Effect of protein, cholesterol, and phosphatidylglycerol on the surface activity of the lipid–protein complex reconstituted from pig pulmonary surfactant

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Abstract Lipid–protein complexes reconstituted from pig surfactant lipids and proteins were investigated for surface adsorption, minimum surface tension, stability index, and surface compressibility. Lipid constituents remained unchanged with procdures for the reconstitution. An apoprotein with a nominal molecular weight of 15,000 daltons significantly accelerated the lipid–protein complex to absorb the air–water interface. A 34,000-dalton apoprotein slightly modified the surface adsorption and the surface activity when it was incorporated into the lipid–protein complex formed from lipids and 15,000-dalton apoprotein. No significant surface adsorption was found in lipid vesicles even with the same lipid constituents as in the lipid–protein complex. In the lipid–protein complex prepared by changing the content of cholesterol and phosphatidylglycerol, cholesterol affected both the minimum surface tension and the surface compressibility while phosphatidylglycerol had little effect on the surface activity of the complex.—**Suzuki Y.** Effect of protein, cholesterol, and phosphatidylglycerol on the surface activity of the lipid–protein complex reconstituted from pig pulmonary surfactant. *J. Lipid Res.* 1982. **23:** 62–69.

Supplementary key words apoprotein • surface adsorption • minimum surface tension • stability index • surface compressibility

Pulmonary surfactant, essential for the normal mechanical properties of the lung as an antiatelectatic substance, is a complex of lipids and proteins (1, 2). Phospholipids, including dipalmitoylphosphatidylcholine (DPPC) (the major lipid) and cholesterol, were identified in isolated surface-active material (SAM) from alveolar wash or lung homogenates (1, 2). Significant amounts of protein were included in the isolated SAM and attempts were made to reconstitute the lipid–protein complex with DPPC or lipids from SAM and apoproteins (3–5). By changing the constituents, various lipid–protein complexes could be prepared and the action of those various components on the surface activity, in a form more nearly natural than that of lipids alone, could be predicted.

In the present study, we investigated the manner in which proteins, cholesterol, and phosphatidylglycerol (PG) affected the surface activity of the reconstituted lipid–protein complex.

MATERIALS AND METHODS

Isolation of SAM

SAM was isolated from alveolar wash (SAM-A) or homogenates (SAM-H) of fresh pig lung, according to the method described by Suzuki, Nakai, and Ohkawa (5).

Preparation of lipids and protein fractions from SAM for reconstitution studies

The precise method for the preparation of lipid and protein fractions was described elsewhere (5). Briefly, lipids were extracted from SAM-A with ethanol–ether 3:2 (v/v) at −10°C and the solvent was evaporated to dryness. The lipid residue was purified according to the method of Folch, Lees, and Sloane Stanley (6) and stored at −20°C in chloroform. Protein fraction A was extracted with 0.005 M sodium borate buffer (pH 10.0) and contained mainly a 34,000-dalton apoprotein together with albumin and globulin. Fraction B was extracted with 30 mM sodium deoxycholate solution from the precipitate after extraction of fraction A and contained mainly a 15,000-dalton apoprotein. Both protein fractions were prepared from the dried residue of SAM delipidated as described above. SAM-A was used in experiment I and

Abbreviations: SAM, surface-active material; DPPC, dipalmitoylphosphatidylcholine; PL, phospholipid; PC, phosphatidylcholine; PG, phosphatidylglycerol; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; TLC, thin-layer chromatography; NPL, neutral phospholipid.

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SAM-H was used in experiment II. Proteins were kept at 4°C until processing.

Fractionation of lipids

Crude lipids were separated into neutral lipids and phospholipids (PL) by silicic acid column chromatography. The neutral lipids were separated into triglycerides, fatty acids, and free cholesterol by thin-layer chromatography (TLC) (Kieselgel H, Merck, Darmstadt, West Germany). Free cholesterol, the major neutral lipid, was used in reconstitution experiment II. PL was further separated into neutral and acidic fractions by DEAE-cellulose acetate column chromatography, according to the method of Rooney, Canavan, and Motoyama (7). Acidic phospholipids were further fractionated into a crude PG fraction and a phosphatidylserine (PS) + phosphatidylinositol (PI) fraction by silicic acid column chromatography. They were further purified by TLC (four times) using chloroform–methanol–water 65:25:4 or chloroform–methanol–concentrated ammonium hydroxide 65:35:5. The neutral PL contained 96.1% phosphatidylcholine (PC), 3.4% phosphatidylethanolamine (PE), and 0.6% sphingomyelin (SPH) (this fraction is referred to as NPL). The isolated acidic PL fractions were stored in chloroform after treatment with 0.5 M Na_2SO_4.

