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Synthesis of Cholesterol Derivatives with Amino Acid as Hydrophilic Group and the Vesicles Prepared Therefrom

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Abstract: Three single chain amphiphilic cholesterol derivatives with amino acid as hydrophilic groups, **I**, **II** and **III**, were synthesized. When they were hydrated in water, they can all form lamellae structures as evidenced by DSC measurement. The lamellae states of **II** and **III** can be converted to vesicles upon ultrasonication of the diluted aqueous solutions. The vesicles showed very slow release rate of the encapsulated water-soluble dyes, indicating that they are stable and belong to the least permeable vesicles.

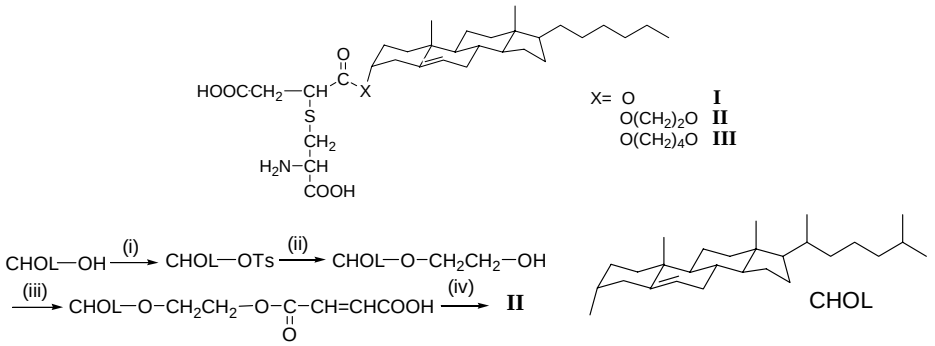
Keywords: Vesicles, amphiphiles, amino acid, cholesterol.

Cholesterol is one of the main constituents of membrane lipids and has been considered to govern the membrane fluidity and permeability of solutes[1]. In the course of study on liposomes constructed by phospholipids as model biomembrane or drug delivery system, it was also found that the stability of liposomes was enhanced by incorporation of cholesterol into the lipid bilayer[2,3]. All these properties of cholesterol in membrane chemistry are caused by its unique structure, in which the rigid and flat steroidal ring shows good compatibility with lipid. Due to the extraordinary functions of cholesterol in biomembrane, it has been interested in synthesizing amphiphiles based on cholesterol, for example, polymerizable and non-polymerizable quaternary ammonium derivatives and polyoxyethylene cholesteryl ethers[4-9]. Recently, there have also been some reports on cholesterol-polyamine carbamates[10,11]. Vesicles and other types of molecular assemblies based on these cholesterol derivatives provide a convenient source to prepare therma-dynamically stable molecular assemblies. We have been interested in synthesis and vesicles of amphiphiles with amino acid as hydrophilic groups. The polycondensation of amino acid at the membrane surface can result in the formation of polypeptide vesicles, which have high stability and controlled permeability *via* the variation of polycondensation degree[12]. We report here on the synthesis of a new type of amphiphilic cholesterol derivatives, **I**, **II** and **III**, with amino acid as the hydrophilic groups, the vesicular properties of these amphiphiles have also been investigated.

The synthesis of **II** is illustrated as an example, which is obtained according to the following procedure. 2-O-Cholesteryl ethanol was synthesized according to the published procedures[6,13]. Reaction of the alcohol with maleic anhydride in toluene for

10 h. yielded maleic acid mono (2-O-cholesterylethyl) ester, which was purified by silica

Scheme 1



Reagents and conditions*:* (i) TsCl, pyridine, 0-5℃, 72 hrs., 80%, (ii) ethylene glycol, dioxane, reflux, 10 hrs., 70%, (iii) maleic anhydride, toluene, reflux, 10 hrs, 53%; (iv) cysteine hydrochloride, $NaHCO_3$, *iso*-propanol/H_2O, 50℃, 8 hrs., 50%.

column chromatography with $CHCl_3/CH_3OH$ (10/1, v/v) as eluent. The preparation of **II** was according to the published procedure [12]. **I** and **III** were synthesized in the same way, all the intermediates and final products were characterized by MS, IR and NMR measurements.

Hydration of I, II and III in water and DSC measurement of the aggregates in lamellae state

I, **II** and **III** are difficult to dissolve or disperse in water at room temperature. In order to examine the properties of these amphiphiles in the hydrated state, small portions of them were put into aluminum pans for differential scanning calorimetry (DSC) measurement, then a small amount of water was added into the pans and the pans were tightly sealed. The samples were allowed to hydrate at 70℃ for 10 hr. before the thermal transition was detected under nitrogen atmosphere on a Shimadzu DSC-50 differential scanning calorimeter at a heating rate of 5℃/min. Here DSC measurement is applied, because it is an important technique to study the phase transition of phospholipid bilayer membrane, it can not only give information of heat evolution of a certain transition, but it can also show the cooperativity of the transition itself. For comparison, the thermal transition of the pure amphiphiles was also recorded on the same instrument. The results are shown in **Table 1**.

