Chapter 5

Design of cyclodextrin-based systems for intervention execution

Introduction

Cyclodextrins (CDs) are cyclic (α-1,4)-linked oligosaccharides of α-D-glucopyranose [1]. The most common forms of CDs are α-, β-, and γ-CDs. They are made up of six, seven, and eight α-D-glucopyranose units, respectively (Fig. 5.1) [2,3]. The high content of hydroxyl groups renders CDs soluble in water. Yet, the aqueous solubility of CDs in general is lower than that of the comparable linear dextrins, owing to the relatively high crystal energy of CDs [4]. CDs were documented first by Villiers, who isolated a crystalline substance, namely "cellulosine," from a bacterial digest of starch. That substance has been found to resist acid hydrolysis [5]. It has later been known as "cyclodextrin." The basic physicochemical features of CDs (including chemical structure, reactivity, cavity size, solubility, and inclusion complexation capacity) have also been subsequently documented by Cramer in his book *Einschlussverbindungen* [6].

Over the last several decades, CDs have exhibited promising practical potential in diverse areas, ranging from controlled drug release [7−12] to chiral separation of basic drugs [13]. These applications are largely mediated by the ability of CDs to form host−guest complexes. In fact, compared to other conventional host molecules (such as cucurbiturils, pillararenes, crown ethers, and calixarenes), CDs display distinctive features that make them attractive in structural design and engineering of polymeric vectors [14]. For instance, while many other host molecules have to be synthesized via multistep synthetic procedures before use, CDs are commercially available and "ready-made" molecular entities. In addition, the native forms of many host molecules display
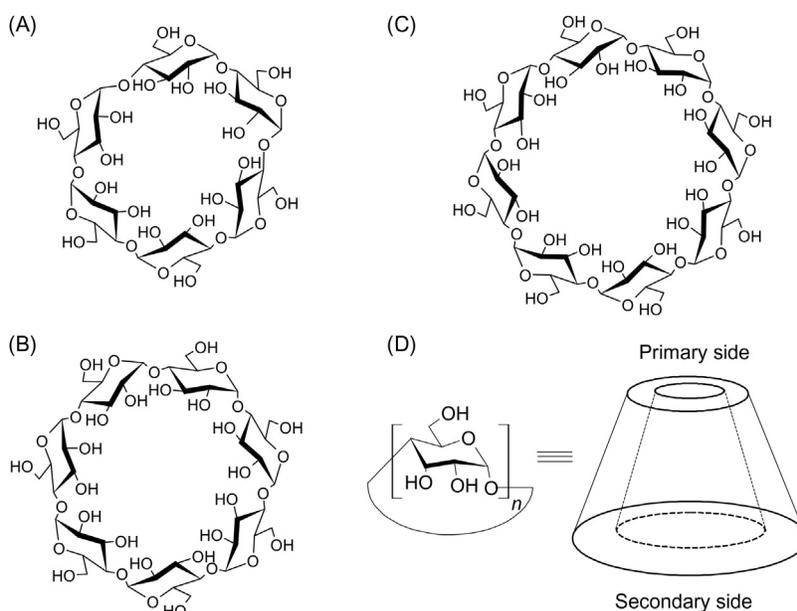
FIGURE 5.1 The structures of (A) α-CD, (B) β-CD, and (C) γ-CD, as well as (D) the torus shape of the CD molecule. The shape of the molecule is not drawn to scale. *Reproduced from W.F. Lai, A.L. Rogach, W.T. Wong, Chemistry and engineering of cyclodextrins for molecular imaging, Chem. Soc. Rev. 46 (2017) 6379−6419 with permission from RSC, [3].*

poor aqueous solubility, whereas native CDs are highly water soluble. This makes the direct use of CDs more convenient. Because of these, CD-based "guest–host chemistry" has gained extensive research interests in the development and modification of polymeric gene carriers. In this chapter, we will first have an overview of the basic properties of CDs, followed by a discussion of incorporation of CDs into the design and modification of polymeric vectors for the execution of biogerontological interventions that involve genetic manipulation.

