Solubilization or Emulsification of Dietary Monoacylglycerols by Bile Salts

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To clarify the roles of bile salts in fat absorption through the unstirred water layer covering epithelial cells, the dispersion of monoolein and beef tallow hydrogenated monoacylglycerol (monoglyceride) was investigated with respect to their states in aqueous solutions of two bile salts, sodium taurochenodeoxycholate and sodium tauroursodeoxycholate. After mixing and incubating solutions of dietary monoacylglycerols and bile salts, each was centrifuged and filtrated using a 0.22 μm membrane filter. The concentration of dissolved monoacylglycerol was then measured. The concentration of unsaturated monoacylglycerol considerably exceeded that of the saturated monoacylglycerol. In bile salt solution, the former dispersed to form an emulsion while the latter solubilized to form mixed micelles. In the case of either monoacylglycerol, the dispersion ability of chenodeoxycholate solution was much stronger than that of ursodeoxycholate solution.

Introduction

A comprehensive understanding of transport mechanisms requires some knowledge of the physico-chemical properties of acylglycerols (glycerides) and bile salts (BS) with reference to their behavior in an aqueous environment. The dietary intake of fat, mainly triacylglycerol (triglyceride) ranges approximately from 50 to 70 g/d in the average Japanese adult. It is known that the absorption of dietary triglyceride is an extremely efficient (> 95%) process. Emulsification, which starts in the stomach by mechanical means prior to absorption, takes place with aid of bile salts in the upper intestine. Since the BS’s themselves are poor emulsifying agents, they are likely to need some cooperation with the other surface active substances for emulsification. Lecithin as well as bilirubin participates in emulsification and also the products of lipolysis, i.e., mono- and diacylglycerols (diglycerides) and fatty acids are considered to play a contributory role. As is well known, lipolysis proceeds parallel to emulsification. An emulsion contains droplets of 0.2~0.5 μm in diameter, which can be seen under the microscope. Droplets require energy for forming and are relatively unstable. Commonly called lipase is secreted by pancreas and it hydrolyzes only ester bonds at positions 1 and (1’) of triglyceride molecule, thereby producing free fatty acids and a 2-monoacylglycerol (2-monoglyceride). Emulsification and lipolysis are followed by micellization or mixed micelle formation. Mixed micelles which contain fatty acids, monoglycerides and BS’s are freely soluble in an unstirred water layer and contact with brush border of epithelial cell of intestine.

In the present study, dissolution or dispersion states of monoglycerides are examined in water with or without addition of BS’s. We focus on comparisons between monoglycerides with a saturated hydrocarbon chain and with an unsaturated hydrocarbon chain; and between chenodeoxycholate and ursodeoxycholate.

Saturated glycerides are in general believed to
be poorly absorbed in comparison with unsaturated glycerides. We employed beef tallow hydrogenated monoglyceride (BTMG) which monopalmitoylglycerol (monopalmitin) 67% and monostearoylglycerol (monostearin) 31% as a typical saturated monoglyceride among those obtained from the most common dietary fats. And monoolein (MO) was examined as a candidate among unsaturated monoglycerides.

We selected two bile salts, sodium taurochenedodeoxycholate (TCDC) and sodium tauroursodeoxycholate (TUDC); in addition, employed sodium chenodeoxycholate (CDC), sodium ursodeoxycholate (UDC), sodium glycochenodeoxycholate (GCDC) and sodium glycourso-deoxycholate (GUDC), for our experiments. Chenodeoxycholate and ursodeoxycholate are the stereochemical isomers. TCDC has 3α- and 7α-OH groups while TUDC has 3α- and 7β-OH groups. The only difference between them is at 7α- and 7β- position. It is interesting to investigate how the stereochemical difference affects the dispersion or dissolution of monoglycerides in water.

