Interaction of Phloretin with Lipid Monolayers: Relationship between Structural Changes and Dipole Potential Change

Richard Cseh and Roland Benz
Lehrstuhl für Biotechnologie, Theodor-Boveri-Institut (Biozentrum) der Universität Würzburg, D-97074 Würzburg, Germany

ABSTRACT Phloretin is known to adsorb to lipid surfaces and alters the dipole potential of lipid monolayers and bilayers. Its adsorption to biological and artificial membranes results in a change of the membrane permeability for a variety of charged and neutral compounds. In this respect phloretin represents a model substance to study the effect of dipole potentials on membrane permeability. In this investigation we studied the interaction of phloretin with monolayers formed of different lipids in the liquid-expanded and the condensed state. Phloretin integrated into the monolayers as a function of the aqueous concentration of its neutral form, indicated by an increase of the surface pressure in the presence of phloretin. Simultaneous recording of the surface potential of the monolayers allowed us to correlate the degree of phloretin integration and the phloretin-induced dipole potential change. Increasing the surface pressure decreased the phloretin-induced shift of the isotherms, but did not influence the phloretin-induced surface potential change. This means that phloretin adsorption to the lipid surface can occur without affecting the lipid packing. The surface potential effect of phloretin is accompanied by a change of the lipid dipole moment vector dependent on the lipid packing. This means that the relation between the surface potential change and the lipid packing cannot be described by a static model alone. Taking into account the deviations of the surface potential change versus molecular area isotherms of the experimental data to the theoretically predicted course, we propose a model that relates the area change to the dipole moment in a dynamic manner. By using this model the experimental data can be described much better than with a static model.

INTRODUCTION

Phloretin is known as a molecule affecting the electrical properties of lipid monolayers and the permeability of membranes. Both effects are the result of its adsorption to the surfaces of lipid monolayers and bilayers where it decreases the dipole potential. This reduces the conductance for anions and increases that for cations on artificial and biological membranes (Andersen et al., 1976; Franklin and Cafiso, 1993). LeFevre and Marshall (1959) investigated the glucose transport system in the human red blood cell membrane and found that phloretin in its uncharged form powerfully inhibits glucose transport. Moreover, phloretin affects membrane transport of glycerol, urea, chloride, and a great number of further charged and neutral substances (Macey and Farmer, 1970; Owen, 1974; Gunn et al., 1975; Jennings and Solomon, 1976; Forman et al., 1982; Krupka, 1985; Toon and Solomon, 1987; Fuhrmann et al., 1992) and affects mitochondrial oxidative phosphorylation acting as an uncoupler (de Jonge et al., 1983).

The dipole potential of membranes and monolayers is caused by the uniform orientation of the dipolar lipid molecules where the ester carbonyls of the fatty acid side chains play a major role (Paltauf et al., 1971; Haydon and Myers, 1973; Pickar and Benz, 1978; Vogel and Möbius, 1988; Brockman, 1994). This confirms the role of dipole moments attached to the lipid molecules as responsible for the observed change in surface (dipole) potential of monolayers. Furthermore, the orientation of the lipids may alter the orientation of water dipoles, which contribute also to the dipole potential (Gawrisch et al., 1992). It is several hundred millivolts positive inside the membrane (Paltauf et al., 1971; Haydon and Meyers, 1973; Pickar and Benz, 1978; Brockmann, 1994). The primary effect of phloretin changing the electrical conductance and the membrane permeability of certain substances is caused by the decrease of the dipole potential of the lipid layer, which is the result of uniformly aligned phloretin dipoles in opposite direction to the lipid ones (Andersen et al., 1976; Melnik et al., 1977; Reyes et al., 1983). This means that the positive end of the adsorbed phloretin dipole is directed toward the aqueous phase and the negative one toward the hydrocarbon layer. The change of membrane conductance is then the result of an increased partition coefficient of cations in the membrane interior and a decreased partition coefficient of anions (Andersen et al., 1976). The adsorption of phloretin to the lipid layer as a function of its aqueous concentration shows saturation, which has been described by a Langmuir adsorption isotherm (De Levie et al., 1979, Reyes et al., 1983). Recently, we have shown that the effects of phloretin on lipid monolayers and bilayers can be understood on a more quantitative basis when besides the Langmuir adsorption isotherm the effect of the dipole-dipole interaction between lipid layer and phloretin is also taken into account (Cseh and Benz, 1998). Although it is widely accepted that the primary effect of phloretin is based on its lipophilicity paired with a large dipole moment (5.6 D, Andersen et al., 1976) there still exist some contradictory results for the permeation of

Received for publication 9 November 1998 and in final form 28 May 1999.
Address reprint requests to Dr. Roland Benz, Lehrstuhl für Biotechnologie, Theodor-Boveri-Institut (Biozentrum) der Universität Würzburg, D-97074 Würzburg, Germany. Tel.: +49-931/8884501; Fax: +49-931/8884509; E-mail: roland.benz@mail.uni-wuerzburg.de.

0006-3495/99/09/1477/12 $2.00

phloretin itself, the nature of its binding site(s) at the lipid layer and/or proteins, and about the active form of phloretin (Jennings and Solomon, 1976; Verkman and Solomon, 1982; Antonenko and Bulychev, 1991; Pohl et al., 1997).

In this study we investigated the effects of phloretin on structural and electrical properties of lipid monolayers: measurements of the surface pressure versus area per lipid molecule (Π-A) isotherms on a Langmuir trough permitted to determine whether the adsorption of phloretin led to its integration into the monolayers formed of different lipids. Furthermore, the phloretin integration was quantified as a function of the surface pressure by compressing/expanding the monolayer (Ibdah and Phillips, 1988; Bürner et al., 1993). We varied the pH of the aqueous phase to investigate to what extent the charged/uncharged form of phloretin affects the area per lipid molecule in a monolayer. We investigated the effect of phloretin on different saturated lipids exhibiting different phase transition behaviors on the Langmuir trough because the adsorption and integration of phloretin may differ as a function of the liquid-expanded or the liquid-condensed state of the lipids. Simultaneous measurements of the surface potential change versus area per lipid molecule ($\Delta\Psi$-A) isotherms allowed to relate the surface active property of phloretin directly with its potency to change the surface potential of the lipid monolayers.

MATERIALS AND METHODS

Materials

Diphytanoylphosphatidylcholine (DPHPC), dimyristoylphosphatidylcholine (DMPC), distearoylphosphatidylcholine (DSPC), and dipalmitoylphosphatidylcholine (DPPC) were obtained from Avanti Polar Lipids (Alabaster, AL). Phloretin was obtained from Sigma (St. Louis, MO). Chloroform and all salts were analytical grade (Merck, Darmstadt, Germany). Ultrapure water was obtained by passing deionized water through a Milli-Q filter (Millipore, Bedford, MA).

Buffers and solutions

The lipids were dissolved in chloroform (2% w/v). The subphase of the monolayers contained 0.1 M NaCl and 20 mM NaH_2PO_4 dissolved in ultrapure water. Phloretin was dissolved in 1 M NaOH and added to the subphase of the monolayers in a final concentration up to 100 μM. The pH of the subphase was adjusted to 5, 7, and 9. The experiments were performed at 22°C throughout.

