Diffusivity of Bile Salt/Phospholipid Aggregates in Mucin

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Purpose. The objective of this study is to evaluate the effect of the mucous layer on the transport of the drug-solubilizing bile salt/phosphatidylcholine (BS/PC) aggregates.

Methods. The self-diffusion coefficient of BS/PC aggregates in bovine submaxillary mucin (BSM) was measured by Fourier-transform pulsed-field gradient spin-echo (FT-PGSE) ^{1}H NMR spectroscopy.

Results. In spite of the complexity of the mixture, the FT-PGSE technique allowed the unambiguous determination of the diffusivity of PC and $^1H^2HO$ (HDO, natural abundance in D_2O). With a series of BS/PC total lipid concentrations ranging from 1 to 7 g/dl, a progressive decrease in the effective diffusivity of HDO was observed with an increase in the both the BSM and total lipid concentration. The effective diffusivity of PC decreased with increasing lipid concentrations in the presence of mucin, while in the controls it increased. After correcting the effective diffusivity of PC for the obstruction effect of mucin, the size of the BS/PC mixed micelle was assessed. It appears that PC associates with BSM resulting in a decrease in the available PC for micellization. This reduces the average size of the mixed micelle within the mucous layer.

Conclusions. The aggregation state of BS/PC micelle is altered by the presence of mucin which would have a direct impact on the transport of dietary lipid and solubilized drug through the aqueous boundary layer of the intestinal tract.

KEY WORDS: bile salt; phospholipid; diffusion; mucin; NMR.

INTRODUCTION

Mucus is a complex epithelial secretion which resides at the mucosal surface of the gastrointestinal tract (1, 2). The principal organic constituent of mucus is mucin, a secretory glycoprotein also known as the mucous glycoprotein. Mucin is responsible for the viscoelastic and gel forming properties of mucus and is present in a soluble form in the luminal secretions as well as in the tenacious gel adherent to epithelial surfaces (1, 2).

Bile salt/phospholipid (BS/PC) aggregates are present as concentrated, anionic colloids in the gallbladder (3). They are excreted into the intestine to assist in the solubilization and transport of dietary fat (4, 5). For this reason, these aggregates can also enhance the absorption of poorly water soluble drugs (6–9). The other aspect of absorption is the transport of BS/PC aggregates through the aqueous boundary layer (10). These aggregates must diffuse through the mucous blanket that separates the intestinal surface from the well-mixed lumen. Often transport through this layer is assumed to be equivalent to that through a stagnant layer of water. However, this hydrated, negatively charged glycoprotein network may present a significant barrier through obstruction and electrostatic effects, especially for colloidal particles. In addition, the aggregation behavior of bile salt/phospholipid can be altered by lipid association with mucin (11–16). Thus, for understanding the role of BS/PC micelles in the absorption of drug, measures of the diffusion of BS/PC mixtures in the presence of mucus are needed.

To address the complicated problem of diffusion in the mucous layer, Fourier-transform pulsed-field gradient spin-echo (FT-PGSE) ^{1}H NMR spectroscopy has been applied. Recently, this technique has been applied to an investigation of the diffusivity and release rate of drug from extended-release dosage forms (17, 18). In principle, FT-PGSE ^{1}H NMR can simultaneously provide the self-diffusion coefficients of all constituent molecules of complex multicomponent systems (19). Moreover, information about the distribution of the components between aggregate and the bulk phase may be obtained in a few minutes without isotopic labeling (19). The obstruction and excluded volume effects on the diffusion of the lipid aggregates may also be readily addressed.

MATERIALS AND METHODS

Materials

The experiments were conducted with sodium taurocholate (NaTC) (Sigma Chemical Co., St. Louis, MO) after recrystallization from ethanol/ethyl acetate (20). The recrystallized surfactant was stored in Teflon-lined screw cap test tubes. Egg phosphatidylcholine (egg PC) was obtained from Avanti Polar Lipids (Alabaster, AL). Both moved as single spots on silica plates with a mobile phase of 65:35:5 chloroform:methanol:water. Bovine submaxillary mucin (BSM) was purchased from Sigma Chemical Co. It contains approximately 5% bound sialic acid. Deuterium oxide (99.9 atom %) used in diffusion measurements was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA) and was used as supplied. Great care was taken to exclude moisture from the samples and to prevent contact with water in order to minimize any proton NMR signal from the exchange reaction with deuterium oxide. For example, the D_2O solution was saturated with argon, all the sample preparation procedures were operated under argon whenever possible, and the prepared solutions were also saturated with argon. All glassware was dried in the oven overnight before use.

