CYCLODEXTRINS – FIELDS OF APPLICATION. PART II¶

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Abstract. This paper represents an analysis of potential and current applications of cyclodextrins as biologically active substances in medicine. The main applications described here include use of cyclodextrins as agents that form inclusion complexes with endogenous substances (membrane lipids, cellular cholesterol), agents that form inclusion complexes with exogenous substances with their man role as guest molecules (sugammadex, FBCx), agents that block endogenous and exogenous macromolecules (ion channels, anthrax toxin, α-hemolysin), and agents which activity is based on the chemical nature of them and of their derivatives (cyclodextrin polysulphate derivatives). The first classification for medically important biological activity of cyclodextrins has been proposed.

Keywords: cyclodextrins, pharmaceuticals, medicine, inclusion complexes.

Introduction

Since the very beginning of the cyclodextrins' research and application in the pharmaceutical products, they have mainly been used as excipients that increase the dissolution rate of the active ingredients, increase their stability, mask their unpleasant taste or odor, etc. Nevertheless, an important number of studies suggested that cyclodextrins and their derivatives can be used as active drug substances in the treatment and prevention of different diseases. According to our knowledge, this is the first review that is trying to deal with all the potential and approved ways of application of the cyclodextrins due to their pharmacological properties. A similar review written by Prof. Otero-Espinar and co-authors [1] has only described the therapeutic activity of the CD's in the treatment of several host-pathogen infections.

The biological effects of the cyclodextrins important for their use in medicine can be classified as follows:

1. effects based on the ability of cyclodextrins to form inclusion complexes with endogenous substances (membrane lipids, cellular cholesterol) with their main role as guest molecules (methyl-β-cyclodextrin);
2. effects based on the ability of cyclodextrins to form inclusion complexes with exogenous substances with their man role as guest molecules (sugammadex, FBCx);
3. effects based on the ability of cyclodextrins to block endogenous and exogenous macromolecules (ion channels, anthrax toxin, α-hemolysin);
4. effects based on the chemical nature of cyclodextrins and their derivatives (cyclodextrin polysulphate derivatives).

Further, all these groups will be presented with a brief description of the most used or recently developed applications of the cyclodextrins and their derivatives from each of them.

1. Biologically active cyclodextrins that form complexes with endogenous substances

A whole range of studies *in vitro* and *in vivo* have shown reduction or inhibition of entry of different infections in host cells due to the presence of cyclodextrins. This effect has been shown for the *Plasmodium* species [2], *Campylobacter jejuni* [3], poliovirus [4], human T cell leukemia virus type I (HTLV-1) and HTLV-I envelope pseudotyped lentivirus particles [5], pseudorabies herpesvirus [6], varicella-zoster [7], *Leishmania donovani* [8], hepatitis *B* virus [9], *HIV-1* [10], and many others. Many of these effects appear as a result of lipid raft disruption by cholesterol depletion in the membrane of the host cell through its extraction by cyclodextrins.

Being ideal chelators of cholesterol, cyclodextrins have been proposed for the treatment of Niemann–Pick type C (NP-C) that is a lysosomal storage disease associated with mutations in NPC1 and NPC2 genes. As a result of this disease cholesterol and glycolipids accumulate in lysosomes. This affects different internal organs and the central nervous system. The lipid-binding ability of cyclodextrins enables the sequestered lysosomal lipid to flow into the cytosolic pool in NP-C animals [11] and cells [12], relieving the cellular burden of lipids. The main cyclodextrin derivatives proposed for this purpose were 2-hydroxypropyl-β-cyclodextrin and methyl-β-cyclodextrin (fig. 1). It has been demonstrated that methyl-β-cyclodextrin is more potent than hydroxypropyl-β-cyclodextrin in reducing both cholesterol and bis(monoacylglycerol) phosphate accumulation in NPC mutant fibroblasts [13].