Measurements of monolayer surface pressure

Monolayer experiments were performed with a commercial Lauda FW1 Langmuir trough (MGW Lauda, Lauda, Germany) providing a maximum surface area of 712 cm^2, which can be decreased by a movable barrier. The speed of the barrier was 1.7 cm/min corresponding to a decrease of the surface area of 25.5 cm^2/min or ~0.07 nm^2/lipid molecule per min, respectively. This speed was chosen to minimize hysteresis effects (Albrecht et al., 1978). Hysteresis (at maximum 2 mN/m depending on magnitude of surface pressure) was similar in experiments with and without phloretin. The surface pressure was measured with an inductive force detection unit provided with the Lauda trough. Both the surface pressure signal and the corresponding surface area signal were digitized using a 12-bit A/D-Board (Keithley Instruments, Taunton, MA) installed in a personal computer. Before each measurement the Teflon-coated trough was cleaned with acetone and rinsed with ultrapure water. After filling the trough with the subphase its surface was cleaned by moving the barrier over the surface to remove all surface-active material. The different lipids were spread on the surface with organic solvents using a Hamilton microsyringe (Hamilton, Bonaduz, Switzerland). After evaporation of the solvent and calibration of the force detection unit with a well-defined weight the lipid monolayer was compressed. The area per molecule was reduced until the monolayer collapsed or the subphase spilled over the edge of the trough. The reproducibility of the surface pressure at the same area per molecule was within $\pm$2 mN/m when the experiments were repeated under identical conditions (same amount of lipid spread on the subphase at the same temperature).

Measurements of monolayer surface potentials

Surface potential measurements of the monolayer were performed using the vibrating plate method originally introduced by Kelvin and improved by Yamins and Zisman (1933). This method has previously been described in detail (Gaines, 1966; Brockman, 1994). We used a 2-cm-diameter, gold-plated disk electrode adjusted in $<$1 mm from the air-water interface. The plate vibrated at ~416 Hz and the signal was measured with a laboratory-built lock-in amplifier (Bürner et al., 1994). The surface potential was referenced to an Ag/AgCl electrode in the water phase. The surface pressure and the surface potential were measured simultaneously using a holding device for the Kelvin apparatus that was mounted at the Langmuir trough. At the beginning of each experiment the potential of the aqueous phase was measured, then the plate was raised and the lipid was spread. After evaporation of the solvent the plate was lowered to the same distance from the interface as before spreading. The potential signal was recorded continuously during the compression/expansion phase of the lipid monolayer using a 12-bit A/D-Board (Keithley Instruments, Taunton, MA) installed in a personal computer. The change of the surface potential results from the difference between the actual potential and the reference. The reproducibility of the surface potential was within $\pm$10 mV at a given area per molecule when the experiments were repeated under identical conditions (same amount of lipid spread on the subphase at the same temperature).

RESULTS

Phloretin-induced surface pressure changes of monolayers

Molecules in monolayers can exist in different states, in analogy to three-dimensional liquids, solids, or gases. The three main states, gaseous, liquid (often described as liquid-expanded), and condensed, are well-characterized together with the phase transitions gaseous-liquid and liquid-condensed (Adam, 1938; Gaines, 1966). In a pure lipid monolayer spread on the water-air interface the phase transition depends on the temperature and the surface pressure. At a given temperature certain lipids are in liquid-expanded or condensed state throughout while compressing them on a Langmuir trough, whereas others show phase transition (Phillips and Chapman, 1968). We examined the effect of the amphiphilic molecule phloretin on lipids, which show different phase behavior on the Langmuir trough at 22°C: DMPC with a main phase transition temperature, T_m, of 23°C is in the liquid-expanded state throughout the measured isotherm, DSPC (T_m = 58°C) is in the condensed state, whereas DPPC (T_m = 42°C) shows phase transition in a temperature range from 15°C to 40°C (Albrecht et al.,

1978). In additional experiments we used the half-synthetic lipid DPHPC, which shows isotherms similar to lipids being in the liquid-expanded state. However, there does not exist general agreement in the literature about the phase condition(s) of DPHPC. Lindsey et al. (1979) have reported that DPHPC does not show any gel-to-liquid-crystalline phase transition over a temperature range from −120 to 120°C, whereas Hsieh et al. (1997) have found indications for phase transitions. Our own differential scanning calorimetry (DSC) experiments have shown that the phase transition, if there is any, must be below 0°C (Cseh, Hetzer, Bayerl, and Benz, unpublished results). Since the phase transition temperature is normally correlated with the phase state of a monolayer, we regard DPHPC as a fluid lipid under room temperature.

Amphiphilic molecules dissolved in the water phase in contact with a lipid phase tend to insert into the lipid layer. This influences the lipid packing and leads to an increased area per lipid molecule, or, respectively, changes the lateral surface pressure at constant area per lipid molecule. We determined the surface pressure (Π) as a function of the area per lipid molecule in the presence of phloretin and compared it with the corresponding surface pressure versus area per lipid (Π-A) isotherms of control experiments. Most experiments were performed at a phloretin concentration of 100 μM, but we studied also the concentration dependence (see below). Fig. 1 shows monolayer measurement with DPHPC. Curve P_0 represents the isotherm of the control monolayer spread on the buffered subphase, and curve P_{100} that of the isotherm, where 100 μM phloretin was added to the subphase. The gaseous phase of the monolayer, which shows very low surface pressures (<0.1 mN/m; Gaines, 1966), cannot be detected with our equipment and is therefore characterized by Π = 0 mN/m. Compression of the monolayer led to a phase transition to the liquid-expanded state at an area of ~1.3 nm^2 per lipid molecule (*curve* P_0), which can be noticed by an increase of Π. Curve P_{100} demonstrates that the presence of phloretin in the subphase led to a considerable shift of the Π-A isotherm. This result indicated that phloretin integrated into the DPHPC monolayer, thus increasing the area per lipid molecule at a given surface pressure. Interestingly, this effect was greater at low surface pressures. At a surface pressure of 3 mN/m the increase was ~0.3 nm^2 per lipid molecule and at 40 mN/m it was only 0.08 nm^2. This means that the interaction between phloretin and lipid molecules depended on the surface pressure of the monolayers.

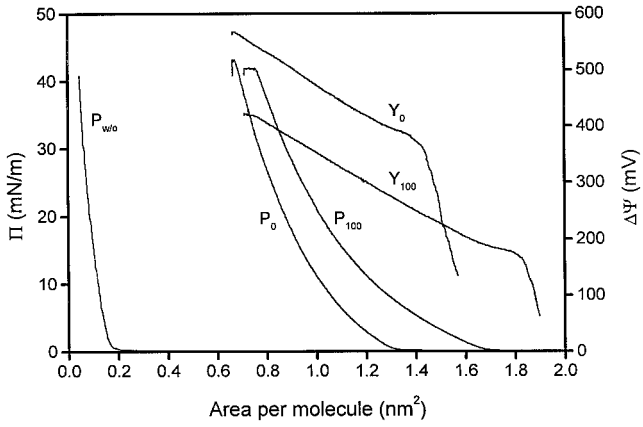

FIGURE 1 Surface pressure (Π) versus area per lipid molecule isotherms (P), and surface potential change ($\Delta\Psi$) versus area per lipid molecule isotherms (Y) of DPHPC monolayers. Indices indicate the phloretin concentration in the subphase (in μM). Curve $P_{w/o}$ represents a Π-A isotherm with 100 μM phloretin in the subphase without lipid. Note that the abscissa unit "area per lipid molecule" does not apply for curve $P_{w/o}$ (no lipid was present), but represents instead the absolute area at the Langmuir trough. The curves P_0, P_{100}, and $P_{w/o}$ are in the same ratio to the absolute area. The subphase contained 100 mM NaCl and 20 mM NaH_2PO_4, pH 7; the temperature was 22°C.

It is noteworthy that phloretin also forms monolayers at the water-air interface without lipid. Fig. 1, curve $P_{w/o}$ shows a Π-A isotherm with 100 μM phloretin in the subphase. However, the effect of phloretin on the surface pressure was noticeable only at a much smaller surface area compared with the isotherms where lipid was spread.

