Surface Activity of the Lipid Products Hydrolyzed with Lipase and Phospholipase A-2

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We examined the *in vitro* surface activity, immersional wettability and adhesional wettability shown by aqueous solutions of soy lysophospholipid (SLP)/monoglyceride (MG)/fatty acid (FA), SLP/FA and SLP/MG, and found that many lipid mixtures showed significant surface activity when their MG and FA components consisted of polyunsaturated FA and/or medium chain FA. The more unsaturated the FA, the higher the surface activity. A mixture of SLP/medium chain fatty acid MG (medium chain MG)/medium chain FA showed the highest surface activity, and was comparable to an Aerosol-OT surfactant, the most effective wetting agent. SLP/polyunsaturated FA monoglyceride (polyunsaturated MG)/polyunsaturated FA, SLP/medium chain FA, and SLP/polyunsaturated FA, SLP/medium chain MG, and SLP/polyunsaturated MG also showed a high degree of activity. Wettability decreased rapidly when the amount of saturated, long chain FA moieties increased. It is recognized that the degrees of unsaturation and the chain length of FAs in the lipid mixtures have a decisive influence on surface activities. Higher ratios of MG and FA to SLP gave higher activity; and solubilizers such as bile salts were necessary to dissolve them in water.

KEY WORDS: **Hydrolyzed lipid product, lipase, lysophosphatidylcholine, lysophospholipid, medium chain fatty acid, monoglyceride, phospholipase A-2, surface activity, unsaturated fatty acid.**

Soy phospholipid (soya lecithin) is a by-product obtained in the production of soybean oil. Recently commercial lecithin has been improved by the addition of phospholipase A-2, leading to wider applications of these modified materials for oil in water (o/w) emulsions (1–3). There are few reports about the utilization of enzymatically modified phospholipids containing a large amount of lysophospholipids except for emulsifying use. We studied the synergistic effects of lysophospholipids with other surfactants (4,5) and with monoglycerides (MGs) and/or fatty acids (FAs). It was found that aqueous solutions of mixtures of lysophospholipid/MG/FA, lysophospholipid/FA and lysophospholipid/MG showed significant surface activity (e.g., wettability) when their MG and FA components consisted of polyunsaturated FAs and/or medium chain FAs (6,7). There are some reports giving physical properties of egg lysophosphatidylcholine (LPC) (8–19). However, we could not find any report showing physical properties of soy LPC or soy mono- and diacylphospholipids, or surface activity shown by the products of partially hydrolyzed lipids. The objective of the present study was to investigate the surface properties of mixtures of lysophospholipid/MG/FA with or without sodium taurocholate (STC), lysophospholipid/FA and lysophospholipid/MG, and to compare the influence of different FAs and components of lysophospholipid, MG, and FA in various ratios on the surface activities.

MATERIALS AND METHODS

Materials. Soy LPC and egg LPC were supplied by Nihon Shyoji K.K. (Osaka, Japan). Soy phospholipid was prepared from commercial lecithin by acetone extraction (Table 1). MGs were all obtained from Riken Vitamin K.K. (Tokyo, Japan) (Table 1). The FAs were: 18:0, >97%; 14:0, >99%; 12:0, >99%; 10:0, >99%; 8:0, >99%; oleic acid, >99%; linoleic acid, >99%; linolenic acid, 85.5%; eicosapentaenoic acid, 90.8%; docosahexaenoic acid, 90.3%; erucic acid, >88%; and coconut oil FA (12:0, 48%; 14:0, 20%); and were from the Nippon Oil and Fat Company (Tokyo, Japan). The FAs 16:0 (> 99%) and 6:0 (>99%) were from Asahi Denka Kogyo K.K. (Tokyo, Japan), and the *trans* FA, elaidic acid (99%) was from Sigma Chemical Co. (St. Louis, MO). Sodium taurocholate (STC) (>70%) was purchased form Difco Laboratories (Detroit, MI).

Preparation of mono- and diacylphospholipids. One-hundred parts of commercial crude soy phospholipid, from Ajinomoto K.K. (Tokyo, Japan), with an addition of 20 parts of water and 0.2 parts of pancreas phospholipase A-2 (Novo Lecithase 10-L), were incubated at 60°C. Different degrees of deacylated phospholipids were obtained by varying the reaction times. Oil and FAs were removed from the reaction mixture with acetone (20). The mono- and diacylphospholipids were analyzed by a modified thin-layer chromatography-flame ionization detector (TLC-FID) method (21) with an Iatroscan TH-10, Iatron Co., Ltd. (Tokyo, Japan). Analytical data for soy mono- and diacylphospholipids (SLPs) containing 60–80% (w/w) of lysophospholipids (SLP) are shown in Table 1 (abbreviations: SLP 60, SLP 66 and SLP 80). SLP 40 and SLP 50 were made by mixing soy phospholipid and SLP 80.