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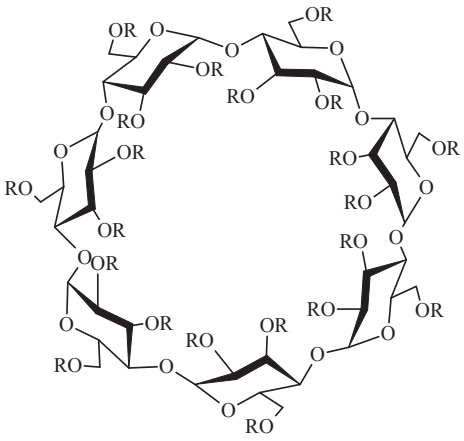


methyl-β-cyclodextrin: R= -CH_3 or -H

2-hydroxypropyl-β-cyclodextrin: R= -CH_2-CH(OH)-CH_3 or -H

Fig. 1. Structures of methyl-β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin

2. Biologically active cyclodextrins that form complexes with exogenous substances

Cyclodextrin derivatives are used as a new class of selective relaxant binding agents (SRBAs). One of the first SRBAs introduced in pharmaceutical market by Organon International, a part of Schering-Plough Corporation, is sugammadex sodium (fig. 2). Sugammadex is used for a consistent and rapid termination of neuromuscular blockade induced by neuromuscular blocking agents. During the induced neuromuscular blockade, the intravenous administration of sugammadex creates a concentration gradient favoring the movement of neuromuscular blocking agent molecules from the neuromuscular junction back into the plasma, which results in a fast recovery of neuromuscular function [14].

One of the main advantages of sugammadex is a low number of side effects. It is biologically inactive, does not bind to plasma proteins, and appears to be safe and well tolerated. Additionally, it has no effect on acetylcholinesterase or any receptor system in the body [14].

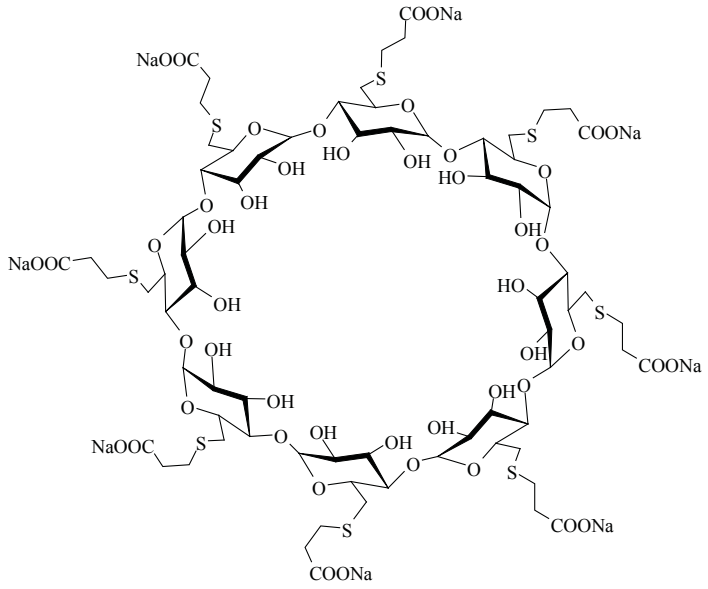


Fig. 2. Structure of sugammadex sodium molecule

The mechanism of action of the drug is based on direct encapsulation of neuromuscular blocking agents (NMBAs) as it is shown in example with rocuronium (fig. 3) [14]. The studies performed with sugammadex have shown a high level of its selectivity towards aminosteroidal NMBAs [15], and its better performance as compared to neostigmine [16].

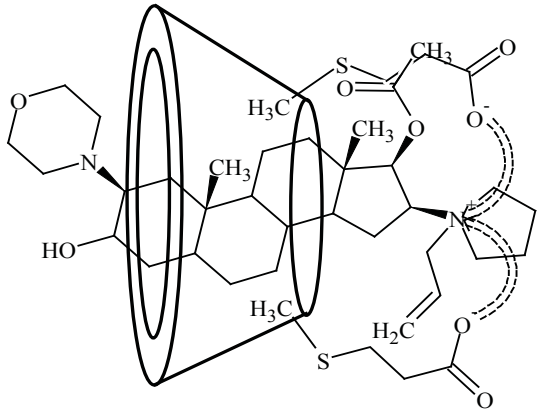


Fig. 3. Inclusion complex of sugammadex-rocuronium

α-cyclodextrin has been proposed as naturally derived fiber for the treatment of high blood lipid levels and obesity of humans. A double-blind study on obese patients with types 2 diabetes has shown that the total cholesterol had reduced by 8,2% after a 30-day treatment at the patients taking α-CD as compared to 5,2% increase in the group of patients taking placebo [17]. Another double-blind research on healthy overweight individuals has shown a decrease in total blood cholesterol by 5,3% and low-density lipoproteins by 6,7% during the two-month period. At the same time, apo-lipoprotein B levels have decreased by 5,6% and insulin levels by 9,5%, while blood glucose and leptin levels did not change [18].