Basic properties of cyclodextrins

CDs have the apolar cavity interiors and the hydrophilic cavity exteriors. Because of this unique structure, CD molecules can provide a microenvironment for encapsulation and solubilization of hydrophobic guest molecules [15,16]. This makes CDs a favorable candidate to be exploited as excipients in pharmaceutical formulation. In addition to delivering chemical drugs, since the turn of the last century more and more efforts have been directed to exploiting the use of CDs in delivering nucleic acids [17,18]. For instance, Agrawal's group has examined the possible use of CDs (and their analogs) in enhancing cellular internalization of oligonucleotides [19,20]. Abdou and coworkers have also evaluated the capacity of various native and derivatized CDs to enhance the activity of an 18-mer phosphodiester oligodeoxynucleotide (ODN) (which is complementary to the initiation region of a messenger RNA (mRNA) molecule coding for the spike protein, and possesses the intergenic consensus sequence of an enteric coronavirus) against viral growth in human adenocarcinoma cells [21]. They have found that upon complexation with an β-CD derivative, namely 6-deoxy-6-S-β-D-galactopyranosyl-6-thio-cyclomalto-heptaose, in a molar ratio of 1:100, the ODN has induced up to 90% of viral inhibition. This is much higher than that achieved (12%–34%) by using the naked ODN [22–26]. This, along with other studies [26,27], has established the foundation on which subsequent research on CD-mediated gene delivery can be built.

CDs exhibit a binding affinity with nucleic acids [17,28], and can also attenuate the cytotoxicity of other nucleic acid carriers. The latter has been revealed by an earlier study [29], which has linked diamino-CD monomers with diimidate comonomers to generate a number of linear cationic β-CD-based polymers. In BHK-21 cells, the IC_{50} of the polymers produced has been found to be much lower than that of the CD-lacking polyamidines [29]. This has suggested that, by incorporating CD moieties into the backbone of a cationic polymer, the cytotoxicity of the polymer can possibly be reduced. Apart from this, CDs can facilitate the efficiency of

virus-mediated nucleic acid delivery by enhancing viral binding and internalization into a host cell. This has been illustrated by the successful use of CDs to improve adenoviral-mediated gene transfer to the rat jejunum [30].

Strategies for structural engineering of cyclodextrins

Until now, a wide diversity of CD derivatives (whose OH groups have undergone alkylation, esterification, or even random derivatization) have been reported. Through chemical modification of the OH groups, charged groups can usually be incorporated into the molecular structure, causing changes in the solubility of CDs in water or organic solvents [31]. Over the years, different methods have been adopted to functionalize CDs. These methods generally take place at the OH groups located either in the upper rim (primary side) or in the lower rim (secondary side). The reactivity of hydroxyl groups in the CD ring varies with the positions of those groups. In general, there are secondary hydroxyl groups at 2- and 3-positions, and primary hydroxyl groups at the 6-position of the glucopyranose ring [32]. Among them, 3-OHs are the least accessible, whereas 2-OHs are the most acidic. Compared to the hydroxyl groups at the other two positions, 6-OHs are the most nucleophilic, and can be readily converted into other functional groups. These reactivity differences among hydroxyl groups at different locations have been exploited extensively for the development of strategies for selective chemical functionalization of CDs [32,33].

At the moment, most of the functionalization approaches involve the primary hydroxyl group at the 6-position. A typical functionalization method is to use a nucleophilic substitution reaction, during which mono-6-(p-toluenesulfonyl)-6-deoxy-CD (Ts-CD) is first synthesized as a precursor, whose tosyl group then undergoes nucleophilic displacement by selected nucleophiles (amines, azide, carboxylate, hydroxylamine, iodide, polyamines, and thiols) to form a monofunctionalized CD [34]. The process of tosylation can be performed by using tosyl chloride in dry pyridine [35], or in water along with either NaOH [36] or $CuSO_4$ [37]. Either 6-monotosylate or a mixture of 6-polytosylates can be formed via this process, and the direction of the process depends mainly on the molar ratio between CDs and tosyl chloride. Furthermore, the formation of regioisomers during the process may increase the difficulties of subsequently isolating pure tosylates. This problem can be alleviated by using mesitylenesulfonyl chloride or other oversized sulfonyl chlorides. This method has been shown to be able to limit the number of regioisomers formed [35].
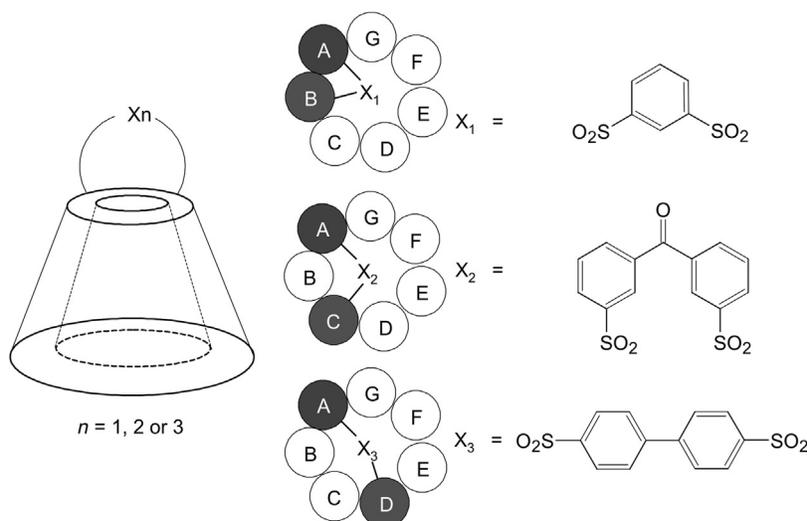
FIGURE 5.2 A schematic diagram showing the structures of the AB, AC, and AD isomers of 6-disulfonates of β-CD. *Reproduced from W.F. Lai, A. L. Rogach, W.T. Wong, Chemistry and engineering of cyclodextrins for molecular imaging, Chem. Soc. Rev. 46 (2017) 6379−6419 with permission from RSC, [3].*

