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Review article

# A short review, effect of dimethyl-β-cyclodextrin on the interaction between *Helicobacter pylori* and steroidal compounds

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ABSTRACT

The 2,6-di-*O*-methyl-β-cyclodextrin (dMβCD) is an amphiphilic annular compound consisting of seven dimethyl-glucose molecules. This compound is well known as a solubilizer of lipophilic compounds. Especially, dMβCD extracts cholesterol from the plasma membrane of mammalian cells and releases the cholesterol to the aqueous solution. The experimental use of dMβCD, therefore, serves to investigate the role of cholesterol in the mammalian cell membrane. It is, however, unclear as to how dMβCD extracts cholesterol incorporated into the glycerophospholipid biomembrane. Meanwhile, dMβCD acts as a beneficial compound for *Helicobacter pylori* and is used as the standard component for supporting the growth of this bacterium in the serum-free culture. However, the detailed mechanism of dMβCD for supporting the growth of *H. pylori* is still to be clarified. *H. pylori* is a Gram-negative microaerophilic bacillus recognized as a pathogen concerned with gastrointestinal diseases in human. Previous studies by our group have successfully obtained the *H. pylori* strains culturable without dMβCD and demonstrated the distinct effects of dMβCD on the interaction between *H. pylori* and exogenous steroidal compounds. For instance, dMβCD promotes and inhibits the absorption of cholesterol and several steroidal compounds respectively into the biomembranes of *H. pylori*. In this study we summarized behaviors of dMβCD toward steroidal compounds relevant to *H. pylori*.

## 1. Introduction

A cyclic compound 2,6-di-*O*-methyl-β-cyclodextrin (dMβCD) is comprised of seven dimethyl-glucose molecules that are linked by α(1→4) bonding and takes the conformation like a bottomless cup of trapezoid (Figure 1). This compound is amphiphilic and solubilizes lipophilic compounds in aqueous solution. Lipophilic compounds such as flavonoid glycosides, sodium salicylate and ibuprofen are considered to be embedded into the inside of ring of dMβCD molecule and to form the molecular inclusion complexes at the molar ratio of 1:1 [1, 2, 3, 4]. In particular, cholesterol is well known as the most suitable "guest molecule" for dMβCD [5, 6, 7, 8, 9]. A previous study by other group has demonstrated that the molecular inclusion complexes comprising of cholesterol and dMβCD are more predominant at the molar ratio of 1:2 rather than 1:1 [10]. As one of the behaviors of dMβCD toward the mammalian cell membrane, this annular compound extracts cholesterol from the plasma membrane lipid constituents. The experimental use of dMβCD, therefore, serves to investigate the role of cholesterol in the mammalian cell membrane, although it is unclear as to how dMβCD extracts cholesterol incorporated into the glycerophospholipid biomembrane.

Of the βCDs, hydroxypropyl-β-CD (HPβCD) has been attracting attention as a therapeutic medication for Neiman-Pick disease, type C1 (NPC1) [11]. NPC1 is an autosomal recessive inherited disease and causes hepatosplenomegaly and progressive neuropathy in childhood. Patients (children) with NPC1 abnormally accumulate nonesterified

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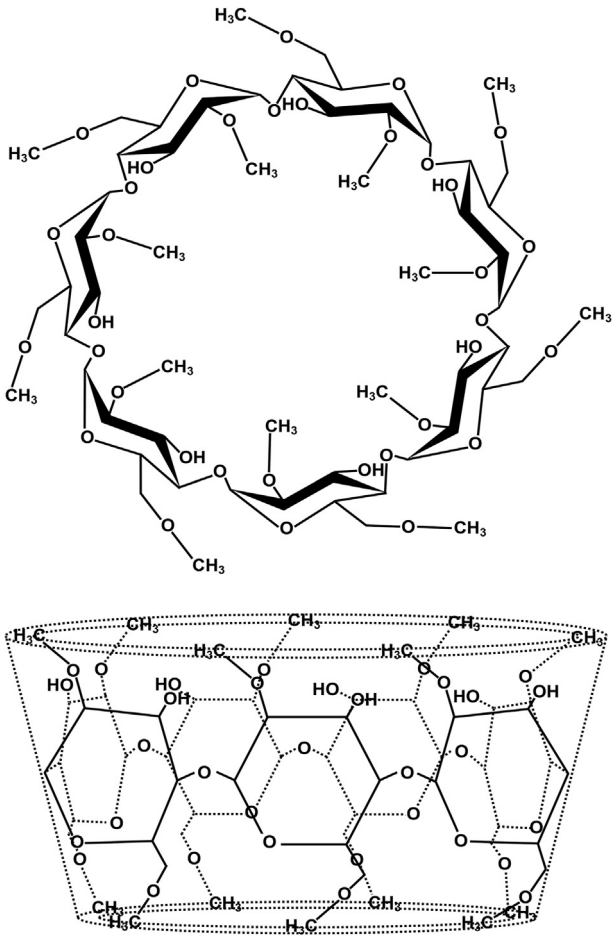

**Figure 1.** Chemical structure of dMβCD.

cholesterol and sphingoglycolipids to the intracellular lysosome. HPβCD decreases neurological disease progression in NPC1 though its detailed mechanism is still to be clarified. In addition, lactosyl-β-CD (LacβCD) has been demonstrated to effectively reduce the intracellular cholesterol levels in NPC-like HepG2 cells [12]. Differing from dMβCD, HPβCD and LacβCD turned out to be useful for the treatment of patients with NPC1.

