creased up to 1-octanol, which then showed a decrease. The decrease in the solubility coefficient for 1-octanol simply may reflect that the number of "holes" in the polymer to accommodate the larger 1-octanol molecule is less than for the smaller molecular weight alcohols.

Since the permeability coefficient is a product of the diffusion coefficient and the solubility coefficient or $P = DS$, the permeability coefficients for the group of alcohols were calculated (Table IV). This simple relationship holds for the permeation process when D obeys Fick's diffusion law and S obeys Henry's law (5, 9). It is not certain with the penetrant–polymer system used in this study if in fact one or both laws are actually obeyed, particularly since it is known that penetrant–polymer interactions generally lead to variable D values. Therefore, the P values shown in Table IV should be considered as estimates of the permeability coefficients.

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Interactions of Dimethyl Sulfoxide with Lipid and Protein Monolayers

N. D. WEINER▲, M. Y. LU, and M. ROSOFF

Abstract □ Phospholipids, cholesterol, protein, and mixed phospholipid–protein monolayers were used as models to study interactions between dimethyl sulfoxide and cell membranes. Surface pressure measurements were used to study these dimethyl sulfoxide–film interactions. While films of dipalmitoyl lecithin, egg lecithin, and cholesterol showed no interaction with dimethyl sulfoxide, films of bovine serum albumin exhibited a significant condensation effect. The degree of film condensation was found to be a function of pH and dimethyl sulfoxide concentration in the subphase. It was postulated that the observed effect of dimethyl sulfoxide on albumin films is due to the loss of protein from the surface. Lipid–protein films showed only small condensation effects in the presence of dimethyl sulfoxide, indicating that the phospholipids protect the protein from attack by dimethyl sulfoxide. These studies indicate that the remarkable penetration abilities of dimethyl sulfoxide may be due to some alteration of protein structure as a result of dehydration at the biomembrane.

Keyphrases □ Lipid monolayer films—interactions with dimethyl sulfoxide, surface pressure–surface area curves □ Protein monolayer films—interactions with dimethyl sulfoxide, surface pressure–surface area curves □ Lipid–protein monolayer films—interactions with dimethyl sulfoxide, surface pressure–surface area curves □ Dimethyl sulfoxide—interactions with lipid, protein, and lipid–protein monolayer films, surface pressure–surface area curves □ Monolayers, lipid and protein—interactions with dimethyl sulfoxide, surface pressure–surface area curves

Although dimethyl sulfoxide has been reported to possess a number of desirable therapeutic properties (1–3), the potential medical hazards associated with its use (4) have limited its widespread use in humans. There have also been numerous reports concerning the ability of dimethyl sulfoxide to penetrate rapidly human skin and to enhance the percutaneous absorption of materials dissolved therein (5, 6). While the utility of dimethyl sulfoxide in promoting percutaneous absorption is well documented, few studies have appeared concerning its mechanism of action upon the barrier to absorption through the skin. While it has been suggested that dimethyl sulfoxide exerts its effect by causing a swelling or expansion of the protein fibers of the skin barrier (7), a lipid extraction effect (8) also has been implicated to account for the rapid penetration effects caused by dimethyl sulfoxide.

Monomolecular films have been widely used to elucidate interactions that occur between various substances and membrane components. The mechanisms of action of polyene antibiotics (9), anesthetics (10), air pollutants (11), and other agents have been elucidated, to some degree, at the cellular level by the use of monomolecular films.

Since the activity of dimethyl sulfoxide seems to be related to its ability to permeate cell membranes (12, 13), it is of interest to investigate the effects of dimethyl sulfoxide on monolayers of components found in biological membranes. This paper presents data on the interactions of dimethyl sulfoxide with monolayers of lipids, protein, and lipid–protein mixed films.