Besides forming monofunctionalized CDs, disubstituted CD derivatives can be generated using appropriate disulfonyl chlorides, whose geometry can be exploited to control the regiochemistry and to generate AB, AC, or AD isomers (Fig. 5.2) [32].

Example protocols for experimental design

The method below is an example protocol for preparing Ts-CD. This protocol is based on the one previously reported by Tang and Ng [34].

1. Fit a three-necked, round-bottomed flask, into which a magnetic stir bar is added, with a Liebig condenser and a pressure equalizing addition funnel.
2. Fit the condenser with a rubber septum.
3. Degas the reaction setup by applying three cycles of pumping and nitrogen refilling.
4. Fill the flask with nitrogen.
5. Add 400 mL of freshly dried pyridine and 25 g of β-CD into the flask.
6. Turn on the magnetic stirrer.
7. Cool the flask to 0°C in an ice-water bath.
8. Fit a two-necked, round-bottomed flask, into which a magnetic stir bar is added, with a rubber septum.
9. Degas the flask by applying two cycles of pumping and nitrogen refilling.
10. Fill the flask with nitrogen.
11. Add 4 g of *p*-toluenesulfonyl chloride into the two-necked flask.
12. Add 30 mL of freshly dried pyridine into the flask using a glass syringe fitted with a hypodermic needle.
13. Dissolve *p*-toluenesulfonyl chloride in pyridine under magnetic stirring.

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14. Add the obtained solution to the addition funnel (which has been fitted with the three-necked flask in Step 1) for one hour under reflux and magnetic stirring, during which the temperature of the reaction flask should be kept at 0°C.
15. Remove the water bath.
16. Keep the setup at ambient conditions for 24 hours.
17. Perform vacuum distillation under reduced pressure to remove most of the pyridine from the reaction mixture.
18. Add 600 mL of acetone into the flask with vigorous stirring for 30 minutes.
19. Filter the reaction mixture to obtain solid residues.
20. Wash the solid residues with acetone three times.
21. Use hot water to recrystalize the solid.
22. Dry the solid overnight in a vacuum oven at 60°C to obtain the product.

When functionalization of the larger rim is required, sulfonation or tosylation of the C-2 position is a vital step. To obtain 2-tosyl-β-CD, one possible approach is via a transesterification reaction using *m*-nitrophenyl tosylate in a DMF-water solution at the basic pH [38]. The reaction yield is, however, far from satisfactory. More recently, Teranishi has postulated a synthetic route to obtain disulfonated CDs at the C-2 position. Similar to the situation of 6-disulfonates as mentioned earlier, the geometry of the sulfonating agent has been exploited to control the regioselectivity of the sulfonation reaction [39,40]. Furthermore, strategies have been reported to generate 3-polyfunctionalized CDs [41], among which

amino derivatives of CDs have received extensive research efforts. In particular, monoamine derivatives at the 3- or 6-position have been adopted to generate a series of CD derivatives with side chains via coupling reactions [42−46].

Roles in vector design

Native CDs have rarely been used directly as a nucleic acid carrier, particularly because of their failure to form stable complexes with nucleic acids for mediating transfection [47]. Derivatization of CDs is, therefore, usually performed prior to applications in nucleic acid transfer. One example of CD derivatives developed for nucleic acid delivery is the polycationic amphiphilic CD. This derivative is constructed by modifying the facial anisotropy of the truncated-cone CD torus through instillation of hydrophobic and cationic elements in the "jellyfish" or "skirt" configuration [48,49]. Its DNA complexation capacity and transfection efficiency can be tuned by modulating various parameters, including the nature of the functional groups, charge density, hydrophilic-hydrophobic balance, and spacer length [50−52]. More examples of CD derivatives are presented in Fig. 5.3. They have been prepared by incorporating β-CD with an alkylimidazole group, a

methoxyethylamino group, a pyridylamino group, or a primary amine group at the 6-position of the glucose units [47]. Compared to native β-CD, these derivatives have been shown to be more effective in facilitating the uptake of the transgene [47]. In addition to functioning as nucleic acid carriers, CDs can be used as structural modifiers during vector development.