Sample Preparation

The samples were prepared by first lyophilizing egg PC from an ethanol/cyclohexane solution. From the measured dry weight, the appropriate weight of NaTC in 0.9% NaCl/D_2O solution was added to achieve 100 mM NaTC:100 mM egg PC. The sample was purged with argon and equilibrated in excess of 24 hours at room temperature. After equilibration, the 100 mM NaTC:100 mM egg PC solution was further diluted with 0.9% NaCl/D_2O to make 80 mM:80 mM, 60 mM:60 mM, 40 mM:40 mM, and 20 mM NaTC:20 mM egg PC solutions. The solutions were purged with argon and equilibrated in excess of 24 hours at room temperature. The stock solution and the series of dilutions were then further diluted with 1% sodium azide/0.9% NaCl/D_2O solutions which contained 0, 0.5, 1, 1.5,

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and 2% of mucin to make a series of 50 mM:50 mM, 40 mM:40 mM, 30 mM:30 mM, 20 mM:20 mM, and 10 mM NaTC:10 mM egg PC solutions with different mucin concentrations. The solutions were purged with argon and equilibrated in excess of 24 hours at room temperature before the NMR experiments.

NMR Spectroscopy

The Fourier-transform pulsed-field gradient spin-echo (PGSE) ^{1}H NMR diffusion measurements were performed on nonspinning samples in thin-wall 5-mm tubes on a Nicolet 300 spectrometer (Nicolet Magnetic Corporation, Madison, Wiscosin) equipped with a pulse field gradient NMR proton probe (Doty Scientific, Inc., Columbia, SC). The sample temperature was controlled at 37 ± 0.2 °C by a versatile digital PID temperature controller (T-3000 Cryo Controller, Tri Research, Inc., St. Paul, MN). The magnetic field gradient pulses were generated by a home-built electronic apparatus. The standard spin echo pulse sequence was used, and the transformed intensity was analyzed by the following equation (19):

$$A(2\tau) = A(0) \exp(-2\tau/T_2) \exp\{-(\gamma G\delta)^2 D(\Delta - \delta/3)\} \quad (1)$$

where $A(2\tau)$ is the peak intensity at time, 2τ, $A(0)$ is the peak intensity at time, 0, τ is the time interval between 90° and 180° pulses, T_2 is the spin-spin relaxation time, γ is the gyromagnetic ratio, G is the strength of field gradient, δ is the duration of field gradient, D is the diffusion coefficient and Δ is the time interval between the first and second gradient pulses. The diffusion experiments were performed at constant τ (11 ms), Δ (11 ms) and G value. A series of 10 δ values ranging from 0.2 to 2 ms were used. To obtain absolute values for self-diffusion coefficients, the field gradient strength was calibrated from measurements on reference H_2O, D_2O and SDS samples at 37 °C. The spectra were evaluated off-line utilizing nonlinear least-squares fitting of peak heights using the MacNMR 5.0 with NMRscript® software (Tecmag, Inc., Houston, TX) on a personal computer. The average and standard deviation were calculated from different proton groups in a particular molecule.

DATA ANALYSIS

In the mucin solutions, there is a distribution of water between free water molecules and water bound to mucin. Therefore, the observed self-diffusion coefficient, D_w, can be expressed with a simple two-site model (21):

$$D_w = D_f(1 - f_b) + D_b f_b \quad (2)$$

where D_f is the diffusivity of free water molecules in the presence of mucin, D_b is the diffusivity of mucin-water complex, and f_b is the fraction of water bound to mucin. The diffusivity of free water in the presence of mucin is reduced due to the obstruction effect. The obstruction effect originates from the presence of impenetrable particles leading to an increase in the path length of the diffusing species.

The factors that influence the obstruction effect of mucin include the size and shape of the diffusing species, the mucin radius, the volume fraction of mucin, and the mucin persistence length. The obstruction contributions for nonflexible polymer chains and spherical molecules can be described by a model developed by Johansson *et al.* (22, 23):

$$D_f/D_f^\circ = e^{-\alpha} + \alpha^2 e^{\alpha} E_1(2\alpha) \quad (3)$$

$$\alpha = \Phi_m(R_s + r)^2/r^2 \quad (4)$$

$$E_1(2\alpha) = \int_{2\alpha}^{\infty} e^{-u}/u \, du \quad (5)$$

where D_f is the self-diffusion coefficient of the diffusing species in the mucin solutions in the absence of strong attractive interactions between the diffusing species and mucin, D_f° is the self-diffusion coefficient of the diffusing species at infinite dilution, Φ_m is the volume fraction of mucin, R_s is the radius of the diffusing species, r is the radius of mucin, and E_1 is the exponential integral.

