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Self-assembled drug delivery systems 2. Cholesteryl derivatives of antiviral nucleoside analogues: Synthesis, properties and the vesicle formation

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Abstract

Self-assembled drug delivery systems (SADDS) are defined as the self-aggregates of amphiphilic prodrugs. Prodrug, molecular self-assembly and nanotechnology are involved in SADDS manufacturing. But the knowledge of the self-assembly of amphiphilic prodrugs and the formation rules of SADDS is very limited. In this paper, five cholesteryl derivatives of antiviral nucleoside analogues were synthesized, involving antiviral acyclovir, didanosine and zidovudine, and the different acyl linkers, succinyl, adipoyl and phosphoryl. The derivatives are typical amphiphiles with nucleosides as polar heads and long-chained lipids as hydrophobic tails. The derivatives showed the similar soluble behavior, and the solubility highly depended on the types of solvents. Two forces, hydrogen bonding and hydrophobic interaction in alcohol solutions could improve the derivatives dissolving. However, the molecular self-assembly of derivatives could prefer to happen in the noncompetitive solvents including chloroform and tetrahydrofuran (THF) based on the intermolecular hydrogen bonding between nucleobase moieties, which could greatly increase their solubility. The derivatives formed nanosized vesicles based on hydrophobic interaction after injecting their THF solutions into water. The volume ratios of polar heads and hydrophobic tails of amphiphiles could determine the vesicle size, and the amphiphiles with large ratios would prefer to form small vesicles. The self-assembled vesicles would likely become SADDS.

Keywords: Antiviral; Cholesteryl; Molecular self-assembly; Nucleosides; Prodrugs; Vesicles

1. Introduction

In our previous researches, the long-chained lipid derivatives of acyclovir were prepared and one of them (the glyceride derivative) formed self-assembled nanoparticles (SAN) in water due to its amphiphilic property (Jin et al., 2005). After i.v. administration to rabbits, the SAN showed the significant mononuclear phagocyte system (MPS, mainly including liver, lung and spleen) specific distribution and drug sustained-release effect in the targeted sites (Jin et al., 2006a). The novel drug delivery systems have many unique properties different from traditional drug carriers such as liposomes and nanoparticles, which are defined as self-assembled drug delivery systems (SADDS). The combination of prodrug, molecular self-assembly and nanotechnology in SADDS could take them many unique advantages over traditional carriers. They can deliver themselves in vivo, and show high drug loading, very low drug leakage in preservation and circulation, drug targeting and controlled-release.

The knowledge about SADDS is very limited up to now although the preliminary researches have been done (Jin et al., 2005, 2006a,b; Jin, 2007). It was known that a polar drug head such as acyclovir and a long-chained glyceride-type lipid other than double-chained lipids were necessary to prepare an appropriate amphiphilic prodrug that would then

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form nanosized self-aggregates. The physicochemical properties including molecular self-assembly of amphiphilic prodrugs must be known before manufacturing SADDS, and the obtained enough information can help to prepare ideal SADDS. In the previous case of the glyceride derivative of acyclovir, the bilayers were first formed based on the hydrophobic interaction between lipid chains, and then overlapped layer-by-layer to form cuboid-shaped nanoparticles in water (Jin et al., 2005). However, a lot of researches on different drugs, lipids and their various combinations are necessary to obtain the deep and extensive knowledge of SADDS.

Nucleoside analogues could become important active agents such as antivirals (Simons et al., 2005), anticancer agents (Lech-Maranda et al., 2006) and oligonucleotide antisense agents (Stahel and Zangemeister-Wittke, 2003). Due to a relatively strongly polar group (nucleobase) and one or more reactive groups (hydroxyl or amino) in molecules, nucleoside analogues are appropriate model drugs to study their amphiphilic prodrugs and SADDS. And after the research, both the general self-assembling rules and the potential pharmacotherapeutic agents would be obtained. Five cholesteryl derivatives of antiviral nucleoside analogues containing representative nucleobase groups were synthesized and investigated on their physicochemical properties including solubility and molecular self-assembly in the paper. The obtained useful information benefits to well understand the intermolecular interaction and molecular self-assembly of amphiphilic antiviral nucleosides, and prepare optimal SADDS in future.