In the next set of experimental conditions we studied the influence of increasing concentrations of phloretin on Π-A isotherms. Fig. 2 *A* shows these isotherms of DMPC monolayers at pH 5 and at three different phloretin concentrations (*curves* P_{10}, P_{30}, and P_{100}) and the corresponding control (*curve* P_0). Phloretin integrated into the monolayer dependent on its aqueous concentration, which is indicated by a concentration dependent shift of the Π-A isotherms (Fig. 2 *A*). As already shown for DPHPC monolayers, this effect was greater at low surface pressures. At a surface pressure of 3 mN/m the increase was 0.06, 0.16, and 0.3 nm^2 per lipid molecule at phloretin concentrations of 10, 30, and 100 μM, respectively. The corresponding area increases at Π = 40 mN/m were only 0.03, 0.06, and 0.13 nm^2 per lipid molecule, respectively. This means that the interaction between phloretin and lipid molecules depended on the surface pressure of the monolayers. Similar results have been obtained for the interaction between abscisic acid and different lipids (Bürner et al., 1993) and the adsorption of apolipoprotein A-I to lipid monolayers (Ibdah and Phillips, 1988). Increasing the surface pressure of lipid monolayers obviously reduces the integration of certain surface active agents, which may be explained by the increased lipid packing. The amphiphilic molecules inserted into the lipid monolayer are "squeezed out" at higher surface pressures (Bürner et al., 1993; Heckl et al., 1987). We will adopt this term although it may be somewhat misleading, it means precisely expressed a decrease of the partition coefficient for phloretin between the aqueous phase and the lipid monolayer caused by the increased surface pressure in the monolayer (see Discussion).

Effect of pH on Π-A isotherms in the presence of phloretin

The effect on the dipole potential of monolayers and bilayers is observed only with the neutral forms of phloretin and

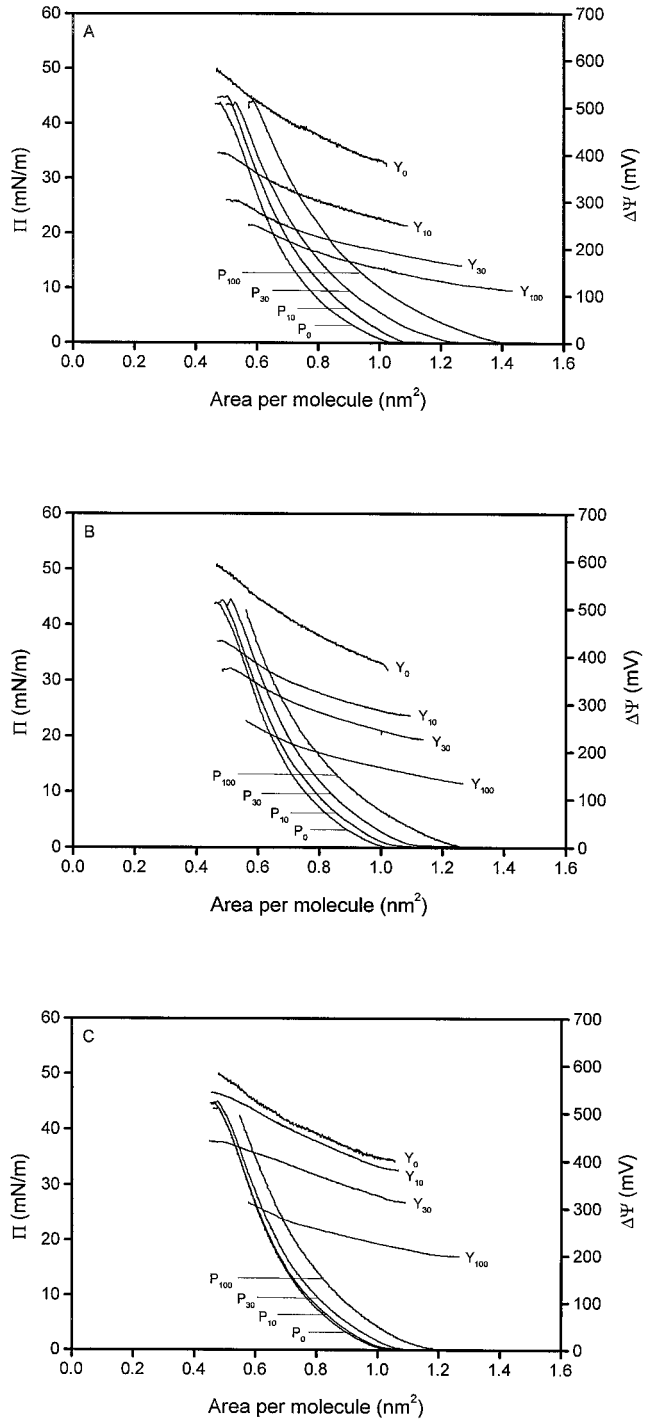

FIGURE 2 Surface pressure (Π) versus area per lipid molecule isotherms (P), and surface potential change (ΔΨ) versus area per lipid molecule isotherms (Y) of DMPC monolayers. Indices indicate the phloretin concentration in the subphase (in μM). The subphase contained 100 mM NaCl and 20 mM NaH_2PO_4 besides phloretin. The pH was 5 (*A*), 7 (*B*), and 9 (*C*); the temperature was 22°C.

its analogs (Andersen et al., 1976; Reyes et al., 1983) and only the neutral form of phloretin is able to adsorb to human red cell membranes (LeFevre and Marshall, 1959). The influence on the insertion of the charged/neutral form of phloretin into lipid monolayer was investigated within the pH range of 5 to 9. The percentage of neutral phloretin (pK_a = 7.35; Reyes et al., 1983) varies within this pH range from >99% to 2%, respectively; i.e., the charged form predominates at higher pH. Fig. 2 *B* shows the results of measurements that were performed at pH 7. The Π-A isotherms at different phloretin concentrations show some qualitative agreement with those of pH 5 (Fig. 2 *A*) but the increase of the area per lipid molecule was somewhat smaller at the different phloretin concentrations indicating that indeed its neutral form was responsible for the effect of phloretin on the Π-A isotherms. At pH 5 (Fig. 2 *A*), where almost all phloretin is in the neutral form, the greatest shift of the isotherms could be observed followed by a decrease of the molecular area increment at pH 7 (Fig. 2 *B*) and to a greater extent at pH 9 (Fig. 2 *C*), where only ~2% of the phloretin in the subphase remains undissociated. In particular, at pH 9 the Π-A isotherm at 10 μM was similar to the control.

Another indication that indeed the neutral form of phloretin is the active component was obtained when we kept the concentration of the neutral form in the subphase constant at different pH. At phloretin concentrations of 2 μM at pH 5 and 100 μM at pH 9 the concentration of the neutral form is ~2 μM. In both cases we found virtually identical Π-A and ΔΨ-A isotherms (data not shown). However, the pH-dependent shift of the isotherms was not always linearly correlated with the concentration of neutral phloretin in the subphase. This means that an increase of its concentration by a factor of ~50 (decreasing the pH from 9 to 5 increases the fraction of neutral phloretin from 2% to 99%) does not shift the isotherms by a similar factor (Fig. 2). This is caused by saturation effects similar to those previously observed for the adsorption of phloretin to monolayers and bilayers and for surface potential measurements at high phloretin concentration in the aqueous phase (LeFevre and Marshall, 1959; Andersen et al., 1976; Reyes et al. 1983; Cseh and Benz, 1998). It is noteworthy that we did not observe any significant pH dependence (within the given pH range from 5 to 9) for the reference isotherms (compare Fig. 2, *A–C*).