When CDs are applied as structural modifiers, they can be used in two ways. One is to be adopted as threading devices. This is exemplified by the case of supramolecular polyrotaxanes, in which around 12 α-CD rings can be found in each molecule of the poly((ethylene oxide)-ran-(propylene oxide)) (P(EO-r-PO)) random copolymer, with the rings being located selectively on EO segments [53]. The polyrotaxanes have displayed higher transfection efficiency than PEI 25 kDa in HEK293 cells [53], and may warrant further development as gene carriers for in vivo use. The second method of using CDs as structural modifiers is as pendants. A good example of nucleic acid carriers developed using this approach is the PAMAM dendrimer conjugates containing α-, β-, and γ-CDs. The conjugates can condense plasmids and protect the plasmids from DNase I-mediated degradation [54]. In vitro studies have shown that the conjugate with α-CD, namely α-CDE, is more effective in transfection
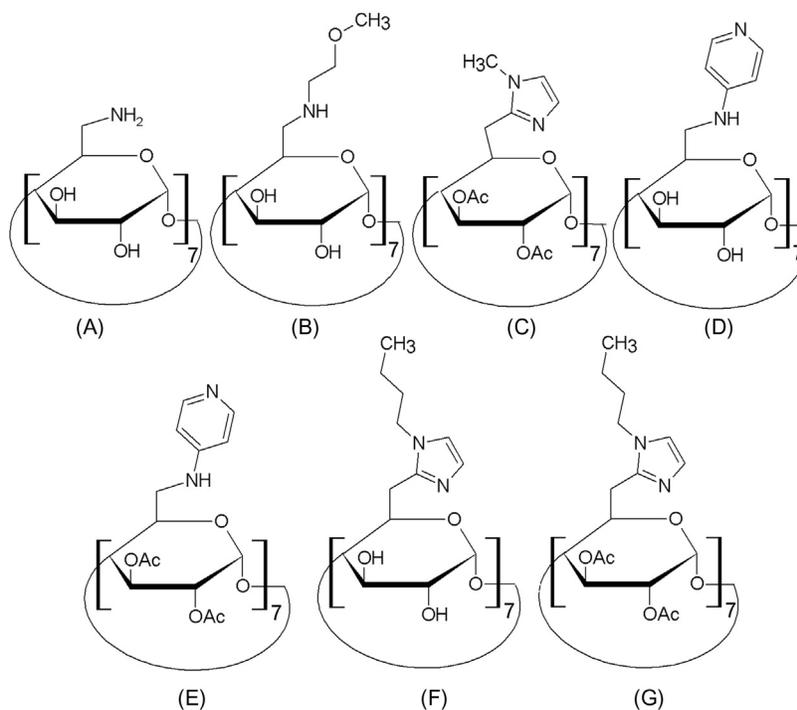
FIGURE 5.3 Structures of some CD derivatives. These derivatives include (A) heptakis(6-amino-6-deoxy)-β-CD, (B) heptakis(6-deoxy-6-methoxyethylamino)-β-CD, (C) heptakis[2,3-di-O-acetyl-6-deoxy-6-(1-methyl-1H-imidazol-2-yl)]-β-CD, (D) heptakis(6-deoxy-6-pyrid-4-ylamino)-β-CD, (E) heptakis(2,3-di-O-acetyl-6-deoxy-6-pyrid-4-ylamino)-β-CD, (F) heptakis[6-(1-n-butyl-1H-imidazol-2-yl)-6-deoxy]-β-CD, and (G) heptakis[2,3-di-O-acetyl-6-(1-n-butyl-1H-imidazol-2-yl)-6-deoxy]-β-CD. *Reproduced from W.F. Lai, Cyclodextrins in non-viral gene delivery, Biomaterials 35 (2014) 401−411 with permission from Elsevier B.V., [2].*

than those with β- and γ-CDs [54]. Its transfection efficiency in RAW264.7 and NIH3T3 cells has also been found to be much higher than that achieved by Lipofectin and the unmodified dendrimer [54]. To enhance the transfection efficiency of the conjugate, an earlier study has attempted to modulate the structural parameters by generating conjugates with different dendrimers. Results have revealed that α-CDE generated from the G3 dendrimer has exhibited higher transfection efficiency than those generated from G2 and G4 dendrimers [55]. In addition, conjugates having different degrees of substitution (DS) of α-CD have shown variations in cytotoxicity, transfection efficiency, and membrane-disruptive ability [56]. Compared with those having DS values of 1.1 and 5.4, the one having a DS value of 2.4 has been found to be more effective in transfection in NIH3T3 and HepG2 cells, and to show higher efficiency in delivering plasmids to liver, spleen, and kidney after i.v. administration [56]. These results have demonstrated the importance of structural optimization of a synthetic vector for nucleic acid transfer.