3. Biologically active cyclodextrins that block endogenous and exogenous macromolecules

A group of β-cyclodextrin derivatives have been studied on their ability to inhibit anthrax lethal toxin by blocking the trans-membrane pore formed by its protective antigen (PA) subunit [19]. Most of the tested compounds have shown protective activity against anthrax lethal toxin at low or submicromolar concentrations. The activities of the derivatives in both cell protection and channel blocking have been found to depend on the length and chemical nature of the substituent groups.

A hepta-6-substituted β-cyclodextrin derivative IB201 (fig. 4) has been shown to prevent alpha-toxin mediated hemolysis of rabbit red blood cells (rRBCs), a cell type that is highly sensitive to the lytic action of the toxin. α-hemolysin (Hla) has been proved to play important role in the pathogenesis of *Staphilococcusaureus*, a bacteria that is causing severe forms of pneumonia. Under its monomeric form, Hla binds to susceptible host cell membranes and assembles into a stable transmembrane pore with a 2-nm internal diameter after a series of intra- and intermolecular interactions. Mechanism of action of IB201 is based on blocking of ion conductance through the assembled α-hemolysin pore afterlodging in it. The drug has been proved to have a high potency being active in the low micromolar concentration range. *In vivo* investigation in a murine model has detected that IB201is able to prevent mortality associated with *S. aureus pneumonia* [20].

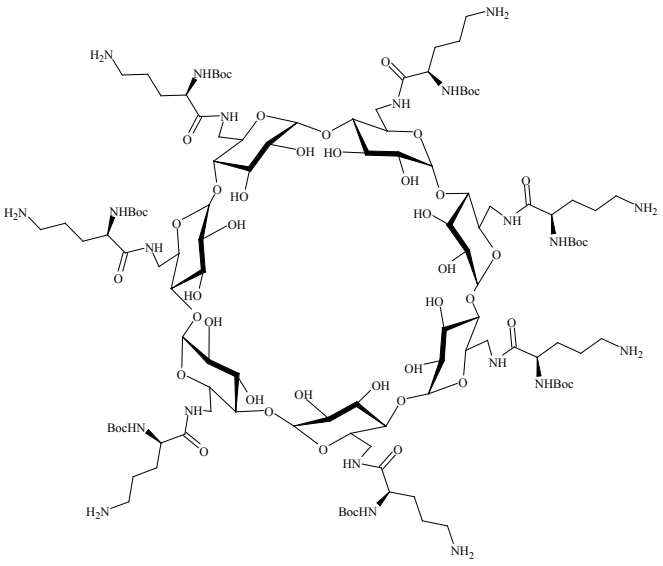


Fig. 4. Structure of IB201 molecule

Comparison of structurally related cationic cyclodextrins on ability of inhibition of *Bacillus anthracis* lethal toxin and *Staphylococcus aureus* α-hemolysin has shown that both β- and γ-cyclodextrin derivatives effectively inhibited anthrax toxin action. On the other hand, α-hemolysin was selectively blocked only by β-cyclodextrin derivatives, demonstrating that both symmetry and size of the inhibitor and the pore are important [21].

Another group of researchers [22] has designed and successfully tested *in vitro* and *in vivo* a group of heptavalent anthrax toxin inhibitors, in which β-cyclodextrin plays the role of a biocompatible core (fig. 5).