It can be seen that all of the three cholesterol derivatives show broad phase transitions, indicating that these molecules might be liquid crystal due to the coexistence

Table 1. Phase transition temperatures of cholesterol and its derivatives, and those of their lamellae states in water*

	CHOL-OH	**I**	**II**	**III**
T_1(℃) [a]	143-144	78.5-162.3	66.6-153.3	63.5-153.8
T_2(℃)[b]	-----	57.1	65.0	58.2

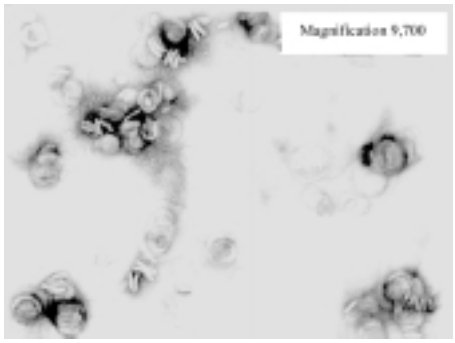
* Peak temperature as detected by DSC
a: Phase transition temperatures of cholesterol and its derivatives
b: Phase transition temperatures of the hydrated samples in water

of steroidal ring and chiral amino acid groups. The hydrated samples of **I**, **II** and **III** also show distinct and broad phase transitions under the present conditions. This means that these three cholesterol derivatives can all self-organize into lamellae structures. Introducing of amino acid into the cholesterol molecules as hydrophilic groups has altered the hydrophobic-hydrophilic balance of these amphiphiles. This is the premise to use them to construct stable spherical vesicles in aqueous media. The highest phase transition temperature of **II** suggests a well organized state of this molecule in the aggregates.

TEM observation of the dilute aqueous dispersions

Upon dispersal in water or phosphate buffer solution (conc. 10mg/dm^3) either by ultrasonication or vortexing at temperatures above the phase transition (*e.g.* 65℃), **I**，**II** and **III** gave translucent suspensions. However, cholesterol can not be dispersed in water even upon longer sonication at this temperature. The suspension of **I** is not stable, after being stored at room temperature for even one hour, **I** precipitated from the suspension, so direct observation of the solution under transmission electron microscope (TEM) is impossible. Observation of the supernatant solution with TEM (JEOL 100CXII) showed no regular assembly structure. Examination of aqueous dispersions of **II** and **III** by TEM with negative staining revealed the existence of closed vesicular structures in these systems. One of the microphotographs of **II** was shown in **Figure 1**. The diameters of the

Figure 1. Electron microsphotograph of **II** in phosphate buffer solution (pH=7.4)

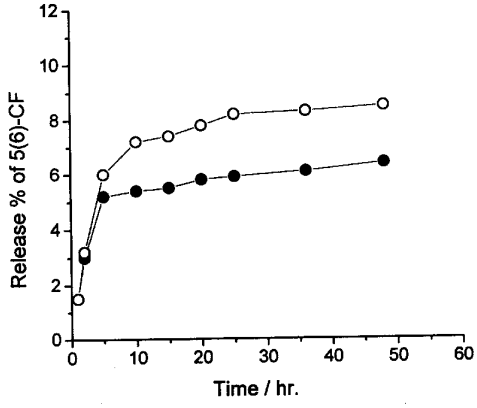


vesicles can be measured in the range of 300-400 nm, they belong to the large vesicles, presumably caused by the flat and rigid hydrophobic chain. Since the head group in **I** is directly linked with a steroidal ring, though its hydrophobic-hydrophilic balance enables it to self-organize in water, the solution itself is unstable, the relative rigid hydrophobic chain restrains its well organized state as closed vesicles. However, in the case of **II** and **III**, the additional two methylene and four methylene groups not only alter the hydrophobic-hydrophilic balance, they also act as spacers to regulate the organization of the steroidal ring in the aggregates, therefore, they can form stable vesicles.

Release of encapsulated 5(6)-CF from the vesicles of II and III

The same method as reported was used to evaluate the permeability of the vesicles

Figure 2. Release of 5(6)-CF *vs* time from vesicles of **II** (O) and **III** (●) (pH=6.8)



formed from **II** and **III**[12], the encapsulated concentration of 5(6)-carboxyfluoresein (5(6)-CF) was 50 mM. The encapsulation efficiencies of the vesicles were determined to be 0.1~1.0 v/v% depending on the sizes of vesicles from **II** or **III**. The release of the encapsulated 5(6)-CF from vesicles of **II** and **III** is shown in **Figure 2**. It can be seen that the release from both of the vesicles are very slow, this is due to the well-packing of the steroid ring in the bilayer region, which hinders the permeation of CF from the bilayer. When **II** and **III** are compared, the release from **III** is slower. This can be accounted for that the hydrophobic chain of **III** is two methylene groups longer than that of **II**.

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