Apart from being used as structural modifiers, CDs can function as linking agents to covalently link other polymers together to form larger molecular constructs. For instance, linear CD-linked polymers have previously been generated from difunctionalized CDs and difunctionalized comonomers [57]. Among the polymers generated, the linear polymer containing 6 methylene units has displayed the highest transfection efficiency in BHK-21 cells. The efficiency of those containing 5, 4, 7, 8, 10 methylene units has been found to be only 6%, 22%, 50%, 64%, and 10% of the efficiency of the one containing 6 methylene units, respectively [29]. These results have shown that different levels of CD incorporation can influence the efficiency of the generated polymer in transfection. Another example of polymeric nucleic acid carriers developed by using CDs as linking agents is PEI-β-CD, which can be synthesized by first using tosyl chloride to generate amine-reactive tosyldeoxy-β-CD, followed by the reaction with PEI [24]. In vitro studies have demonstrated that PEI-β-CD exhibits higher transfection efficiency than unmodified PEI, and shows negligible toxicity in HEK293 cells at its working concentration for plasmid delivery [24]. Such high efficiency of PEI-β-CD in transfection can be further escalated upon conjugation of the polyplexes with human insulin that has been derivatized with a hydrophobic palmitate group [24]. Here it is worth mentioning that proper optimization of the grafting ratio of CDs is a prerequisite to the development of an effective PEI-β-CD-based carrier. This has been demonstrated by the fact that modification of 5%, 10%, and 16% of the amine groups in PEI with CDs may lead to a reduction in the luciferase activity by 1, 2, and 4 orders of

magnitude, respectively [25]. This reduction has been attributed to the changes in the pKa values of the PEI amines, leading to a decrease in the efficiency in endosomal escape of the polyplexes. This hypothesis has been supported by the observation that the pH buffering capacity of PEI-β-CD is much lower than that of PEI [25].

Example protocols for experimental design

The method below is an example protocol for preparing PEI-β-CD. This protocol is based on the one previously reported by Forrest and coworkers [24].

1. Disperse 10 g of β-CD in 25 mL of H_2O.
2. Adjust the pH to 13 using a NaOH solution.
3. Stir the solution vigorously at 0°C in an ice-water bath.
4. Recrystallize 2.23 g of tosyl chloride from chloroform with petroleum ether, and then dissolve it in 5 mL of acetonitrile.
5. Add the solution dropwise to the CD solution under magnetic stirring.
6. Readjust the pH of the CD solution to 13.
7. Allow the mixture to react for 5 minutes.
8. Adjust the pH of the mixture to 5.5 using HCl.
9. Keep the reaction mixture at ambient conditions for 1 hour under vigorous stirring.
10. Filter the mixture to obtain the white precipitate.
11. Recrystallize the precipitate from boiling water.
12. Lyophilize to obtain tosyldeoxy-β-CD.
13. Dissolve 0.2 g of PEI 25 kDa in 6 mL of DMSO.
14. Add triethylamine into the PEI solution to reach a concentration of 100 mM.
15. Dissolve 368 mg of tosyldeoxy-β-CD in 1 mL of DMSO.
16. Add the solution in Step 15 into the PEI solution.
17. Keep the reaction mixture at ambient conditions for 12 hours.
18. Remove the solvent in vacuo at elevated temperature to obtain the product.

Besides native CDs, derivatives of CDs can be adopted as linking agents. This has been demonstrated by Huang et al.'s study, which has cross-linked PEI by using (2-hydroxypropyl)-β-CD (2-hy-β-CD) and (2-hydroxypropyl)-γ-CD (2-hy-γ-CD) [58]. The generated polymers have been found to be less cytotoxic than PEI 25 kDa, with the transfection efficiency in SKOV-3 cells being around 2 and 20 times higher than that achieved by PEI 25 kDa and PEI 600 Da, respectively. Apart from hydroxyl-alkylation, β-CD has been converted into the carboxymethyl-β-CD sodium salt, which has subsequently been combined with quaternized CS to generate a DNA carrier [59]. The carrier has been found to adsorb plasmids at a polymer/DNA mass-to-mass ratio of 4:1, and has reached 40% of the efficiency achieved

by liposomes in transfection [59]. All polymers discussed above, however, have not been compared with those generated with native CDs. The effects of hydroxy- and carboxy-alkylation of CDs on the performance of the polymeric carriers still have not yet been fully elucidated. More recently, methyl β-CD has also been exploited as a crosslinking agent to generate a nucleic acid carrier from lPEI [60]. The product has exhibited a low membrane-disruptive capacity ex vivo and high transfection efficiency both in the presence and absence of serum [60]. It may be further developed as a promising delivery system for genetic manipulation.