Combining Eq. 2 with Eq. 3, the following expression is obtained:

$$D_w = D_f^\circ[e^{-\alpha} + \alpha^2 e^{\alpha} E_1(2\alpha)](1 - f_b) + D_b f_b \quad (6)$$

where D_f° is the diffusivity of water at infinite dilution (3.04×10^{-5} cm^2/s) (24). Since the diffusivity of mucin-water complex is considerably lower than that of free water, it was assumed to be negligible compared to D_w. In addition, f_b and Φ_m can be expressed as

$$f_b = N_h(C_{mucin}/C_{water}) \quad (7)$$

$$\Phi_m = C_{mucin}(\rho_{mucin} + N_h\rho_{water}) \quad (8)$$

where ρ_{mucin} and ρ_{water} are the densities of mucin (1.42 ml/g) (25) and water (1.0067 ml/g), respectively, and N_h is defined as the grams of water molecules associated with a gram of mucin which move as a kinetic entity. Since the radius of a water molecule is much smaller than that of mucin (100 Å) (25), α is approximately equal to Φ_m. Thus, from the obstruction effect of mucin, the hydration and volume fraction of mucin were calculated.

RESULTS AND DISCUSSION

^{1}H NMR Spectra

^{1}H NMR spectra of bile salt (sodium taurocholate), bile salt/phospholipid, mucin, and bile salt/phospholipid/mucin are given in Figure 1. The inserts are the magnifications of the spectra. The proton resonances of bile salt and phospholipid have been assigned by *Stark et al.* (26). The proton peaks at 3.13 and 3.64 ppm are from the methylene groups of bile salt at position 25 and 26, respectively, and those at 0.91 and 1.21 ppm are from the methyl groups of bile salt at position 18 and 21, respectively. The proton peaks at 3.40, 3.81, 4.45 and 5.47 ppm are from the $N(CH_3)_3$, CH_2N, choline CH_2OP and glycerol CHO groups of phospholipid, respectively. The proton spectrum of mucin has also been assigned by Gerken (27). The resonances were assigned as follows: 1.02 ppm, CH_3 of Val, Ile and Leu; 1.45 ppm, nonglycosylated Thr γ-CH_3; 1.78 ppm, H3a of sialic acid; and 2.09 ppm, N-acetyl methyl of the residual N-acetylgalactosamine residues. The unresolved spectral region between 3 and 5 ppm was assigned to the protons of amino acids and sialic acid.

It is obvious in Figure 1 that the proton peaks of mucin did not interfere with those of BS and PC at the mucin concentrations studied in this study. Therefore, the complicated problem of diffusion of BS/PC aggregates in mucin can be readily addressed by applying FT-PGSE ^{1}H NMR spectroscopy.

FT-PGSE ^{1}H NMR Spectra

A stacked plot of FT-PGSE ^{1}H NMR spectra on the BS/PC/mucin system as a function of the magnetic field gradient