Effect of phloretin on the phase transition of monolayers

We also studied the influence of phloretin on the phase transition of lipid monolayers. For this we used lipids, which undergo phase transitions during compression on the Langmuir trough. Pure DPPC exhibits phase transition at a surface pressure of ~8 mN/m (Fig. 3, *A* and *B, curves* P_0) under the experimental conditions described above. In the presence of 100 μM phloretin in the subphase at pH 5 the surface pressure at the phase transition increased to ~25 mN/m (Fig. 3 *A, curve* P_{100}). Similar to the results with DPHPC and DMPC, we also found a considerable increase of the area per lipid molecule at a given surface pressure as

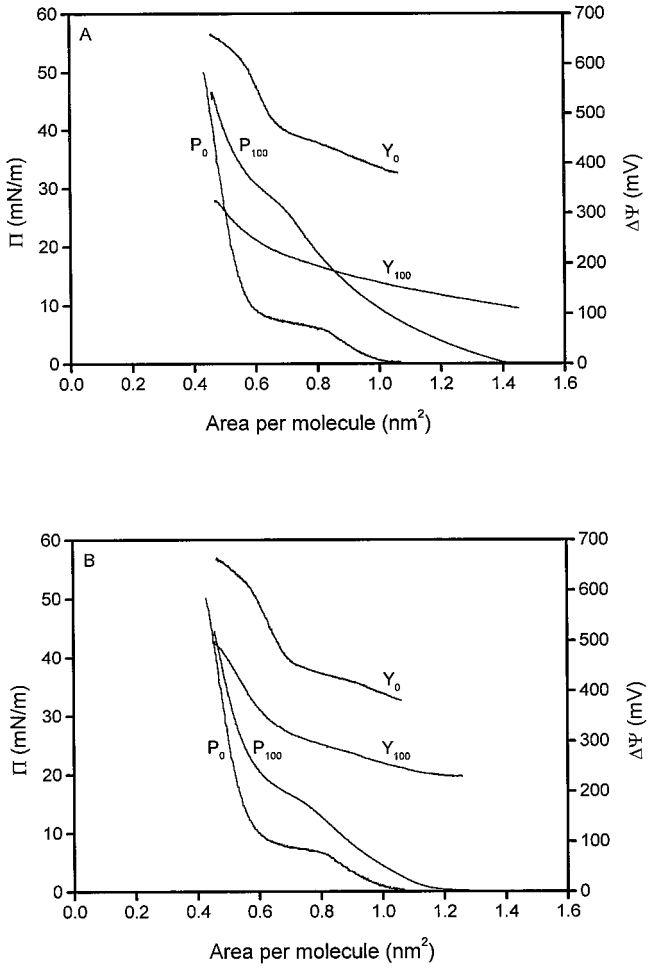

FIGURE 3 Surface pressure (Π) versus area per lipid molecule isotherms (P), and surface potential change (ΔΨ) versus area per lipid molecule isotherms (Y) of DPPC monolayers. Indices indicate the phloretin concentration in the subphase (in μM). The subphase contained 100 mM NaCl and 20 mM NaH_2PO_4 besides phloretin. The pH was 5 (*A*) and 9 (*B*); the temperature was 22°C.

compared with the reference isotherm. This applied also to the condensed state of the monolayers (above ~30 mN/m); however, the increase was much smaller in this state than in the liquid-expanded state.

In additional experiments we studied the effect of pH on the phase transition in the presence of phloretin. Fig. 3 *B* shows the Π-A isotherms for DPPC with and without 100 μM phloretin at pH 9. It is noteworthy that we found a similar pH dependence as compared with fluid lipids; i.e., the lower the pH, the larger the increase per lipid molecule (Fig. 3, *A* and *B, curves* P_{100}). The amount of phloretin that integrated into the monolayer obviously increased with increasing aqueous concentration of neutral phloretin in the subphase (see Discussion). The Π-A isotherms of lipid monolayers taken at different temperatures (Phillips and Chapman, 1968; Albrecht et al., 1978; Blume, 1979) show some similarities to those observed here for DPPC in the presence of phloretin at the two different pH values (Fig. 3, *A* and *B, curves* P_{100}). Although we kept the temperature constant in our experiments, decreasing pH, which corresponded to increasing concentration of neutral phloretin in the subphase, increased the surface pressure at which phase transition occurred. This means that the phase state of the lipid monolayers was influenced by the integration of phloretin into them. As a consequence the phase transition temperature changed.

The experiments with DSPC monolayers confirmed the results gained with DMPC and DPPC: phloretin led to a pH-dependent shift of the Π-A isotherms. Monolayers from pure DSPC do not show a liquid-expanded state at a temperature of 22°C. Independent from the area per lipid molecule or the surface pressure, the gaseous phase was immediately followed by the condensed state of the monolayer (Fig. 4, *A* and *B, curves* P_0), indicated by a strong increase of the surface pressure starting with an area of ~0.6 nm^2 per molecule. Interestingly, in the presence of 100 μM phloretin in the subphase we found at pH 5 (Fig. 4 *A, curve* P_{100}) and

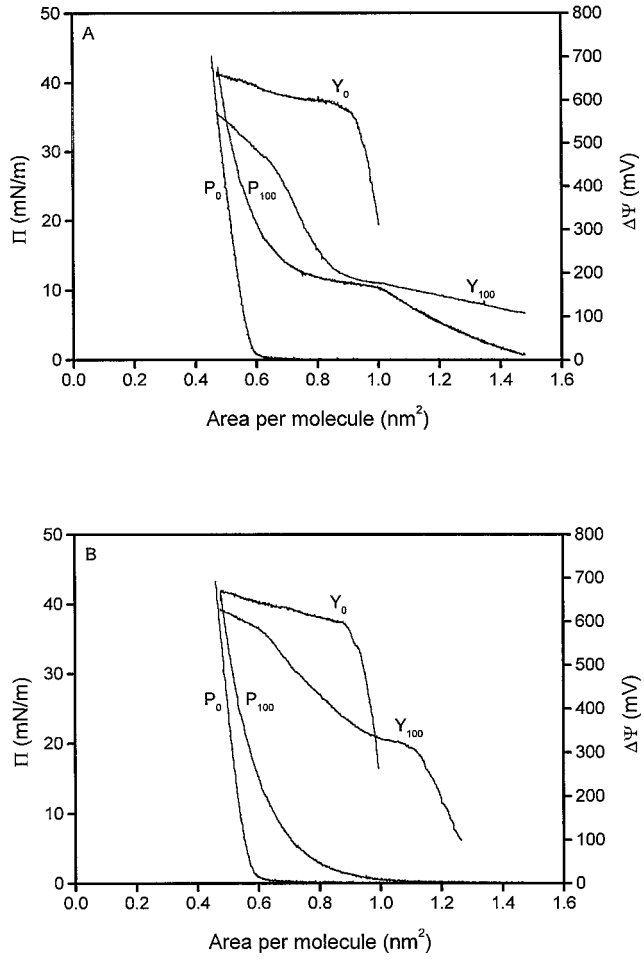

FIGURE 4 Surface pressure (Π) versus area per lipid molecule isotherms (P), and surface potential change (ΔΨ) versus area per lipid molecule isotherms (Y) of DSPC monolayers. Indices indicate the phloretin concentration in the subphase (in μM). The subphase contained 100 mM NaCl and 20 mM NaH_2PO_4 besides phloretin. The pH was 5 (*A*) and 9 (*B*); the temperature was 22°C.

pH 7 (data not shown) isotherms, which were subjected to phase transitions from the liquid-expanded to the condensed state. This represents another indication for the phloretin-induced change of phase transition temperature. At pH 9 (Fig. 4 *B, curve* P_{100}) no liquid-expanded state was observed, but the transition from the gaseous to the condensed state was smoother than in the reference isotherm. Furthermore, as shown for the other lipids, the phloretin-induced increase of the area per lipid molecule decreased with increasing surface pressure.

Phloretin-induced surface potential changes of monolayers

Detailed investigations of the dipole potential changes as a function of the phloretin adsorption have shown that phloretin decreases the dipole potential of monolayers and bilayers (Cousin and Motais, 1978; De Levie et al., 1979; Reyes et al. 1983; Cseh and Benz, 1998). In brief, the change in dipole potential, $\Delta\Psi$, of a monolayer that consists of uniformly aligned dipolar molecules is a function of their surface density, Γ:

$$\Delta\Psi = \frac{4\pi\mu N_A \Gamma \sin\Theta}{\epsilon} \quad (1)$$

where μ is the dipole moment of a single molecule, Θ the angle between the direction of the dipole moment vector and the water/lipid interface, N_A Avogadro's number, and ϵ the effective dielectric constant within the dipole plane. Equation 1 is also applicable in the case when dipole molecules such as phloretin adsorb to the monolayer and reduce the dipole potential by their alignment in the direction opposite to the lipid ones (Cseh and Benz, 1998).