**Experimental**

**Materials**

The monoglycerides employed for the present experiment, BTMG and MO were gifts from Kao Co. and used as received. The major difference between these lipids lies in their melting points. The representative chemical properties of BTMG and MO are listed in Table-1. All the bile sodium salts, taurochenodeoxycholate (TCDC), tauroursodeoxycholate (TUDC), chenodeoxycholate (CDC), ursodeoxycholate (UDC), glycochenodeoxycholate (GCDC), and glycourso-deoxycholate (GUDC) were purchased from Calbiochem, CA, USA and used without further purification. Inorganic salts were analytical grades and used as received. Water as solvent was purified by two distillations.

**Methods**

Excess but fixed amounts of monoglycerides were incubated in saline or bile salt-saline solution with successive shaking (150 min⁻¹) at 37°C. Aqueous turbid solutions were sampled at the same time intervals and centrifuged to eliminate solid substances at 3000 rpm for 10 min. Afterwards, still turbid solutions were filtrated by a millipore filter with pore size of 0.22 μm. As reported previously, the filtrate which could pass through 0.22 μm microfilter could also pass through the 0.05 μm microfilter almost 100%. The 0.05 μm pore size is similar to the minimum size of the intermicrovillous spaces. Because we were concerned that the monoglycerides would be hydrolyzed spontaneously through the experimental procedures, we determined the concentration of monoglycerides by applying the acetylacetone method (Triglyceride-Test, Wako Pure Chem. Ind., Ltd.) which determines substantially the concentration of glycerol⁶. Measurements were carried out by the use of JASCO UVIDEC-320 Spectrophotometer. The total amount of monoglyceride A in the sampled solution is equal to the sum of B and C as shown in Fig. -1, where B is the concentration of monoglyceride hydrolyzed spontaneously during incubation and C is the concentration of dispersed monoglycerides themselves. Therefore, C is known from determinations of A and B.

**Results and discussion**

The dissolution was at first examined as a
function of incubation time (day unit) for BTMG in the presence or absence of different BS’s (5mM) at 37°C and a fixed pH, where the phosphate buffer solution of pH 9.5 was employed as the solvent for each BS solution.

As shown in Fig.-2, the dissolution of BTMG seems to continue to increase even after 9 d incubation. Measured points go up along the curve for the solution with no addition of BS’s, and apparently the addition of BS’s itself seems little to contribute to BTMG dissolution, although the group of chenodeoxycholates (CDC, GCDC and TCDC) shows in general a little more contribution compared with that of ursodeoxycholates (UDC, GUDC and TUDC) or the solutions of the latter group show rather lower values than that with no addition of BS. The result shown in Fig.-2 suggests that BTMG is automatically hydrolyzed by the catalyst of OH⁻ ion to convert into glycerol and fatty acid and then to form soap micelles or mixed micelles of soap with BS. Here, it is important to define and discuss the term “dissolution”. “Dissolution of BTMG” involves all solution states such as singly dispersed BTMG molecules, hydrolyzed species of singly dispersed and micellar forms, and solubilized and/or emulsified BTMG molecules with or without aid of BS’s. Therefore the word “dissolution” does not mean simply the thermodynamically defined “solubility” of BTMG but includes various states of dispersion in solution. So instead of dissolution, the term “dispersion” is used hereafter.

As has been shown above, the automatic hydrolysis of monoglyceride takes place and this does not allow us to correctly evaluate the ability of each BS in the dispersion of monoglycerides in aqueous media. Thus, it is desirable to investigate to what extent the pH acts on the hydrolysis or the dispersion of BTMG. We prepared three phosphate buffer solutions; pH = 4.0, 9.5 and 11.0 and one saline solution (0.15M NaCl) as solvents. In Fig.-3 are given the plots of incubation time (d) vs. the concentration of dispersed BTMG.
pH 11.0 still shows a conspicuously increasing trend even though 9 d have gone by, and the curve at pH 9.5 also shows a continuous increase. In contrast, both the systems in saline and in buffer of pH 4.0 show to reach their dispersion equilibrium within 1 d, and give very low equilibrium values compared to the systems of pH 9.5 and pH 11.0. As pH is raised, saponification (hydrolysis) of BTMG is promoted and thus more auto-solubilization is caused by produced soap micelles. In the neutral pH range, the saponification is found to be restricted to a small extent. Taking into account the above results, we decided to employ the saline solution as the solvent hereafter in order to evaluate the dispersion power of each BS.