In eukaryote cells, dMβCD is considered to be a toxic compound that induces the membrane lipid conformational disorder by dissociating cholesterol from the plasma membrane [13]. Meanwhile, dMβCD is a beneficial compound for *Helicobacter pylori* that is a pathogen responsible for peptic ulcers and gastric cancers in human [14]. In the serum-free culture, *H. pylori* actively grows in the medium supplemented with dMβCD (0.1–0.2%) [15, 16]. Though the detailed mechanism of dMβCD for supporting the growth of *H. pylori* is not fully understood, dMβCD is considered to scavenge toxic lipophilic compounds contained in the culture medium [17].

A previous study by our group successfully obtained the *H. pylori* strains that had been acclimatized to the culture medium without either serum or dMβCD, and we invented the interesting effects of dMβCD on the membrane responses of *H. pylori* to exogenous steroidal compounds using those strains [18, 19, 20, 21]. In this review we summarize the unique effect of dMβCD on the interaction between *H. pylori* and exogenous steroidal compounds based on the results obtained so far.

## 2. Interaction of dMβCD with lipophilic compounds

### 2.1. Phosphatidylethanolamine

The biomembranes of Gram-negative bacteria including *H. pylori* are constructed with the inner and outer membranes. In general the inner membrane is comprised of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and cardiolipin (CL). Meanwhile, the outer membrane is comprised of PE, PG, CL, and lipopolysaccharide (LPS). LPS is a glycolipid consisting of hydrophobic portion called lipid A, inner core saccharide-chain, outer core saccharide-chain, and *O*-polysaccharide-chain. LPS localizes to the outermost layer of the outer membrane [22, 23, 24, 25, 26]. The *O*-polysaccharide-chain takes role of directly contact with outside the bacterial cells and regulates the membrane permeability of hydrophobic compounds. PE is the most predominant glycerophospholipid of Gram-negative bacterial membrane lipid constituents. A previous study by our group has revealed that the fatty acid composition of PE of *H. pylori* considerably differs from that of the typical Gram-negative bacterial PE such as *Escherichia coli* PE. After the total lipids were extracted from the biomembranes of either *H. pylori* or *E. coli* by the organic solvent distribution method, PE was purified from the total lipids by the silica gel-column chromatography, and the fatty acid composition of the purified PE was analyzed by the gas chromatography-mass spectrometry (GC-MS). As a consequence, the saturated fatty acid side-chain of *H. pylori* PE is myristate (14:0) whereas the saturated fatty acid side-chain of *E. coli* PE is palmitate (16:0) [21, 27]. In addition, the high performance liquid chromatography-mass spectrometry (LC-MS) analysis showed that PE molecular species of *H. pylori* are almost composed of the three PE molecular species with the fatty acid combination of myristate/19:0 cyclopropanoic acid (34.5%), myristate/myristate (28.9%), and myristate/oleate (18:1) (15.0%). Meanwhile, the predominant PE molecular species of *E. coli* were comprised of the fatty acid combination of palmitate/17:0 cyclopropanoic acid (25.7%), palmitate/oleate (14.7%), and palmitate/-palmitoleate (16:1) (14.7%). Moreover, we have demonstrated that dimyristoyl-PE as one of the most prevalent PE molecular species of *H. pylori* selectively interacts not with cholesterol ester but with cholesterol [21].

After cholesterol-free *H. pylori* and *E. coli* at $10^9$ colony-forming units (CFU)/ml were incubated for 4 h in the presence of dMβCD (5 mM) in the culture medium (5 ml) and were washed three times with PBS, the dMβCD bound to either *H. pylori* or *E. coli* was quantified using the phenol-sulfuric acid method. As a consequence, the detection levels of dMβCD in the cholesterol-free *H. pylori* cells were approximately 6.5 nmol/$10^9$ CFU. However, the detection levels of dMβCD in the *E. coli* cells were less than 1 nmol/$10^9$ CFU. In sum, *H. pylori* cells turned out to tightly bind to dMβCD [21]. We next examined the binding-affinity of dMβCD for either *H. pylori* PE or *E. coli* PE using paper disks that had fixed several amounts of PE (100–300 μg). After the PE-fixed paper disks were soaked for 4 h in the buffer (2 ml) containing dMβCD (5 mM) and were washed six times with distilled water, dMβCD extracted from the PE-fixed paper disks were quantified using phenol-sulfuric acid method. As a consequence, the amounts of dMβCD increased along with the increase of *H. pylori* PE amount fixed to the paper disk. However, the amounts of dMβCD were negligible regardless of the increase of *E. coli* PE amount fixed to the paper disk. In sum, dMβCD turned out to show high binding-affinity not for *E. coli* PE but for *H. pylori* PE (Figure 2). Though the selective binding-affinity of dMβCD for *H. pylori* PE may be relevant to the myristate of the PE molecular species, the detailed mechanism of it is unknown.

