Surfactant protein D (SP-D) counteracts the inhibitory effect of surfactant protein A (SP-A) on phospholipid secretion by alveolar type II cells

Interaction of native SP-D with SP-A

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The surfactant proteins SP-A and SP-D were obtained from rats given intratracheal instillation of silica. SP-D was isolated from the 33000 *g* supernatant of rat bronchoalveolar lavage fluids, and we examined whether SP-D affects surfactant secretion by alveolar type II cells. Native SP-D affected neither basal secretion nor stimulated secretion by type II cells. However, native SP-D counteracted the inhibitory effect of SP-A on surfactant secretion in a concentration-dependent manner; however, SP-D failed to counteract the inhibitory effect of concanavalin A. The activity of SP-D was unaffected by inclusion of excess methyl α -mannoside. Excess native SP-D competed with ^{125}I -SP-A for high-affinity binding to type II cells. Heat treatment of SP-D and antibody against SP-D both decreased SP-D activity. Butanol extraction of native SP-D was most effective at destroying SP-D activity and attenuated the ability of the protein to compete with labelled SP-A for binding to type II cells. The butanol-soluble fraction of SP-D possessed the ability to alter the inhibitory effect of SP-A to the same extent as native SP-D. Direct binding of ^{125}I -SP-A on nitrocellulose sheets demonstrated that SP-A could bind native SP-D, but not butanol-extracted SP-D. We conclude that native SP-D alters SP-A activity in type II cells through interaction with it via SP-D-associated lipids.

INTRODUCTION

Pulmonary surfactant lines the alveolar spaces and functions to keep the alveoli from collapsing at expiration. The major protein component of the surfactant, SP-A, is a glycoprotein with molecular mass of 26–38 kDa in the rat (Kuroki *et al.*, 1988a). This protein is characterized by the striking feature of collagen-like sequences (Sano *et al.*, 1987) and a carbohydrate-binding property (Haagsman *et al.*, 1987). SP-A has been shown to function as an inhibitor of surfactant secretion by primary cultures of alveolar type II cells (Dobbs *et al.*, 1987; Rice *et al.*, 1987) and to facilitate the uptake of phospholipid liposomes by type II cells (Wright *et al.*, 1987). SP-A also interacts with surfactant-like phospholipids (King *et al.*, 1983; Ross *et al.*, 1986), specifically binds dipalmitoyl phosphatidylcholine (dipalmitoyl-PtdCho) (Kuroki & Akino, 1991) and causes phospholipid aggregation (Hawgood *et al.*, 1985). Recent studies have demonstrated that SP-A binds a cell surface receptor expressed on type II cells (Kuroki *et al.*, 1988b; Wright *et al.*, 1989).

Another hydrophilic surfactant protein, SP-D, is a glycoprotein with a monomeric molecular mass of 43 kDa which is synthesized and secreted by alveolar type II cells (Persson *et al.*, 1988, 1989). SP-D shows similarities to SP-A in its collagenous structure and in having a carbohydrate-binding property (Persson *et al.*, 1989, 1990), suggesting that SP-D may possess a function similar to that of SP-A. In the present study, we isolated SP-D and examined its effect on surfactant secretion by type II cells. We show that native SP-D can interact with, and alter the activity of, SP-A.

MATERIALS AND METHODS

Bronchoalveolar lavage and surfactant preparation

Bronchoalveolar lavage fluids were obtained from Sprague–Dawley rats given intratracheal instillation of 10 mg of silica/rat in saline 28 days before lung lavage, according to the method of Dethloff *et al.* (1986). Lungs were lavaged 10 times with 5 mM-Hepes buffer (pH 7.4) containing 150 mM-NaCl. The lavage fluids were centrifuged at 150 *g* for 10 min to remove cell debris. CaCl_2 was added to the supernatant at a final concentration of 5 mM and the whole was centrifuged at 33000 *g*_{av.} at 4 °C for 16 h. The supernatant obtained was used for the isolation of SP-D. The resulting pellet was dispersed in 5 mM-Hepes buffer (pH 7.4) containing 150 mM-NaCl and 1.64 M-NaBr for surfactant preparation by the method of Hawgood *et al.* (1985). This suspension was centrifuged at 100000 *g*_{av.} at 4 °C overnight. The pellicle obtained was resuspended in 5 mM-Hepes buffer (pH 7.4) containing 150 mM-NaCl and centrifuged at 60000 *g*_{av.} for 1 h. The surfactant pellet obtained was used for isolation of SP-A.