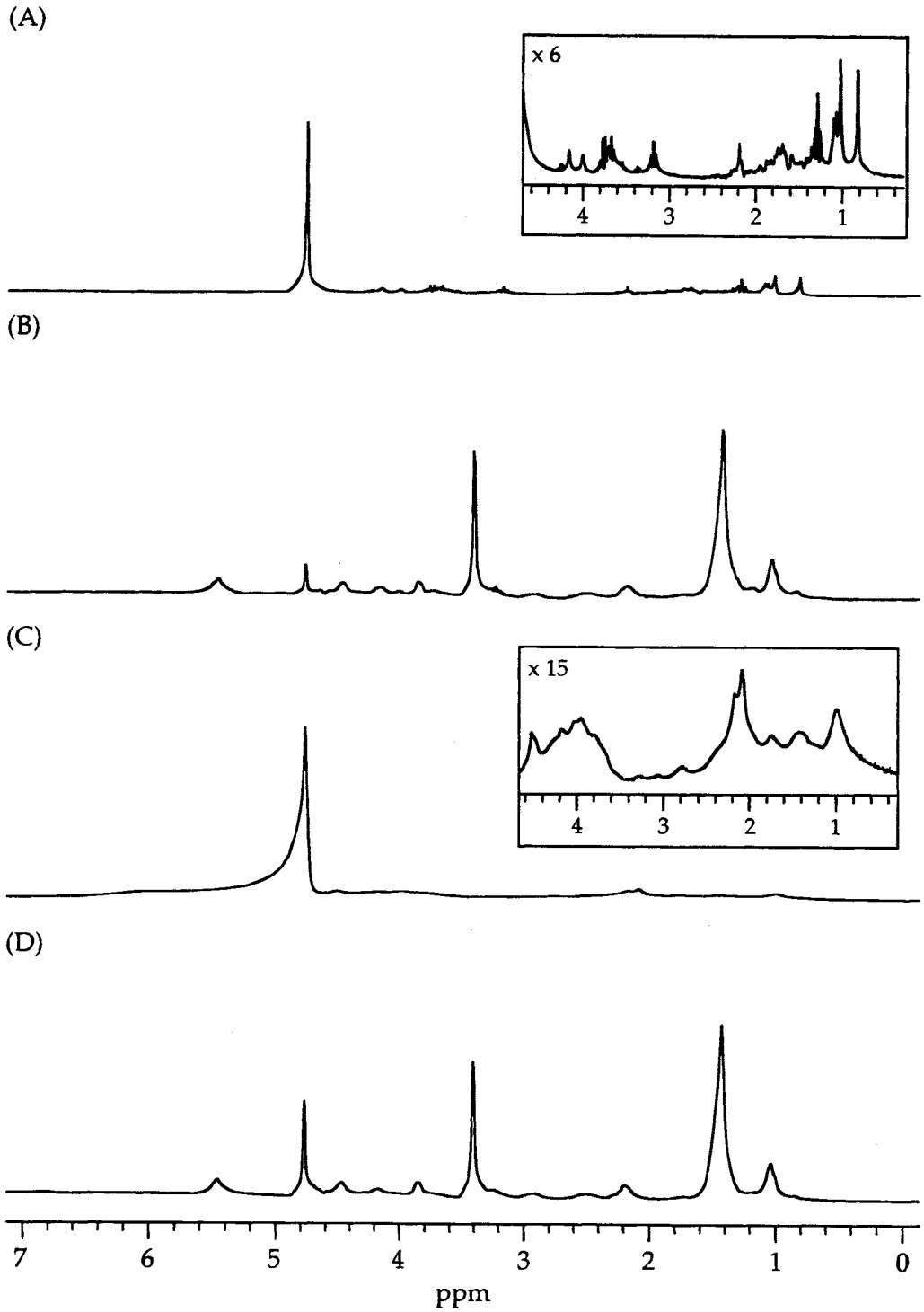

Fig. 1. ^{1}H NMR spectra of (A) bile salt (sodium taurocholate), (B) sodium taurocholate/egg phospholipid, (C) mucin, and (D) bile salt/phospholipid/mucin. The inserts show the magnifications of the spectra.

duration is shown in Figure 2. The HDO peak (at 4.76 ppm) decays much faster than the BS, PC, and BS/PC mixed peaks due to the faster self-diffusion of water as compared with BS and PC. The decays of BS, PC and BS/PC mixed peaks are observed at longer field gradient duration. The average diffusion coefficients of phospholipid were calculated from the echo signals of the protons on the $N(CH_3)_3$, CH_2N, choline CH_2OP and CHO groups.

It is obvious that FT-PGSE ^{1}H NMR can simultaneously provide the self-diffusion coefficients of all constituent molecules of complex multicomponent systems in a few minutes without isotopic labeling (Figure 1 and 2). Moreover, information about the distribution of the components between aggregate and the bulk phase may be obtained (19). Finally, the obstruction and excluded volume effects on the diffusion of the lipid aggregates may be readily addressed.

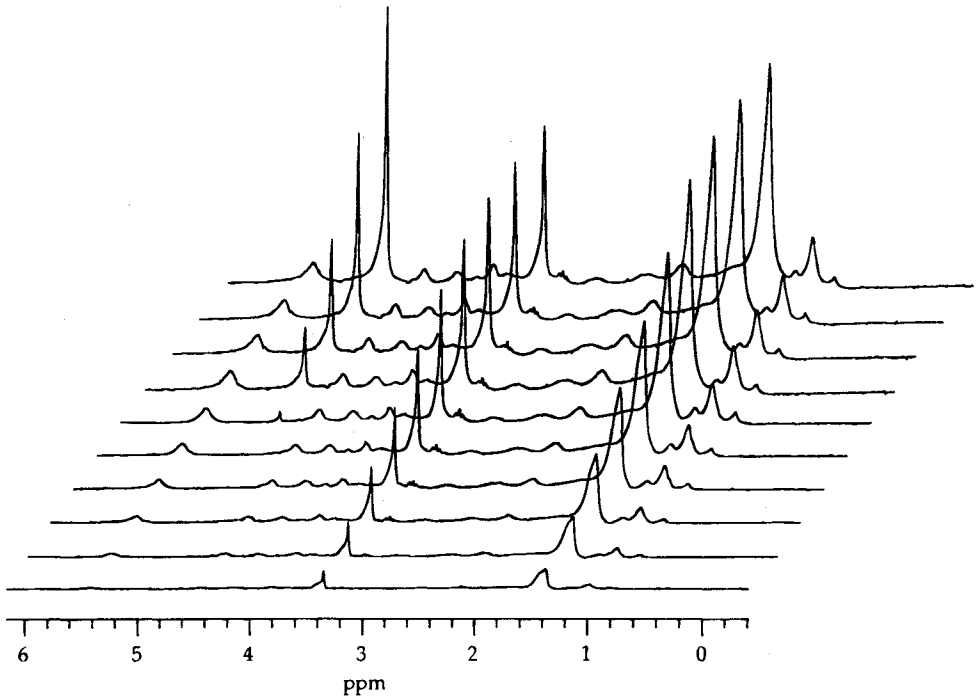

Fig. 2. A stacked plot of FT-PGSE ^{1}H NMR spectra on BS/PC/mucin system is shown as a function of the magnetic field gradient duration. The diffusivity of HDO is determined by the decay of the peak intensity at 4.76 ppm. The average diffusivity of PC is calculated from the decays of peak intensities at 3.40 $(N(CH_3))_3$, 3.81 (CH_2N), 4.45 (choline CH_2P) and 5.47 (glycerol CHO) ppm.