We at first obtained the dispersion curves of BTMG and MO saline solutions with and without addition of BS’s as a function of incubation time, and confirmed that the dispersion reached an equilibrium within 20~30 h. The concentration changes in dispersion of BTMG and MO in the various solutions are plotted as a function of incubation time as shown in Figs. 4 and 5 respectively, the concentration of bile salts being the upper value (10 mM) and the lower value (5 mM) of small intestine in digestion.

It should be again noted that the monoglycerides of both saturated and unsaturated fatty acids are spontaneously (even without addition of catalyst for the hydrolysis) hydrolyzed during the experiments and that the concentration of monoglyceride with saturated fatty acid solubilized by TUDC is almost similar to the concentration of those hydrolyzed spontaneously in saline (see Fig. 4). The concentration of 5 mM TUDC is close to its cmc (critical micelle concentration). This means that TUDC itself is very poor in solubilizing monoglyceride having a saturated hydrocarbon chain. However, as is seen in Fig. 5, 5 mM TUDC solution can solubilize MO clearly more than saline solution (with no BS) even though this solution exhibits the lowest solubilizing power among the solutions with addition of bile salts. This result suggests that TUDC micelles interact rather well with the unsaturated hydrocarbon chain whose double bond is slightly hydrophilic compared with the saturated hydrocarbon chain of BTMG, and that TUDC is likely to be weak in solubilizing strong hydrophobic substances. This may be related to the previous studies reporting that TCDC is a good solubilizer of cholesterol while the cholesterol solubility in TUDC solution is very low.

The solubilization for cholesterol monohydrate (ChM) and that for acid form of each BS have been compared between CDC and UDC and/or between TCDC and TUDC in terms of the molecular number of each BS required to solubilize a solubilize molecule. We have introduced a measure of solubilizing power (Sp) defined as $Sp = \frac{dw}{d(C-cmc)} = \frac{dw}{dC}$, where, is the molar concentration of solubilized species and $C$ is the total concentration of BS. In the concentration range sufficiently higher than cmc (above ca. 20 mM), the plot of $w$ vs. $C$, in most cases, shows a linear relation. This fact,
therefore, may allow us to compare the \(Sp\) values estimated by different researchers at different concentrations (20 mM–100 mM) of BS with each other. In Tables-2 and 3 are tabulated the literature values of \(Sp^{-1}\) and solubilizing or dispersing power ratios of TCDC/TUDC and CDC/UDC. These tables clearly show that the solubilizing power reflects not only the difference in the interaction between host (bile salt) molecules in micelles but also that in host-guest (solubilize) interaction. From the tables, in addition to the fact that \(Sp\) values of CDC and TCDC are generally superior to those of UDC and TUDC, the mode of solubilization by BS is found to be divided into two groups in terms of power ratio; one is the group having a long hydrocarbon chain whose ratio is around 3 and the other is the steroid skeleton group whose ratio ranges from 10 to 20. This suggests that the micellar structure composed of BS and solubilize molecules may be governed by their molecular structure, and accordingly that the mechanism of mixed micelle formation differs depending on a difference in molecular structure.