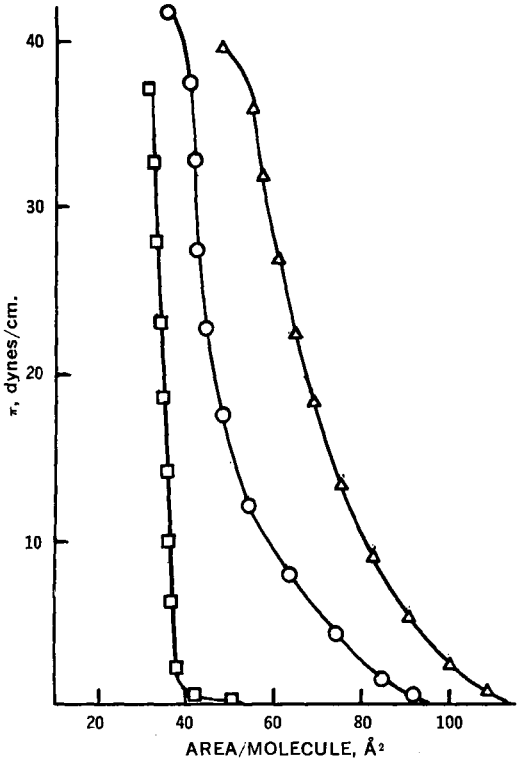

Figure 1—*Surface pressure–surface area* (π–A) *curves of dipalmitoyl lecithin* (○), *egg lecithin* (△), *and cholesterol* (□) *on an aqueous subphase.*

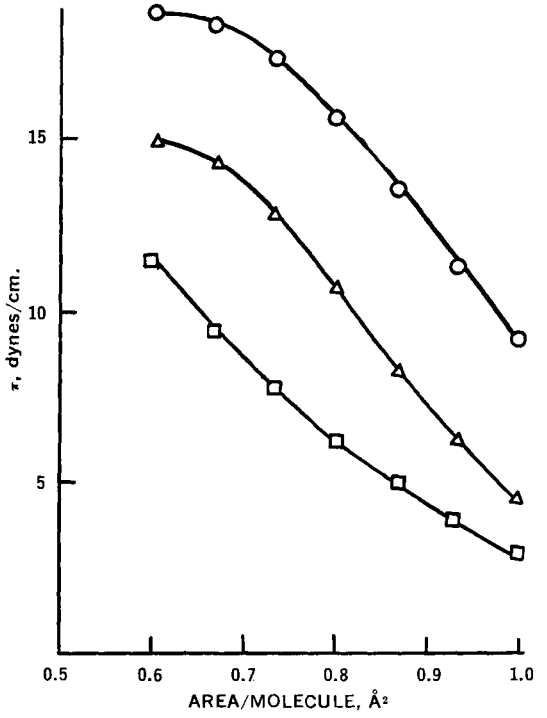

Figure 2—*Surface pressure–surface area* (π–A) *curves of bovine serum albumin on subphases at pH 2.8 containing no dimethyl sulfoxide* (○), *10% dimethyl sulfoxide* (△), *and 25% dimethyl sulfoxide* (□).

EXPERIMENTAL

Egg lecithin[1], dipalmitoyl lecithin[2], cholesterol[3], and bovine serum albumin[4] were used for the film studies. These compounds were tested for purity by TLC, using samples of at least 250 mcg. (14). Single spots were observed for all compounds tested. Dimethyl sulfoxide[5], certified spectroanalyzed grade, was purified by passage through a silica gel–alumina column.

The organic solvents used in the experiments were spectrograde and were checked for the presence of surface-active impurities by compression of the film after addition of small amounts of these solvents onto the subphase. No surface pressure was observed for any solvent used. All inorganic chemicals were of reagent grade. Water used in all experiments was deionized by being passed through a mixed resin bed of a demineralizer[6] and then distilled in an all-glass apparatus.

Solutions of lipids and proteins used in the film studies were always freshly prepared. Egg lecithin (39.8 mg./100 ml.) was dissolved in a 1:99 v/v ethanol–hexane solvent mixture. Dipalmitoyl lecithin (50 mg./100 ml.) was dissolved in a 5:95 v/v ethanol–hexane solvent mixture. Cholesterol (28.9 mg./100 ml.) was dissolved in *n*-hexane. Bovine serum albumin (45 mg./100 ml.) was dissolved in water. Generally, 0.07 ml. of each of the spreading solutions was used to prepare the films.

