Communications to the Editor

Molecular Assembly of Cholesterol-Bearing Poly(allylamine) for Binding Bile Salts in Water

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Water soluble and/or naturally occurring polymers modified with hydrophobic substituents have been of great interest both for understanding self-organization and molecular recognition of lipids, proteins, and nucleic acids and for a variety of biological and medical applications, such as mimicking the function of cell membranes, a material for immobilizing enzymes, a coating reagent for erythrocytes, or a drug delivery system.[1] Many researchers have investigated the relationship between the molecular structure and the aggregation properties in water of polymer amphiphiles.[2] Some of them are, for example, hydroxyethyl cellulose (HES) modified with long alkyl chains,[3] pyrene-labeled hydroxypropyl cellulose,[4] and polysaccharides bearing palmitoyl and cholesteryl moieties.[5] The polymers form self-aggregates in aqueous solutions like biological lipid membranes.

Bile salts are amphipathic steroids with detergent properties,[6] which are formed from cholesterol in the liver. Binding bile salts in aqueous solutions has been a challenging subject for biochemists and pharamacologists because the lowering of intestinal bile salt concentrations results in reducing the cholesterol level in the blood and, thereby, preventing atherosclerosis. Like other detergents, bile salts tend to self-aggregate in water with increasing concentration.[7] The self-aggregation of bile salts has been extensively studied for several decades;[8] however, there have been no reports on the complexation between the self-aggregate of hydrophobized water soluble polymers (polymer amphiphiles) and bile salts. In this communication, we describe a novel polymer amphiphile based on a synthetic water soluble polymer bearing alkyl cholesteryl groups that forms complexes with bile salts by self-aggregation. This might be a unique strategy for designing a hypocholesterolemic molecule with binding properties superior to the previous ones based on 1:1 complexation.[9]

Poly(allylamine) (PAA) obtained by the radical polymerization of allylammonium chloride[10] was employed as a base skeleton because of its simple chemical structure, good solubility in water and other polar solvents, great facility for chemical modification, and low toxicity.[11] The polymer substituted with x mol % 6-cholesterylcarbamoyl groups (PAA-C; x = 1.6−9.2) was prepared by the reaction of cholesteryl *N*-(6-isocyanatohexyl)carbamate with PAA composed of 59

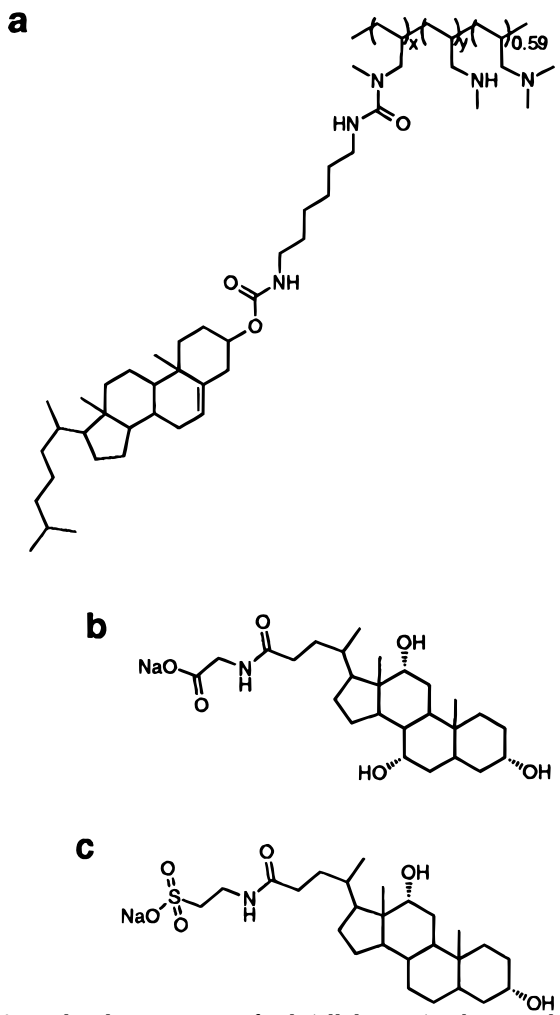

Figure 1. Molecular structure of poly(allylamine) substituted with 6-cholesterylcarbamoyl groups (PAA-C) (x = degree of substitution by cholesteryl group; $x + y = 0.41$) (a), sodium glycocholate (GC) (b), and sodium taurodeoxycholate (TDC) (c).