Diffusion of Water in Mucin

The diffusivities of water in mucin solutions (0.25, 0.5, 0.75, and 1% w/w) and in the absence of lipid are given in Table I. It decreased with the mucin concentration. Therefore, mucin has a significant obstruction effect on the diffusion of water molecules.

Different theoretical approaches have been pursued in order to gain a fundamental understanding of the events occuring in the polymer systems. In this study, the theory by Johansson *et al.* (22, 23) was used to account for the obstruction effect of mucin. It was derived using the so-called cylindrical cell model. This model consists of an infinite cylindrical cell, containing solvent and polymer. The polymer is represented as a rod centered in the cell and is composed of rather stiff chains. Since mucins are elongated rod-shaped molecules whose central core is a linear polypeptide (100 to 250 kDa) called apomucin, the use of the theory is reasonable. In addition, the theory was developed for the diffusion of spherical solutes in a network which applies to the water molecule.

Table I. The Diffusivity of Water (cm^2/s), Water of Hydration (g Bound Water/g Mucin), Volume Fraction of Mucin, and Fraction of Bound Water as a Function of Mucin Concentration (w/v)

Mucin conc.	Diffusivity of water	Water of hydration	Volume fraction of mucin	Fraction of bound water
0%	3.04×10^{-5}[a]	—	—	—
0.25%	2.64×10^{-5}	30	0.078	0.074
0.50%	2.55×10^{-5}	18	0.10	0.093
0.75%	2.45×10^{-5}	15	0.12	0.11
1.00%	2.37×10^{-5}	13	0.15	0.13

[a]Data is taken from reference 24.

From the diffusivity of water in the mucin solutions, the water of hydration, volume fraction of mucin, and fraction of bound water were obtained (Table I). The fraction of bound water increased with mucin concentration from 7.4% to 13%. The water of hydration of mucin was much higher than that found for other proteins. It suggests that mucin is much more hydrated. This may be due to the large water-holding capacity of mucin-bound carbohydrates and the high charge density from sialic acid residues. In addition, the water of hydration decreased with increasing mucin concentration. This may be explained by understanding the polymerization and gelation of mucin (28). Mucin molecules in solution can cross-link to form aggregates via hydrogen bonds, electrostatic and hydrophobic interactions, and Van der Waals forces. Increasing the number of cross-links leads to the formation of a gel. The gelation of mucin arises from its entanglement properties. High molecular weight polymer solutions are considered dilute if the individual chains do not overlap. The overlap concentration of mucin is very low because of its high molecular weight. Mucin solutions at concentrations of 2–4 mg/ml (0.2%–0.4%) are already in the semi-dilute regime where polymer chains are overlapped. At the higher mucin concentrations typically found *in vivo* (>20 mg/ml), the extensively entangled monomers will behave like a transient gel. Such close contact between neighboring molecules greatly increases the formation of noncovalent interactions that stabilize the entangled network and provides the rheological characteristics of a weak, reversible gel. Since the mucin concentrations (0.25%–1%) in the present work are in the range

where the polymer chains are overlapped, the extent of overlapping increases over mucin concentrations of 0.25% to 1%. Therefore, less water is accessible to the hydrophilic glycosylated regions of mucin molecules.

Diffusion of Water in Lipid and Mucin

The diffusivities of water in the BS/PC solutions with 0, 0.25, 0.5, 0.75, and 1% mucin are shown in Figure 3. The total lipid concentration accounts for both the bile salt and phospholipid. A progressive decrease in the effective diffusivity of HDO was observed with an increase in the both the mucin and total lipid concentration.

Diffusion of BS/PC Aggregates

The main focus of this paper was to determine the effect of mucin on the diffusivity of BS/PC micelle. Before the results are discussed, it is necessary to describe the nature of the BS/PC association. There are two premises (29). The first is that the equivalent hydrodynamic radius measured in the mixed micellar solution is uniquely determined by the bile salt to phospholipid ratio within the micelles. The larger the BS/PC ratio, the smaller the micellar size is. Second, the intermicellar concentration (IMC) is also dependent on the equivalent hydrodynamic radius and temperature but attains the same value regardless of the concentration of the mixed micelles. Therefore, if there is binding of lipid to mucin, the aggregation state of the micelles would be altered which, in turn, might affect the solute-carrying capacity of the micelles. It has been demonstrated by several investigators that there is an association of lipids with rat small-intestinal mucus, dog and rat gastric mucus, purulent sputum, and bovine gallbladder mucin (11–16). However, the consequences of binding of lipid to mucin on the size and capacity of BS/PC mixed micelles have not been considered.