2. Materials and methods

2.1. Materials

Acyclovir (ACV) was purchased from Zhejiang Jiayuan Pharmaceutical Co. Ltd., China. Zidovudine (AZT) and didanosine (ddI) were from Zhang Jiang Desano Science and Technology Co. Ltd., Shanghai, China. Organic solvents were of analytical grade. Other chemicals were of reagent grade. Water was distilled. UV spectra, ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded respectively on a Shimadzu UV-2501PC spectrophotometer, a JNM-ECA-400 NMR spectrometer, and FAB-MS and ESI-MS were respectively recorded on a Micromass ZabSpec high-resolution mass spectrometer and a Thermo LCQ Advantage mass spectrometer.

2.2. Synthesis of the cholesteryl derivatives of antiviral nucleoside analogues

2.2.1. Cholesteryl-succinyl acyclovir (1, CSA, $C_{39}H_{59}N_5O_6$)

Succinyl acyclovir (SACV) was synthesized according to the literature (Colla et al., 1983). SACV (1 eq.) and cholesterol (3 eq.) were dissolved in *N*,*N*-dimethyl formamide/tetrahydrofuran (DMF/THF, 1:1, v/v), and 4-dimethylaminopyridine (DMAP, 0.2 eq.) and dicyclohexylcarbodiimide (DCC, 1.5 eq.) as catalysts were added. The solution was sealed and agitated at room temperature for about 30 h. Most solvents were removed under vacuum, and the remained solution was poured into the saturated $NaHCO_3$ solution. The white suspension was filtered. The residual was dried, recrystallized from 2-propanol and isolated by silica gel column chromatography. Yield 55%; TLC: chloroform/methanol, 8.5:1.5 (v/v), $R_f = 0.65$; UV (methanol): $\lambda_{max} = 252.8$ nm; δ_H (400 MHz; $CDCl_3$) 0.66–2.06 (43H cholesteryl), 2.31, 2.62 (m, 4H, $OCCH_2CH_2CO$), 3.75 (s, 2H, OCH_2CH_2OCO), 4.00 (t, 2H, $J = 11.7$ Hz, OCH_2CH_2CO), 4.25 (m, 1H, OCH cholesteryl), 5.35 (d, 1H, $J = 5.6$ Hz, $CCHCH_2$ cholesteryl), 5.46 (s, 2H, NCH_2O), 6.72 (s, 2H, NH_2), 7.76 (s, 1H, NCHN guanine), 11.70 (s, 1H, OCNHC guanine); δ_C (100 MHz; $CDCl_3$) 11.8–27.7, 28.9–56.6 (24C cholesteryl), 28.0, 28.2 ($OCCH_2CH_2CO$), 63.4 (CH_2CH_2O), 67.1 (CH_2CH_2O), 72.7 (NCH_2O), 74.4 (OCH cholesteryl), 116.5 (C-5 guanine), 122.7 (CH cholesteryl), 137.9 (CH-8 guanine), 139.5 (C cholesteryl), 151.8 (C-6 guanine), 153.8 (C-2 guanine), 156.8 (C-4 guanine), 171.7, 172.4 ($OCCH_2CH_2CO$); FAB-MS: 695.2 ($M+H^+$).