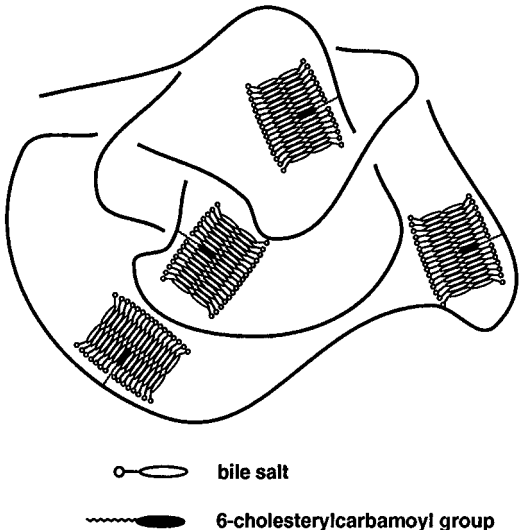

Figure 2. Schematic representation of bile salt binding by PAA-C.

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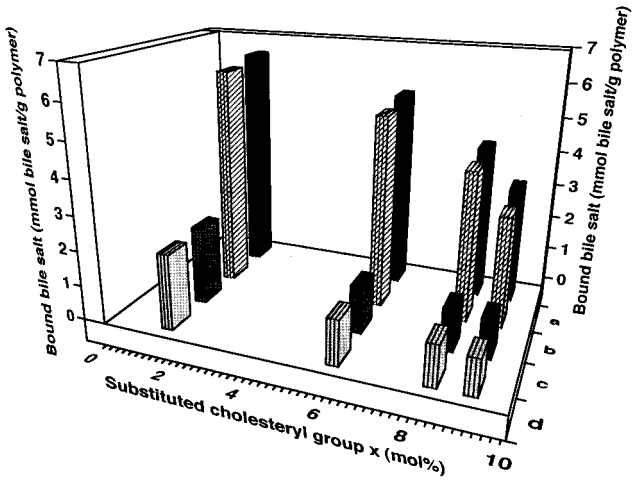

Figure 3. Bile salts binding by PAA-C in phosphate buffer. Binding of TDC at pH = 6.0 (a) and after the dissociation test at pH = 8.0 (b). Binding of GC at pH = 6.0 (c) and after the dissociation test at pH = 8.0 (d).

mol % *N*,*N*-dimethylallylamine units and 41 mol % *N*-methylallylamine units (Figure 1a). PAA-C was characterized by ^{1}H NMR and IR spectra. The resulting PAA-C is a white powder and is soluble in polar aprotic organic solvents, such as dimethyl sulfoxide and *N*,*N*-dimethylformamide. A small amount of substitution of hydrophobic cholesteryl groups (1.6 mol %) is sufficient for making the polymer insoluble in water.

The complexation between the polymer and bile salts[12] (GC and TDC, Figure 1b,c) was examined by the bile salt-binding property of PAA-C in water.[13] In 0.3 M phosphate buffer at pH = 6.0, nonsubstituted PAA does not show bile salt binding because there is no interaction between the polymer main chain and the bile salts. In buffered aqueous solutions, part of the amino groups of the polymer are protonated; however, the results show that ionic interaction would not be important for bile salt binding. The introduction of a cholesteryl side group onto the PAA contributes to the bile salt binding by complexation, that is, the formation of their comicelles based on hydrophobic interaction (Figure 2). PAA-C substituted with a 1.6 mol % cholesteryl group (PAA-C1.6) binds 6.4 mmol of TDC/g or 2.2 mmol of GC/g, which means that one cholesteryl side group binds 36 TDC or 12 GC molecules (Figure 3). These values are close to the association number of TDC (32 in 0.3 M aqueous NaCl) and GC (9 in 0.3 M aqueous NaCl) at their critical micellar concentration (cmc). It is thought that the higher binding value of TDC than GC is due to the former's lower cmc of 0.9 mM than that of the latter (10 mM in 0.3 M aqueous NaCl). The formation of the comicelle of TDC is easier than that of GC. An increase in the substitution degree of the cholesteryl group results in a decrease in bile salt binding from 6.4 mmol of TDC/g (PAA-C1.6) to 3.3 mmol of TDC/g (PAA-C9.2), probably because the cholesteryl groups attached to the polymer can aggregate by themselves and form stable micelles in PAA-C9.2. The comicelle once formed is stable and not disintegrable even after removing the remaining free bile salt solution and soaking in 0.3 M phosphate buffer at pH = 8.0. The