Isolation of SP-D and SP-A

SP-D was obtained from the supernatant of bronchoalveolar lavage fluids after centrifugation at 33000 *g*_{av.}. The method used for SP-D isolation was adapted from that described by Persson *et al.* (1990). The supernatant obtained as described above was applied to an affinity column of mannose–Sepharose 4B (Pharmacia Fine Chemicals) prepared by the method of Fornstedt & Porath (1975). The affinity column was then washed with 5 mM-Tris buffer (pH 7.4) containing 150 mM-NaCl and

Abbreviations used: SP-D, surfactant-associated protein D; SP-A, surfactant-associated protein A; DMEM, Dulbecco's modified Eagles medium; PtdCho, phosphatidylcholine; PMA, phorbol 12-myristate 13-acetate.

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1 mM- CaCl_2 . SP-D bound to the affinity matrix in the presence of Ca^{2+} was isolated by eluting with 5 mM-Tris buffer (pH 7.4) containing 150 mM-NaCl and 2 mM-EDTA. The isolated SP-D was dialysed against 5 mM-Tris buffer (pH 7.4) containing 150 mM-NaCl to remove EDTA. In some experiments the SP-D preparation was extracted with butan-1-ol.

SP-A was isolated as previously described (Kuroki *et al.*, 1988b). Surfactant was delipidated by extraction with butan-1-ol (98 ml of butanol/2 ml of surfactant suspension in water) and the lipids were separated from the protein precipitate by centrifugation. The protein was suspended in 5 mM-Tris buffer (pH 7.4) and dialysed against the same buffer. The insoluble materials were removed by centrifugation at $130\,000\ g_{av}$ at 4°C for 1 h and the supernatant was applied to the mannose-Sepharose 4B column in the presence of Ca^{2+} . SP-A was isolated by eluting with 5 mM-Tris buffer (pH 7.4) containing 2 mM-EDTA, followed by Bio-Gel A5m (Bio-Rad) column chromatography. Human SP-A was isolated in the same fashion as rat SP-A.

Secretion of PtdCho by alveolar type II cells

Alveolar type II cells were isolated from adult male Sprague-Dawley rats by tissue dissociation with elastase (Worthington Biochemical Corp.) and metrizamide density gradients (Dobbs & Mason, 1979). Type II cells were seeded at a concentration of 2×10^6 cells per 35 mm dish (Falcon) in 2 ml of Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum and [^3H]choline ($0.5\ \mu\text{Ci/ml}$) (New England Nuclear) and cultured for 20 h. The cells were washed with 10 ml of DMEM containing 1.0 mg of BSA/ml to remove non-adherent cells and unincorporated radiolabel. Next, fresh medium (without radiolabels), surfactant proteins and phorbol 12-myristate 13-acetate (PMA; $0.1\ \mu\text{M}$) were added, and incubation was carried out for 3 h. Secretion was measured using [^3H]PtdCho as a marker for surfactant and was expressed as % secretion (radioactivity in medium/radioactivity in medium plus radioactivity in cells) (Sano *et al.*, 1985). The lactate dehydrogenase activity released into the medium did not exceed 3% of the total cellular content in any experiment.

^{125}I -SP-A binding to alveolar type II cells

Rat SP-A was iodinated by the method of Bolton & Hunter (1973). In all preparations, more than 96% of the radioactivity was precipitated by treatment with 10% (w/v) trichloroacetic acid. The specific radioactivity of the ^{125}I -SP-A used ranged between 100 and 314 c.p.m./ng.