2.2.2. Cholesteryl-succinyl didanosine (2, CSD, $C_{41}H_{60}N_4O_6$)

The synthesis of cholesteryl hemisuccinate (CHS) was referred to as the literature with a little modification (Deng et al., 2001). Cholesterol (1 eq.), succinic anhydride (3 eq.) and DMAP (0.1 eq.) were dissolved in dichloromethane (DCM), and refluxed at 55 °C for 3 days. The solvent was removed under vacuum. The residual was dissolved in ethanol and then poured into the ice solution containing 15% NaCl. The obtained suspension was adjusted to pH 2.0 by adding 1 M HCl solution. The suspension was filtered and washed to neutral by water. The dried solid residual was recrystallized from ethanol/ethyl acetate (10:1, v/v) with the CHS yield near 100%. CHS (2 eq.), ddI (1 eq.), DCC (1.2 eq.) and DMAP (1 eq.) were dissolved in DMF/THF (1:1, v/v), sealed and agitated at 50 °C for 2 days. Most solvents were removed under vacuum, and the remained solution was poured into the saturated $NaHCO_3$ ice solution. The white suspension was filtered and the residual was dried. CSD was recrystallized from methanol and isolated by silica gel column chromatography. Yield 65%; TLC: chloroform/methanol/ammonia, 85:15:5 (v/v/v), $R_f = 0.70$; UV (methanol): $\lambda_{max} = 249.6$ nm; δ_H (400 MHz; $CDCl_3$) 0.66–2.17 (43H cholesteryl), 2.31, 2.65 (m, 4H, $OCCH_2CH_2CO$), 4.36 (m, 1H, OCH cholesteryl), 5.35 (d, 1H, $J = 3.9$ Hz, $CCHCH_2$ cholesteryl), 6.29 (t, 1H, $J = 4.5$ Hz, NCH), 8.11 (s, 1H, C(8)H hypoxanthine) 8.14 (s, 1H, NC(2)HN hypoxanthine), 12.71 (s, 1H, NH); δ_C (100 MHz; $CDCl_3$) 11.8–27.7, 29.0–56.7 (24C cholesteryl), 28.0, 28.2 ($OCCH_2CH_2CO$), 65.0 (OCH_2), 79.5 (OCH cholesteryl), 85.7 (NCH), 122.7, 138.4, 139.6, 148.2, 160.0 (5C hypoxanthine), 125.3, 144.5 (2C cholesteryl), 171.6, 172.3 ($OCCH_2CH_2CO$); FAB-MS: 705.5 (M^+).

2.2.3. Cholesteryl-adipoyl didanosine (3, CAD, $C_{43}H_{64}N_4O_6$)

Cholesterol hemiadipate (CHA) was synthesized like CHS except for using DCM as solvent. The immediate product had the yield near 100%. CAD was also synthesized like CSD. Yield 60%; TLC: chloroform/methanol/ammonia,

7:1:0.5 (v/v/v), $R_f = 0.58$; UV (methanol): $\lambda_{max} = 249.6$ nm; δ_H (400 MHz; $CDCl_3$) 0.63–2.40 (43H cholesteryl), 1.53, 1.64 (m, 4H, $OCCH_2CH_2CH_2CH_2CO$), 2.26, 2.52 (m, 4H, $OCCH_2CH_2CH_2CH_2CO$), 4.37 (m, 1H, OCH cholesteryl), 5.33 (s, 1H, $CCHCH_2$ cholesteryl), 6.26 (t, 1H, $J = 2.8$ Hz, NCH), 8.08 (s, 1H, C(8)H hypoxanthine) 8.19 (s, 1H, NC(2)HN hypoxanthine), 13.10 (s, 1H, NH); δ_C (100 MHz; $CDCl_3$) 11.9–27.9, 31.0–56.7 (24C cholesteryl), 28.1, 28.3 ($OCCH_2CH_2CO$), 65.0 (OCH_2), 79.6 (OCH cholesteryl), 85.9 (NCH), 122.7, 138.3, 139.7, 148.3, 159.2 (5C hypoxanthine), 125.3, 145.0 (2C cholesteryl), 172.7, 173.1 ($OCCH_2CH_2CO$); ESI-MS: 733.6 ($M+H^+$).

2.2.4. Cholesteryl-succinyl zidovudine (4, CSZ, $C_{41}H_{61}N_5O_7$)

Succinyl zidovudine (SAZT) was synthesized according to the literature (Giammona et al., 1998). SAZT (1 eq.), cholesterol (3 eq.), DMAP (0.2 eq.) and DCC (1 eq.) were dissolved in THF, and refluxed at 65 °C for 10 h. Most solvents were removed under vacuum, and the remained solution was poured into the saturated $NaHCO_3$ solution. The white suspension was filtered and the residual was dried. CSZ was recrystallized from 2-propanol and isolated by silica gel column chromatography. Yield 70%; TLC: chloroform/methanol/ammonium, 9:1:0.5 (v/v/v), $R_f = 0.65$; UV (methanol): $\lambda_{max} = 265.2$ nm; δ_H (400 MHz; $CDCl_3$) 1.11–1.98 (43H cholesteryl), 2.44, 2.71 (m, 4H, $OCCH_2CH_2CO$), 4.02 (m, 1H, N_3CHCH), 4.27 (m, 1H, OCH cholesteryl), 6.16 (t, 1H, $J = 6.2$ Hz, NCHO), 7.33 (s, 1H, NCHCO thymine), 8.15 (s, 1H, OCNHCO thymine); δ_C (100 MHz; $CDCl_3$) 12.6–26.0, 30.7–55.2 (24C cholesteryl), 29.3, 29.6 ($OCCH_2CH_2CO$), 63.0 (OCH_2), 76.7 (OCH cholesteryl), 77.3 (NCHO), 81.8 ($OCHCH_2O$), 111.4 (C-5 thymine), 135.4 (C-6 thymine), 150.2 (C-2 thymine), 163.7 (C-4 thymine), 170.6, 173.1 ($OCCH_2CH_2CO$); FAB-MS: 736.2 (M^+).