In Buffer

The BS/PC solutions in the absence of mucin served as controls. The observed diffusivities of PC in the BS/PC solutions are shown in Figure 4. They increased with increasing lipid concentrations. The solid curve was drawn by eye. The BS/PC aggregates have been proposed to be disk-like or rod-like. However, for the purpose of comparison, the diffusivities of PC were converted to hydrodynamic radii assuming the micelles are spherical. The hydrodynamic radii were then calculated to be 206, 104, 93, 86, 83 Å for the total lipid concentration of 1.28, 2.56, 3.80, 5.15, and 6.42 g/dl, respectively.

It is clear that micelles grow when the system is diluted with normal saline. This can be understood by a closer examination of the physical picture of micelle formation. If a solution of a mixed micelles is diluted with a solvent containing no bile salts, it must reestablish the IMC. To do this, bile salt molecules must leave the mixed micelles, thereby increasing the ratio of phospholipid to bile salt in the mixed micelles which in turn, necessitates an increase in mixed micellar size.

In Mucin

The observed diffusivities of PC in the BS/PC solutions with 0.25, 0.5, 0.75, and 1% mucin are also shown in Figure 4. The change in the effective diffusivity of PC with increasing lipid concentrations showed a different pattern in the presence of mucin. The observed diffusivity of PC in the solutions of total lipid concentration of 1.28 and 2.56 g/dl increased with increasing mucin concentration, whereas the observed diffusivity of PC in the solutions of total lipid concentration of 5.15 and 6.42 g/dl decreased with increasing mucin concentration. The observed diffusivity of PC in the solution of total lipid concentration of 3.80 g/dl remained constant with increasing mucin concentration.

Theoretically, if there is no binding of lipid to mucin, the decrease in the diffusivity of PC due to the obstruction effect of mucin can be predicted from Eq. 3 with the size of micelle in buffer and the volume fraction of mucin (Table I). Thus, the dashed curves in Figure 4 were calculated relative to the solid curve by applying the obstruction factor. It is apparent that experimentally observed diffusivities of PC were not consistent with the theoretically predicted diffusivities of PC in the presence of mucin (Figure 4). This demonstrates that mucin not

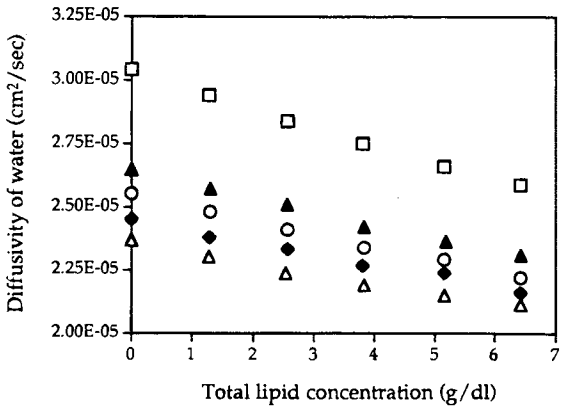

Fig. 3. Effective diffusivity of water (HDO) as a function of total lipid concentration. □, control (0% mucin); ▲, 0.25% mucin; ○, 0.5% mucin; ◆, 0.75% mucin; △, 1% mucin.

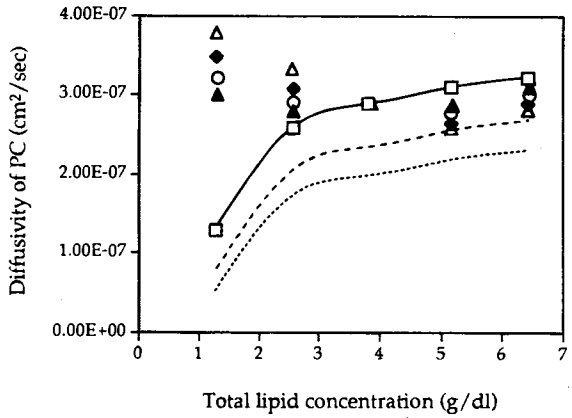

Fig. 4. Observed diffusivities of phospholipid in the BS/PC solutions. □, control (0% mucin); ▲, 0.25% mucin; ○, 0.5% mucin; ◆, 0.75% mucin; △, 1% mucin. The solid curve is drawn by eye based on the control data. The predicted diffusivities of PC in the presence of mucin (assuming no binding of lipids to mucin) is calculated relative to the solid curve by Eq. 3. – – –, 0.25% mucin; ------, 1% mucin.