A receptor-binding study was performed as previously described (Kuroki *et al.*, 1988c). After alveolar type II cells had been cultured for 20 h, each cell monolayer was washed with 10 ml of DMEM containing 1.0 mg of BSA/ml. Next, 1.6 ml of DMEM containing 10% fetal calf serum and 0.5 or 1.0 μg of ^{125}I -SP-A/ml were added and incubated in the absence or the presence of the indicated amount of unlabelled proteins at 37°C for 5 h. The cell monolayers were washed on ice with ice-cold buffer (50 mM-Tris buffer, pH 7.4, containing 0.15 M-NaCl, 2 mM- CaCl_2 and 1 mg of BSA/ml), and the amount of ^{125}I -SP-A bound to the cells was determined using a γ -radiation counter. Values are established after subtracting background binding (binding to the dish without cells). Non-specific binding was determined as the amount of ^{125}I -SP-A bound in the presence of 50 μg of unlabelled SP-A/ml when 1 μg of ^{125}I -SP-A/ml was used. Non-specific binding was between 4.5 and 11%.

^{125}I -SP-A binding on nitrocellulose

Direct binding of SP-A to SP-D was carried out using ^{125}I -SP-A as a probe. Various amounts of SP-A and SP-D were spotted

on to 1 cm^2 of nitrocellulose sheets. Each nitrocellulose sheet was incubated with 50 mM-Tris buffer (pH 7.4) containing 0.15 M-NaCl, 2 mM- CaCl_2 and 20 mg of BSA/ml (binding buffer) for 30 min to block non-specific binding, and then further incubated with 2 μg of ^{125}I -SP-A/ml in the binding buffer at room temperature for 2 h. Next, nitrocellulose sheets were washed with 50 mM-Tris buffer (pH 7.4) containing 0.15 M-NaCl, 2 mM- CaCl_2 and 1.0 mg of BSA/ml, and the radioactivity bound to each sheet was determined. Values were established after subtracting background binding (binding to nitrocellulose sheets not spotted with proteins).

Other methods

The protein content was estimated by the method of Lowry *et al.* (1951). SDS/PAGE was performed according to the method of Laemmli (1970). The phospholipid content was determined by measuring phospholipid phosphorus (Bartlett, 1959). Antibody was prepared against purified rat SP-D by immunization in New Zealand White rabbits.

RESULTS

Effect of SP-D on surfactant secretion by type II cells

SP-D was isolated from the lavage supernatant obtained after centrifugation at $33\,000\ g_{av}$ for 16 h by mannose-Sepharose 4B column chromatography, and analysed by SDS/PAGE (Fig. 1). The result showed that SP-D had been isolated at a high purity from the soluble fraction of the lavage fluids; there appeared to be no band of SP-A in the SP-D preparation.

The effect of SP-D on [^3H]PtdCho secretion by alveolar type II cells was studied (Table 1). SP-D inhibited neither basal secretion nor PMA-stimulated secretion by type II cells. On the other hand, SP-A inhibited PMA-stimulated secretion as previously reported (Kuroki *et al.*, 1988a), while SP-D counteracted the inhibitory effect of SP-A on surfactant secretion.

The effects of varying the concentration of added SP-D in the incubation medium containing 1.0 μg of SP-A/ml and PMA are shown in Fig. 2. The addition of increasing concentrations of SP-D increased surfactant secretion, indicating that the effect of SP-D on SP-A activity was dependent upon SP-D concentration.

SP-A derived from individuals with alveolar proteinosis has previously been reported to inhibit phospholipid secretion by rat

Fig. 1. Electrophoretic analysis of SP-D isolated from the 33000 g supernatant

Proteins were analysed using a 13% polyacrylamide gel under reducing conditions and stained with Coomassie Brilliant Blue. Lane a, molecular mass standards; lane b, SP-D; lane c, SP-A.

Table 1. Rat SP-D counteracts the inhibitory effect of rat SP-A on lipid secretion by alveolar type II cells

Cells were incubated with [3 H]choline for 20 h. The cells were washed and surfactant proteins and PMA were added, and secretion of PtdCho was measured after 3 h, as described in the Materials and methods section. Results are expressed as percentage secretion of [3 H]PtdCho (means $\pm$ s.d., $n = 7$) by alveolar type II cells. * $P < 0.001$ compared with SP-A + PMA.