2.2.5. Cholesteryl-phosphoryl zidovudine (5, CPZ, $C_{37}H_{58}N_5O_7P$)

Cholesteryl phosphorochloridate (CPC) was synthesized by the phosphorochloridate chemistry referred to as the literature (D'Cruz et al., 1999). The process was described as follows. Phosphoryl chloride (ca. 3 eq.) was dissolved in ethyl ether, and agitated with incubation in ice bath. Cholesterol (1 eq.) and triethylamine (TEA, 1.2 eq.) was also dissolved in ethyl ether, and transferred into the above phosphoryl chloride solution in drops with agitation. After the reaction had proceeded for 1 h, the solvent was removed under vacuum and the crude CPC (slight yellow solid) was obtained. CPC (ca. 3 eq.) was dissolved in DCM. AZT (2.5 eq.) was dissolved in DCM followed by adding TEA (3 eq.), and then the solution was dropped into the above CPC solution with agitation. After the reaction had proceeded at room temperature for about 3 h, most solvents were removed under vacuum. The remained solution was mixed with the 50-fold-volume water followed by thoroughly shaking. The organic phase was collected after departing from the aqueous phase, which was then dried by anhydrous Na_2SO_4 and then filtered. The solvent in the filtrate was removed under vacuum to obtain unpurified cholesteryl phosphorochloridate zidovudine (CPCZ). CPCZ was dissolved in THF containing 3% water and refluxed at 70 °C for about 1 h to be transformed to CPZ by hydrolysis. Most THF was removed under vacuum. The remained solution was mixed with appropriate water followed by extraction with an equal volume of DCM for three times. The departed organic phase was dried by anhydrous Na_2SO_4 and then filtered. The solvent in the filtrate was removed under vacuum to obtain CPZ that was then recrystallized from 2-propanol and isolated by silica gel column chromatography. Yield 50%; TLC: cyclohexane/acetone, 1.1:0.9 (v/v), $R_f = 0.71$; UV (methanol): $\lambda_{max} = 263.6$ nm; δ_H (400 MHz; $CDCl_3$) 0.68–1.95 (43H cholesteryl), 4.16 (d, 2H, $J = 2.8$ Hz $N_3CHCHCH_2$), 4.30 (m, 1H, OCH cholesteryl), 4.80 (m, 1H, POH), 5.42 (s, 1H, $CCHCH_2$ cholesteryl), 7.39 (s, 1H, NCHCO thymine), 8.29 (s, 1H, OCNHCO thymine); δ_C (100 MHz; $CDCl_3$) 11.8–56.6 (24C cholesteryl), 65.8 (OCH_2), 76.7 (OCH cholesteryl), 77.3 (NCHO), 81.0 ($OCHCH_2O$), 111.9 (C-5 thymine), 135.2 (C-6 thymine), 150.3 (C-2 thymine), 163.5 (C-4 thymine); ESI-MS: 716.4 ($M+H^+$).

2.3. Determination of solubility

The derivatives were respectively added to the varied solvents of appropriate amount, and agitated at 37 °C for at least 24 h until the residual solid did not disappear. After the saturated solutions were filtrated through 0.45-μm membranes, the filtrates were determined using the ultraviolet spectrophotometry. The high-concentrated solutions needed to be diluted with methanol before determination.

2.4. Preparation of self-aggregates in water

An injection method was used to prepare the self-aggregates of the derivatives in water. The solutions in the water-miscible organic solvent THF containing 5 mg/ml derivatives were injected slowly and continually into vortexed water under the surface via a 100-μl micro-syringe until a homogeneous and slightly opalescent suspension was obtained. The suspensions were subsequently incubated in a 37 °C water bath under vacuum to remove solvents.