Example protocols for experimental design

The method below is an example protocol for crosslinking lPEI with methyl β-CD.

1. Dissolve 0.05 g of methyl β-CD in 1 mL of degassed DMSO.
2. Dissolve 0.05 g of CDI in 1 mL of degassed DMSO.
3. Inject the two solutions into a flask that has been filled with nitrogen.
4. Add 100 μL of triethylamine into the flask.
5. Keep the reaction mixture in darkness under magnetic stirring for 3 hours.
6. Dissolve 0.67 g of lPEI (2.5 kDa) in 1.5 mL of H_2O and 11.5 mL of DMSO.
7. Heat the PEI solution at 60°C−70°C under vigorous stirring until complete dissolution.
8. Add the PEI solution into the reaction mixture.
9. Keep the reaction mixture at 60°C for 1 day under magnetic stirring in darkness.
10. Dialyze the reaction mixture (molecular weight cut-off = 12 kDa) against doubly deionized water for 2 days.
11. Lyophilize to obtain the product.

Cyclodextrin incorporation for multifunctionality

In preceding sections, we have discussed how CDs can be used in the design and modification of polymeric carriers. As a matter of fact, CDs show great potential for use in concomitant delivery of multiple agents. The possibility of this has been demonstrated by Hu et al. [61], who have conjugated tegafur to PEI-β-CD to generate a prodrug of tegafur for drug/gene codelivery. They have observed that at an optimal polymer−DNA ratio, the conjugate has condensed plasmids into complexes at the nanoscale; however, as primary amine groups on PEI have participated in the conjugation reaction, the DNA condensation capacity, pH buffering capacity, and transfection efficiency of PEI-β-CD have been reduced after incorporation with tegafur [61]. The 5-fluoro-2′-deoxyuridine (FdUrd)-PEI-β-CD conjugate, which has been synthesized from PEI-β-CD and FdUrd, is another example of CD-based carriers for gene/drug codelivery [62]. Compared to FdUrd, this polymer has displayed higher cellular internalization efficiency, higher cytotoxic effects, and stronger anti-proliferative activities in glioma cells [62]. Despite this, before PEI-β-CD-tegafur and FdUrd-PEI-β-CD can be adopted as prodrugs, their pharmacokinetic and pharmacodynamic profiles should be studied to examine the effect of PEI-β-CD conjugation on the properties of tegafur and FdUrd. Recently, through radical copolymerization of 2-vinyl-4,6-diamino-1,3,5-triazine (VDT) with PEG methacrylated β-CD, a hydrogen bonding strengthened hydrogel has been developed [63]. The hydrogel has been successfully loaded with ibuprofen. Furthermore, its surface has been found to anchor plasmids through hydrogen bonding between the DNA base pairs and diaminotriazine, thereby enabling reverse gene transfection in COS-7 cells cultured on the gel surface. This multifunctional feature enables the hydrogel to be exploited as a tissue engineering scaffold for drug/gene codelivery.

Apart from codelivering chemical drugs with nucleic acids, CDs can be incorporated with imaging agents to enable the fate of a carrier to be tracked. One example of these agents is cyanine dyes, which possess structures based on two aromatic or heterocyclic rings linked via a polymethine chain with conjugated carbon−carbon double bonds [64]. These compounds exhibit both colorimetric and fluorescent properties. Not only can they cover all wavelengths in the visible spectrum [65], but they also display high molar absorptivity and have narrow absorption bands [65]. They therefore have emerged as promising fluorescent probes for optical imaging [66,67]. In as early as the 1990s, synthesis of cyanine-β-CD derivatives has already been reported in an attempt to generate a fluorescent labeling reagent with enhanced photostability [68]. These compounds have later been successfully used as spectroscopic probes to recognize colorless guest molecules (e.g., 1-adamantanol, and vitamin B6) [69]. More recently, synthesis of two water-soluble cyanine dye/β-CD derivatives has been performed under simultaneous ultrasound/microwave irradiation via Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition, in which monoazido CD derivatives have been linked together by using a 1,2,3-triazole moiety [70]. The stability constants for doxorubicin complexes with cyanine/β-CD derivatives have been found to be four orders of magnitude larger than the constants reported for those with native β-CD [70]. If the derivative is incorporated as part of a CD-containing polymeric carrier, it is plausible that the resulting polymer may enable the execution of imaging during gene/drug codelivery. We have reviewed the use of CDs in molecular imaging in detail elsewhere [3]. Readers may refer to that article for reference.