Reconstitution of surface-active material

Crude lipids obtained from alveolar SAM (experiment I) or mixtures of isolated lipids (experiment II) were reconstituted into lipid–protein complexes or protein-free liposomes with or without protein fractions (5). Crude lipids contained 86.6% PC, 4.2% PS + PI, 5.7% PG, and 3.7% PE. The cholesterol–PC ratio was 0.06 (w/w). To investigate the effect of PG on surface adsorption, PG was added to one sample up to 15.6% of the total PL of crude lipids in experiment I. In experiment II, mixtures of isolated lipids were prepared by changing the relative amount of NPL, PG, PS + PI, and cholesterol. The PL–protein fraction B ratio in the starting mixtures was 19.4–23.0 in these experiments. As shown in a previous study (5), the recovery of PL and protein was maximal at these ratios.

Chemical analysis

Protein was determined according to the method of Bensadoun and Weinstein (8), and total phosphorus was measured according to the method of Bartlett (9). Each PL was separated by two-dimensional TLC after fractionation into neutral and acidic fractions by DEAE-cellulose acetate column chromatography, and then quantitated according to the method of Kahovkova and Odavic (10).

Surface adsorption and surface tension measurements

Surface adsorption rate was measured as follows. SAM-H, reconstituted lipid-protein complex, or lipid vesicles without protein, each containing 500 μg of PL, were added to 50 ml of 0.145 M NaCl buffered with 0.01 M Tris–HCl (pH 7.4) containing 0.001 M EDTA, in a Teflon beaker (diameter 5.4 cm), and constantly stirred for 5 min with a magnetic stirrer (240 rpm). After the cessation of stirring, suction was applied to the sur-

TABLE 1. Phospholipid, protein, and cholesterol content of crude lipids, lipid vesicles, reconstituted lipid–protein complexes, and surface-active material from lung homogenate

	Phospholipids					
Materials	PC	PE	PS + PI	PG	Protein/PL	Cholesterol/PC
		mol %			*w/w*	*w/w*
Original lipids[a]	86.6[b]	3.7	4.2	5.7		0.06
LIP[c]	85.5	2.4	6.3	6.2		0.07
LIP-PG[d]	79.5	4.1	3.2	13.3		0.06
LA[e]	88.8	2.5	3.5	5.2	0.01	0.06
LB[f]	87.4	2.6	4.6	5.5	0.03	0.06
LBA[g]	86.1	2.6	5.7	5.8	0.10	0.06
SAM-H[h]	86.0	4.2	7.5	2.4	0.17	0.09

[a] Crude lipids for reconstitution obtained from alveolar surface active material.
[b] Mean of two determinations.
[c] Lipid vesicle prepared by dialysis without protein from crude lipids.
[d] Lipid vesicle prepared by dialysis without protein from crude lipids with extra PG added.
[e] Lipid–protein complex prepared from crude lipids and protein fraction A.
[f] Lipid–protein complex prepared from crude lipids and protein fraction B.
[g] Lipid–protein complex prepared from LB and protein fraction A.
[h] Surface-active material from lung homogenate (purification procedure was repeated twice).

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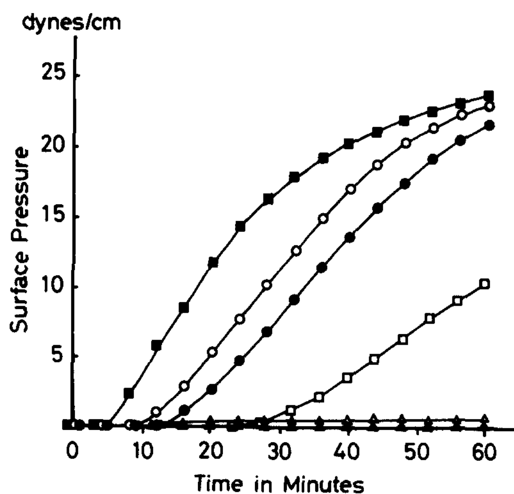

Fig. 1. Surface adsorption of surface-active material from lung homogenate (■); LA (□), prepared from lipids and protein fraction A; LB (●), prepared from lipids and protein fraction B; LBA (○), prepared from LB and protein fraction A; lipid vesicles containing low phosphatidylglycerol content (▲); and vesicles with high phosphatidylglycerol content (△). Each sample was added to the subphase solution in the amount of 10 μg of phospholipids/ml and each point represents the mean of two determinations. Surface pressure increase was measured continuously after suctioning off lipids absorbed to the surface (see text).

face to remove lipids absorbed during stirring and a platinum plate was immersed to measure the surface tension. The decrease of surface tension was recorded for 1 hr.