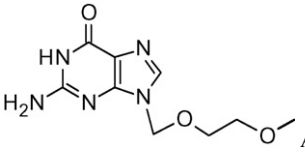
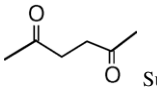
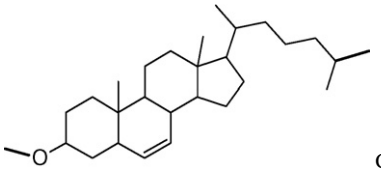
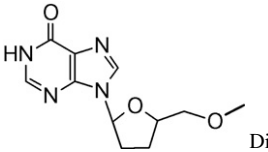
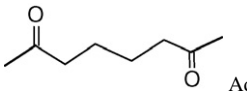
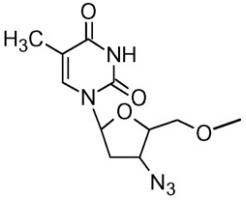
2.5. Transmission electron microscopy (TEM)

The self-aggregates of derivatives in water were observed using a Philips CM120 80-kV transmission electron microscope. They were stained with 2% sodium phosphotungstate on carbon-coated copper nets and air-dried at room temperature before observation.

2.6. Size measurement by light scattering

A laser light scatting instrument (NANOPHOX Particle Size Analysis, Sympatec GmbH System-Partikel-Technik, Germany) was used to analyze the particle size of self-aggregates in water at 25 °C. Before analysis, the suspensions were diluted 1:10 with distilled water.

Table 1
Structural information of the cholesteryl derivatives of antiviral nucleoside analogues

Derivative	Nu	L	R	Polar head	Length of hydrophobic tail[a] (Å)
CSA	 Acyclovir	 Succinyl	 Cholesteryl	Guanine and methoxy	24
CSD	 Didanosine	Succinyl	Cholesteryl	Hypoxanthine and tetrahydrofuran cycle	24
CAD	Didanosine	 Adipoyl	Cholesteryl	Hypoxanthine and tetrahydrofuran cycle	27
CSZ	 Zidovudine	Succinyl	Cholesteryl	Thymine, tetrahydrofuran cycle and azide	24
CPZ	Zidovudine	 Phosphoryl	Cholesteryl	Phosphoryl zidovudine	15

[a] The length of hydrophobic tail was computed out using ChemSketch 10.0 after 3D structural optimization.

3. Results and discussion

3.1. Structural characterization of the derivatives

The synthesized cholesteryl derivatives of antiviral nucleoside analogues are typical amphiphilic molecules. They have purin-type nucleobase heads (guanine in CSA; hypoxanthine in CSD and CAD) or pyrimidine-type nucleobases head (thymine in CSZ and CPZ), and a conjugated long-chained lipid group containing cholesteryl moiety (Table 1). It was known that the lipid derivatives of nucleobases including the cholesteryl derivatives could self-assemble (Sivakova and Rowan, 2005). Therefore, the design of the cholesteryl derivatives of antiviral nucleoside analogues is rational with the theoretic and experimental basis. In fact, the molecular structures of amphiphiles determine the physicochemical properties. However, besides the structural aspect, other factors could also affect the preparation of SADDS, such as manufacturing methods and formulated additives. ACV, ddI and AZT were selected as model drugs because they were most-used anti-HSV or anti-HIV agents in clinics and had typical nucleobase groups.

3.2. Solubility of the derivatives

These derivatives showed the similar soluble behavior, and the solubility highly depended on the types of solvents. They were very freely soluble in chloroform and THF, slightly or very slightly soluble in methanol, octanol and cyclohexane, and almost insoluble in water (Table 2). It is well known that the solubility is generally determined by the intermolecular interaction in the solute–solvent system. The cholesteryl moiety can improve the derivatives dissolving in organic solvents. Moreover, intermolecular hydrogen bonds could be formed between the derivatives and the solvents because nucleobase moieties had hydrogen donors and acceptors. In alcohol solutions, two forces could simultaneously occur between the derivatives and the solvents, i.e. hydrogen bonding and hydrophobic interaction, which were respectively resulted from the interaction between the hydroxyl and alkyl moieties of alcohol molecules and the nucleobases and cholesteryl-containing lipid chains of the derivatives. The multi-types of forces would then improve the amphiphilic derivatives dissolving in alcohols. However, in cyclohexane solutions, no hydrogen bonding could occur between solutes and solvents. Therefore, the derivatives generally showed the better solubility in alcohols than in cyclohexane except for CAD that had a longer lipid chain than the others (Table 1). Certainly the strong hydrophobicity of cholesteryl moiety led to the derivatives insoluble in aqueous media. But surprisingly, they showed the very high solubility of more than 100 mg/ml or 0.15 M in chloroform and THF, almost unlimited. The special phenomena of very high solubility were interesting. The mechanism analysis was showed in the next section.