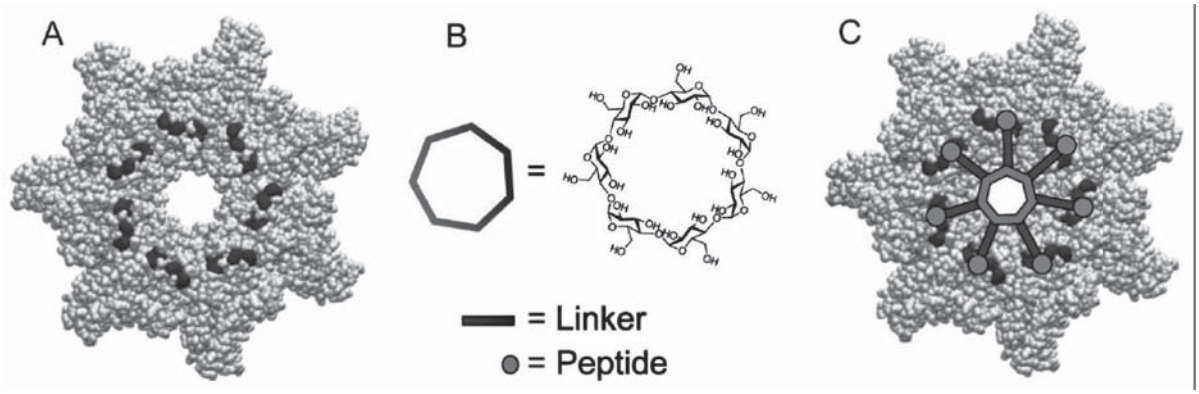


Fig. 5. Structure-based design of heptavalent anthrax toxin inhibitors. A) Structure of the L Fbinding face of [PA63]7. Residues 184, 187, 197, and 200, which form part of the peptide binding site are shown in purple. B) Structure of the core, β-cyclodextrin. C) Scheme illustrating the binding of a heptavalent inhibitor, synthesized by the attachment of seven inhibitory peptides to the β-cyclodextrin core via an appropriate polyethylene glycol linker, to [PA63]7 [22].

The process of the anthrax toxin inhibitors obtaining according to [22] is based on copper-catalyzed azidealkyne cycloaddition (click-chemistry) to facilitate the attachment of seven copies of the inhibitory peptide to a β-cyclodextrin core via a polyethylene glycol linker of an appropriate length, as it is presented in the scheme of synthesis (fig. 6).