Before spreading the film-forming solutions, the surface was checked for cleanliness by taking surface pressure readings at the full trough area and then compressing the film to two-thirds the trough area. The readings were the same in each case, indicating the absence of insoluble surface-active impurities.

Solutions of phospholipids and cholesterol were spread on water as well as on 10 and 25% dimethyl sulfoxide in water. Spreading was accomplished with the aid of a micrometer syringe[7]. Ten minutes was permitted to pass to allow for complete evaporation of spreading solvent from the surface before manual compression of the film was initiated.

Solutions of bovine serum albumin were spread on buffer solutions of various pH values as well as on 10 and 25% dimethyl sulfoxide in the various buffer solutions. Spreading was accomplished with the aid of the micrometer syringe by the method of Trurnit (15, 16). The method consists of allowing the drops to fall at a slow, steady rate onto the hemispherical top of a clean glass rod placed in the trough. The syringe was always held directly above the top of the glass rod, which was positioned so that it could remain in place during the compression cycle. Fifteen minutes was permitted to elapse to allow the system to reach equilibrium before compression of the film was initiated.

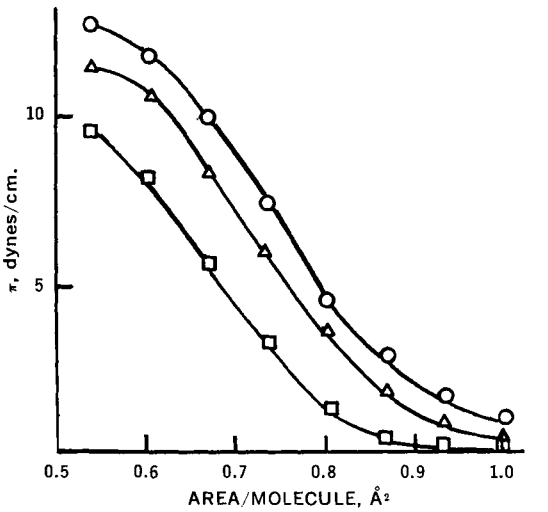

Figure 3—*Surface pressure–surface area* (π–A) *curves of bovine serum albumin on subphases at pH 4.7 containing no dimethyl sulfoxide* (○), *10% dimethyl sulfoxide* (△), *and 25% dimethyl sulfoxide* (□).

[1] Sylvana Co., Milburn, N. J.
[2] Mann Research Laboratories, New York, N. Y.
[3] Applied Science Laboratories, Inc., State College, Pa.
[4] Pierce Chemical Co., Rockford, Ill.
[5] Fisher Scientific Co., Fair Lawn, N. J.
[6] Bantam.
[7] Agla.

Table I—Apparent Percentage of Bovine Serum Albumin Lost (*PL*) from the Surface at Various pH Values in the Presence of Dimethyl Sulfoxide

Concentration of Dimethyl Sulfoxide in Subphase, %	—Apparent Percentage Protein Lost— pH 2.8	pH 4.7	pH 7.2
10	15.5	4.4	14.7
25	33.0	14.5	42.7

Mixed films of dipalmitoyl lecithin–bovine serum albumin and egg lecithin–bovine serum albumin were prepared by using the Trurnit method to spread the protein onto the previously spread phospholipid. These films were spread on a water subphase as well as on 10 and 25% dimethyl sulfoxide in water. Fifteen minutes was permitted to elapse to allow the system to reach equilibrium before compression of the film was initiated.

Solutions of lipids or protein were spread onto the subphase contained in the trough of a film balance[8]. This balance, which was described previously (17), consists essentially of a rectangular Teflon trough with a surface area of 315 × 10^{15} Å^2 and a movable Teflon barrier, which permits a manual, incremental compression of a surface film. The trough area was decreased by 5.0-cm.^{2} increments, and surface pressure readings were taken immediately after each area change. Approximately 30 sec. elapsed between each reading. The surface pressure, defined as the difference between the surface tension of the subphase and that of the film-covered subphase, was determined at different surface areas by the Wilhelmy plate method (18) using a thin, roughened, 5.0-cm. platinum plate attached to a torsion balance[9]. All experiments were carried out at room temperature (25 ± 2°).