Here let us consider a problem of hydrophobicity and hydrophilicity which must be closely related to aggregation and dispersion in water. Since CDC and UDC are different from each other just in terms of the hydroxyl group configuration at the 7th position in steroid skeleton, \(i.e., 7\alpha-OH\) or \(7\beta -OH\), HLB (hydrophilic–lipophilic balance) is the same between them (according to Kawakami’s empirical expression\(^{15}\), HLB = 1.27). As has been reported, however, they show a conspicuously different behavior with respect to lipid solubilization, surface activity etc. This indicates that the HLB cannot be the case for bile salts, that is, it does not work as a measure of the hydrophilicity or lipophilicity. Instead of HLB, Miyajima et al.\(^{16}\) have proposed the “Hydrophobic Index (HI)” which was evaluated from the ratio of computed areas of hydrophobic and hydrophilic surfaces. For instance, HI of CDC is 7.27 (\(\alpha\)-side, 0.80; \(\beta\)-side, 6.47; total, 7.27), while HI of UDC is 5.48 (\(\alpha\)-side, 0.96; \(\beta\)-side, 4.52; total, 5.48). The larger the HI value, the stronger the hydrophobicity, so that CDC should exhibit a stronger hydrophobic behavior compared with UDC.

This has been confirmed in various aspects as the following. By employing high-performance-reversed-phase liquid chromatography (HPLC), Carey et al.\(^{10}\) measured the relative hydrophilic–hydrophobic properties of the BS’s and found that chenodeoxycholates showed a larger normalized retention time (mobility\(^{-1}\)) compared with ursodeoxycholates where the normalized retention time can be used as a measure of hydrophobicity and also that the rank order is; free species > glycine conjugates > taurine conjugates. The most important finding in the HPLC study is that

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<th>Table-2 Solubilization data of TCDC and TUDC.</th>
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<td>ChM(^{(a)})</td>
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<td>((S_{TCDC})^{-1})</td>
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<td>((S_{TUDC})^{-1})</td>
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<td>((S_{TCDC})/(S_{TUDC}))</td>
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(a) Igimi et al. (1981) and Carey et al. (1981) estimated in 100 mM bile salt solution at 37°C, 0.15 M Na\(^+\), pH 7.0
(b) Present work estimated in 10 mM bile salt-saline solution at 37°C, pH 7.0

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<th>Table-3 Solubilization data of CDC and UDC.</th>
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<td>ChM</td>
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(a) Igimi et al. (1981) and Carey et al. (1981) in 100 mM BS at 37°C, pH 10.0
(b) Montet et al. (1987) in 30 mM BS at 37°C, pH 9.5
(c) Nagadome et al. in 10 mM BS at 37°C, pH 10.0; unpublished data
(d) Montet et al. (1987) in 20 mM BS at 25°C; recalculated from their \([A^-]/[HA]\) data
cholesterol solubilizing power is closely correlated with the mobility\(^1\). In addition to those mentioned above, a lot of other measures to evaluate hydrophobicity of surfactants are available. Above all, lowering degree of surface tension and cmc are important measures. We have examined in detail the surface activity change with concentration for CDC-UDC bile salt mixture and respective pure systems by application of the drop volume method. The results clearly showed that CDC had a lower cmc and a stronger surface activity or a higher degree of surface tension depression as was predicted by the HI values\(^ {15} \) (See Footnote).

Turning to the structure formation of aggregates with guest molecules, in the case of CDC and UDC with cholesterol, Carey et al.\(^ {10} \) derived the simplest possible scheme from a thermodynamic consideration. In their model, surface adsorption of cholesterol occurs by hydrocarbon-hydrocarbon contact onto the hydrophilic face of the bile salt micelle. This interaction may also involve OH-OH group interactions between cholesterol and the bile salt. However they did not necessarily deny a possible model analogous to the idea of a palisade layer, into which a cholesterol molecule is inserted, as is seen in common surfactant systems.

The equilibrium values of dispersion in various saline solutions are shown in Fig.-6. TCDC is superior to TUDC in solubilization for both BTMG and MO. We can say that MO is much more dispersed than BTMG is in both TCDC and TUDC solutions. It is noteworthy that the solutions in which BTMG was dissolved by an incubator were always clear by naked eye even after the filtration, while MO solutions were slightly turbid always even after the filtration by 0.22 \(\mu\)m microfilter. These facts suggest that the saturated monoglycerides are solubilized in the course of forming mixed micelles while the unsaturated monoglycerides are dispersed in forming emulsion. It may correspond to the fact that vegetable oil containing much unsaturated fatty acids is more easily digested than animal fat.