We measured the change of the surface (dipole) potential, $\Delta\Psi$, during compression of the lipid monolayers in the presence and the absence of phloretin. Fig. 1, curve Y_0, shows the potential change of a DPHPC monolayer during compression, and curve Y_{100} the corresponding potential change when 100 μM phloretin was added to the subphase. Fig. 1 clearly demonstrated that the surface potential change of DPHPC monolayers is considerably reduced under the influence of 100 μM phloretin in the subphase. The point of phase transition from gaseous to liquid-expanded state of the monolayer is indicated by an abrupt change of slope of the curves Y_0 and Y_{100}. This can be attributed to the reorientation of the lipid molecules in the liquid-expanded state to a uniform upright alignment leading to an dipole angle, $\Theta > 0$ (see Eq. 1) and therefore to a surface potential change. The surface potential increased at a much smaller rate when the area per lipid molecule was below the phase transition point. This also applied to curve Y_{100} in the presence of 100 μM phloretin; however, the point of phase transition from gaseous to liquid-expanded state was, in this case, at a much larger area per lipid molecule (see Note 1 at end of text). Unless the Π-A isotherms undergo phase transition from liquid-expanded to condensed state (see below) we found similar curve shapes for all $\Delta\Psi$-A isotherms. The relation between $\Delta\Psi$ and the area per lipid molecule will be discussed in detail in the Discussion section.

It is noteworthy that the phloretin-induced shift of the $\Delta\Psi$-A isotherms (and also the Π-A isotherms, see above) are not dependent whether phloretin was added to the subphase before or after the formation of the lipid monolayer. When we added phloretin after compression of the monolayer to a certain surface pressure (data not shown), $\Delta\Psi$ (and Π) reach the same value within a few minutes as when phloretin was added before the compression. This result was independent from the surface pressure where phloretin was added. When we continued the compression of the monolayer we got isotherms similar to those shown in the figures, although the standard deviations were somewhat larger, which could be attributed to problems in reaching a homogeneous distribution of phloretin in the subphase. To prevent destruction of the monolayer we only slightly stirred the subphase after addition of phloretin.

In additional experiments we studied the concentration and the pH dependence of the effect of phloretin on the $\Delta\Psi$-A isotherms. The results are summarized in Fig. 2 *A* (pH 5), Fig. 2 *B* (pH 7), and Fig. 2 *C* (pH 9). Compared with the reference curves without phloretin that are not significantly affected by pH, we noticed some influence of pH on the phloretin-mediated decrease of $\Delta\Psi$. Increasing the concentration of phloretin resulted in a further decrease of $\Delta\Psi$. The decrease was also dependent on pH and was smallest at pH 9, where the addition of 10 μM phloretin had almost no influence on the surface potential, whereas the same concentration decreased the potential by 150 mV at pH 5. This result suggested that the effect of phloretin on the surface potential of the monolayers was indeed dependent on the concentration of the neutral form of the molecule. The comparison of Figs. 1 and 2 *B* shows that 100 μM phloretin has a greater effect on $\Delta\Psi$ of DMPC compared with that of DPHPC, although both lipids are in the same phase state and have the same headgroup. This means probably that also the hydrocarbon chains have a certain influence on the adsorption of phloretin to the lipid and on the parameters determining $\Delta\Psi$ (see Eq. 1). The distance between the headgroups is larger than that of DMPC (Lindsey et al. 1979), probably caused by the branched hydrocarbon chains of DPHPC.

Similar results were also obtained for monolayers from other lipids. Fig. 3, *A* and *B*, curves Y_0 show the $\Delta\Psi$-A isotherms of DPPC monolayers at pH 5 and pH 9, respectively. At an area of $\sim$0.6–0.8 nm^2 per lipid molecule, where phase transition between the liquid-expanded and the condensed phase occurs, these curves show a shape similar to the Π-A isotherms, which means that their courses also reflect the phase transition (Gaines, 1966). Similar curves were obtained in the experiments with 100 μM phloretin in the subphase at pH 5 and pH 9 (Fig. 3, *A* and *B, curves* Y_{100}), but the surface potential change decreased by $\sim$250

mV (pH 5) and 150 mV (pH 9) compared with the control experiments.

Surface potential measurements were also performed with monolayers from DSPC. The surface potential change of the control experiments showed a plateau already before phase transition from gaseous to condensed state (sublimation) took place (Fig. 4, *A* and *B, curves* Y_0). The area per lipid molecule where $\Delta\Psi$ reached the plateau was ~0.9 nm^2, whereas the surface pressure indicated a phase transition at ~0.6 nm^2. This result suggested that the lipid dipoles are already uniformly aligned in this range, similar to conditions in the liquid-expanded or condensed state, and therefore created an almost maximum surface potential. Again, the surface potential change was considerably smaller in the presence of 100 μM phloretin (Fig. 4, *A* and *B, curves* Y_{100}) as compared with the control experiments. The $\Delta\Psi$-A isotherms also reflected the phase transitions, which have been observed at the corresponding Π-A isotherms at pH 5 and pH 7 (data not shown). Interestingly, at pH 9 (Fig. 4 *B, curve* Y_{100}) the $\Delta\Psi$-A isotherm indicated a phase transition at a molecular area of ~0.7 to 1 nm^2 (marked by the slope change of the curve) although it was not detectable in the corresponding Π-A isotherm.

DISCUSSION

Phloretin integrates into lipid monolayers

The Π-A isotherms suggested that phloretin integrates into the lipid monolayers. So far it is not clear whether adsorption and integration were equivalent to one another, i.e., whether the effects of phloretin on the surface potential of monolayers are necessarily combined with a change of lipid packing. The term "adsorption" in its usual meaning does not distinguish between the meanings. However, in the following we define the term "integration" as a change of the lipid packing (i.e., increase of the area per lipid molecule) caused by the interaction of surface active molecules with the monolayer (wherever the exact location of the molecules within the monolayer). In contrast to this we define adsorption as the close contact of a molecule to the monolayer that does not necessarily require integration in the sense above, i.e., does not require a change of lipid packing. This differentiation will become important when we compare the effects of phloretin on the Π-A isotherms with those on the surface potential change (see below).

The results of the monolayer measurements clearly demonstrated that phloretin increased the area per lipid molecule (i.e., affected the lipid packing) but this effect is counteracted by increasing surface pressure. We proposed that the effect on the molecular area increase at higher surface pressure is due to a "squeezing out" of phloretin molecules. However, we cannot completely exclude that the intermolecular forces in the monolayer are dependent on the surface pressure under influence of phloretin. This means that phloretin molecules could remain in the monolayer and realign during compression, changing the balance between the lateral attraction/repulsion forces of the components. However, we regard such a conceivable interaction as small and negligible because of the following reasons: it has been shown that the molecules in a condensed monolayer are arranged in nearly their closest possible packing and the molecular areas correspond to that found for the appropriate projection of a molecular model (Gaines, 1966). If the amount of integrated phloretin does not change during compression of the monolayer, then we would expect an increase of the molecular areas in the condensed phase due to the additional space needed for phloretin molecules. This additional space cannot be neglected, since phloretin partitions to a high degree to the lipid layer (Verkman and Solomon, 1982). The Π-A isotherm with phloretin and the corresponding reference curve should take a parallel course in the condensed state. This was not observed because the isotherms approximate the reference at high surface pressure (they would coincide if the surface pressure could be high enough). This convergence can be attributed to a "squeezing out" of molecules (Heckl et al., 1987), thus excluding that the amount of phloretin in the monolayer is constant during compression.