Preparation of solutions. Each component was dissolved in a small amount of hexane/ethanol (1:1, v/v) and blended. After evaporation of the solvent in vacuum, the mixtures were dissolved in deionized water with a sonicator.

Measurement of surface activity. Surface tension was measured by Wilhelmy's plate method (22) with a platinum plate—CBVP A-3, Kyowa Kagaku K.K. (Tokyo, Japan). Immersional wettability (penetrating power) was measured by the modified canvas disk method (23), the wettability test was carried out by measuring the sinking time of a one-inch diameter No. 6 canvas disk at 25°C and at 37°C. Adhesional wettability (contact angle) was measured on bee's wax and Japan wax with a type 13 contact angle meter (Elmar Kogaku K.K., Tokyo, Japan).

RESULTS

Effect of mixtures of SLP/MG/FA/ (with or without) STC

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TABLE 1

Analytical Data of Phospholipids and FA-Monoglycerides used in the Present Study [% (w/w)]

Lipid	Abbreviation	Analytical data							FA composition				
		LPC	LP	IV	MG	DG	TG	FFA[a]	16:0	18:0	18:1	18:2	18:3
Soy lysophosphatidylcholine	Soy LPC	>99	>99						24	7	11	51	5
80% Soy lysophospholipid	SLP 80	30	80						29	6	10	47	6
66% Soy lysophospholipid	SLP 66	29	66				4	5	35	7	9	41	6
60% Soy lysophospholipid	SLP 60	22	60						35	8	9	39	5
Soy phospholipid		3	—				4	2	19	4	11	58	7
Hydrogenated tallow FA MG	MG (18:0)			1.6	98	1.5	—	0.1	30	64			
Safflower oil FA MG	MG (18:2)			116	95	0.9	—	0.9	8	3	13	74	
Cottonseed stearin FA MG	MG (18:1–2)			72	93	3.0	0.1	1.1	37	2	14	47	
Oleic safflower oil FA MG	MG (18:1)			67	93	0.6	—	0.9	5	2	76	3	16:1=7
Hydrogenated coconut oil FA MG	MG (HCoco)			2.2	86	10	0.4	2.2	C8 = 7; C10 = 6; C12 = 51; C16 = 8				
Lauric acid MG	MG (12:0)			1.7	92	4	—	—	C12 = 98				
Capric acid MG	MG (10:0)			1.7	84	10	0.6	—	C10 = 98				
Caprylic acid MG	MG (8:0)			1.8	86	9	—	—	C8 = 97 C10 = 2				

[a]FFA, free fatty acid.

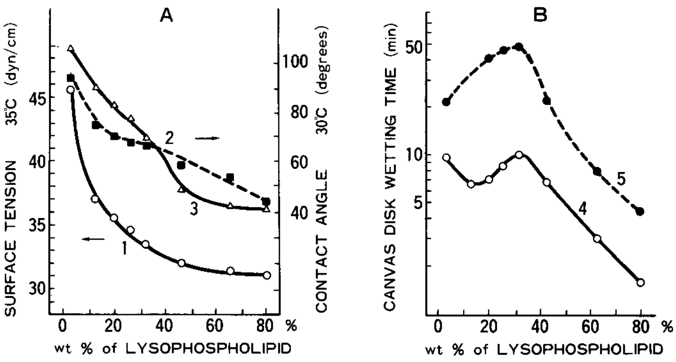

FIG. 1. **Surface activity of soy phospholipids containing lysophospholipids in various amounts. Measured on 0.5% (w/w) aqueous solutions. A: 1, surface tension at 35°C, ○━○; 2, contact angle on bees wax, at 30°C, △━△; 3, contact angle on Japan wax, at 30°C, ■– –■; B: Canvas disk wetting time, 4 at 37°C, ○━○; 5, at 25°C, ●– – –●.**

on surface activity. The degree of deacylation of soy phospholipids by phospholipase A-2 caused the surface activity of the aqueous solutions of partially deacylated SLPs to change (Fig. 1). When these SLPs were blended with MGs and FAs, aqueous solutions of these mixed lipids (total 0.5%, w/w) showed a significant change in surface activity as compared to the original SLPs at the same concentrations. Higher ratios of MG and FA to LPC or SLP gave bigger changes. Lysophospholipids solubilized MGs and FAs into micellar solutions but, in the higher ratios of (MG + FA)/lysophospholipid, the water solubility of the mixture decreases. Therefore, the addition of a solubilizer, such as bile salt, is necessary to dissolve them in water.