### 2.2. Cholesterol

*H. pylori* aggressively absorbs exogenous cholesterol into the biomembranes and uses the cholesterol as one of the membrane lipid constituents [19, 28, 29, 30, 31, 32, 33, 34, 35]. The assimilation of cholesterol into the biomembranes is a unique biological feature of this bacterium. In a previous study we examined the effect of dMβCD on the cholesterol absorption in *H. pylori* [21]. Cholesterol-free *H. pylori* ($10^6$ CFU/ml) was cultured for 24 h in the presence of cholesterol-coating beads (250 μM as cholesterol) in the medium (30 ml) supplemented with or without dMβCD (0.2%). After the bacterial cells ($10^8$ CFU) were

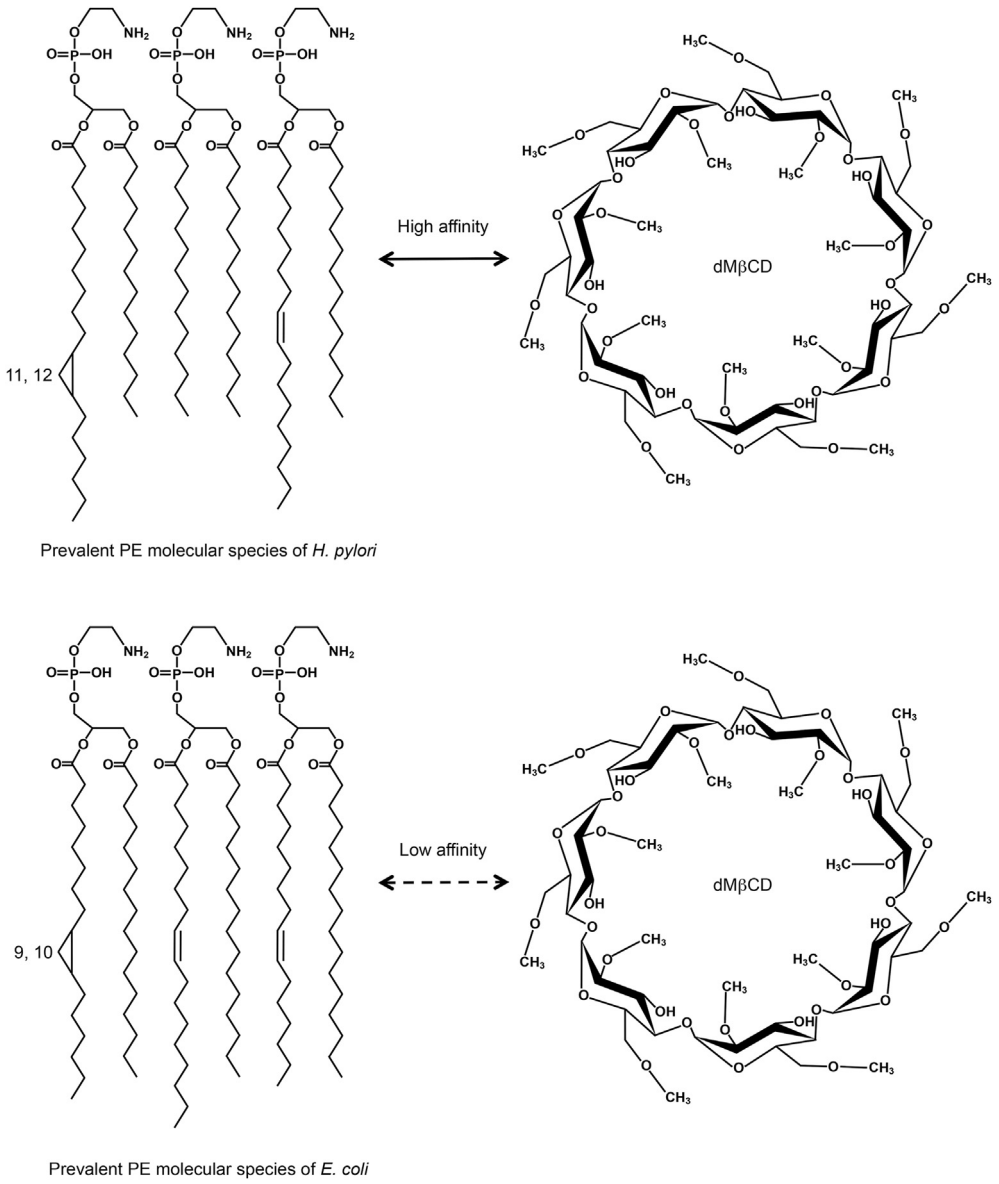


**Figure 2.** Interaction of dMβCD with the membranal PE of either *H. pylori* or *E. coli*.

recovered, the lipids were extracted from the bacterial cells by the organic solvent distribution method, and cholesterol was quantified using the ferrous chloride-sulfuric acid method. Intriguingly, the cholesterol contents were only approximately 2% of whole lipids (excluding LPS) in *H. pylori* cultured in the absence of dMβCD whereas were approximately 10.5% of whole lipids (excluding LPS) in *H. pylori* cultured in the presence of dMβCD. These results indicate that the cholesterol-inclusion dMβCD delivers cholesterol to *H. pylori* through the intermediation of the membranal myristoyl-PE and promotes the cholesterol absorption of *H. pylori* (Figure 3).