A surface tension-area diagram was obtained as previously reported (11) by using a saline suspension of lipid–protein complex or SAM-H. Stability index (12) and surface compressibility (S.C. = $1/A \times dA/d\gamma$) at 15 dynes/cm of the compression limb were calculated. All the above measurements were performed at 25°C.

Statistical analysis

Difference between means was statistically evaluated by using Student's t test.

RESULTS

Experiment I

The PL composition of crude lipids, reconstituted lipid–protein complexes (LA, LB and LBA), lipid vesicles, and SAM-H are shown in **Table 1.** LA is a lipid–protein complex formed from lipids and protein fraction A and it contained the 34,000- and 15,000-dalton apoproteins with a very low relative ratio of protein to PL. LB is a lipid–protein complex formed from lipids and protein fraction B and it had mainly a 15,000-dalton apoprotein. LBA is a lipid-protein complex formed from LB and protein fraction A and it had both the 15,000- and 34,000-dalton apoproteins selectively incorporated from protein fraction A. The PL composition of the lipid vesicles and the reconstituted lipid-protein complexes remained the same except when additional PG was added to the crude lipids for reconstitution (LIP-PG). Therefore, the main difference between the lipid vesicles and the lipid–protein complexes could be assessed from the standpoint of whether or not they contained protein. The difference between SAM-H and lipid-protein complexes was the content of protein, PG, and cholesterol. In SAM-H, the content of protein and cholesterol was high and that of PG was low.

The surface adsorption rates of SAM-H, LA, LB, LBA, and two types of lipid vesicles, LIP and LIP-PG, are shown in **Fig. 1.** SAM-H had the most rapid surface absorption reflected by the highest rate of increment in surface pressure; LBA and LB were next in order. The surface absorption rate of LA was about half that of SAM-H, LBA, and LB at 1 hr after initiation of the experiment. LIP and LIP-PG showed no remarkable surface adsorption. LIP-PG contained 13.3% PG in total PL and demonstrated an increase of surface pressure of 0.5 dynes/cm at 1 hr after initiation of the experiment. Protein fraction A (1.4 μg/ml) showed a surface pressure

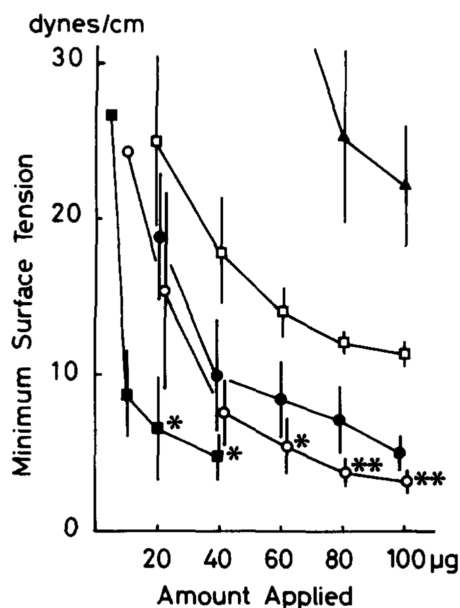

Fig. 2. Minimum surface tension of various amounts of LIP (▲), lipid vesicles without protein; LA (□), lipid-protein complex prepared from lipids and protein fraction A; LB (●), a complex prepared from lipids and protein fraction B; LBA (○), a complex prepared from LB and protein fraction A; and SAM-H (■), surface-active material from lung homogenate. Vertical bars indicate standard deviation of means and (*) and (**) show the statistically significant difference at $P < 0.05$ and $P < 0.01$, respectively. Minimum surface tension of SAM-H was significantly lower even with small amounts of PL than other preparations, and minimum surface tension of LBA was lower with large amounts of PL than that of LA and LB. For preparation of LBA, the ratio of protein fraction A to the protein in LB was 20 in the second dialyzing mixture.