RESULTS AND DISCUSSION

The surface pressure–surface area (π–A) curves of dipalmitoyl lecithin, egg lecithin, and cholesterol on an aqueous subphase are shown in Fig. 1. The addition of 10 and 25% dimethyl sulfoxide to the subphase resulted in π–A curves that were identical to those obtained in the absence of dimethyl sulfoxide. The lack of discernible interaction between dimethyl sulfoxide and these compounds indicates that the remarkable penetration powers of dimethyl sulfoxide into membranes is not the result of its interaction with membrane lipids. Figures 2–4 show the π–A curves of bovine serum albumin spread on aqueous subphases at pH values of 2.8, 4.7, and 7.2, respectively. In each case, π–A curves are shown for protein spread on subphases containing no dimethyl sulfoxide, 10% dimethyl sulfoxide, and 25% dimethyl sulfoxide. In all cases, it was observed that condensation of the protein films occurred in the presence of dimethyl sulfoxide in the subphase. The surface pressure decreased at any given surface area as the concentration of dimethyl sulfoxide increased in the subphase, and the apparent condensation of the protein monolayers due to the presence of dimethyl sulfoxide was considerable. For example, at pH 7.2 (Fig. 4), when A = 0.7 m.^{2}/mg., π = 8 dynes/cm. in the absence of dimethyl sulfoxide but decreases to 2.2 and 0.3 dynes/cm. when 10 and 25% dimethyl sulfoxide, respectively, are added to the subphase.

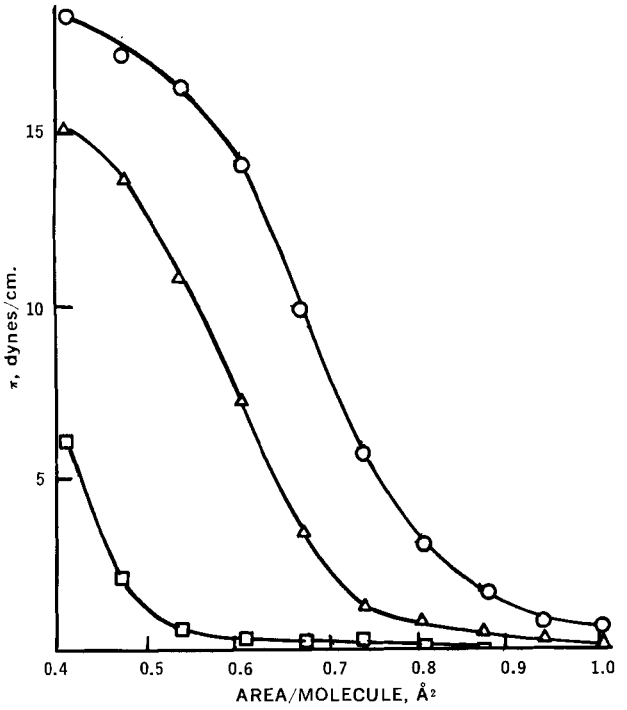

Figure 4—*Surface pressure–surface area* (π–A) *curves of bovine serum albumin on subphases at pH 7.2 containing no dimethyl sulfoxide* (○), *10% dimethyl sulfoxide* (△), *and 25% dimethyl sulfoxide* (□).