### 2.3. 3β-hydroxyl steroids

In addition to cholesterol, *H. pylori* also assimilates the 3β-hydroxyl steroids such as pregnenolone and dehydroepiandrosterone into the biomembranes [18]. These steroidal compounds are also considered to be incorporated into the *H. pylori* biomembranes at least through the intermediation of the dimyristoyl-PE [21]. However, the interaction of dMβCD with the 3β-hydroxyl steroids completely differs from that of dMβCD with cholesterol. Either cholesterol-free *H. pylori* or *E. coli* at $10^9$ CFU/ml was incubated for 4 h together with cholesterol (100 nmol)-fixed paper disk in the presence or absence of dMβCD (5 mM) in the culture medium (5 ml). After the bacterial cells were recovered and were washed three times with PBS, the lipids were extracted from the bacterial cells by the organic solvent distribution method, and cholesterol contained in the lipids was quantified using the ferrous chloride-sulfuric acid method. Intriguingly, cholesterol (approximately 4.5 nmol) was detected only in the lipids of *H. pylori* incubated with cholesterol-fixed paper disk in the presence of dMβCD. These results indicate that *H. pylori* is incapable of directly extracting cholesterol from the paper disk, and that dMβCD solubilizes cholesterol fixed to the paper disk and delivers the cholesterol to *H. pylori* through the intermediation of the membranal myristoyl-PE.

The same experiments as mentioned above were carried out using either pregnenolone-fixed paper disk or dehydroepiandrosterone-fixed paper disk in place of cholesterol-fixed paper disk [21]. Surprisingly, pregnenolone and dehydroepiandrosterone were detected at approximately 10 nmol and 7 nmol respectively in the lipids of *H. pylori* ($10^9$ CFU) not in the presence of dMβCD (5 mM) but in the absence of it. These results indicate that *H. pylori* absorbs those steroids spontaneously eluted from the paper disks, and that the membrane absorption of those steroids