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TABLE 2. Stability index and surface compressibility of lipid vesicles, reconstituted lipid–protein complexes, and surface-active material from lung homogenate

Materials[a]	Total Phospholipids Applied			
	40	60	80	100
		μg		
LIP (3)[b]				
S.I.[c]	0.37 ± 0.50[d]	0.63 ± 0.31	0.84 ± 0.14	1.03 ± 0.11
LIP-PG (3)				
S.I.	0.62 ± 0.45	0.71 ± 0.33	0.79 ± 0.29	0.86 ± 0.23
LA (4)				
S.I.	1.13 ± 0.15	1.25 ± 0.08	1.34 ± 0.04	1.37 ± 0.04
S.C.[e]			0.059 ± 0.030	0.039 ± 0.010
LB (5)				
S.I.	1.38 ± 0.19	1.44 ± 0.15	1.50 ± 0.13	1.55 ± 0.11
S.C.	0.044 ± 0.012	0.041 ± 0.014	0.052 ± 0.020	0.054 ± 0.023
LBA (7)				
S.I.	1.47 ± 0.13	1.60 ± 0.14	1.71[f] ± 0.06	1.73[f] ± 0.05
S.C.	0.041 ± 0.024	0.043 ± 0.020	0.036 ± 0.008	0.041 ± 0.013
SAM-H (4)				
S.I.	1.69[g] ± 0.10			
S.C.	0.019[h] ± 0.009			

[a] Lipid vesicles, reconstituted lipid–protein complexes and surface-active material from lung homogenate listed in Table 1.
[b] Number of determinations.
[c] Stability index calculated by the formula: S.I. = $2(\gamma max - \gamma min)/(\gamma max + \gamma min)$, (12).
[d] Mean ± S.D.
[e] Surface compressibility at 15 dynes/cm calculated by the formula: S.C. = $1/A\ (dA/d\gamma)$.
[f] Significantly different from the same amount of PL of LB at $P < 0.01$ and from LA at $P < 0.001$.
[g] Significantly different from LBA and LB at $P < 0.05$ and from LA at $P < 0.01$.
[h] Significantly different from LB at $P < 0.05$.

increase, 9 dynes/cm at 1 hr, starting from 12 min after the initiation of the experiment (data not shown).

Minimum surface tensions obtained with various amounts of lipid–protein complexes and SAM-H are shown in **Fig. 2.** SAM-H showed a low minimum surface tension even at smaller amounts (less than 10 μg of PL was necessary to obtain 10 dynes/cm) than the reconstituted lipid–protein complexes (35–40 μg of PL was required to obtain the same minimum surface tension in LB and LBA). Slightly but significantly lower minimum surface tension was obtained in LBA than in LB with large amounts of the materials. Minimum surface tension below 10 dynes/cm was not obtained even with the largest amount in LA and LIP. A relatively higher minimum surface tension was obtained in LIP-PG than in LIP (not shown in the figure).

Stability indices and surface compressibility of these materials are shown in **Table 2.** High stability index (statistically significant at $P < 0.05$) and relatively low surface compressibility (not statistically significant, $P < 0.07$) were obtained with a small amount of material in SAM-H, as compared to LBA and LB. Significantly higher stability indices were obtained in lipid–protein complexes than in lipid vesicles without protein and, especially in LBA, in which the 34,000-dalton apoprotein was incorporated into LB, stability indices were highest among the three types of lipid–protein complexes. However, the apoprotein had no effect on surface compressibility.

Experiment II

Various lipid mixtures were prepared with PL and cholesterol compositions listed in **Table 3(A).** Nine kinds of lipid–protein complexes (corresponding to LBA in experiment I) were prepared from these mixtures; they had the PL, protein, and cholesterol content shown in Table 3 (B). The lipid composition of A and B was not significantly different although a slight increase in the percentage of PC and cholesterol and a slight decrease in the percentage of PS + PI in B were observed. The amounts of protein incorporated decreased slightly according to the cholesterol content. The recovery of PL in the reconstitution of LB at the first dialyzing step, however, decreased markedly, as the content of cholesterol in the original lipid mixtures increased (70% in the absence of cholesterol, 57% at a cholesterol-PC ratio of 0.05, and 31% at a ratio of 0.20), but it did not depend on PG content. About 30% of the PL and protein in LB

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TABLE 3. Phospholipid, protein, and cholesterol content of lipids for reconstitution and reconstituted lipid–protein complexes