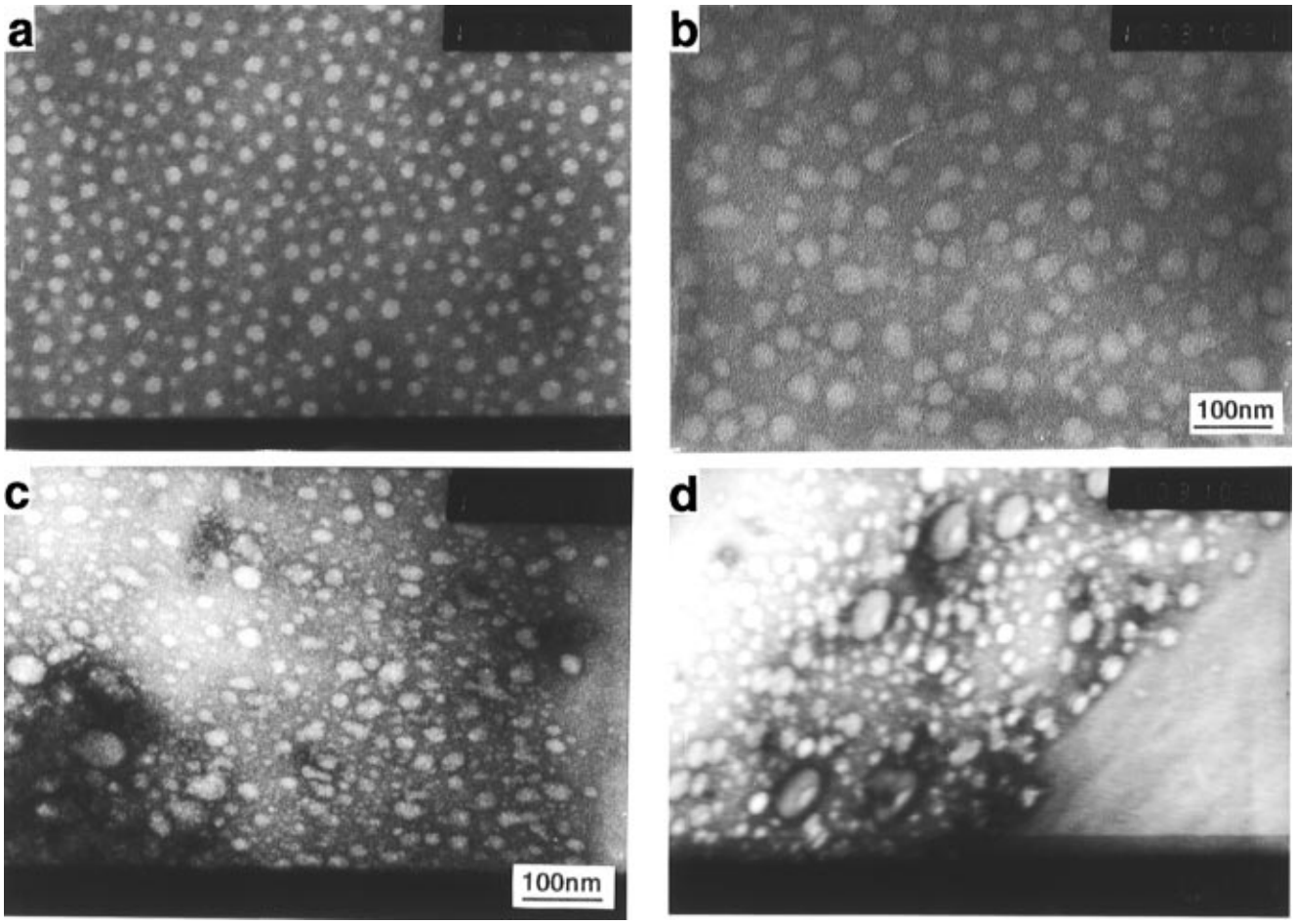

Figure 4. Transmission electron micrographs (negatively stained by 0.38 wt % uranyl acetate): 0.16 g/mL TDC aqueous solution (a), 1.0 mg/mL PAA-C1.6 (b), 1.0 mg/mL PAA-C1.6 mixed with TDC (cholesteryl group/TDC = 4/1 by mol) (c), and 1.0 mg/mL PAA-C1.6 mixed with TDC (cholesteryl group/TDC = 1/1 by mol) (d).

dissociation test reveals that only <6 mol % of both bile salts bound to the polymer integrates into the solution. The concentration of bile salts in the polymer domain would be kept higher than their cmc even in the solution containing no bile salts. The binding potency of PAA-C1.6 is much stronger than that of cholestyramine (2.9 mmol of TDC/g, 1.2 mmol of GC/g), which is a commercial anion exchange resin based on cross-linked polystyrene bearing a trimethylammonium group and is well-known to be an effective hypocholesterolemic agent.[14]

Spherical particles of the comicelle of TDC and the pendant cholesteryl group with a relatively uniform size were confirmed by negatively stained electron-microscopic observation. PAA-C1.6 forms nanoparticles, ca. 45 nm in diameter (Figure 4b), which are larger than those of TDC (ca. 25 nm, Figure 4a). The addition of TDC to PAA-C1.6 solutions induced an enlargement of the particles (40−100 nm, Figure 4c,d), which supports the formation of the comicelle.

Bile salt binding studies with PAA-C indicate that the polymer exhibits excellent binding ability by self-aggregation and the possibility warranting their exploration as a hypocholesterolemic agent under biological conditions. Although the elimination of cholesterol in animal blood is also related to other factors, these in vitro assay results suggest the possibility of an in vivo effect in which PAA-C would bind conjugated bile salts in the acidic animal intestine and not release the bound bile salts in the basic duodenum. The reduced level of bile salts in the intestine prompts aprotein B catabolism and promotes the conversion of cholesterol to bile acids in the liver. These studies may also explore a variety of applications, such as a liposomal drug delivery system (DDS)[15] or polymer surfactants.