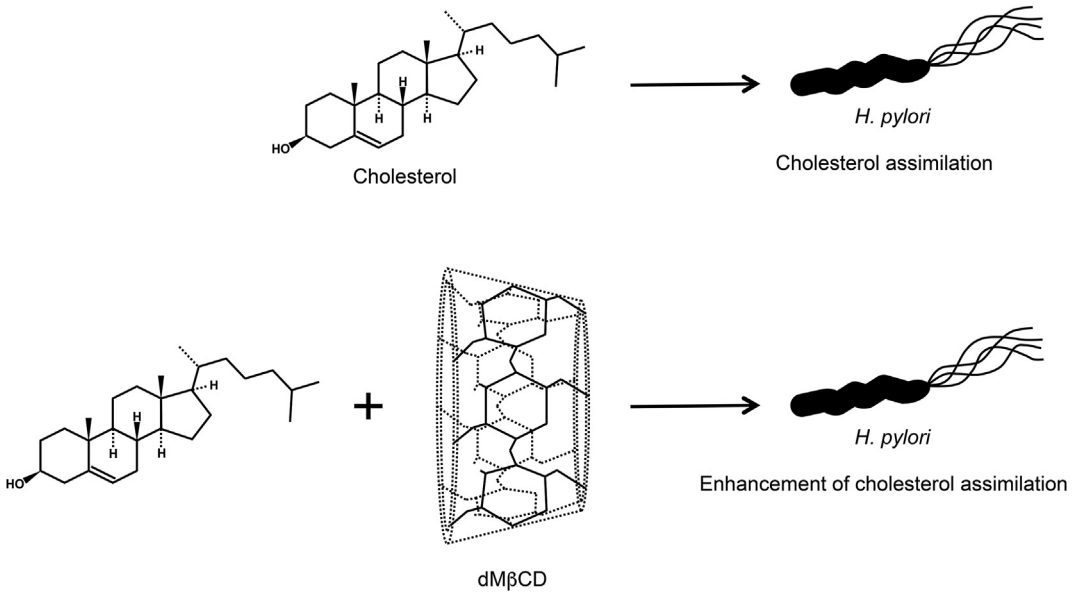


**Figure 3.** Relationship between cholesterol, dMβCD, and *H. pylori*.

is completely inhibited by the action of dMβCD (Figure 4). Though it is unclear as to why dMβCD promotes the cholesterol absorption of *H. pylori* and inhibits the 3β-hydroxyl steroid absorption of this bacterium, the differences of molecular weights of the guest molecules for dMβCD may affect the membrane absorption of steroidal compounds in *H. pylori*. The molecular weights of cholesterol, pregnenolone, and dehydroepiandrosterone are 386.654, 316.4776, and 288.424, respectively. On this basis, relatively low-molecular-weight steroids may be more deeply embedded into the inside of ring structure of dMβCD than cholesterol and may be difficult to dissociate from dMβCD. In addition, the steroid-inclusion dMβCD may be unable to bind to the myristoyl-PE of the *H. pylori* outer membrane by a certain factor such as the conformational structure of molecular inclusion complexes. Further investigations will need to solve the enigma as for the relationship between *H. pylori* myristoyl-PE and cholesterol-inclusion dMβCD or steroid-inclusion dMβCD.

### 2.4. Progesterone and its derivatives

Previous studies by our group have demonstrated that progesterone, 17α-hydroxyprogesterone caproate, and 17α-hydroxyprogesterone linoleate confer the selective bactericidal action to *H. pylori*. The minimum inhibitory concentrations (MIC) of progesterone, 17α-hydroxyprogesterone caproate, and 17α-hydroxyprogesterone linoleate for the cholesterol-free *H. pylori* strain measured by the conventional agar-plate dilution method were the 50, 3, and 1 μM, respectively [19, 20, 34]. In addition, when the minimum bactericidal concentrations (MBC) were estimated by the conventional serial-dilution method, the MBC of

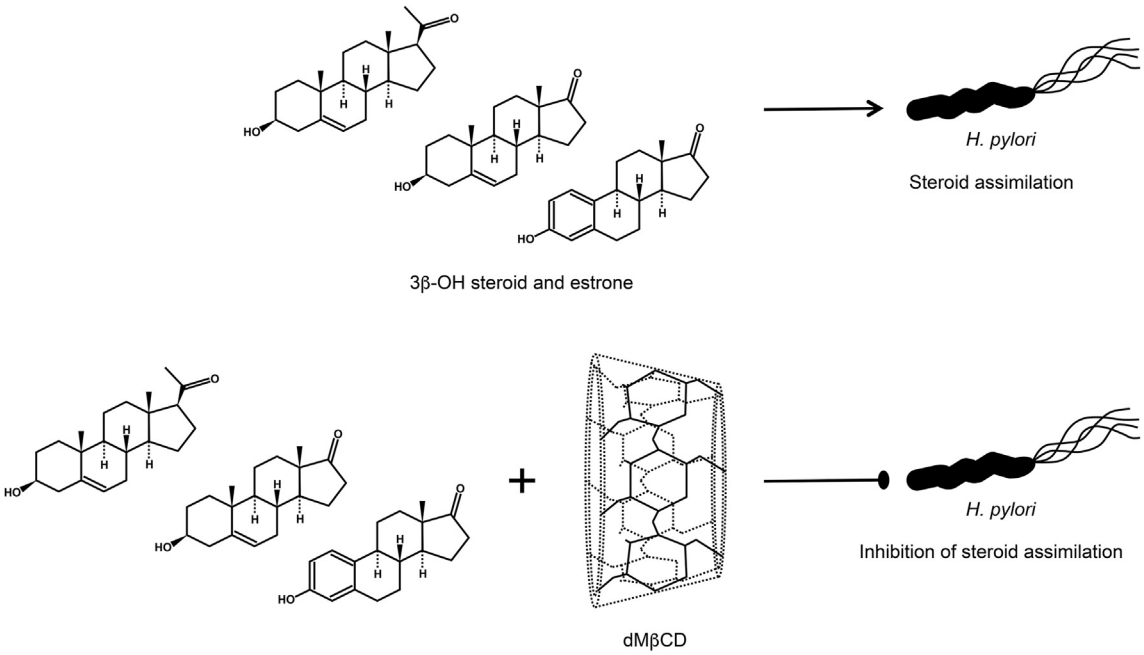


**Figure 4.** Relationship between 3β-OH and 3-OH steroids, dMβCD, and *H. pylori*.

17α-hydroxyprogesterone linoleate for the three cholesterol-absorbing *H. pylori* strains that had been continuously acclimatized to the culture medium supplemented with cholesterol (30 μM) were the ranging of 4–24 μM [27]. Meanwhile, these progestins have no influence on the viability of representative bacteria such as *Enterobacteriaceae* bacteria (the five bacterial species), *Pseudomonas aeruginosa*, and genus *Staphylococcus* [19, 20, 27, 34]. Therefore, the MIC or MBC of those progestins for the above bacterial species were greater than the 100 μM. Progesterone and its derivatives are considered to act on the myristoyl-PE of *H. pylori* biomembranes and to induce the bacteriolysis to this bacterium [27].