Table 2
Solubility of the cholesteryl derivatives of antiviral nucleoside analogues

Derivative	Solubility[a] (mM)		
	Methanol	Octanol	Cyclohexane
CSA	10.07	12.25	1.64
CSD	1.49	2.52	0.91
CAD	0.57	2.32	1.20
CSZ	3.26	0.56	0.35
CPZ	3.99	7.64	2.00

[a] All the derivatives had the very high solubility in chloroform and THF (over 150 mM), and they were hardly soluble in water.

3.3. Molecular self-assembly in solutions

The nucleobase-containing chemicals preferred to form intermolecular hydrogen bonding between themselves in non-competitive solvents (the solvents having poor hydrogen bonding donors and acceptors) such as chloroform and dioxane (Lutz et al., 2006). The derivatives in this paper are also nucleobase-containing amphiphiles. Therefore, the intermolecular hydrogen bonding between the derivatives in chloroform and THF should prefer to happen, and then lead to form self-aggregates (Fig. 1). The derivatives containing guanine (CSA) and hypoxanthine moieties (CSD and CAD) might adopt linear association (Sivakova and Rowan, 2005; Giorgi et al., 2003), although the thymine-containing derivatives (CSZ and CPZ) could likely form dimmers (Mamdouh et al., 2006).

The molecular self-assembly of the derivatives in solutions might be continuous and dynamic, also possibly unlimited, which resulted in a high apparent solubility in the solvents. The self-assembly phenomena also appeared in some lipophilic nucleosides though no solubility data were given (Sivakova and Rowan, 2005; Giorgi et al., 2003). In other solvents like alcohols, the self-assembly of the derivatives based on hydrogen bonds might occur but probably weak and substituted by the hydrogen bonds between the derivatives and the solvents. In addition, a gel-like viscous liquid was observed on the surface of their high concentrated chloroform solutions, which could be organogels (high concentrated molecular self-assemblies in organic solvents). The long-chained glyceride derivative of acyclovir also showed the similar soluble behavior, probably based on the same mechanism (Jin et al., 2005).

3.4. Formation of vesicles

The aqueous suspensions of the derivatives obtained by the injection method could keep stable for about a week or more. Well-defined nanosized vesicles were observed in the suspensions from the TEM images (Fig. 2). In the vesicle formation process, when the organic solvent THF was mixed with water molecules, the derivatives began to produce the hydrophobic interaction between themselves after meeting water molecules, and the intermolecular hydrogen bonds were disrupted by water and further substituted by the hydrogen bonds between the nucleobase moieties and water. The whole process proceeded very rapidly. The amphiphilic derivatives could prefer to self-assemble into bilayers in water with polar nucleoside heads outside and lipid tails inside based on hydrophobic interaction, and then the bilayers further bent to form closed vesicles (Fig. 3).