Surface activities of lipid mixtures containing different FA components are shown in Figure 2, in which the molar ratios of SLP/MG/FA/STC are fixed at 1:3:6 (M/M/M), and the ratio of STC ranged from 1–3.7 M, depending on the solubility of the mixtures. Figure 2 also shows the effect of pure soy LPC and soy mono- and diphospholipid [containing 66% lysophospholipids, (w/w), SLP 66]. Each mixture contained the same molar ratio of monolinolein, and differed only in FA component. The concentration of each aqueous solution is the same 0.2% (w/w), except for the amount of STC. Stearic and palmitic acids were not completely solubilized. FA (14:0) showed the minimum surface tension, while FA (12:0) and FA (18:2) showed the minimum values (maximum activity) in wetting time and contact angle. There was almost no difference in surface activity between the two mixtures containing soy LPC and SLP 66.

The canvas disk wetting time of different concentrations of mixtures is given in Figure 3. Soy LPC/MG (10:0)/FA (10:0)/STC (1:2:3:2, w/w/w/w)had the most activity, and is comparable to sodium dioctylsulfocsuccinate (an Aerosol-OT type surfactant), known as the most powerful industrial penetrating agent (Fig. 3). A mixture of SLP (LPC)/MG (18:2)/FA (18:2)/STC (1:2:3:4, w/w/w/w) had an activity which was comparable to that of polyoxyethylene monylphenolether (Triton X-100 type surfactant) at low concentrations. A comparison of surface activities of the lipid mixtures and commercial surfactants is shown in Table 2. The lipid mixture containing capric acid showed lower values for surface tension and contact angle on bee's wax than those of sodium dioctylsulfosuccinate.

Mixtures of soy lysophospholipid/MG/FA/(STC), with FA or MG that are long chain, saturated acids (such as stearic, palmitic or behenic) had surface activities that were much lower than those of the same lysophospholipid alone. However, when the FAs and MGs of the mixtures had polyunsaturated FA and/or medium chain FA, the aqueous solutions showed significant surface activity.

The addition of STC to the mixtures showed almost no effect on surface tension and contact angle, but its addition substantially increased their penetrating power (data not shown).

Figure 4 shows the effect of the FA content in lipid mixtures on their surface activity. These experiments were performed by changing the ratios of FA/MG from 0:1 to 2.5:1 (w/w), while keeping the ratio of SLP/MG constant. The mixture having FA (18:2)/MG (18:2) in ratios above about 1.5 did not increase in surface activity. Maximum surface activities for the mixture having FA