We examined the effect of dMβCD on the bactericidal activity of the three progestins against *H. pylori*. When cholesterol-free *H. pylori* ($10^{5.5}$ CFU/ml) were cultured for 24 h in the presence or absence of progesterone (50 μM) in the medium (1 ml) supplemented with dMβCD (0.2%), progesterone had no influence on the viability of *H. pylori* and therefore the bacteria grew to $10^8$ CFU/ml even in the presence of progesterone (50 μM) as similar to the absence of it [18]. Meanwhile, the cholesterol-free *H. pylori* in the absence of dMβCD succumbed to the bactericidal action of progesterone (50 μM), and therefore the viability of the bacteria decreased from $10^{5.5}$ CFU/ml to $10^4$ CFU/ml [19]. In sum, dMβCD turned out to completely inhibit the bactericidal activity of progesterone against *H. pylori* (Figures 5 and 6). Similarly, when the cholesterol-free *H. pylori* ($10^{6.5}$ CFU/ml) was cultured for 24 h in the presence of 17α-hydroxyprogesterone caproate (6 μM) in the medium (1.5 ml) supplemented with or without dMβCD (30 μM), *H. pylori* without dMβCD was almost completely eradicated by the action of 17α-hydroxyprogesterone caproate [34]. However, dMβCD protected the cholesterol-free *H. pylori* from the bactericidal action of 17α-hydroxyprogesterone caproate, and the viability of *H. pylori* was maintained the $10^{6.5}$ CFU/ml that is the baseline CFU levels immediately before the start of experiment. In contrast, dMβCD had no effect on the bactericidal action of 17α-hydroxyprogesterone linoleate against the cholesterol-free *H. pylori* (Figure 7). Hence, 17α-hydroxyprogesterone linoleate (6 μM) completely eradicated *H. pylori* ($10^{6.5}$ CFU/ml) by 24 h regardless of the presence or absence of dMβCD (30 μM). However, dMβCD did not involve in the augmentation of the bactericidal activity of 17α-hydroxyprogesterone linoleate against *H. pylori*.

We next examined the effect of dMβCD on the interaction between *H. pylori* PE and 17α-hydroxyprogesterone caproate or 17α-hydroxyprogesterone linoleate [27, 34]. The *H. pylori* PE (200 μg)-fixed paper disk was soaked for 2 h in the presence of dMβCD (3 mM) in the buffer (2 ml) containing either 17α-hydroxyprogesterone caproate (100 μM) or 17α-hydroxyprogesterone linoleate (100 μM or 30 μM). After the PE-fixed paper disk was washed six times with distilled water (2 ml), the 17α-hydroxyprogesterone caproate and 17α-hydroxyprogesterone linoleate bound to the PE fixed to the paper disks were analyzed by thin-layer chromatography. As a consequence, 17α-hydroxyprogesterone linoleate was detected at high density whereas no 17α-hydroxyprogesterone caproate was detected. These results indicate that dMβCD inhibits the binding of 17α-hydroxyprogesterone caproate to *H. pylori* PE but has no inhibition effect on the binding of 17α-hydroxyprogesterone linoleate to *H. pylori* PE. On this basis, dMβCD is considered to inhibit the bactericidal action of progesterone and 17α-hydroxyprogesterone caproate against *H. pylori* by preventing the binding of those progestins to the myristoyl-PE of the biomembranes.

The molecular weight of progesterone is 314.46 and is lower than the molecular weight of cholesterol. Therefore, progesterone may be unable to interact with the myristoyl-PE of *H. pylori* biomembranes by being deeply embedded to the inside of ring of dMβCD molecule. In addition, the progesterone-inclusion dMβCD as similar to the other steroid-inclusion dMβCD also may be unable to tightly bind to the myristoyl-PE of *H. pylori* biomembranes. However, 17α-hydroxyprogesterone caproate was excluded from the above hypothesis, because the molecular weight (428.6041) of this progesterone derivative is higher than that (386.654) of cholesterol. As mentioned earlier, the inhibition effect of dMβCD (30 μM) was incomplete on the bactericidal action of 17α-hydroxyprogesterone caproate (6 μM) against *H. pylori* ($10^{6.5}$ CFU/ml) since the bacteria were not eradicated and maintained the baseline CFU levels ($10^{6.5}$ CFU/ml), though dMβCD (3 mM) inhibited the interaction between *H. pylori* PE (200 μg) and 17α-hydroxyprogesterone caproate (100 μM). These results may mean that the short alkyl chain (6:0) of the 17α-hydroxyprogesterone caproate is an important structure that affects the dissociation of its molecule from the dMβCD molecules and inhibits the tightly binding of the inclusion complexes to the myristoyl-PE of *H. pylori* biomembranes. Further investigations will be necessary to elucidate the detailed mechanism of dMβCD for inhibiting the bactericidal action of progesterone and 17α-hydroxyprogesterone caproate against *H. pylori*. Meanwhile, the molecular weight of 17α-hydroxyprogesterone linoleate is 576.892 and is higher than that of cholesterol.