Additions	[3 H]PtdCho secretion (%)
None	1.2 $\pm$ 0.5
SP-D (3 μ g/ml)	1.6 $\pm$ 0.6
PMA (0.1 μ M)	14.2 $\pm$ 3.5
SP-A (1 μ g/ml) + PMA	1.3 $\pm$ 0.4
SP-D (3 μ g/ml) + PMA	12.8 $\pm$ 3.6
SP-D (3 μ g/ml) + SP-A (1 μ g/ml) + PMA	4.9 $\pm$ 0.9*

Fig. 2. Effect of various concentrations of SP-D on the inhibitory activity of SP-A on PMA-stimulated secretion

The secretion study was performed in the presence of 1 μ g of SP-A/ml, 0.1 μ M-PMA and 0–10 μ g of SP-D/ml as described in the Materials and methods section. Values are expressed as means $\pm$ s.d. ($n = 4$). * $P < 0.05$, ** $P < 0.001$ compared with SP-A + PMA.

type II cells (Dobbs *et al.*, 1987). Concanavalin A also effectively inhibited lipid secretion by type II cells (Rice & Singleton, 1988; Kuroki *et al.*, 1988c). Thus we examined whether rat SP-D alters the effects of inhibitors other than rat SP-A (Table 2). SP-D counteracted the inhibition by human SP-A of lipid secretion by type II cells. However, SP-D failed to counteract the similar inhibitory effect of concanavalin A. Even when the secretion study was performed in the presence of 0.2 M-methyl α -mannoside, SP-A was able to inhibit PMA-stimulated secretion as reported previously (Kuroki *et al.*, 1988c), and SP-D was still able to alter SP-A activity (Table 3).

These data demonstrate that SP-D can counteract the inhibitory effect of SP-A on surfactant secretion by type II cells, that the effect of SP-D is specific for SP-A, and that a lectin property against mannoside ligands does not appear to be required for SP-D activity.

SP-D competes with 125 I-SP-A for high-affinity binding to type II cells

The cell surface binding activity of SP-A is directly related to its capacity to inhibit surfactant lipid secretion by type II cells (Kuroki *et al.*, 1988c). Thus we examined whether excess SP-D

Table 2. Rat SP-D counteracts the inhibitory effect of human SP-A upon surfactant secretion but not that of concanavalin A (ConA)

Results are expressed as percentage secretion of [3 H]PtdCho (means $\pm$ s.d., $n = 4$) by alveolar type II cells. * $P < 0.05$ compared with human SP-A + PMA; †not significant when compared with ConA (1 μ g/ml) + PMA; ‡not significant when compared with ConA (10 μ g/ml) + PMA.

Additions	[3 H]PtdCho secretion (%)
None	1.7 $\pm$ 0.2
PMA (0.1 μ M)	15.2 $\pm$ 3.1
Human SP-A (1 μ g/ml) + PMA	0.9 $\pm$ 0.5
SP-D (3 μ g/ml) + human SP-A (1 μ g/ml) + PMA	4.3 $\pm$ 1.8*
ConA (1 μ g/ml) + PMA	4.9 $\pm$ 1.0
ConA (10 μ g/ml) + PMA	2.8 $\pm$ 1.8
SP-D (3 μ g/ml) + ConA (1 μ g/ml) + PMA	5.0 $\pm$ 1.1†
SP-D (3 μ g/ml) + ConA (10 μ g/ml) + PMA	3.2 $\pm$ 0.5‡

Table 3. Methyl α -mannoside does not alter the effect of SP-D on the inhibitory activity of SP-A

Methyl α -mannoside (0.2 M) was added to all incubations. Results are expressed as percentage secretion of [3 H]PtdCho (means $\pm$ s.d., $n = 3$) by alveolar type II cells. * $P < 0.02$ compared with SP-A + PMA.

Additions	[3 H]PtdCho secretion (%)
None	1.5 $\pm$ 0.1
PMA (0.1 μ M)	8.2 $\pm$ 1.0
SP-A (1 μ g/ml) + PMA	1.9 $\pm$ 0.7
SP-D (3 μ g/ml) + PMA	8.8 $\pm$ 0.9
SP-D (3 μ g/ml) + SP-A (1 μ g/ml) + PMA	3.6 $\pm$ 0.6*

competes with 125 I-SP-A for high-affinity binding to type II cells. Iodinated SP-A (1 μ g/ml) was incubated with type II cells in the absence or the presence of 5, 10 and 40 μ g of unlabelled SP-A or SP-D/ml, and the amount of labelled SP-A bound to the cells was determined. As shown in Fig. 3, SP-D was effective at competing with 125 I-SP-A for binding to type II cells. This result raised the possibilities that SP-D may bind the SP-A receptor or that the interaction of SP-D with SP-A may result in the decrease of 125 I-SP-A binding to type II cells.