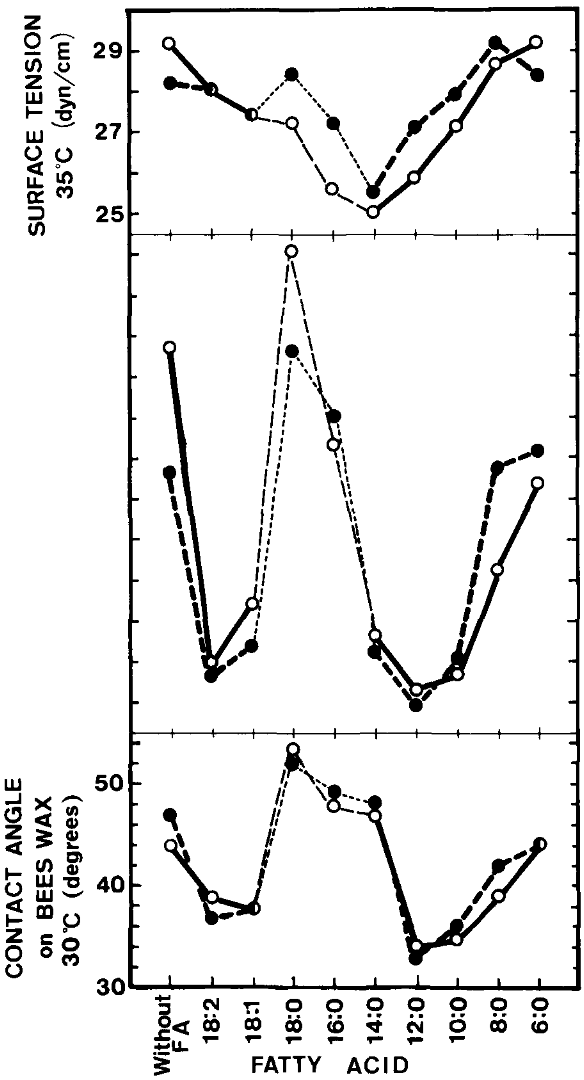

FIG. 2. Surface properties of lipid mixtures (soy phospholipid/MG/FA/STC) containing different FA components. The mixtures consisted of soy lysophospholipid/MG(18:2)/different FA/STC in the ratios of 1:3:6:1–3.7(M/M/M/M), STC molarities were: FA (16–18), 3.7; FA (10–14), 2; FA (6–8). 1. Soy LPC, ○━━○, and SLP66, ●– – –●, were used, and the concentration of the mixture was 0.2% (w) except for the amount of STC. When the FA component of a mixture was stearic or palmitic acid, a small part of the FA could not be solubilized at 37°C, even if the concentration of STC was increased to 5 times that of the summed value of the three components.

(10:0)/MG (10:0) were obtained in the range of 1.0–1.5 (w/w) ≅ 2:1, M/M. The canvas disk sank immediately in mixtures having MG (8:0–12:0) and FA (8:0–12:0).

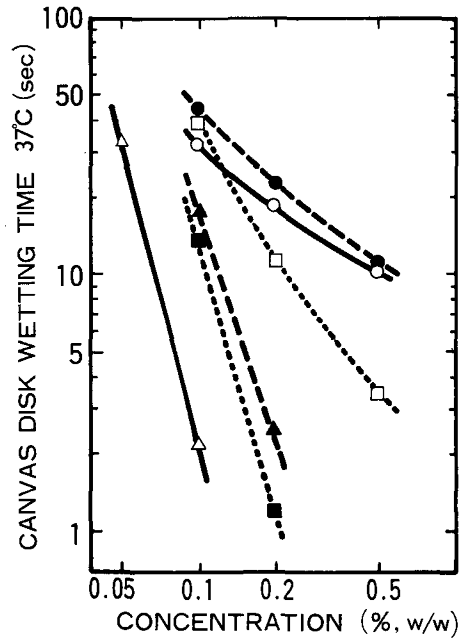

FIG. 3. Canvas disk wetting time vs concentration for aqueous solution of lipid mixtures and industrial surfactants. Canvas disk wetting time was measured for these mixtures: 1: soy LPC/MG(18:2)/FA(18:2)/STC = 1:2:3:3 (w), ○━━○; 2: soy LPC/MG (10:0)FA(10:0)/STC = 1:2:3:2 (w), △━━△; 3: SLP66/MG(18:2)/FA(18:2)STC = 1:2:3:4 (w), ●– – –●; 4: SLP66/MG(10:0)/FA(10:0)/STC = 1:2:3:4 (w), ▲– – –▲; 5: polyoxyethylene nonylphenolether, ADK TOL NP-700, a Triton X-100 type surfactant, Asahi Denka Kogyo K.K. (Tokyo, Japan), □-----□; and 6: sodium dioctylsulfosuccinate, ADK COL EC-4500, Aerosol-OT type surfactant, Asahi Denka Kogyo K.K. (Tokyo, Japan), ■------■, at 37°C.

Effect of mixtures of lysophospholipid/FA on surface activity. During the course of phospholipid hydrolysis with phospholipase A-2 in an aqueous solution, vesicular dispersion of phospholipids causes the solution to become clear and change into a micellar solution. To simulate this phenomenon, the transmittancy of several aqueous solutions of soy PC/soy LPC/FA in various ratios were examined. Medium chain FAs are not combined at the *sn*-2 position of PC, while polyunsaturated FA are combined at this position. The amount of solubilized FAs by LPC was much larger with medium chain FAs than with polyunsaturated FAs. FA 10:0 was solubilized by LPC at the molar ratio of 2:1. FA 18:2 and 18:0 were solubilized by LPC at the molar ratio of 1:0.75 and 1:1, respectively (data not shown).