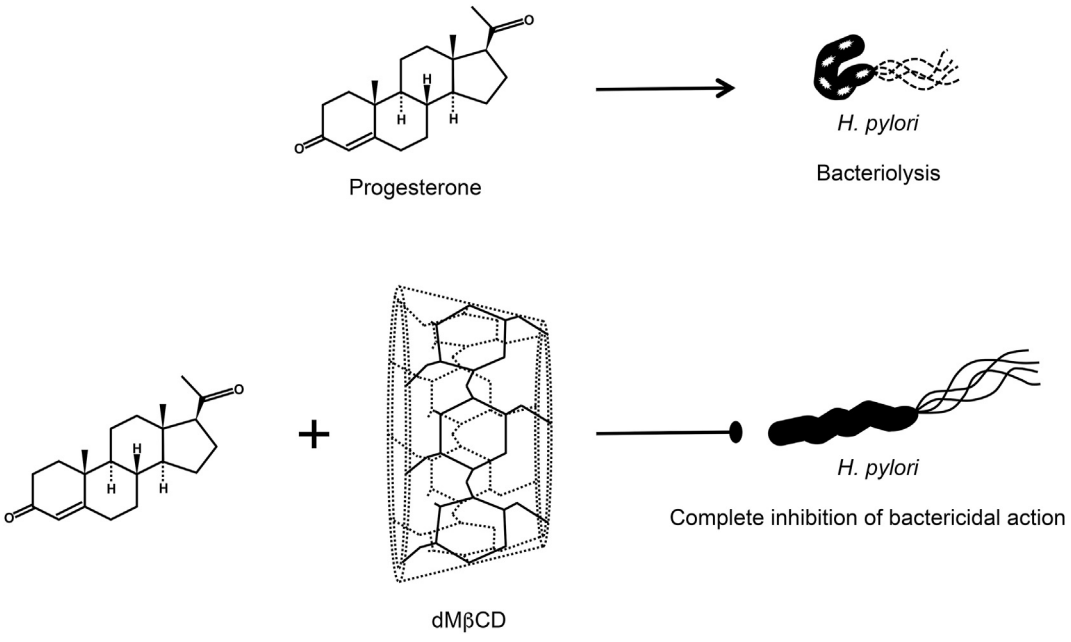


**Figure 5.** Relationship between progesterone, dMβCD, and *H. pylori*.

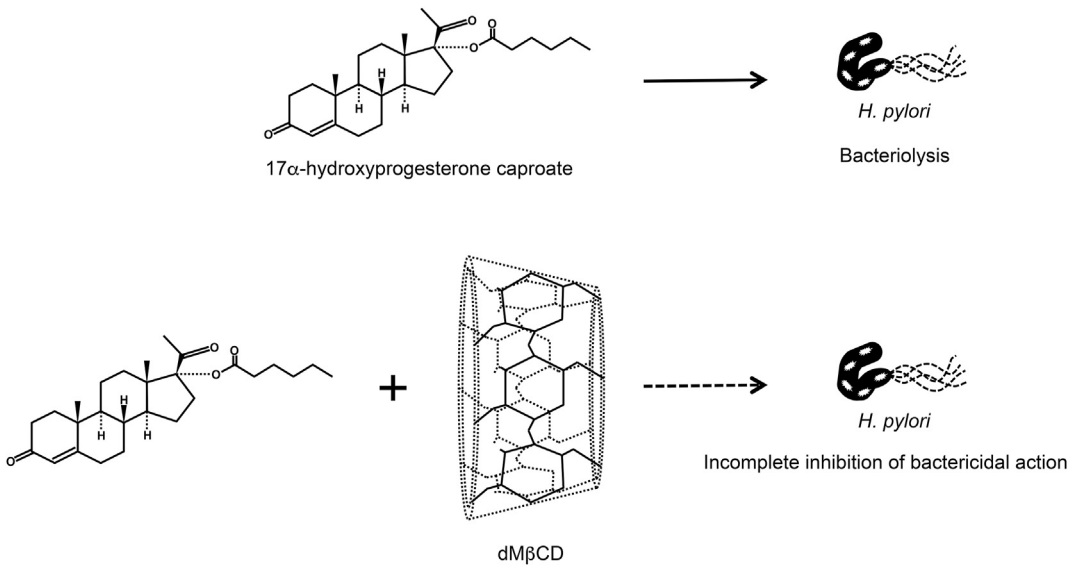


**Figure 6.** Relationship between 17α-hydroxyprogesterone caproate, dMβCD, and *H. pylori*.

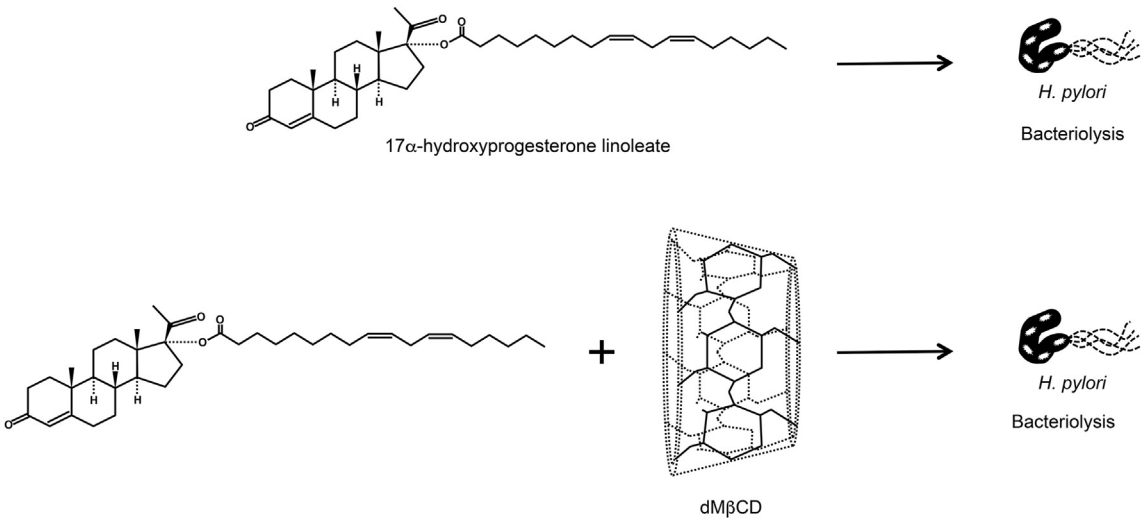


**Figure 7.** Relationship between 17α-hydroxyprogesterone linoleate, dMβCD, and *H. pylori*.

This suggests the possibility that dMβCD delivers 17α-hydroxyprogesterone linoleate to *H. pylori* by forming the baggy inclusion complexes due to the high molecular weight of its progesterone derivative.

## 3. Conclusions

In this review we described the following interactions between dMβCD, steroidal compounds, and *H. pylori* myristoyl-PE: (1) dMβCD tightly binds to the myristoyl-PE of *H. pylori* biomembranes; (2) dMβCD promotes the absorption of cholesterol into the *H. pylori* biomembranes; (3) dMβCD inhibits the absorption of relatively low-molecular-weight steroids into the *H. pylori* biomembranes; (4) dMβCD protects *H. pylori* from the bactericidal action of progesterone and 17α-hydroxyprogesterone caproate; (5) dMβCD has no effect on the bactericidal action of 17α-hydroxyprogesterone linoleate against *H. pylori*. However, there are still a number of enigmas as to either how dMβCD delivers the steroidal compounds to the biomembranes of *H. pylori* or how dMβCD selectively prevents the binding of several steroidal compounds to the biomembranes of *H. pylori*.

Earlier studies by our group have demonstrated that progesterone and its derivatives confer the selective bactericidal action to *H. pylori* without affecting the viability of typical bacterial species [27]. These results suggest that anti-*H. pylori* drugs are capable of being developed using progesterone as the fundamental structure. However, as a number of steroidal compounds is hydrophobicity, it