Effects of modified SP-D and antibody against SP-D

The effects of modified SP-D on SP-A activity as an inhibitor of lipid secretion and the effects of anti-SP-D on SP-D activity are shown in Table 4. Heat treatment of SP-D and the addition of antibody against SP-D both decreased the inhibitory effect of SP-D on SP-A action. Butanol extraction of SP-D decreased SP-D activity most effectively. The butanol-soluble material obtained (presumably lipids) appears to possess the ability to alter SP-A activity as much as native SP-D. Next, the effect of the butanol extraction of SP-D on its ability to compete with 125 I-SP-A for type II cell binding was examined. The result showed that butanol-extracted SP-D failed to compete with labelled SP-A (Table 5). These results show that lipids associated with SP-D appear to be essential for SP-D activity.

125 I-SP-A binding to nitrocellulose sheets

Next, to examine whether SP-A binds SP-D, 125 I-SP-A binding to nitrocellulose sheets, on which various amounts of SP-A and

Fig. 3. Competition of SP-D with ^{125}I -SP-A for high-affinity binding to type II cells

Type II cells were incubated with $1\text{ }\mu\text{g}$ of ^{125}I -SP-A/ml in the presence of $0\text{--}40\text{ }\mu\text{g}$ of unlabelled SP-A (○) or SP-D (●)/ml, and the amount of SP-A bound to the cells was determined as described in the Materials and methods section. Values are expressed as percentage of labelled SP-A bound in the absence of unlabelled proteins (mean $\pm$ S.D., $n = 4$), which was $87.6 \pm 17.2\text{ ng/dish}$.

Table 4. Blocking the effect of SP-D on the inhibition of surfactant secretion by SP-A

Results are expressed as percentage secretion of [^3H]PtdCho secreted (means $\pm$ S.D., $n = 3$) by alveolar type II cells. For heat treatment, SP-D was treated at $100\text{ }^\circ\text{C}$ for 10 min. The butanol-soluble fraction of SP-D contained 2.0 nmol of phospholipid/ml. * $P < 0.02$, ** $P < 0.001$ compared with native SP-D. † $P < 0.05$ compared with control IgG + native SP-D. ‡Not significant compared with native SP-D.

Additions	[^3H]PtdCho secretion (%)
SP-A ($1\text{ }\mu\text{g/ml}$) + PMA ($0.1\text{ }\mu\text{M}$)	1.5 ± 0.1
+ Native SP-D ($3\text{ }\mu\text{g/ml}$)	3.4 ± 0.3
+ Heat-treated SP-D ($3.0\text{ }\mu\text{g/ml}$)	$2.1 \pm 0.2^*$
+ Antibody ($100\text{ }\mu\text{g/ml}$) + native SP-D	$1.5 \pm 0.3^\dagger$
+ Control IgG + native SP-D	3.1 ± 0.9
+ Butanol-extracted SP-D ($3\text{ }\mu\text{g/ml}$)	$1.0 \pm 0.1^{**}$
+ Butanol-soluble fraction of SP-D	$3.6 \pm 1.3^\ddagger$

Table 5. Butanol-extracted SP-D fails to compete with ^{125}I -SP-A for binding to type II cells

Alveolar type II cells were incubated with $0.5\text{ }\mu\text{g}$ of ^{125}I -SP-A/ml at $37\text{ }^\circ\text{C}$ for 5 h in the absence or the presence of $20\text{ }\mu\text{g}$ of unlabelled proteins/ml, and then the amount of SP-A bound to the cells was calculated after subtracting that which bound to the dish without cells. Results are expressed as means $\pm$ S.D. ($n = 3$). * $P < 0.005$ when compared with control (no excess protein).