The canvas disk wetting times and contact angles on bee's wax measured on solution of various molar ratios of soy PC/soy LPC/FA are shown in Figures 5A and 5B, respectively. Their surface activities reach maximal values when the FA/LPC values are about 1:1, M/M. The effects of different FAs in equimolar solutions of FA/lysophospholipid on surface tension, immersional wettability and adhesional wettability are demonstrated in Figure 6. FA 14:0 gives the minimal surface tension for saturated FAs, as was the case for a mixture of lysophospholipid/MG/FA and STC (Fig. 2). The equimolar solution with FA 12:0 showed the maximum immersional and adhesional wettability. These phenomena also occurred in the case of the three component mixtures of lysophospholipid/MG/FA (and STC) (Fig. 2).

In a two component mixture of lysophospholipid and FA, the degrees of unsaturation and chain lengths of the FAs have a decisive influence on surface activity. Among saturated FAs, FA 12:0 is the most active, though this combination is nonexistent in living cells. The mixture of lysophospholipid/polyunsaturated FA, FAs 18:3 and 20:5 showed the maximum surface activity, whereas mixtures with saturated long chain FAs, long chain monoenoic FAs, or a *trans* monoenoic FA (e.g., stearic, erucic, and elaidic acid) showed low activity. Palmitic and oleic acid showed almost the same degrees of activity. The mixture

TABLE 2

Comparison of the Surface Properties of Lipid Mixtures and Commercial Surfactants, on 0.2% (w/w) Aqueous Solutions

Surfactant	Surface tension 35°C, dyne/cm)	Canvas disk wetting time, (37°C, seconds)	Contact angle on bees wax, (30°C)
Na-dioctylsulfosuccinate[a]	27.8	1.2	34
POE (10mol) nonylphenolether[b]	29.7	11	38
Soy LPC[c]	38.0	194	62
Soy LPC/MG(18:2)/FA(18:2)/STC = 1:2:3:3 (w/w/w/w)	28.2	10	40
Soy LPC/MG(10:0)/FA(10:0)/STC = 1:2:3:2 (w/w/w/w)	24.7	1	20
SLP 66[d]	31.4	2060	64
SLP66/MG(18:2)/FA(18:2)/STC = 1:2:3:4 (w/w/w/w)	28.3	11	33
SLP66/MG(10:0)/FA(10:0)/STC = 1:2:3:4 (w/w/w/w)	24.8	2.5	20

[a] ADK COL EC-4500 (Aerosol- OT type surfactant), Asahi Denka Kogyo K.K. (Tokyo, Japan).

[b] ADK TOL NP-700 (Triton X-100 type surfactant), Asahi Denka Kogyo K.K. (Tokyo, Japan).

[c] Soy LPC 99% (w/w).

[d] Soy PLs containing 66% PLs (w/w).

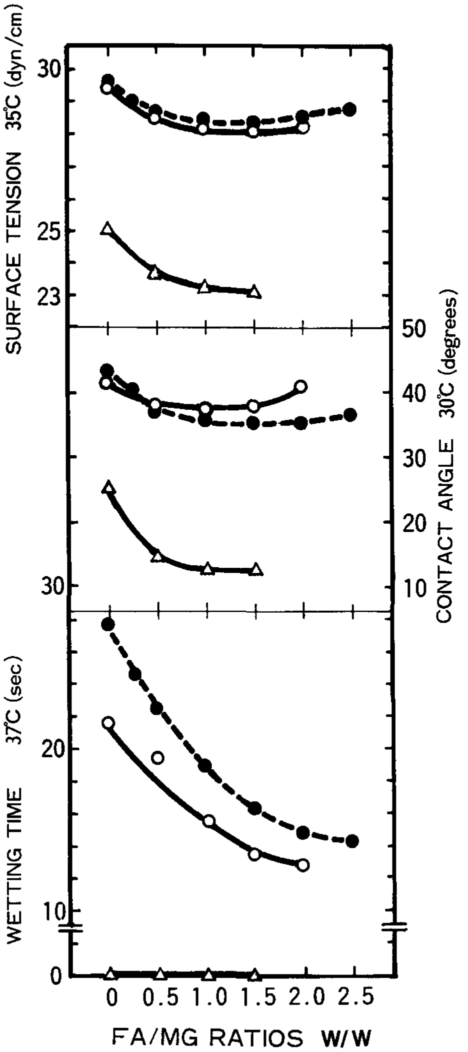

FIG. 4. Effect of fatty acid content on the surface properties of lipid mixtures. A, surface tension, B, contact angle on bee's wax; C, canvas disk wetting time. Mixtures: SLP66/MG(18:2)/FA(18:2)/STC = 100:100:X:100, ●- - -●; SLP66/MG(18:2)/FA(18:2)/STC = 50:100:X:150, ○━━○; SLP80/MG(10:0) /FA(10:0)/STC = 25:100:X, △━━△. The ratios of FA/MG were changed from 0 to 2.5, and surface properties were measured on 0.5% (w/w) solutions, the concentration value excepts for STC.