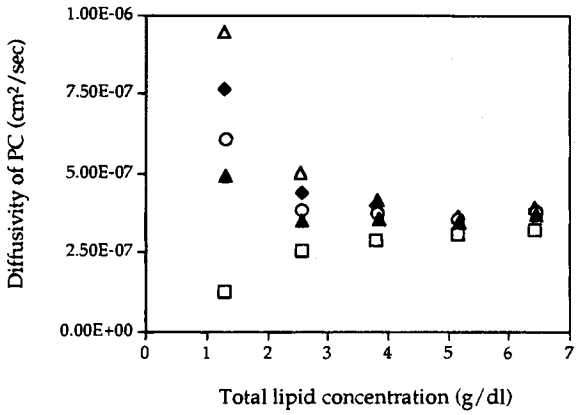

Fig. 5. Observed diffusivities of phospholipid (PC) corrected for the obstruction effect of mucin as a function of total lipid concentration. □, control (0% mucin); ▲, 0.25% mucin; ○, 0.5% mucin; ◆, 0.75% mucin; △, 1% mucin.

only obstructs the diffusion of the micelles but also affects their aggregation state. However, the theory of Johansson *et al.* was developed for the diffusion of spherical solutes in a network which was not the case of BS/PC aggregates. A more sophisticated theory needs to be applied to understand the diffusion of bile salt/phospholipid micelles through mucin.

Correcting the experimentally observed diffusivity for the obstruction effect of mucin (Eq. 3) leads to the results in Figure 5. The diffusivity of PC increased with increasing mucin concentration. In other words, the size of micelle decreased with increasing mucin concentration (Table II). It is therefore evident that phospholipid molecules bind to mucin. As mentioned above, the IMC decreases with increasing micellar size and the micellar size decreases with increasing BS/PC ratio. As the phospholipid leaves the mixed micelle, both the BS/PC ratio and the intermicellar concentration increase (29). The IMCs are 4, 4.25, 4.5, 5, 6, and 8 mM for the micellar size of 80, 70, 60, 50, 40, and 30 Å at 40 °C, respectively (29). The IMC stays at 4 mM when the micellar size is larger than 80 Å (29). From the size change in the presence of mucin (Table II), only a small amount of bile salt was dissociated from the micelles. Therefore, the bile salt to phospholipid ratio within the micelle increases and thus the size of the micelle decreases.

In summary, it has been demonstrated that the aggregation state of BS/PC mixed micelles is altered by the presence of mucin. This would have a direct impact on the transport of dietary lipids and solubilized drugs through the aqueous boundary layer of the intestinal tract.

Table II. The Effect of Mucin on the Hydrodynamic Radius (Å) of BS/PC Aggregates

	Total lipid concentration (g/dl)				
Mucin conc.	1.28	2.56	3.80	5.15	6.42
0%	206	104	93	86	83
0.25%	54	76	76	77	72
0.50%	44	69	72	76	70
0.75%	35	61	67	75	69
1.00%	28	53	64	73	68

CONCLUSIONS

This study has been carried out to investigate the effect of mucin on the diffusivity of BS/PC aggregates. A progressive decrease in the effective diffusivity of HDO was observed with an increase in the both the BSM and total lipid concentration. The effective diffusivity of PC decreased with increasing lipid concentrations in the presence of mucin, while in the controls it increased. After correcting the effective diffusivity of PC for the obstruction effect of mucin, the size of the BS/PC mixed micelle was assessed. It appears that PC associates with BSM resulting in a decrease in the available PC for micellization. This reduces the average size of the mixed micelle within the mucous layer. Thus, the aggregation state is altered by the presence of mucin which has implications for the transport of dietary lipid and solubilized drug through the aqueous boundary layer of the intestinal tract.