Fig. 5 shows that the area change per lipid molecule under the influence of phloretin was dependent on the surface pressure at DMPC monolayers. The molecular area change decreases with increasing surface pressure, and approximates asymptotically to a minimum molecular area change, which depends on the pH, respectively, on the concentration of neutral phloretin in the subphase. This suggests that even at the highest possible surface pressure, at the collapse point, some phloretin remains in a liquid-expanded monolayer, which means that only part of the integrated phloretin is squeezed out. The integration of phloretin has also been observed in egg phosphatidylcholine

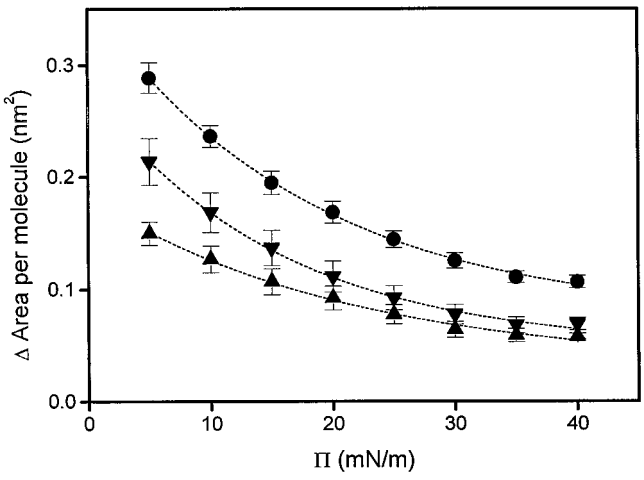

FIGURE 5 Area per lipid molecule changes versus surface pressure (Π) at DMPC monolayer in presence of 100 μM phloretin at pH 5 (●), pH 7 (▼), and pH 9 (▲). The data points correspond to the average of at least five measurements. The subphase contained 100 mM NaCl and 20 mM NaH_2PO_4 besides phloretin; the temperature was 22°C.

multilayers, where it increases the area per lipid molecule (Jendrasiak et al., 1997).

Phloretin changes the phase behavior of lipid monolayers

The Π-A isotherms in the presence of phloretin differ considerably from those of the control experiments. In particular, in the case of lipid monolayers, which exhibit phase transitions from the liquid-expanded to the condensed state or are always in the condensed state, phloretin led to dramatic changes. Fig. 3 demonstrates that the phase transition of DPPC monolayers is shifted to a considerably higher surface pressure, which suggested in an analogy to previous investigations (Phillips and Chapman, 1968; Albrecht et al., 1978; Blume, 1979) that the phase transition temperature changed. Experiments with multilamellar lipid vesicles using DSC (Cseh, Hetzer, Bayerl, and Benz, unpublished results) showed a decrease of the main phase transition temperature in the presence of phloretin, which confirmed the monolayer results. Similarly, the Π-A isotherms of pure DSPC did not show any indication for a phase transition from the liquid-expanded to the condensed state (Fig. 4). When 100 μM phloretin was added to the subphase, phase transitions occurred at pH 5, pH 7, and probably also at pH 9, as the ΔΨ-A isotherms suggested (see Fig. 4). These results agree with those obtained from monolayers from fluid lipids and imply indeed that phloretin influenced the packing of the lipid molecules in the monolayers. This means that phloretin also integrated into monolayers in the condensed state, as shown in the experiments with DSPC, but to a much smaller degree at high surface pressures, where it was squeezed out. From simple thermodynamics it can be derived that the partition coefficient for a substance soluble in two mutually nonsoluble phases changes if the pressure and therefore the chemical potential of one phase (in our case the lipid phase) changes. Our observation of a surface pressure-dependent integration of phloretin into lipid monolayers is consistent with a thermodynamic point of view. However, we have confined our view to a qualitative description, which means that a detailed theoretical model still has to be worked out.

Adsorption of phloretin to monolayers can occur without affecting lipid packing

The adsorption of phloretin to monolayers is accompanied by a decrease of ΔΨ, which is due to the addition of uniformly aligned phloretin dipoles to the lipid layer (Andersen et al., 1976; Melnik et al., 1977; Reyes et al., 1983). This is confirmed by the fact that the dipole potential change is dependent on the dipole moment of the adsorbed molecules: phloretin analogs with lower dipole moments cause smaller dipole potential changes (Reyes et al., 1983). The question arises how the effects of phloretin on the lipid packing are related to the decrease of the surface potential. The Π-A isotherms with phloretin in Fig. 3, curves P_{100} suggest a change in the lipid packing at lower surface pressures compared with the reference, whereas it is nearly unchanged at $\Pi > 40$ mN/m, i.e., the isotherms converge to the reference. This means that phloretin virtually does not affect the lipid packing at these high surface pressures. When we assume that the decrease of ΔΨ is exclusively dependent on the change of lipid packing, we would expect a similar convergence of the ΔΨ-A isotherms (Fig. 3, *curves* Y_{100}) to the corresponding references. This means that the effect of phloretin on the dipole potential should disappear at high surface pressures due to the unchanged lipid packing. Figs. 3 and 7 *B* demonstrate that this is not the case: the reduction of ΔΨ is almost independent on the lipid packing. However, for the effect of phloretin on ΔΨ at least its contact to lipid, i.e., adsorption, is required. This means its effect on the dipole potential cannot be attributed exclusively to its integration. We therefore favor a model that combines both aspects: adsorption of phloretin as the primary effect leads to a decrease of ΔΨ, but only leads to major integration when the surface pressure is sufficiently low.

On the one hand, the experiments with lipids in the liquid-expanded state (Figs. 1 and 2) agree with these considerations, even though the influence on the area increase per lipid molecule (i.e., the lipid packing) is less pronounced throughout the Π-A isotherms. On the other hand, the experiments with DSPC (Fig. 4) seem to contradict our conclusion. In this case, the ΔΨ-A isotherms tend to converge to the reference, at least the differences between ΔΨ are considerably lower at high surface pressures. However, the curves with DSPC have to be considered under the aspect of the phase transition induced under the influence of phloretin, which means that the ΔΨ-A isotherms are in a different phase state from the reference curves.

The differentiation between adsorption and integration becomes important when we consider the location of phloretin molecules at the lipid-water interface. A simple but descriptive model of the different possible locations is shown in Fig. 6. Molecule *a* is in close contact (adsorbed) to the monolayer but leaves its packing unchanged. Molecule *b* integrates into the headgroup region of the monolayer (noticeable by the additional space between the lipid molecules). It is likely that phloretin covers both aspects due to

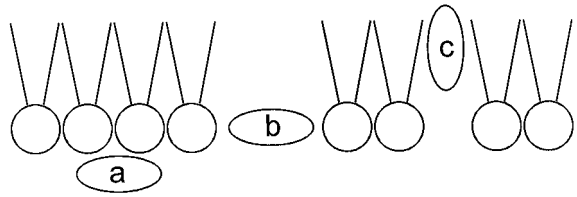

FIGURE 6 Illustration of possible interactions of adsorbed molecules with a lipid monolayer. Only the integration of molecules *b* and *c* affects lipid packing, whereas molecule *a* adsorbs but leaves the structure of the monolayer essentially unchanged.

the effects on the Π-A and ΔΨ-A isotherms discussed above. The effects of phloretin on the headgroups of the lipid (depicted in the section below) are an indication that a pure lipophilic interaction (as shown for molecule *c* in Fig. 6) is unlikely (which is also confirmed by an only weak broadening of DSC endotherms in the presence of phloretin; Cseh, Hetzer, Bayerl, and Benz, unpublished results).

As already mentioned above, phloretin does not only affect electrolyte permeability of membranes but is also a potent inhibitor of nonelectrolyte transport (Macey and Farmer, 1970; LeFevre and Marshall, 1959). According to the considerations above, phloretin should not integrate into lipid layers to a significant extent at surface pressures comparable to those of bilayers. Therefore, it is unlikely that an altered membrane structure is the reason for a changed permeability for nonelectrolytes. Andersen et al. (1976) have suggested that the inhibition of urea and glucose transport in biological membranes results from the same effect of phloretin on electrolyte transport, namely the reduction of the dipole potential. This reduction might be responsible for a changed activity of the translocators for these molecules. According to this suggestion, the modified transport properties for nonelectrolytes also seem to be primarily dependent on the electrical effects of phloretin rather than on its integration into the membrane.