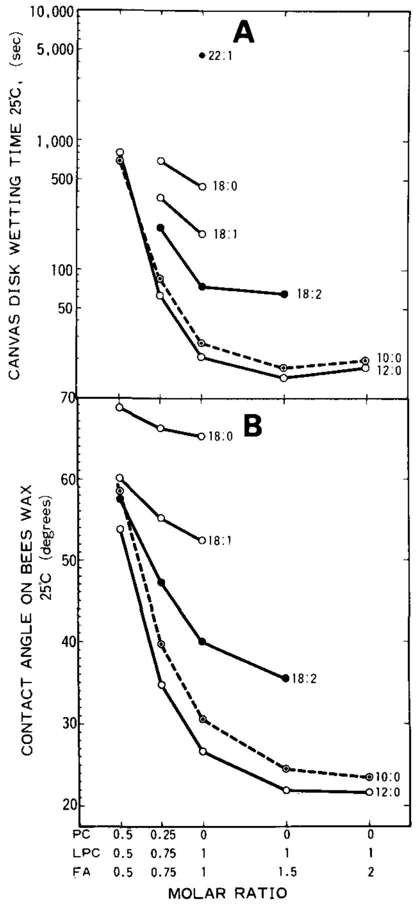

FIG. 5. Effect of molar ratios of soy PC/soy LPC/FA in lipid mixtures on the surface properties of their 0.2% (w/w) aqueous solutions, at 25°C. A, canvas disk wetting time; and B, contact angle on bee's wax.

of soy LPC/coconut oil FAs showed a little higher activity than that of soy LPC/FA (14:0); the mean chain length of coconut FAs is about 13:0. The critical micellar concentration values of several equimolar mixtures of soy LPC/FA at 25°C in % (w/v) were as follows: LPC/FA (18:0), 7.5 × 10^{-3}; LPC/FA (18:2), 2.7 × 10^{-3}; LPC/FA (20:5), 1.8 × 10^{-3}; LPC/FA (12:0), 7.4 × 10^{-3}; and they

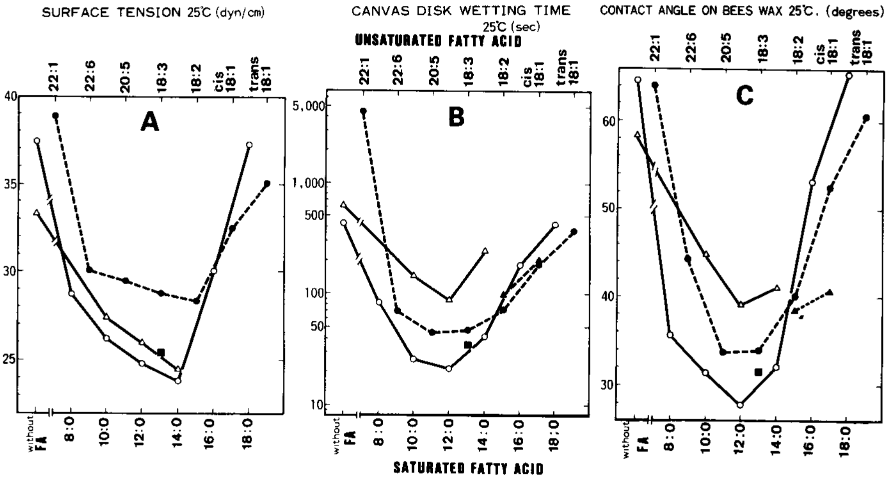

FIG. 6. Effect of various FAs on the surface properties of equimolar solution of soy lysophospholipid and FA. Surface tension, A; canvas disk wetting time, B; and contact angle on bees wax, C; values were measured on both 0.3 mM of component aqueous solutions. Mixtures: soy LPC/saturated FA, ○▬○; soy LPC/unsaturated long chain FA, ●– – –●; SLP66/saturated FA, △▬△; SLP 66/unsaturated long chain FA, ▲– – –▲; and soy LPC/coconut oil FA, ■. The measurement was done at 25°C.