Material[a] No.	Phospholipids				Protein/PL	Cholesterol/PC
	PC	PE	PS + PI	PG		
		mol%			*w/w*	*w/w*
1 (A)[b]	89.7	3.2	7.0	0.1		0
(B)[c]	93.3	2.4	4.1	0.1	0.12	0
2 (A)	83.8	3.0	6.9	6.3		0
(B)	88.3	2.3	4.2	5.2	0.11	0
3 (A)	78.9	2.8	6.5	11.8		0
(B)	84.3	1.8	3.9	10.1	0.10	0
4 (A)	89.7	3.2	7.0	0.1		0.05
(B)	91.0	2.7	6.3	0	0.10	0.08
5 (A)	83.8	3.0	6.9	6.3		0.05
(B)	85.8	2.5	5.9	5.9	0.10	0.08
6 (A)	78.9	2.8	6.5	11.8		0.05
(B)	84.0	2.3	4.7	9.0	0.10	0.08
7 (A)	89.7	3.2	7.0	0.1		0.20
(B)	92.1	2.0	5.7	0.2	0.08	0.22
8 (A)	83.8	3.0	6.9	6.3		0.20
(B)	91.8	2.4	3.8	2.0	0.09	0.21
9 (A)	78.9	2.8	6.5	11.8		0.20
(B)	85.4	2.3	4.0	8.3	0.09	0.22

[a] Lipid–protein complex prepared from lipids and protein fraction A and B.
[b] Constituents of lipids for reconstitution.
[c] Constituents of reconstituted lipid–protein complex.

was lost during the second dialysis and centrifugation in the preparation of LBA, but this loss was roughly the same among all lipid–protein complexes; that is, it was irrespective of the cholesterol composition (these recovery data are not included in Table 3).

Minimum surface tension of the various lipid–protein complexes is depicted in **Fig. 3.** The cholesterol content significantly affected the minimum surface tension and 10 dynes/cm could not be obtained even at the largest amount of PL applied (i.e., 100 μg) when the cholesterol-PC ratio was 0.2. As shown in **Table 4,** stability indices decreased by addition of cholesterol at the weight ratio of 0.08 to PC; the PG content did not affect the stability indices. Among complexes with a very low PG content (0–0.2%), surface compressibility was significantly larger in the complex with a cholesterol–PC ratio of 0.2. Larger surface compressibility according to the cholesterol content was also found in other complexes with different PG content, but the differences were not statistically significant because of larger standard deviations.

DISCUSSION

The present results have clearly demonstrated that proteins in SAM are essential for rapid absorption to the air–liquid interface and for high surface activity.

We found that LB had a remarkably faster surface adsorption than did the lipid vesicles. These vesicles (LIP and LIP-PG) showed negligible surface adsorption compared to lipid–protein complexes, although one containing a high proportion of PG (LIP-PG) had a slightly faster adsorption than LIP with low PG content. Since the chemical compositions of lipids in LB and LIP were similar, it was reasonable to assume that the protein in LB, the main component of which was 15,000-dalton apoprotein, was responsible for the faster surface adsorption in LB than in LIP. Whether this was due to contamination by the 34,000-dalton apoprotein or to some structural difference of the liposomes induced by the incorporation of the 15,000-dalton apoprotein should be examined. The former possibility is unlikely since only a trace of the 34,000-dalton apoprotein was seen compared to the 15,000-dalton apoprotein, even when a higher amount of LB was analyzed by SDS-polyacrylamide gel-electrophoresis.

King and MacBeth (4) reported that a DPPC-apolipoprotein complex showed a faster adsorption to the surface than a suspension of DPPC and they used the 35,000 to 45,000-dalton apoprotein for their experiment. The surface pressure increase was remarkably different from our reconstituted lipid–protein complex. However, our results support their findings inasmuch as LBA,