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Supporting Information Available: Experimental procedures and ^{1}H NMR and IR spectral data for PAA-C (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(11) LD_{50} = 1600 mg/kg (mouse) as a hydrochloric salt. See: Harada, S. *Kinouzairyou* **1985**, *5*, 29.

(12) Sodium glycocholate (GC) and sodium taurodeoxycholate were employed because they are the representative bile salts in human bile.

(13) Bile salt binding by the polymer was analyzed as follows. GC (10 mM) and TDC (15 mM) solutions in 0.3 M phosphate buffer (pH = 6.0) were prepared. Samples (10 mg) of PAA-C were accurately weighed and placed into 10 mL vials together with a 4.5 mL portion of the bile salt solution. All vials were securely closed and mechanically shaken at 25 °C until equilibrium was established. Equilibration was determined by means of repetitive sampling and was found to occur within a 10 h period. The equilibrated samples were centrifuged at 12 000 rpm for 10 m, and the supernatant solution was assayed for unbound bile salt using an enzymatic reaction method with a bile salt concentration measuring reagent (3α-hydroxysteroid dehydrogenase). The amount of bile salt bound to PAA-C was calculated from the difference between the initial concentration of bile salt introduced into the system and the concentration present in the solution at equilibrium. In the dissociation studies, bile salt binding PAA-C recovered after centrifugation was placed into a 10 mL vial with 4.5 mL of 0.3 M phophate buffer (pH = 8.0). After equilibration, the solution was centrifuged at 12 000 rpm for 10 m and assayed for bile salt concentration.

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