TABLE 3

Canvas Disk Wetting Time for the Mixtures of Various Mono- and Diacylphospholipid and MG Having Different Iodine Values (IV)

Phospholipid	% of Lysophospholipid in total phospholipid	Canvas disk wetting time (min)			
		Phospholipid only	Phospholipid/MG=6/4 (w/w)		
			MG (18:0) IV=1.6	MG (18:1-2) IV=72	MG (18:2) IV=116
Soy LPC	99	2.6	2.5	3.1	
SLP 80	80	4.1	16	5.4	3.5
SLP 60	60	15.6	25	3.2	
SLP 50	50	19.2	26	2.7	1.4
SLP 40	40	37.5	67	3.6	1.5
Soy phospholipid	3	9.9	320	1.2	0.6

The ratio of phospholipid/MG was 6:4, and values were measured on 0.5% (w/w) aqueous solutions at 25°C.

TABLE 4

Comparison of the Surface Activity of the Three Types of Lipid Mixtures and Commercial Surfactants, 0.5% (w) Aqueous Solution at 25°C

Surfactant or lipid mixture	Surface tension (dynes/cm)	Canvas disk wetting time (seconds)	Contact angle on bee's wax (degrees)
Sucrose mono stearate[a]	34.8	438	64
POE (10mol) nonylphenol ether[b]	31.2	4	39
Na-Dioctylsulfosuccinate[c]	28.3	<0.5	35
Soy LPC	37.9	116	56
SLP 80[d]	33.2	288	44
SLP 50[e]	33.8	1140	57
SLP50/MG(18:2)/FA(18:2)/STC=1:2:3:4 (w/w/w/w)	28.4	18	35
SLP50/MG(10:0)/FA(10:0)/STC=1:2:3:4 (w/w/w/w)	25.2	<0.5	22
Soy LPC/FA (18:2) equimolar	27.8	65	33
Soy LPC/FA (10:0) equimolar	26.2	9	32
SLP80/FA (18:2) equimolar	28.8	51	44
SLP80/FA (10:0) equimolar	27.3	37	35
SLP50/MG (18:2)=4:6 (w/w)	28.2	37	42
SLP50/MG (10:0)=4:6 (w/w)	27.6	7	37

[a] Ryoto Ester S-1670, Mitsubishi Kasei Syokuhin K.K. (Tokyo, Japan).
[b] ADK COL EC-4500 (Aerosol-OT type surfactant), Asahi Denka Kogyo K.K. (Tokyo, Japan).
[c] ADK TOL NP-700 (Triton X-100 type surfactant), Asahi Denka Kogyo K.K. (Tokyo, Japan).
[d] SLP 80, Soy phospholipids containing lysophospholipids 80% (w/w).
[e] SLP 50, Soy phospholipids containing lysophospholipids 50% (w/w).

were a little larger than the critical micellar concentration of soy LPC, 1.8×10^{-5} M, 9.5×10^{-4} % (w/v) at 35°C.

Effect of mixtures of lysophospholipid/MGs on surface activity. When the partially deacylated phospholipids, such as soy LPC and soy mono- and diacylphospholipid, are blended with MGs at a ratio of 6:4 (w/w), the wettability of the mixtures change. The mixture with MG (18:0) had a longer canvas disk wetting time than the polyunsaturated FA MG (Table 3). When the canvas disk wetting time of mixtures of SLPs and different MGs at various ratios were examined, the mixture with MG (10:0) or MG (8:0) showed a high level of surface activity (data not shown). The surface tension is much lower with mixtures of MGs having higher iodine values, and mixtures of SLP/medium chain MGs also displayed much higher activity (Table 4).

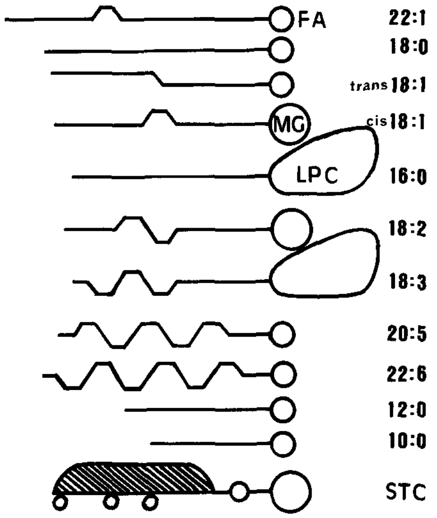

FIG. 7. Schemes of lipid chains in their most extended forms, the lipids of which were used in the present study.