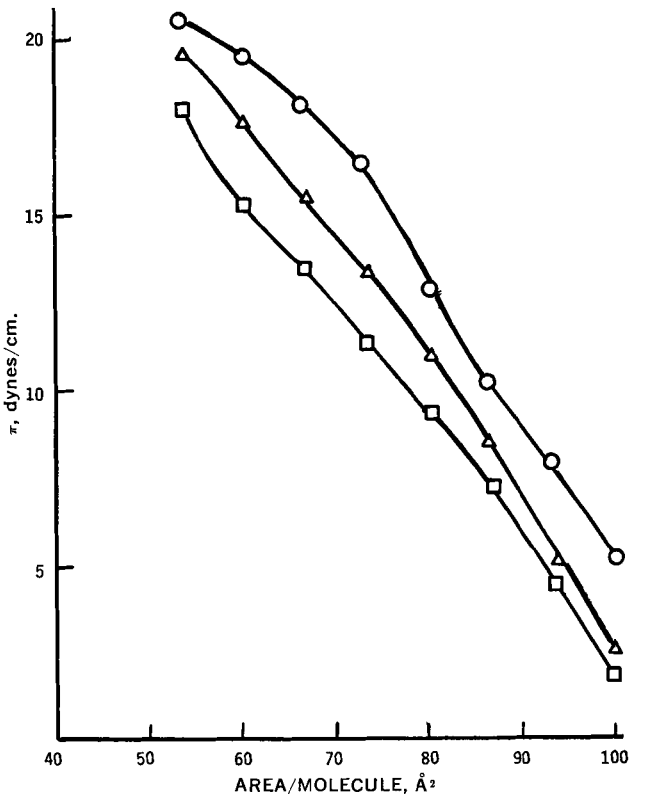

Figure 5—*Surface pressure–surface area* (π–A) *curves of mixed dipalmitoyl lecithin–albumin films on aqueous subphases containing no dimethyl sulfoxide* (○), *10% dimethyl sulfoxide* (△), *and 25% dimethyl sulfoxide* (□).

Interpretation of the results is difficult because little is known about the structure of proteins at the air–water interface. Surface pressure develops from the unfolding of the protein molecules in the surface at the air–water interface in order to reduce surface free energy. The use of the Trurnit method facilitates the process.

The overall shape of the π–A curves in the presence and absence of dimethyl sulfoxide at each pH value tested remains basically the same, and the curves are closely parallel, particularly at smaller areas. Thus, dimethyl sulfoxide simply causes displacement of the curves toward smaller areas while not appreciably affecting the film compressibility in this region. The condensation effect of the protein films produced by dimethyl sulfoxide appears to be the result of the loss of protein from the surface. It is not possible to determine from the data whether the loss in area is due to solubilization, surface precipitation, or surface aggregation.

The appearent percentage protein lost from the surface (*PL*) due to the presence of dimethyl sulfoxide can be calculated from Eq. 1:

$$PL = [(A_0 - A)/A_0]100 \qquad \text{(Eq. 1)}$$

[8] Frater Instrument Co., Corona, N. Y.
[9] Federal Pacific Electric Co., Newark, N. J.