Excess unlabelled protein	SP-A bound (ng/dish)
None	55.4 ± 10.1
SP-A	$6.0 \pm 2.8^*$
Native SP-D	$16.5 \pm 5.8^*$
Butanol-extracted SP-D	44.7 ± 19.5

SP-D were spotted, was measured. ^{125}I -SP-A bound SP-D in a manner dependent upon the amount of native SP-D, but failed to bind the butanol-extracted SP-D (Fig. 4). This result

Fig. 4. ^{125}I -SP-A binding to SP-D

^{125}I -SP-A binding to nitrocellulose sheets, in which there was $0.2\text{--}4\text{ }\mu\text{g}$ of native SP-D (■), butanol-extracted SP-D (□) or SP-A (○), was performed, and the radioactivity bound to each nitrocellulose sheet was determined as described in the Materials and methods section.

demonstrates that SP-D can interact with SP-A via lipids associated with SP-D. In other words a complex of SP-D and lipids can interact with SP-A (Fig. 4).

DISCUSSION

The hydrophilic surfactant proteins SP-A and SP-D are synthesized and secreted by alveolar type II cells. Both proteins are characterized by a collagenous structure and a carbohydrate-binding property. SP-A has recently been demonstrated to inhibit phospholipid secretion by primary cultures of alveolar type II cells (Dobbs *et al.*, 1987; Rice *et al.*, 1987), and to bind a high-affinity receptor expressed on type II cells (Kuroki *et al.*, 1988b; Wright *et al.*, 1989). Similarities between SP-A and SP-D suggested that SP-D may affect surfactant secretion. However, SP-D isolated from the soluble fraction of rat bronchoalveolar lavage fluids affected neither basal secretion nor PMA-stimulated secretion by type II cells. SP-D did however alter the inhibitory activity of SP-A on surfactant secretion in a manner dependent upon SP-D concentration. The study of ^{125}I -SP-A binding in the presence of excess unlabelled SP-D demonstrated that SP-D easily competes with labelled SP-A for receptor binding. These results correlate well with the idea that the action of SP-A upon surfactant lipid secretion by rat alveolar type II cells occurs via a cell surface receptor (Kuroki *et al.*, 1988c), although the relationship between receptor occupancy and biological activity is unclear. The action of SP-D is specific for SP-A, as SP-D failed to counteract the inhibitory effect of concanavalin A on surfactant secretion. Since SP-D has also been reported to be a carbohydrate-binding protein (Persson *et al.*, 1990), and as we utilized this property for its isolation, there was a possibility that a lectin property may be involved in the effect of SP-D on SP-A activity, e.g. binding of SP-D as a lectin to SP-A as a glycoprotein, resulting in the counteraction of the inhibitory effect of SP-A on surfactant secretion. However, SP-D activity was unaffected by inclusion of excess methyl α -mannoside. Thus a lectin property does not appear to be required for SP-D activity.

The mechanism by which SP-D alters SP-A activity in type II cells has been examined. Both heat treatment of SP-D and an antibody prepared against rat SP-D decreased SP-D activity. Heat treatment of SP-D may denature the protein, and the antibody may interfere with the interaction between SP-D and

SP-A. We also extracted SP-D with butan-1-ol, since lipids are associated with SP-D isolated from the soluble fraction of lavage fluids (Y. Kuroki & T. Akino, unpublished work). Butanol extraction was most effective at destroying SP-D activity. A butanol-soluble fraction containing a concentration of phospholipid almost equivalent to that which associates with 3 μ g of native SP-D/ml possessed the ability to counteract the inhibitory effect of SP-A on surfactant secretion as much as did native SP-D. Butanol-extracted SP-D, i.e. delipidated SP-D, also failed to compete with 125 I-SP-A binding to type II cells. These data suggest that the SP-D peptide may be involved in the action of SP-D on SP-A function, but also indicate that lipids are essential for SP-D activity. Direct binding of 125 I-SP-A on nitrocellulose sheets demonstrated that SP-A could bind a complex of SP-D and lipids, i.e. native SP-D but not delipidated SP-D. Thus it is possible to conclude that native SP-D affects SP-A action on type II cells through the interaction of native SP-D with SP-A via SP-D-associated lipids. How SP-D associates with lipids and which component of SP-D-associated lipids binds SP-A remain to be elucidated.

In the present study we used SP-A and SP-D that were isolated from rats by silica-induced lipoproteinosis, since pretreatment of rats with silica increased the yield of apoprotein 20–40-fold. SP-A derived from silica-treated rats was indistinguishable from the protein obtained from normal rats when the capacity to inhibit surfactant lipid secretion was examined (Kuroki *et al.*, 1988a). The ability of native SP-D derived from normal rats to affect the SP-A function should be examined in future, although it is difficult to isolate sufficient quantities of SP-D from normal rat lung lavage.