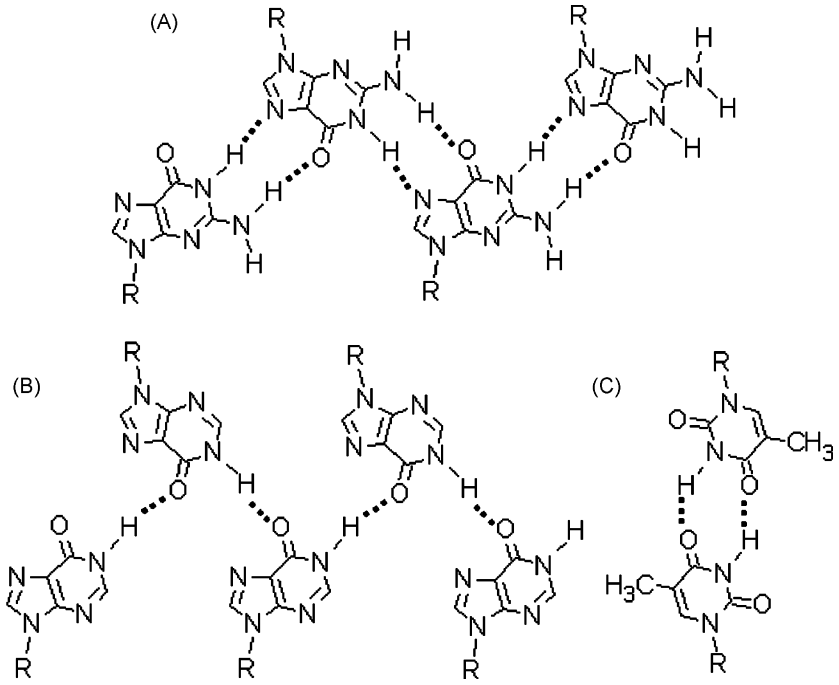


Fig. 1. Hydrogen bonded self-assembly of the cholesteryl derivatives of antiviral nucleoside analogues in chloroform and THF. The derivatives containing acyclovir, didanosine or zidovudine moieties were shown in (A), (B) and (C), respectively.

3.5. Effect of molecular configuration on the sizes of vesicles

Interestingly, the sizes of formed vesicles were greatly different, ranging from 20 nm to about 200 nm. Generally, the dynamic shapes of amphiphiles determine the morphology of self-aggregates, and the volume ratio of polar heads and hydrophobic tails of amphiphiles is the key factor (Israelachvili, 1992). In the light of the structural analysis (Table 1), CSA had a median-volume polar head composed of guanine and methoxy; the larger polar head composed of hypoxanthine and tetrahydrofuran cycle was in the molecules of CSD and CAD. CSZ had a relatively small head composed of thymine, tetrahydrofuran cycle and azide. However, the other zidovudine-conjugate CPZ had a large head of phosphoryl zidovudine. Especially, the phosphoryl preferred to disassociate in water to release hydrogen ions so that the head was negatively charged. The subsequent electrostatic repulsion led to the distance between the adjacent heads of amphiphiles in bilayers broadened. Therefore, CPZ actually possessed an apparently large head. Based on the results of molecular simulation (Table 1), these derivatives had long hydrophobic tails with different length. Also they showed the various volume ratios of polar heads and hydrophobic tails, wherein CSD and CPZ had the largest ratios, CSA had the median, and CAD and CSZ had the smallest. Interestingly, it was found that the vesicle sizes of them changed along with the same turn as the ratios, which were 20 nm (CSD and CPZ), about 100 nm (CSA) and about 200 nm (CAD and CSZ), respectively (Fig. 2). Therefore, it might be concluded that the volume ratios of polar heads and hydrophobic tails of nucleoside-derived amphiphiles could determine the size of formed vesicles, and the amphiphiles with the large ratio would

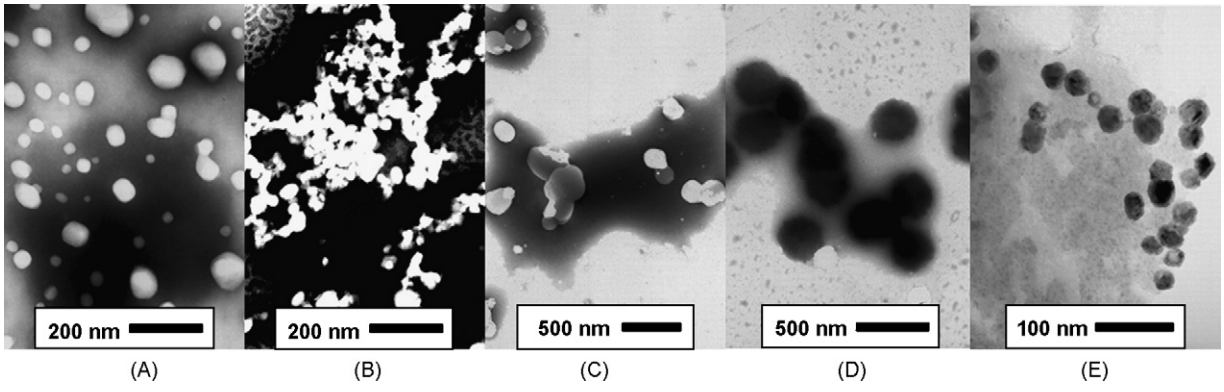


Fig. 2. TEM images of the self-assembled vesicles obtained after injecting the THF solutions of the cholesteryl derivatives of antiviral nucleoside analogues into water. The aggregates were stained with sodium phosphotungstate solution (2%, pH 7.0). Graphs (A)–(E) represent the vesicles prepared from CSA, CSD, CAD, CSZ or CPZ, in turn.