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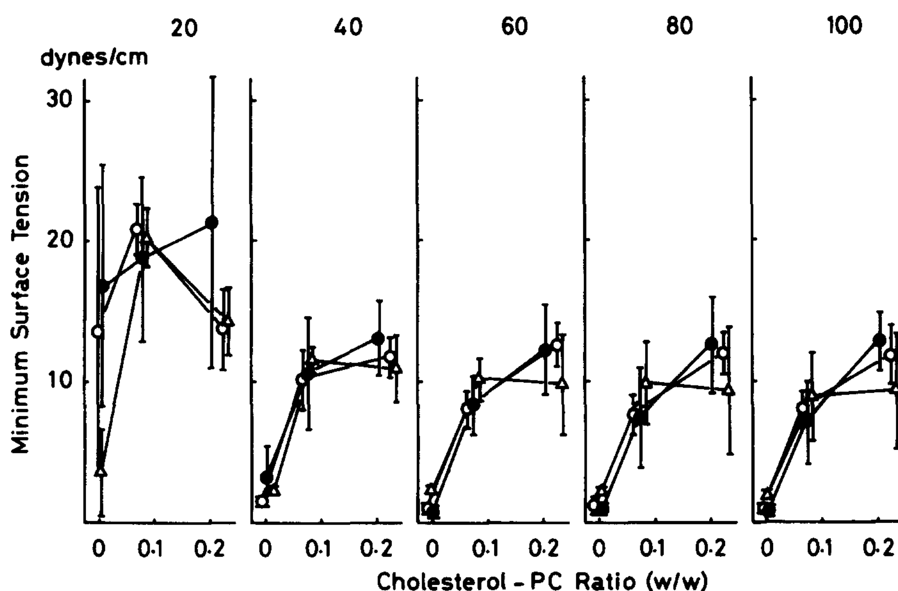

Fig. 3. Minimum surface tension of the reconstituted lipid–protein complex having the various cholesterol and phosphatidylglycerol (PG) contents listed in Table 3. PG contents were 0–0.2 mol% of total phospholipids (○); 2.0–5.9% (●); and 8.3–10.1% (△). The numbers shown above are amounts of phospholipids applied, expressed in μg. Each point represents the mean value from three to four determinations and the vertical bar indicates the standard deviation of the mean. Cholesterol contents are expressed as the ratio to phosphatidylcholine (PC) (w/w). Lipid–protein complex had both the 15,000- and 34,000-dalton apoproteins and corresponded to LBA in experiment I. For preparation of those complexes, the weight ratio of protein fraction A to the protein in the complexes obtained at the first dialysis (corresponding to LB in experiment I) was 11.5 in the second dialyzing mixtures.

which contained the 34,000-dalton apoprotein, had a slightly faster surface adsorption than did LB, which contained only the smaller apoprotein. In addition to this function of acceleration of surface adsorption, the 34,000-dalton apoprotein has a more important role in the surface activity of the lipid–protein complex. Significantly lower minimum surface tension and higher stability indices were obtained in LBA than in LB. This means that the 34,000-dalton apoprotein has an important role for the complete manifestation of surface activity and it may be concluded that the lipid–protein complex having these two apoproteins in sufficient amounts is nearer to SAM with respect to the surface activity than is a complex having only one kind of apoprotein.

By altering the lipid constituents in the reconstituted lipid–protein complex, two definite conclusions were reached. Cholesterol modifies the surface activity, i.e., increases minimum surface tension, decreases the stability index, and increases surface compressibility; and PG does not affect these parameters significantly. The inhibitory action of cholesterol has been reported (13, 14) and the action of cholesterol related to enlargement of the hysteresis curves was also reported by Tabak and Notter (15). The action of PG in SAM has not yet been clarified, although it is suspected to modify the surface activity (16), to regulate surfactant PC (17), or to stabilize the surfactant (18). In our experimental approach, PG exerted no remarkable effects on the surface activity. With respect to surface adsorption, LIP-PG showed a very small but measurable surface pressure increase compared to LIP. Thus, PG may accelerate surface absorption at much higher concentrations than those examined.

In a previous study (11), we reported the change in lipid constituents of rat surfactant induced by the administration of 4-aminopyrazolo(3,4d)pyrimidine. The changes included an increase in cholesterol content and a decrease in PC and PG content in isolated SAM. In light of our present results, the elevated minimum surface tension and larger surface compressibility found in the SAM from treated rats are probably due to the elevated cholesterol content and not to the decreased level of PG.