Manipulation of host−guest complexation

When CDs are incorporated into the design of a carrier, sometimes this is driven by the wish of taking advantage of the ability of CDs for host−guest complexation, either for improving the targeting specificity of the system [71] or for loading chemical drugs into the carrier for gene/drug codelivery [72]. To optimize the structure of these modified carriers, which need to make use of the complexation capacity of CDs, we need to understand properly the electrostatic potentials inside the CD cavity as well as the molecular interactions during inclusion complexation. As far as the complexation thermodynamics of CDs is concerned, it is thought to be contributed by three major mechanisms: (1) penetration of the hydrophobic moiety of a guest molecule into the CD cavity [73], (2) degradation of the guest molecule [73−75], and (3) conformational alternations or strain release experienced by the CD molecule upon complexation [76,77]. Apart from this, thermodynamic quantities of the inclusion complexation process may be influenced by the buffer used, the solvation of the chemical species, and the release of water molecules from the cavity to bulk water [74,78].

In fact, though α-, β-, and γ-CD have a similar height of torus (≈ 7.9 Å) [79], their cavity volumes are very different. As the number of α-D-glucopyranose units comprising the CD molecule increases from 6 for α-CD to 8 for γ-CD, the cavity volume increases from 174 to 427 Å^3 [79]. Taking the change in the size of the hydrophobic CD cavity into consideration, the general trend in the complexation thermodynamics of CDs can be partially explained by using the size-fit concept. The validity of this concept has been shown by Varghese and coworkers [80], who have studied changes in the photophysical properties of 3-naphthyl-1-phenyl-5-(4-carboxyphenyl)-2-pyrazoline (NPCP) when NPCP has drifted into the CD

cavity from bulk water. Results have revealed that an increase in the concentration of β-CD (or γ-CD) in an aqueous solution has led to an increase in the intensity of fluorescence emission from NPCP, along with a shift to the lower wavelength side [80]. This phenomenon has been partly attributed to the deep inclusion of the pyrazoline fragment into the CD cavity (Fig. 5.4), thereby restricting the rotational and vibrational motions of NPCP and hence inhibiting the occurrence of nonradiative decay processes [81]. Under the stoichiometry of 1:1, the association constant K for NPCP/γ-CD complexes (1.3×10^4 mM^{-1}) has been reported to be much higher than that of NPCP/β-CD complexes (5.8×10^3 mM^{-1}). This has shown that the binding of NPCP to the cavity of γ-CD is stronger than that to the cavity of β-CD, and has been hypothesized to be related to the size fitting between the cavity and NPCP [80]. This hypothesis has been supported by semiempirical quantum mechanics calculations, which suggest that the binding energy of NPCP/γ-CD complexation for β-CD is more positive than that for γ-CD, though the process of inclusion complexation for both β-CD and γ-CD is exothermic [80].

In fact, by understanding the complexation thermodynamics of CDs, we can better design or manipulate the process of host−guest inclusion complexation. For instance, the diameter of the cavity of α-CD ($\approx 4.7-5.3$ Å) is much smaller than that of β-CD ($\approx 6.0-6.5$ Å) [79]. Along with the knowledge that the distance of separation is an important factor modulating the induction of van der Waals forces [82], we may expect that forces induced by a straight-chain guest molecule during complexation with β-CD will be smaller (less negative ΔH°) than that with α-CD. We may also anticipate that the situation will go the other way around if we now change the straight-chain guest into an adamantyl guest. Here it is worth mentioning that if the guest molecule fails to be fully accommodated by
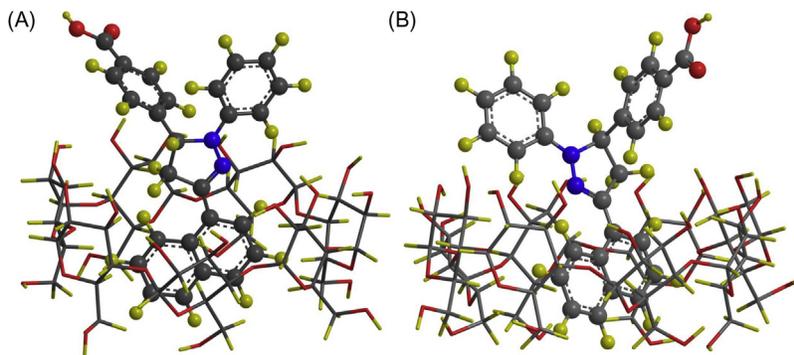
FIGURE 5.4 The most probable structure of the (A) NPCP/β-CD and (B) NPCP/γ-CD complex. The hydrogen, oxygen, carbon, and nitrogen atoms are colored in yellow, red, gray, and blue, respectively. *Reproduced from B. Varghese, S.N. Al-Busafi, F.O. Suliman, S.M.Z. Al-Kindy, Tuning the constrained photophysics of a pyrazoline dye 3-naphthyl-1-phenyl-5-(4-carboxyphenyl)-2-pyrazoline inside the cyclodextrin nanocavities: a detailed insight via experimental and theoretical approach, Spectrochim. Acta A, 173 (2017) 383−389 with permission from Elsevier B.V., [80].*