DISCUSSION

Lindheimer *et al.* (24) reported that the oleic acid micellar solubility in the presence of taurocholate, PC, and LPC or monoolein was strongly decreased by PC and monoolein, but was increased by LPC. It is also known that bile salts solubilize FA, MG, PC, and LPC into micellar solutions (19,25–27), but the role of lysophospholipid (LPC) in the solubilization remains unclear. From Lindheimer's (24) and our results, it is clear that the solubility of FAs in a micelle increases with the presence of lysophospholipid (LPC) and MG. It is also known that the self diffusion coefficients of the mixture (bile salt/MG/FA/phospholipid) containing PC was significantly lower than those observed in the presence of LPC (24). However, until now, there has been no report of detailed interpretations of surface activities shown by aqueous solutions of lipid products digested by lipase and phospholipase A-2.

From a comparison of the surface activity of the three types of lipid mixtures and commercial surfactants, in 0.5% (w/w) aqueous solution at 25°C (Table 4), and from Table 2, it may be understood that these lipid mixtures should be useful as safe and effective biosurfactants for foods, drugs, cosmetics, etc. The values of surface tension (under 25 dynes/cm) and contact angle on bee's wax (<30 degrees) are not shown by ordinary industrial surfactants.

Some of the lipid mixtures showed excellent wettability and good emulsifying ability and dispersibility. Bile salt, PC, FA, and MG in an appropriate physiological ratio and concentration produce extremely small and stable emulsions of triglycerides (28). A mixture of mono- and diacylphospholipid/MG/FA/STC is more effective for emulsification of triglycerides compared to diacylphospholipid/MG/FA/STC. Thus, it is possible to prepare a mixture which is suitable for a specific purpose.

Why do these lipid mixtures exhibit a high degree of surface activity? MGs and FAs having carbon chain, > 12 are swelling lipids, and their water solubility is very low (29), thus, they show poor surface activity. Lysophospholipids and LPC exhibit rather mild surface activity. As mentioned above, the degrees of surface activity shown by the lipid mixtures are closely related to the chain length of FAs and FAs of MGs. Figure 7 shows a scheme of lipid chains that were used in this study in their most extended forms. The length of palmitic acid from C1 to C16 is 19 Å (30). The length of polyunsaturated FAs at temperatures over their melting points remains unclear because their conformations are changing constantly. However, the shortening of polyunsaturated FA is estimated as 0.75 Å per *cis*-olefinic group (31). van Soest has shown by X-ray diffraction patterns of crystalline 2-arachidonyldipalmitolyglycerol, that the arachidonyl group is gently curved in a conformation about 19 Å long. A shortenting of about 0.75 Å per *cis*-olefinic group reduces the arachidonic acid chain to 21 Å, and the slight overall curvature makes the length shorter (31). The most extended chain length of soy LPC, calculated from the results in Table 1, is 20.0 Å. The chain lengths of oleic, linoleic, linolenic, eicosapentaenoic and docosahexaenoic acids are 20.8 Å , 20.01 Å , 19.3 Å , 20.4 Å , and 22.2 Å , respectively. These FAs have nearly the same length, and they may be easily oriented together because of their curvature. Linoleic, linolenic, and eicosapentaenoic acid would be particularly easily oriented in a smooth monolayer with soy LPC. A monolayer or micelle which contains LPC could be formed more easily with MGs and/or FAs when the acyl chain length of the latter two are equal to LPC or are shorter than LPC. Moreover, because of the lower pH value of the micellar solution (around 5.0 for a lysophospholipid/MG/FA; around 6.0 for the same mixture with STC, and about 5.0 for an equimolar solution of lysophospholipid/FA), the degree of swelling of FAs comprised in the micelles is not so high. There are several studies on the micellar conformations of one and two or more components selected from lipids such as LPC, PC, bile salt, MG, and FA (14,15,17–19,25,26). The mixed lipid micelles with bile salt are rather small and their molecular weights are in the range of 15,000–30,000, whereas the micelle of egg LPC is about 95,000. When egg PC is solubilized into LPC micelles, the micellar size becomes as large as 1.5 million (17). Thus, it may be assumed that the micellar sizes in the present study are between 15,000 and 2,000,000.

With unsaturated FAs and MGs, the hydrophobic bonding forces between apolar parts of molecules will probably be decreased because of their kinking chains and bulky conformations. When a mixture consists of lysophospholipid, medium chain MG and/or medium chain FA, it is highly probable that voids in the core of the micelles reduce the forces of attraction among their apolar moieties. Thus the components of these lipid micelles may very easily be reoriented on a newly emerged surface.

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