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REFERENCES

1. J. F. Forstner and G. G. Forstner. Gastrointestinal mucus. In L. R. Johnson (ed.), *Physiology of the Gastrointestinal Tract.* Raven Press, New York, 1994, pp. 1255–1276.
2. M. I. Filipe. Mucins in the human gastrointestinal epithelium: A review. *Invest. Cell Pathol.* **2**:195–216 (1979).
3. C. J. O'Connor and R. G. Wallace. Physical-chemical behavior of bile salts. *Adv. Coll. Interf. Sci.* **22**:1–111 (1985).
4. J. E. Staggers, O. Hernell, R. J. Stafford, and M. C. Carey. Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 1. Phase behavior and aggregation states of model lipid systems patterned after aqueous duodenal contents of healthy adult human beings. *Biochemistry* **29**:2028–2040 (1990).
5. O. Hernell, J. E. Staggers, and M. C. Carey. Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. *Biochemistry* **29**:2041–2056 (1990).
6. V. Bakatselou, R. C. Oppenheim, and J. B. Dressman. Solubilization and wetting effects of bile salts on the dissolution of steroids. *Pharm. Res.* **8**:1461–1469 (1991).
7. T. R. Bates, M. Gibaldi, and J. L. Kanig. Solubilizing properties of bile salt solution. I. Effect of temperature and bile salt concentration on solubilization of glutethimide, griseofulvin, and hexestrol. *J. Pharm. Sci.* **55**:191–199 (1966).
8. L. Martis, N. A. Hall, and A. L. Thakkar. Micelle formation and testosterone solubilization by sodium glycocholate. *J. Pharm. Sci.* **61**:1757–1761 (1972).
9. M. Rosoff and A. T. M. Serajuddin. Solubilization of diazepam in bile salts and in sodium cholate-lecithin-water phases. *Int. J. Pharm.* **6**:137–146 (1980).
10. N. A. Peppas, P. J. Hansen, and P. A. Buri. A theory of molecular diffusion in the intestinal mucus. *Int. J. Pharm.* **20**:107–118 (1984).
11. H. Witas, B. L. Solmiany, E. Zdebska, K. Kojima, Y. H. Liau, and A. Solmiany. Lipids associated with dog gastric mucus glycoprotein. *J. Appl. Biochem.* **5**:16–24 (1983).
12. H. Witas, J. Sarosiek, M. Aono, V. L. N. Murty, A. Solmiany, and B. L. Solmiany. Lipids associated with rat small-intestinal mucus glycoprotein. *Carbohydrate Res.* **5**:16–24 (1983).

13. C. E. Nadziejko, B. L. Solmiany, and A. Solmiany. Most of the lipid in purulent sputum is bound to mucus glycoprotein. *Exp. Lung Res.* **19**:671–684 (1993).
14. B. F. Smith and J. T. LaMont. Hydrophobic binding properties of bovine gallbladder mucin. *J. Biol. Chem.* **259**:12170–12177 (1984).
15. D. Hong, B. Turner, K. R. Bhaskar, and J. T. Lamont. Lipid binding to gastric mucin: protective effect against oxygen radicals. *Am. J. Physiol.* **259**:G681–G686 (1990).
16. L. M. Lichtenberger, T. N. Ahmed, J. C. Barreto, Y. -C. J. Kao, and E. J. Dial. Use of fluorescent hydrophobic dyes in establishing the presence of lipids in the gastric mucus gel layer. *J. Clin. Gastroenterol.* **14**:S82–S87 (1992).
17. P. Gao and P. E. Fagerness. Diffusion in HPMC gels. I. Determination of drug and water diffusivity by pulsed-field-gradient spin-echo NMR. *Pharm. Res.* **12**:955–964 (1995).
18. P. Gao, P. R. Nixon, and J. W. Skoug. Diffusion in HPMC gels. II. Prediction of drug release rates from hydrophilic matrix extended-release dosage forms. *Pharm. Res.* **12**:965–971 (1995).
19. P. Stilbs. Fourier transform pulsed-gradient spin-echo studies of molecular diffusion. *Prog. Nucl. Magn. Reson. Spectrosc.* **19**:1–45 (1987).
20. J. L. Pope. Crystallization of sodium taurocholate. *J. Lipid Res.* **8**:146–147 (1967).
21. P. Stilbs. Fouier transform NMR pulsed-gradient spin-echo (FT-PGSE) self-diffusion measurements of solubilization equilibria in SDS solutions *J. Coll. Interf. Sci.* **87**:385–394 (1982).
22. L. Johansson and C. Elvingson. Diffusion and interaction in gels and solutions. 3. Theoretical results on the obstruction effect. *Macromolecules* **24**:6024–6029 (1991).
23. L. Johansson and J.-E. Löfroth. Diffusion and interaction in gels and solutions. 4. Hard sphere Brownian dynamics simulations. *J. Chem. Phys.* **98**:7471–7479 (1993).
24. R. Mills. Self-diffusion in normal and heavy water in the range 1–45°. *J. Phys. Chem.* **77**:685–688 (1973).
25. A. Silberberg and F. A. Meyer. Structure and function of mucus. *Adv. Exp. Med. Biol.* **144**:53–74 (1982).
26. R. E. Stark, R. W. Storrs, S. E. Levine, S. Yee, and M. S. Broido. One- and two-dimensional NMR relaxation studies of dynamics and structure in bile salt-phosphatidylcholine mixed micelles *Biochim. Biophys. Acta* **860**:399–410 (1986).
27. T. A. Gerken. The solution structure of mucous glycoproteins: Proton NMR studies of native and modified ovine submaxillary mucin. *Arch. Biochem. Biophys.* **247**:239–253 (1986).
28. R. Bansil, E. Standley, and J. T. LaMont. Mucin biophysics. *Annu. Rev. Physiol.* **57**:635–657 (1995).
29. N. A. Mazer, G. B. Benedek, and M. C. Carey. Quasielastic light-scattering studies of aqueous biliary lipid systems. Mixed micelle formation in bile salt-lecithin solutions. *Biochemistry* **19**:601–615 (1980).