Cholesterol significantly affected surface activity but there was a discrepancy in the effect of cholesterol when reconstituted lipid–protein complex and SAM-H were compared; that is, the minimum surface tension was significantly lower despite the relatively high content of cholesterol in SAM-H than in the lipid–protein complex with a lower cholesterol content. In the reconstituted lipid–protein complex, it was necessary to use lipids from which cholesterol had been removed in order to obtain low minimum surface tension and small surface compressibility comparable to SAM-H. It is probable that in SAM-H some mechanism may be operating by which cholesterol does not interfere with the surface activity;

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TABLE 4. Stability index and surface compressibility of reconstituted lipid–protein complexes prepared from various lipid mixtures

Material[a] No.	Chol/PC		Total Phospholipids Applied 40	60	80	100
	w/w		*μg*			
1	0	S.I.[b]	1.89 ± 0.03[c]	1.91 ± 0.01	1.91 ± 0.05	1.93 ± 0.03
		S.C.[d]	0.018 ± 0.004	0.018 ± 0.004	0.017 ± 0.004	0.019 ± 0.005
2	0	S.I.	1.78 ± 0.15	1.91 ± 0.04	1.92 ± 0.02	1.94 ± 0.02
		S.C.	0.015 ± 0.002	0.016 ± 0.001	0.017 ± 0.003	0.017 ± 0.002
3	0	S.I.	1.86 ± 0.02	1.86 ± 0.02	1.85 ± 0	1.87 ± 0.02
		S.C.	0.014 ± 0.002	0.016 ± 0.003	0.016 ± 0.004	0.016 ± 0.004
4	0.08	S.I.	1.40 ± 0.10	1.45 ± 0.11	1.51 ± 0.07	1.46[e]
		S.C.	0.035 ± 0.010	0.027 ± 0.007	0.025 ± 0.003	0.025
5	0.08	S.I.	1.33 ± 0.18	1.38 ± 0.14	1.41 ± 0.10	1.47 ± 0.09
		S.C.	0.025	0.036 ± 0.021	0.031 ± 0.011	0.032 ± 0.014
6	0.08	S.I.	1.21 ± 0.13	1.32 ± 0.10	1.34 ± 0.14	1.42 ± 0.17
		S.C.	0.028 ± 0.007	0.039 ± 0.022	0.038 ± 0.014	0.032 ± 0.012
7	0.22	S.I.	1.35 ± 0.07	1.33 ± 0.06	1.33 ± 0.07	1.33 ± 0.09
		S.C.	0.064 ± 0.005	0.080 ± 0.012	0.078 ± 0.013	0.100 ± 0.037
8	0.21	S.I.	1.26 ± 0.11	1.27 ± 0.12	1.24 ± 0.09	1.25 ± 0.09
		S.C.	0.071	0.088	0.139	0.103
9	0.22	S.I.	1.36 ± 0.11	1.42 ± 0.18	1.45 ± 0.22	1.45 ± 0.22
		S.C.	0.073 ± 0.054	0.069 ± 0.056	0.076 ± 0.070	0.087 ± 0.080

[a] Lipid–protein complex prepared from lipid mixture listed in Table 3 (A).
[b] Stability index calculated by the formula: S.I. = $2(\gamma max - \gamma min)/(\gamma max + \gamma min)$, (12).
[c] Mean ± S.D. from three to four determinations.
[d] Surface compressibility at 15 dynes/cm: S.C. = $1/A(dA/d\gamma)$.
[e] Mean of two determinations.
Irrespective of PG content, S.I. was significantly higher in cholesterol-free lipid–protein complexes but there were only a few occasions of significant differences between cholesterol-containing complexes ($P < 0.05$ at 80 μg of PL, 0–0.2% PG, and 80 and 100 μg of PL, 2–5% PG). Significant increase in S.C. was observed in complexes having a cholesterol–PC ratio of 0.21–0.22 compared to other complexes with 0–0.2% PG. In other complexes having higher contents of PG, significant differences were not found due to the large standard deviations of means among complexes with different amounts of cholesterol.

that is to say, cholesterol is not adsorpbed to the air-liquid interface. The mechanism is not clear at present but hypothetical explanations are presented.

One explanation is the difference in the protein constituents which might affect surface activity; another is that in SAM-H some heterogeneity of liposomes may be present. For example, two different kinds of liposomes may be present, one rich in surface-active lipids, such as DPPC, with proteins and the other rich in cholesterol without or with less apoprotein. Surface activity of the latter material would depend on the liposomes with apoproteins, since liposomes without apoproteins are not adsorbed to the interface but remain in the subphase. Heterogeneity of rat SAM with respect to the protein content was recently presented by Sueishi et al. (19). The above-mentioned hypotheses would have to be verified to elucidate the discrepancy of the effect of cholesterol between reconstituted lipid–protein complex and natural surfactant.

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