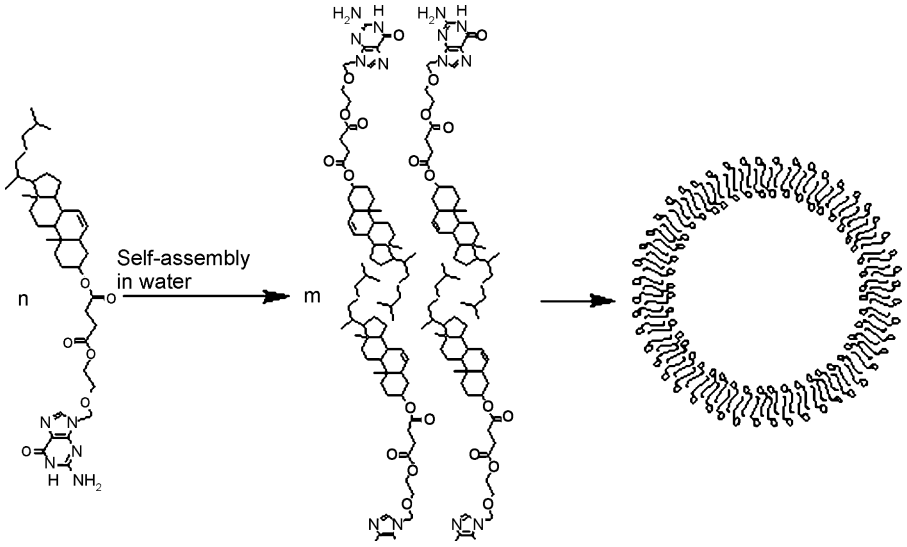


Fig. 3. Illustration of the bilayer and subsequent vesicle formation of CSA in water. The vesicles of the other derivatives (CSD, CAD, CSZ and CPZ) could have the same formation mechanism.

prefer to form small vesicles. This result also agreed with the general rule (Israelachvili, 1992).

According to the size analysis of light scattering, the mean diameters of vesicles were respectively 118 nm (CSA), 160 nm (CSD), 188 nm (CAD), 202 nm (CSZ), and 165 nm (CPZ), which were in accord with the data based on TEM images (Fig. 2) except for CSD and CPZ. Electron microscopy including TEM can yield the most direct information on sizes, size distribution, and shapes of the particles like vesicles though an air-drying procedure adopted (Bootz et al., 2004). The main advantages of light scattering are the short time required to perform the measurements and the relatively low cost of the apparatus. Whereas, the light scattering only determines the hydrodynamic sizes of particles and do not differentiate the morphology of particles. Especially, when particles aggregate, the light scattering can only determine the size of aggregates not single particles. In fact, light scattering is a good method to study particle aggregation (Brunner et al., 1997). From the TEM images (Fig. 2), it is significant that CSD vesicles (Fig. 2B) and CPZ vesicles (Fig. 2E) aggregate because the tiny particles prefer to agglomerate due to very large surface area. Therefore, aggregates were determined by light scattering in the case of CSD or CPZ vesicles.

4. Conclusions

A series of cholesteryl derivatives of antiviral nucleoside analogues were prepared in the paper. The unique structure of the nucleoside-derived amphiphiles determined the type and strength of intermolecular interaction between themselves or between them and surroundings, and the physicochemical properties including self-assembly behavior. The derivatives could form self-assemblies in noncompetitive solvents based on intermolecular hydrogen bonding. The self-assembled vesicles were formed when injecting their organic solutions into water, and the formation mechanism was hydrophobic interaction between amphiphiles. The volume ratios of polar heads and hydrophobic tails of amphiphiles could determine the vesicle size.

The paper provided some basic rules about the physicochemical properties of lipid derivatives of nucleoside analogues. More importantly, the antiviral nucleoside analogues were derived to amphiphilic prodrugs and then the liposome-like self-aggregates in water were obtained. They might act as SADDS to deliver themselves in vivo and release active agents in targets. Because macrophages, the targets of SADDS, are the reservoirs of HIV and other viruses (Aquaro et al., 2002), the antiviral SADDS would be very useful. We will select the proper ones from the derivatives to prepare SADDS and do a deep research.

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