the readily hydrolyzed 6-APA core are potential binding sites for CDs.

The applications of CDs for improved treatment of infectious diseases have been recently reviewed (Imperiale and Sosnik, 2015). In general, use of CDs in the formulation of anti-infectives, increases the solubility, stability and bioavailability of the drugs *via* several routes of administration. Previous NMR studies have revealed weak binding involving the side chain as well as 6-APA moiety (Maffeo et al., 2006). Other studies have confirmed the low to moderate values of the binding constants that depend largely on the pH while more than one type of complexes can be formed in solution (Aki et al., 2009; De Sousa et al., 2012). Recently, new, interesting and relevant properties have been reported for positively charged CDs that demonstrate their great potential since they act (i) as inhibitors of bacterial toxicity due to their ability to block trans-membrane pores located in the cell walls of lethal bacteria such as *Bacillus anthracis* (Karginov et al., 2005; Yannakopoulou et al., 2011); (ii) as disrupting agents of bacterial membranes (Yamamura et al., 2014); (iii) as potentiators of MET against MRSA *in vitro* (Deng, 2013); (iv) as mimics of antimicrobial peptides (Yamamura et al., 2012); (v) as DNA delivery agents (Mourtzis et al., 2007, 2008).

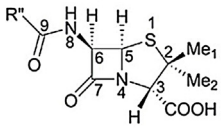
The aim of this study was to design positively charged CDs as optimal hosts for the selected penicillins in order to ensure protection against β-lactamase hydrolysis *in vitro* and enable internalization into cells. The strategy for optimization of the CD structure was based on a combination of cavity size (βCD *vs* γCD) and shape variation (rigid *vs* flexible macrocycle) complemented by the installation of charged functional groups on the narrow side (Table 1a) aiming to maximize electrostatic interactions and augment the cavity space. The host-guest systems were studied in phosphate buffer saline (PBS) at pH 7.4 by NMR spectroscopy and Isothermal Titration Calorimetry (ITC) at 36.75 °C. The optimal host-guest combination was evaluated with *in vitro* biological experiments.

Table 1
Chemical structures of a) the glucopyranose unit and schematic representation of CD macrocycles and b) the penicillins studied.

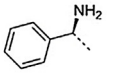
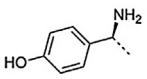
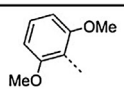
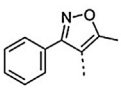
a)



b)



n	R	R'	Label
7	-OH	-H	βCD
	$-SCH_2CH_2NH_2$	-H	βCys
	$-SCH_2CH_2NH_2$	-Me	βCysDM
8	-OH	-H	γCD
	$-SCH_2CH_2COOH$	-H	γPSP
	$-SCH_2CH_2NH_2$	-H	γCys
	$-SCH_2CH_2NH_2$	-Me	γCysDM

	R"	Label
Amino penicillins		AMP
		AMX
Antistaphylococcal penicillins		MET
		OXA