MO was dissolved in the 10 mM TCDC and 10 mM TUDC and in the 5 mM TCDC-5 mM TUDC (total BS concentration : 10mM) mixed solution (see Fig.-7). The MO concentration dispersed in the mixed bile salt solution was found to be just the mean value between those of the 10 mM TCDC and the 10 mM TUDC. This fact suggests that not only our experimental methods were correctly applied, but also all BS’s are mobilized in forming emulsion. In other words, if MO were dispersed only by bile salt micelles, the total concentration less cmc of mixed micellar solution should have been correspondingly related to the dispersion, and thus the mixed bile salt micellar solution could not have shown just the mean value.

In connection with the distinction of emulsion from mixed micellar solution of MO-BS systems, it should be considered that whether emulsification or solubilization occurring in the MO-BS solution depends on the amount of MO given in excess, that is, the extent of excess determines which type of the two solution states occurs. We have previously reported the solubilization and the cmc data for the same MO-BS’s mixed systems\(^ {10} \), while in this paper we are going to present an emphatic contrast. In the previous study, by adding an essential minimum excess amount of MO into the BS

![Fig. -6](image1)

**Fig. -6** Concentration of beef tallow hydrogenated monoglyceride and monoolein dispersed in the various solutions.

![Fig. -7](image2)

**Fig. -7** Mixing effect of two bile salts for dispersion of monoolein.
solutions, the solution state of MO was examined for the solution filtrated by the filter the pore size of which was 0.01 µm. In the previous case, the filtrated solution was clearly transparent although the shaken-and-incubated solution as not yet filtrated was slightly turbid. The turbidity seemed to come from the emulsified particles of MO in small excess. For the transparent solutions, the respective cmc’s of different mixed micellar solutions of BS’s with MO and their solubilization powers were measurable.

On the other hand, in the present study the BS solutions containing MO in large excess were shaken for 20 ~ 30 h. During the shaking-and-incubation the solution changes into a white and comparatively stable emulsion. The use of filters of small pore size did not allow us to obtain filtrates to be examined, but the use of 0.22 µm filter did enable us to do that. Here, it can be regarded that almost all of the BS molecules are adsorbed on the tremendously wide surface of MO droplets and thus little micellization of BS’s takes place.

Fig. 8-(A) is a diagram indicating concentrations of BTMG dispersed in saline and 10 mM TCDC solutions. The bar (1) shows its concentration in saline (with no bile salt). Note that this actually shows the concentration of glycerol, that is, the other half of a monoglyceride molecule which was hydrolyzed spontaneously. Therefore, within this concentration range, only glycerol and fatty acids may exist. The bar (2)-a shows the glycerol concentration in 10 mM TCDC-saline solution without hydrolysis procedure for its determination. The existence of the difference between the bar (2)-a and the bar (1) shows that the spontaneous hydrolysis is enhanced by formation of the mixed micelles of BS’s with fatty acids. The bar (2)-b represents the whole concentration of glycerol, that is, the analytical concentration of monoglyceride in the solution. The bar (2)-c corresponds to the concentration range in which there exist mixed micelles of BS’s, fatty acids and monoglycerides.

Fig. 8-(B) depicts the concentration ranges of the respective solution states of MO dispersed in 10 mM TCDC-saline solution. The bar (1), here, represents the same facts described just above. As mentioned before, these MO solutions were always turbid even without hydrolysis procedure. The range of the bar (2)-a indicates the concentration of spontaneously hydrolyzed fatty acids which are incorporated into the emulsion. Within the range represented by bar (2)-b, the solution contains emulsion particles formed not only by the BS’s and the fatty acids but also by the monoglycerides themselves. Needless to say, all BS molecules must be consumed to form these emulsion particles.

It can be readily appreciated from Fig. 8 that the unsaturated monoglycerides are much more dispersed in the BS solution than the saturated glycerides can be solubilized in forming mixed micelles in the same solution. This difference might be the key to explain the reason why the saturated triglycerides are absorbed less effectively and the unsaturated ones are absorbed more effectively, and also might directly correspond
to the difference in the interfacial tension (wettability) between aqueous media and these glycerides as well as their melting points and densities.