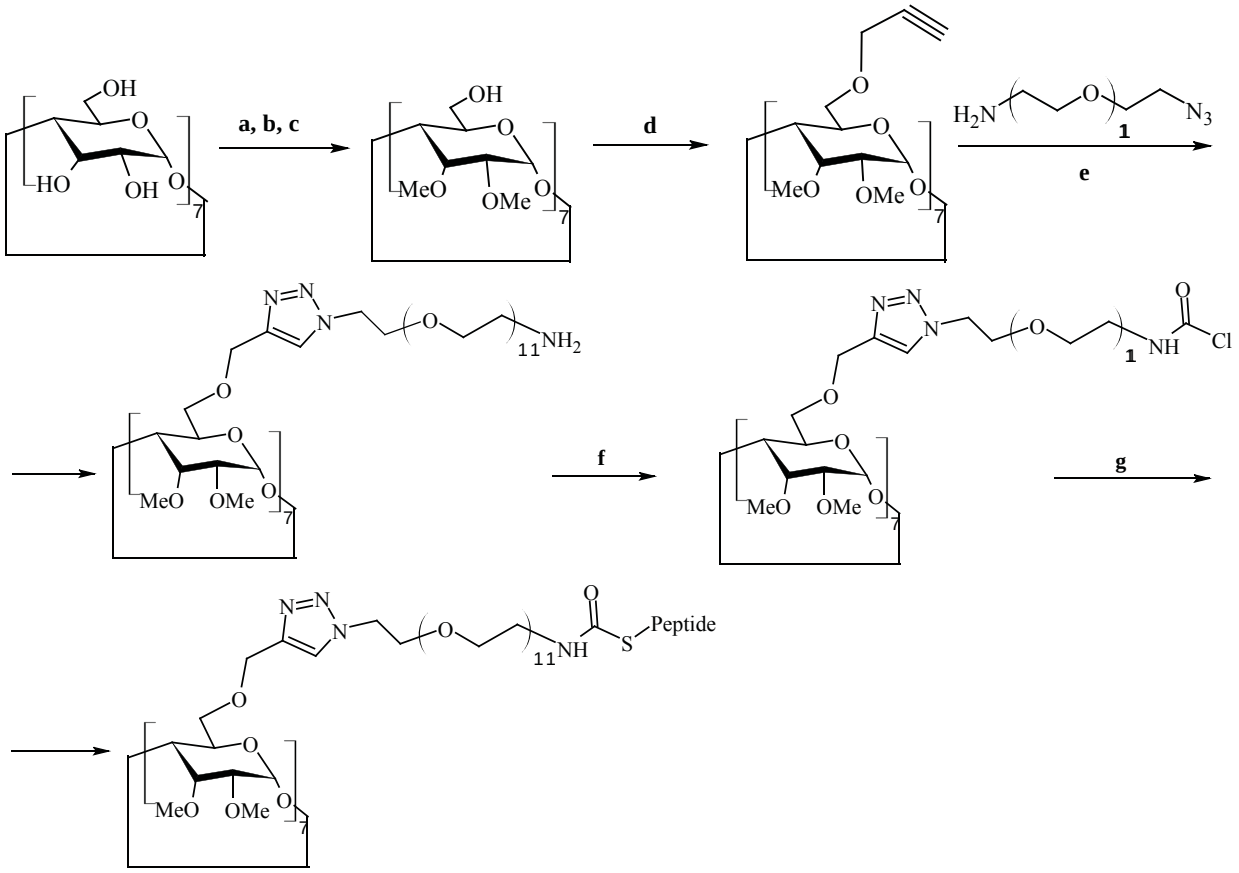


Fig. 6. Scheme of synthesis of heptavalent anthrax toxin inhibitor. (a) TBDMSCl, pyridine, 0°C - rt.; (b) NaH, MeI, THF; (c) NH_4F, MeOH, reflux; (d) NaH, propargyl bromide, DMF, 0°C - rt. (e) $CuSO_4$, sodium ascorbate, THF:H_2O:BuOH (0.5:1:1), 80°C; (f) chloroacetic anhydride, triethylamine; (g) peptide, DMF, DBU, triethylamine [22].

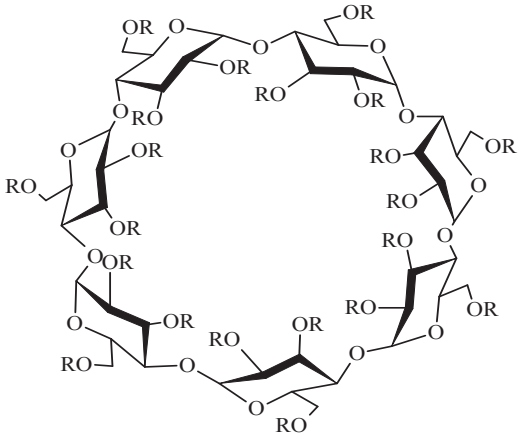
Interestingly, antibacterial activity of cyclodextrins has also been proven to have lysis activity against certain strains of Bacillus, but not against other Gram-positive or Gram-negative bacteria [23]. Another group of Japanese researchers [24] has shown inhibitory effect of dimethylacetyl-β-cyclodextrin on lipopolysaccharide-induced macrophage activation and endotoxin shock in mice.

4. Biologically active cyclodextrins with activity depending on their chemical nature

Cyclodextrin polysulphate (CDPS) has been investigated in with the aim to evaluate its chondroprotective effect in a rabbit model of experimental osteoarthritis (OA) [25]. It has been shown that subcutaneous injections of 1 mg/kg CDPS induced a marked inhibition of osteophyte formation and a significant reduction of cartilage degradation.

In order to alleviate possible heparin-related side effects of CDPS, six alkylated β-cyclodextrin polysulphates have been synthesized (fig. 7) [26]. These compounds have been tested for their capacity to restore cartilage damage *in vitro*. Besides, their effect on blood coagulation and their potency to induce thrombocytopenia through cross-reaction with heparin/PF4 antibodies have been assayed in order to determine possible heparin-like side effects.

These studies have demonstrated that namely poly- but not monosulphated cyclodextrins possess chondrocyte extracellular matrix repair potential. Also, it has been detected that from the polysulphated compounds subjected to studies (2-carboxyethyl)-β-cyclodextrin polysulphates (CE-CDPS) has the best safety profile.



ME-CD-3S: R= $-CH_3$ or $-SO_3H$

ME-CD-6S: R= $-CH_3$ or $-SO_3H$

MA-CDPS: R= $-NH_2$ or $-SO_3H$ or -H

CE-CDPS: R= $-CH_2-CH_2-COOH$ or $-SO_3H$ or -H

HP-CDPS: R= $-CH_2-CH_2-OSO_3CH_3$ or $-SO_3H$

CDPS: R= $-SO_3H$ or -H

Fig. 7. Structures of six polysulphated cyclodextrins. **ME-CD-3S -** 2,6-di-O-methyl-3-sulphate-β-cyclodextrin; **ME-CD-6S -** 2,3-di-O-methyl-6-sulphate-β-cyclodextrin; **MA-CDPS -** 6-monodeoxy-6-monoamino-β-cyclodextrin polysulphate; **CE-CDPS -** (2-carboxyethyl)-β-cyclodextrin polysulphate; **HP-CDPS -** (2-hydroxypropyl)-β-cyclodextrin polysulphate; **CDPS -** β-cyclodextrin polysulphate [26]

5. Conclusions

The main medically important biological activities of the cyclodextrins and their derivatives have been discussed. Classification of these activities has been proposed. For the best of our knowledge, this is the first classification of this type of cyclodextrins' activity proposed so far.

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7. References

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