the CD cavity, the induction of van der Waals interactions can actually be affected by steric hindrance [82]. For this, it is a general trend that if the guest molecule is an acyclic compound, it may fit better into α-CD; whereas a cyclic aliphatic guest may fit better into β-CD. Yet, exceptions to this general trend exist. One example is imidazole, which possesses a five-membered ring and can fit better into the cavity of α-CD than that of β-CD [82]. In addition, if a guest molecule possesses a phenyl moiety, it usually exhibits a stronger affinity with β-CD than with α-CD, although the actual affinity of the guest molecule with CDs may be changed after the phenyl moiety has undergone substitution reactions [82].

The stability of an inclusion complex is affected not only by van der Waals forces but also by hydrogen bonding. The roles played by hydrogen bonding in host−guest inclusion complexation, however, are determined predominately by the type of functional groups present in the guest molecule. This has been suggested by a previous study, which has adopted various structurally related aromatic compounds (either with or without a phenolic hydroxyl group) as guest molecules. The study has revealed that, in general, charged and hydrophilic groups (except phenolic hydroxyl groups) present in the guest molecule stay in the bulk solution even after inclusion complexation [73]. In addition, the standard molar enthalpies and equilibrium constants for inclusion complexation between α-CD and either 1-O-hexyl-β-D-glucopyranoside or 1-hexanol have been observed to be basically the same, despite the difference in the types of hydrophilic groups present in those two guest molecules [83]. This observation has led to a hypothesis that only the hexyl group of these guest molecules can get into the CD cavity, from which both the glucopyranose moiety of hexyl glucopyranoside and the hydroxyl group of 1-hexanol are excluded owing to their high hydrophilicity.

Finally, the thermodynamics of host−guest chemistry is largely affected by the flexibility of a guest molecule. For example, when we see the presence of a double bond in the aliphatic chain residue of a guest molecule, we may expect that the conformational degree of freedom of that molecule will be lower than its saturated counterpart. The process of inclusion complexation with CDs will also become less favorable in the entropic term. This postulation has been supported experimentally by a previous study, which has found that the equilibrium constants of heptanoate and hexanoate for complexation with α-CD are substantially higher than those of 6-heptenoate and *trans*-3-hexenoate [75]. In addition to the flexibility of the molecular structure, the stereoisomerism of a guest molecule should be considered owing to the enantioselective nature of the inclusion complexation process [82]. All these have suggested that when CDs are incorporated into

the design of a carrier in which the host−guest binding capacity is important, careful consideration of the complexation thermodynamics of CDs can help to facilitate carrier design and optimization.

Summary

Nucleic acid delivery is an expanding area of biotechnological research [84−86], partly because of its application potential to mediate genetic manipulation. Over the last several decades, while synthetic polymers have been extensively developed as carriers for nucleic acid transfer [87−92], the possible use of CDs in the design and optimization of these carriers has always been overlooked. This may be partly because native CDs fail to form stable complexes with plasmids [47], thereby being less effective in transfection as compared with other commonly used polymeric carriers, such as CS, PLGA, and PEI. In fact, CDs possess properties (e.g., forming inclusion complexes with chemical drugs for gene/drug codelivery, and modulating the cytotoxicity of other polymers) favorable for use in nucleic acid transfer. They cannot only modulate the performance of existing polymeric carriers, but can also serve as linking agents for the development of new polymers. CDs are, therefore, promising tools to enhance the efficiency of existing polymer-based nucleic acid delivery technologies for genetic manipulation in the future.

Directions for intervention development

CDs can modulate the properties of polymeric carriers, and can also function as linking agents during polymer fabrication. To exploit the possible benefits brought by CDs for genetic manipulation, the following steps may be taken during the design of a polymeric carrier.

1. Exploit the features of CDs and their relevance to the proposed intervention.
2. Consider how those features can facilitate the fabrication of the designed polymer or can improve the efficiency of the intervention.
3. Plan the procedure for the synthesis of the polymer by taking CDs as one of the components.
4. Characterize the structure of the product.
5. Evaluate the performance of the product.
6. Optimize the structure and properties of the product, if necessary, to maximize the efficiency of the proposed intervention.

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