The amount of the SP-D in the lavage fluids is approx. 12% that of SP-A, and approx. 75% of the SP-D of rat bronchoalveolar lavage fluids is present in the supernatant after centrifugation at 33000 g_{av} for 16 h (Y. Kuroki & T. Akino, unpublished work), whereas more than 99% of SP-A is recovered in the surfactant pellet. Thus SP-D may possess functions different from those of SP-A. In the alveoli the soluble form of native SP-D may act as a regulator of SP-A-inhibited surfactant secretion. The action of native SP-D on SP-A activity and interactions between these surfactant-associated proteins may be very important in the physiological sense. Our data support the hypothesis that the control of surfactant secretion may be, in part, regulated by signals coming from alveolar fluids.

This research was in part supported by Grants in Aid for Scientific Research from the Ministry of Education, Japan.

REFERENCES

- Bartlett, G. R. (1959) *J. Biol. Chem.* **234**, 466–468
 Bolton, A. E. & Hunter, W. M. (1973) *Biochem. J.* **133**, 529–539
 Dethloff, L. A., Gilmore, L. B., Brody, A. R. & Hook, G. E. R. (1986) *Biochem. J.* **233**, 111–118
 Dobbs, L. G. & Mason, R. J. (1979) *J. Clin. Invest.* **63**, 378–387
 Dobbs, L. G., Wright, J. R., Hawgood, S., Gonzalez, R., Venstrom, K. & Nellenbogen, J. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 1010–1014
 Fornstedt, J. & Porath, J. (1975) *FEBS Lett.* **57**, 187–191
 Haagsman, H. P., Hawgood, S., Sargeant, T., Buckley, D., White, R. T., Drickamer, K. & Benson, B. J. (1987) *J. Biol. Chem.* **262**, 13877–13880
 Hawgood, S., Benson, B. & Hamilton, R. J. (1985) *Biochemistry* **24**, 184–190
 King, R. J., Carmichael, C. & Horowitz, P. M. (1983) *J. Biol. Chem.* **258**, 10672–10680
 Kuroki, Y. & Akino, T. (1991) *J. Biol. Chem.* **266**, 3068–3073
 Kuroki, Y., Mason, R. J. & Voelker, D. R. (1988a) *J. Biol. Chem.* **263**, 3388–3394
 Kuroki, Y., Mason, R. J. & Voelker, D. R. (1988b) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5566–5570
 Kuroki, Y., Mason, R. J. & Voelker, D. R. (1988c) *J. Biol. Chem.* **263**, 17596–17602
 Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
 Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
 Persson, A., Rust, K., Chang, D., Moxley, M., Longmore, W. & Crouch, E. (1988) *Biochemistry* **27**, 8576–8584
 Persson, A., Chang, D., Rust, K., Moxley, M., Longmore, W. & Crouch, E. (1989) *Biochemistry* **28**, 6361–6367
 Persson, A., Chang, D. & Crouch, E. (1990) *J. Biol. Chem.* **265**, 5755–5760
 Rice, W. R. & Singleton, F. M. (1988) *Biochim. Biophys. Acta* **958**, 205–210
 Rice, W., Ross, G. F., Singleton, F. M., Dingle, S. & Whitsett, J. A. (1987) *J. Appl. Physiol.* **63**, 692–698
 Ross, G. F., Notter, R. H., Meuth, J. & Whitsett, J. A. (1986) *J. Biol. Chem.* **261**, 14283–14291
 Sano, K., Voelker, D. R. & Mason, R. J. (1985) *J. Biol. Chem.* **260**, 12725–12729
 Sano, K., Fisher, J., Mason, R. J., Kuroki, Y., Schilling, J., Benson, B. & Voelker, D. (1987) *Biochem. Biophys. Res. Commun.* **144**, 1010–1014
 Wright, J. R., Wager, R. E., Hawgood, S., Dobbs, L. & Clements, J. A. (1987) *J. Biol. Chem.* **262**, 2888–2894
 Wright, J. R., Borchelt, J. D. & Hawgood, S. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 5410–5414

Received 4 February 1991/22 April 1991; accepted 1 May 1991