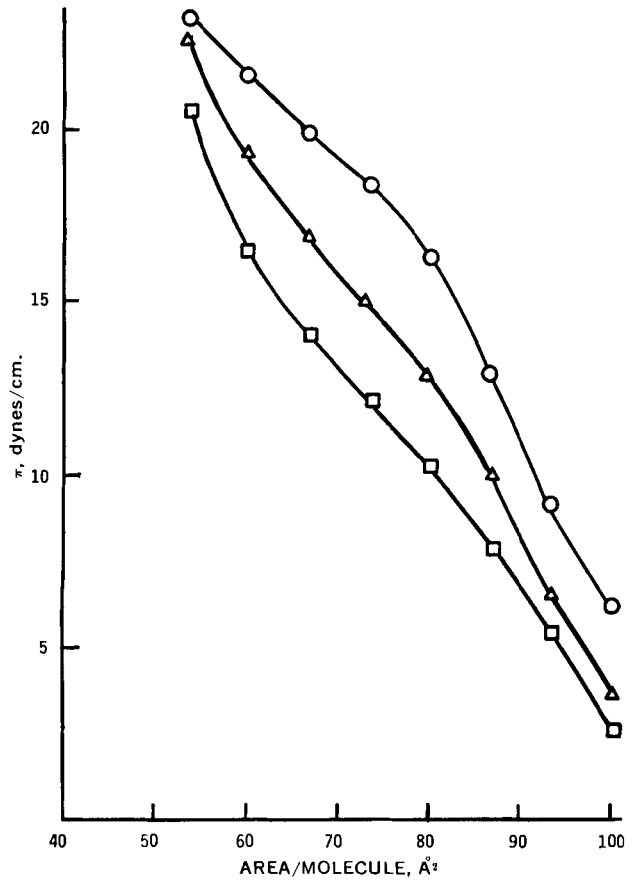

Figure 6—*Surface pressure–surface area* (π–A) *curves of mixed egg lecithin–albumin films on aqueous subphases containing no dimethyl sulfoxide* (○), *10% dimethyl sulfoxide* (△), *and 25% dimethyl sulfoxide* (□).

where A_0 and A represent the surface areas of the protein in the absence and presence of dimethyl sulfoxide, respectively, at a surface pressure of 10 dynes/cm. The value for the surface area of the protein at pH 7.2 in the presence of 25% dimethyl sulfoxide is extrapolated.

It should be pointed out that values of *PL* are not exact, since a conformational change of the protein in the presence of dimethyl sulfoxide may also affect the π–A curves. However, it is reasonable to assume that such an effect would be minimal, since dimethyl sulfoxide is not a particularly good denaturing agent. For example, dimethyl sulfoxide does not affect the α-helix of synthetic polyamino acids, and it is actually used as a solvent for perturbation experiments (19). Hamaguchi and Imahori (20) showed that very high concentrations (about 70%) of dimethyl sulfoxide are needed before this reagent induces a conformational change in bulk solutions of lysozyme.

The percentages of bovine serum albumin lost from the surface at various pH values in the presence of 10 and 25% dimethyl sulfoxide, as calculated from Eq. 1, are shown in Table I. The loss of protein from the surface was lowest at a pH value of 4.7, which is the isoelectric point of bovine serum albumin. These data indicate that although the protein–dimethyl sulfoxide interaction may be complex, the net result is a loss of protein from the surface due to competitive interactions between dimethyl sulfoxide and water with protein at the surface.

It is well known that dimethyl sulfoxide has a great affinity for water and is positively adsorbed at the air–water interface (21). At the isoelectric point, the total number of charges on the protein is a maximum (22–24), yielding the greatest degree of hydration of the film at this pH value. At pH values below and above the isoelectric point, the surface concentration of dimethyl sulfoxide would be expected to be higher due to a lesser degree of hydration of the protein film. Since the concentration of dimethyl sulfoxide in the surface may exceed the concentration for phase separation (about 35%), the protein is preferentially solubilized into the bulk phase, yielding the condensation effect observed.

The observed protein–dimethyl sulfoxide interaction at the surface is not due to a dielectric constant effect, since the presence of dimethyl sulfoxide would lower the dielectric constant of the surface which would enhance the charge repulsion of the protein, particularly at pH 2.8 where the net charge is high (25, 26). If this effect was important, the addition of dimethyl sulfoxide to the subphase would result in film expansion rather than the condensation effect observed.

Similarly, a solubilization effect by dimethyl sulfoxide *via* hydrophobic bonding does not seem to be the predominant mechanism, since the protein is more unrolled at pH 2.8 that at pH 7.2 (25, 26), and a greater degree of solubilization at this pH would be expected due to more exposure of the apolar groups at this pH. Again, this is not the case.

Since the structure of biomembranes is generally accepted to be a biomolecular leaflet of phospholipids with adsorbed protein, it was decided to utilize mixtures of lipids and protein of known composition as spread film models to study further the dimethyl sulfoxide–membrane interactions. Figures 5 and 6 show the π–A curves for the dipalmitoyl lecithin–albumin and egg lecithin–albumin films, respectively, in the absence and presence of dimethyl sulfoxide. Whereas dimethyl sulfoxide caused a condensation effect on the lipid–protein monolayers, this effect was significantly smaller than that of dimethyl sulfoxide on protein films. Therefore, it appears that the phospholipid exerts a protective action on the protein when the latter is exposed to dimethyl sulfoxide.

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