is pharmacologically important to give hydrophilicity to the steroidal drugs designed to more effectively exert the anti-*H. pylori* action. One of the interesting findings is that progesterone derivative with relatively high-molecular-weight acts as a bactericidal agent on *H. pylori* even in the presence of dMβCD. On this basis, the progesterone derivatives covalently linked to dMβCD may have amphiphilic property and act as a novel antibacterial drug for selectively eradicating *H. pylori* without collapsing the balance of intestinal microbiota. A previous study by other group has demonstrated that progesterone has beneficial effect on gastritis of ovarectomized female gerbils infected with *H. pylori* [36]. It was, however, unclear as to whether progesterone has influence on the viability of *H. pylori* colonized stomach of the gerbils. In the future we will synthesize dMβCD-linked progesterone derivatives and need to evaluate the anti-*H. pylori* activity of its newly progesterone derivatives *in vitro* and

*in vivo*. In addition, we will try to examine *in vivo* pharmacokinetics of dMβCD-linked progesterone derivatives using the combinations of the gas chromatography-quadrupole time of flight mass spectrometry (GC-Q-TOF/MS) and the liquid chromatography-quadrupole time of flight mass spectrometry (LC-Q-TOF/MS) [37].

## Declarations

### *Author contribution statement*

All authors listed have significantly contributed to the development and the writing of this article.

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### *Data availability statement*

No data was used for the research described in the article.

### *Declaration of interests statement*

The authors declare no conflict of interest.

### *Additional information*

No additional information is available for this paper.

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