In this current work, we have tried to clarify the molecular movement of monoglyceride in the unstirred water layer. Because monoglycerides are hydrolyzed spontaneously into glycerol and fatty acid which are dispersible in water, it appears easy for monoglyceride to pass through an unstirred water layer on an intestinal mucous membrane. Porter et al. found no impairment of lipolysis even in bile fistula man[7]. These may indicate that the role of BS is not always essential for fat absorption in the intestine as it has so far been considered. However, it should be noted that lipase is known to act more easily for lipid hydrolysis at the oil-water interface of emulsion and micelle which incorporate lipid in state of favorable orientation in their palisade layer. Anyway, more experiments need to be done on the more complex solutions yet. So, we shall have to pay more attention to the behavior of such a biological surfactant as lecithin, and especially to the cooperation of lecithin with BS's for the dispersion of dietary lipids.

Acknowledgements

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References


Footnotes

By citing the cmc data determined from surface tension measurements by Roda et al.[16], Miyajima et al.[14] have shown a clear correlation of HI with bile salts having 3α-OH (3α-BS); dihydroxy cholates 3α, 7α-(CDC); 3α, 12α-(DC); 3α, 12β-; 3α, 7β-(UDC) and trihydroxy cholates (3α, 7α, 12α-; 3α, 7α, 12β; 3α, 7β, 12α-). Here it may be of interest to describe that the HI indicates no correlation with cmc values of bile salts which have 3β-hydroxyl group (3β-BS). That is to say, although their HI values are similar (for dihydroxy cholates of 3β-OH, HI=4.7±0.2 and for trihydroxy cholates of 3β-OH, HI=4.0±0.2), the cmc values widely disperse. This fact is not yet pointed out by Miyajima et al. themselves, but it should be noted here that 3β-OH is likely to play the most leading role for aggregation behavior of 3β-BS's. Conversely speaking, the 3α-OH group of 3α-BS's plays an indispensable screw-and-helm role on the course of aggregate formation. The HI is a trustworthy
measure, because it is exactly true for monosaccharides\textsuperscript{14}. So we can at least say that we need a different measure other than HI given by Miyajima et al. or a new model of aggregation mechanism quite different from common surfactants including 3\(\alpha\)-BS's. One of the present authors is going to propose a model of aggregation formed from such a unit as a dimer whose hydrophobic-hydrophilic area ratio is different from that of a monomer. A correct solution concerning the 3\(\beta\)-BS' problem will never be obtainable without referring to the previous studies\textsuperscript{14,16}, or without creating a new model.

胆汁酸塩水溶液中での食じ(卸)モノアシルグリセリンの分散状態

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脂肪が飽和脂肪が不飽和脂肪がにより消化吸収が異なることに対して、胆汁酸塩がどのように関与しているかを、胆汁酸塩水溶液中でのモノアシルグリセリン（モノグリセリド）の分散状態から研究した。胆汁酸塩にはタウロケノデオキシシコール酸ナトリウム塩とタウロルソデオキシコール酸ナトリウム塩を用い、胆汁酸塩の分子構造において、7位の OH 基の配向の違いによる挙動を検討した。

それらの結果から、（0.22\(\mu\)mフィルターパ過後の水溶液において）飽和モノグリセリドは乳化状態で分散するのに対して、不飽和モノグリセリドは可溶化状態で分散することを示した。また、モノグリセリドに対する分散能は、飽和モノグリセリドと不飽和モノグリセリド両方とも、タウロケノデオキシコール酸ナトリウム塩がタウロルソデオキシコール酸ナトリウム塩より約 3 倍ほど大きかった。さらに、胆汁酸塩が存在しない生理食塩水中でもモノグリセリドが分散していることを考えると、脂肪の消化吸収に対して胆汁酸塩が必要不可欠ではないと推論される。