Relation between surface potential change and lipid packing

Fig. 1, curves Y_0 and Y_{100} show that the slope of the ΔΨ-A isotherms changes abruptly at the phase transition from gaseous to liquid-expanded state. This can be attributed to the alignment of the lipid dipoles. Further compression of the uniformly aligned dipole molecules can be related to the change of their surface density according to Eq. 1. This predicts a linear dependence of ΔΨ on Γ, respectively, a hyperbolic dependence on the area per molecule, A ($\Gamma N_A = 1/A$, see Eq. 1), when μ, Θ, and ϵ are considered as constants. Fig. 7 *A*, curve F_0 shows a fit of ΔΨ using Eq. 1 for a pure DMPC monolayer. The deviation of the theoretical curve from the experimental data suggests that one or more of the parameters used for the calculation changes during compression of the monolayer. The dipole moment, μ, as an essential property of the lipid molecule, is generally taken as constant similar to the permittivity, ϵ (Vogel and Möbius, 1988; Cseh and Benz, 1998). However, the most sensitive parameter in the monolayer system is the dipole angle (Dill and Stigter, 1988). Even small changes can result in large modifications of the dipole moment normal to the surface plane (see Eq. 1).

Fig. 7 *A*, curve Y_0 shows that ΔΨ increases at smaller rate with decreasing molecular area than predicted when a constant dipole angle is assumed (Fig. 7 *A, curve* F_0). This result suggests a rotation of the dipole angle during the compression of the monolayer leading to a decrease of the normal dipole moment. The dipole angle, and therefore the

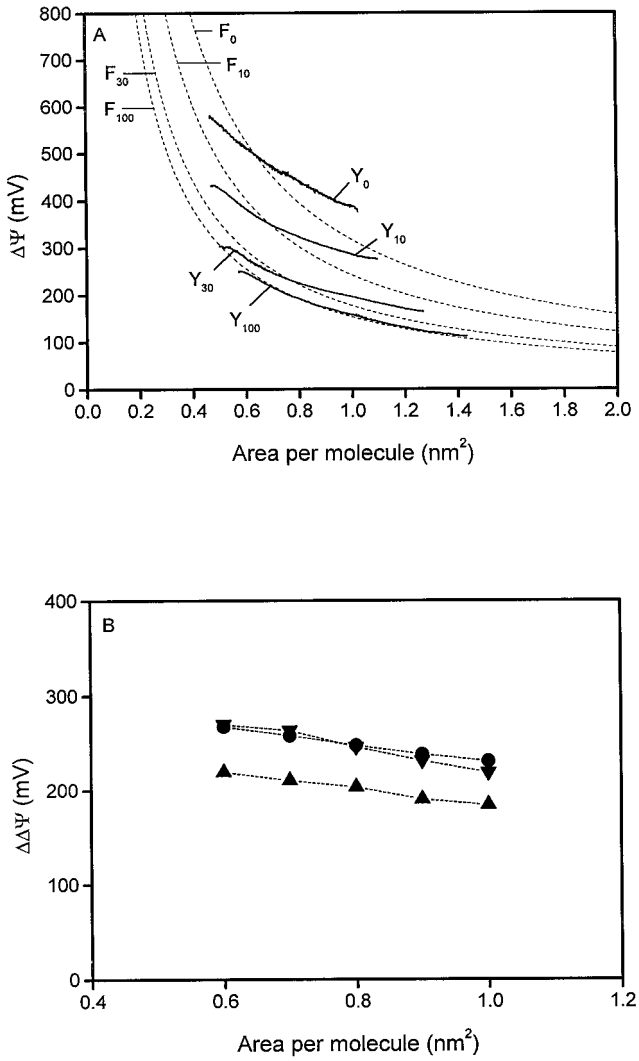

FIGURE 7 (*A*) Surface potential change (ΔΨ) versus area per lipid molecule isotherms (Y) of DMPC monolayers. Indices indicate the phloretin concentration in the subphase (in μM). The subphase contained 100 mM NaCl and 20 mM NaH_2PO_4 besides phloretin. The pH was 5; the temperature was 22°C. The dashed lines (F) represent theoretical curves that were fitted according to Eq. 1 to the experimental data. (*B*) Differences between the surface potential changes without and with 100 μM phloretin (ΔΔΨ) of DMPC monolayers versus area per lipid molecule at pH 5 (●), pH 7 (▼), and pH 9 (▲). The data points correspond to the average of at least five measurements. The subphase contained 100 mM NaCl and 20 mM NaH_2PO_4 besides phloretin; the temperature was 22°C.

normal dipole moment, appears to be dependent on the molecular area. However, the fit of the ΔΨ-A data in the presence of phloretin with Eq. 1 leads to smaller deviations at higher phloretin concentration (Fig. 7 *A, curves* Y_0–Y_{100}, F_0–F_{100}). At a phloretin concentration of 100 μM the corresponding ΔΨ-A isotherm can be well-fitted using Eq. 1 (when Θ is considered as constant). It seems that phloretin counteracts the rotation of the lipid dipoles. The higher the phloretin concentration, the smaller the variations of the ΔΨ-A isotherms from the predicted theoretical curves.

To prove this possibility we used the model proposed by Dill and Stigter (1988), which describes the orientation of

headgroups of PC-lipids by a single degree of freedom, namely the angle of the $^{-}$P-N^{+} dipole that is determined by a balance of electrostatic and hydrophobic forces. The $^{-}$P-N^{+} dipole lies in a small "backward" orientation under room temperature. This means the N^{+} end is closer to the hydrocarbon layer than the P^{-} end (Dill and Stigter, 1988; Bechinger and Seelig, 1991). According to this model, the deviations of the $\Delta\Psi$-A data of a pure lipid monolayer from the theoretical course (see above) can be explained by the rotation of the N^{+} end of the $^{-}$P-N^{+} dipole closer to the water phase while compressing the monolayer; this would result in a decrease of the dipole moment normal to the membrane plane. However, the opposite effect under influence of phloretin has been observed by Bechinger and Seelig (1991). They observed in their ^{2}H-NMR study that phloretin rotates the N^{+} end of the $^{-}$P-N^{+} dipole of PC-lipids closer to the hydrocarbon layer.

Using Eq. 1 we propose a model that relates the dipole potential change, $\Delta\Psi$, to the area per molecule, A, and also takes into account the rotation of the headgroup dipoles as a function of A. In a first step we separated the entire dipole moment of the lipid dipoles into two parts, μ_s and μ_d, where μ_s represents the static contributions of the dipole moments of the hydrocarbon chain (see Note 2), and μ_d represents a dynamic contribution, i.e., the headgroup dipole moment that may change as a function of the lipid packing:

$$\Delta\Psi = \frac{4\pi(\mu_s + \mu_d \sin\Theta)}{\epsilon A} \qquad (2)$$

To fit the experimental data to Eq. 2 we need information about the relationship between the angle of the headgroup dipole, Θ(A), and the area per lipid molecule, A, or at least the relationship between the component of the headgroup dipole moment normal to the monolayer plane, μ_n ($\mu_n = \mu_d \sin\Theta$) and A. As a first approach to this problem we plotted the product $\Delta\Psi$-A for DMPC monolayers (see Fig. 8). Interestingly, we obtained a linear relationship between $\Delta\Psi$-A and the area per lipid molecule A. This means the effect of the rotation of the headgroup dipole while compressing the monolayer can be functionally described by a linear relationship between the headgroup dipole moment, μ_d, and the area per lipid molecule, A. It is noteworthy that the slope of the curves decreases with increasing phloretin concentration. The zero slope at a 100 μM phloretin (Fig. 8, *curve* U_{100}) indicates a constant dipole angle during monolayer compression: at this concentration the tilt of the dipole angle seems to be fully compensated by the adsorption of phloretin. We found similar results by plotting $\Delta\Psi$-A for DPPC and DSPC lipids (data not shown); however, the curves showed deviations of the slopes at the phase transition of the monolayers from the liquid-expanded to the condensed state. We linearized Eq. 2 for the fit of the experimental data for $\Delta\Psi$ as a function of A obtained from DMPC monolayers:

$$\Delta\Psi = \frac{4\pi(\mu_s + \mu_d \omega A)}{\epsilon A} \qquad (3)$$

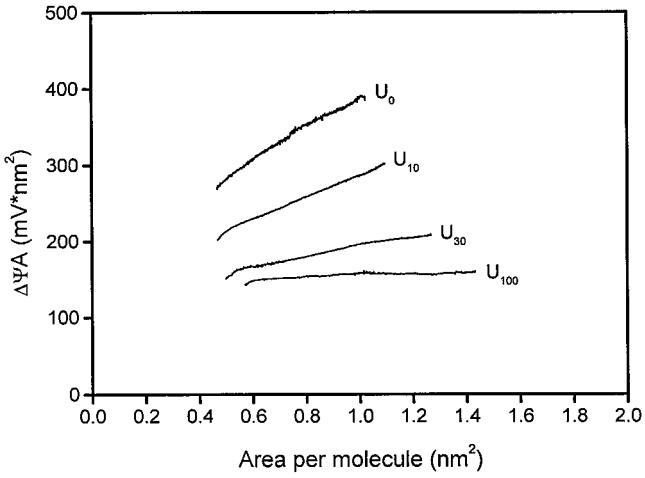

FIGURE 8 Plot of the surface potential change times area per lipid molecule ($\Delta\Psi$-A) versus area per lipid molecule (A) isotherms, (U), of DMPC monolayers. Indices indicate the phloretin concentration in the subphase (in μM). The subphase contained 100 mM NaCl and 20 mM NaH_2PO_4 besides phloretin. The pH was 5; the temperature was 22°C.

where ω represents a scaling factor (unit m^{-2}, ωA must be in the range between 0 and 1, where 0 corresponds to a dipole angle of 0° and 1 to one of 90°). The use of a linearized form of Eq. 2 has often been used to gain some insight in the variations of the dipole moments as a function of the area per lipid molecule (Bürner et al., 1994; Luckham et al., 1993; Vogel and Möbius, 1988; Pickard et al., 1979; Vilallonga, 1968).

To fit the experimental $\Delta\Psi$-A data of DMPC monolayers to Eq. 3 we chose a value of 0.5×10^{-18} m^{-2}, the values for ωA range between 0.25 and 0.75 for the corresponding molecular area between 0.5 and 1.5 nm^2 of the experimental data. The relative dielectric constant may vary between 2 (hydrocarbon region) and 20 (polar headgroups) (Coster and Smith, 1974). We assumed here a medium value of 10 for the relative dielectric constant. It should be noted that the arbitrary choice of ω and ϵ is not crucial for the purpose in this section to find a satisfactory model for the $\Delta\Psi$-A data obtained with various phloretin concentrations; it does not falsify the accuracy of the fits even if the correct values of the dipole moments depend on the correct values for ϵ and ω. Fig. 9 shows the fits of the $\Delta\Psi$-A data using Eq. 3 for DMPC monolayers at pH 5 at the different phloretin concentrations. The fits match the experimental data much better than those where the dipole angle was considered as constant (Fig. 7 *A*; see above). This result indicates that a dynamic model that takes into account an area-dependent change of the dipole moment provides a better description of $\Delta\Psi$(A) than a static one. Furthermore, it suggests that the model can also be used to describe the phloretin-induced change of the surface potential of monolayers as a function of the area per lipid molecule. The corresponding values for the static and the dynamic contribution to the dipole mo-

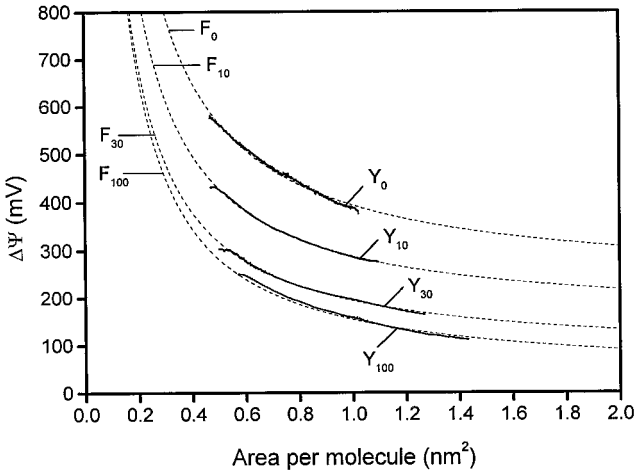

FIGURE 9 Surface potential change ($\Delta\Psi$) versus area per lipid molecule isotherms (Y) of DMPC monolayers. Indices indicate the phloretin concentration in the subphase (in μM). The subphase contained 100 mM NaCl and 20 mM NaH_2PO_4 besides phloretin. The pH was 5; the temperature was 22°C. The dashed lines (F) represent theoretical curves that were fitted according to Eq. 3 to the experimental data.

ments are shown in Table 1. It is possible that the absolute values of the dipole moments are not entirely correct because of the different assumptions involved in their calculation. However, the value for $\mu_s + \mu_d$ of a single DMPC molecule (1.29 D) is in the right order of magnitude because Vogel and Möbius (1988) estimated the dipole moment for a DPPC molecule to be 0.82 D. The values for μ_s are nearly constant, whereas μ_d decreases dramatically with increasing phloretin concentration. This result gives an interesting insight into the mechanism of the interaction of phloretin with the lipid dipoles. On the one hand, phloretin leads to the reduction of the total dipole moment ($\mu_s + \mu_d$), which confirms the model of adsorbed phloretin dipoles in an opposite direction to the lipid ones (Andersen et al., 1976; Melnik et al., 1977; Reyes et al., 1983). On the other hand, phloretin mainly reduces the dynamic part of the lipid dipole, which agrees with the finding of Bechinger and Seelig (1991) that phloretin rotates the $^{-}$P-N^{+} dipole of the lipid molecule closer to the hydrocarbon layer.

TABLE 1 Static and dynamic contribution to the dipole moment of DMPC monolayers in presence of various phloretin concentrations derived from fits of the experimental $\Delta\Psi$-A data to Eq. 3. For details refer to the text.

Phloretin concentration (μM)	μ_s^* (D)	μ_d^* (D)
0	0.36	0.93
10	0.29	0.62
30	0.26	0.3
100	0.27	0.13

*The experimental data are fitted using a scaling factor of 0.5×10^{-18} m^{-2} and a relative dielectric constant of 10 (see text).

CONCLUSION

The interaction of phloretin with lipid monolayers leads to structural changes at the water-lipid interface. This means phloretin adsorbs to the monolayer, affects lipid packing, and changes the phase transition temperature. These effects are strongly dependent on the concentration of the neutral form of phloretin in the subphase. Adsorption to and integration into the monolayer can be distinguished concerning their effects on the lipid packing. Whereas integration strongly depends on surface pressure and the physical state of the lipid, adsorption can occur without changing the lipid packing. The analysis of the surface potential data in terms of compression of the monolayer and in terms of the phloretin effect led to a model that takes into account the variation of the lipid dipole moment vector during compression of the monolayer. The model describes the experimental data better than a static model does.

NOTES

1. Since phloretin also shifts the $\Delta\Psi$-A isotherms to greater molecular areas, and with that the point of phase transition, it looks as though phloretin *increases* $\Delta\Psi$ at a molecular area > 1.5 nm^2. However, the reference isotherm is still in the gas phase at this area range, while the isotherm with phloretin is already in the liquid-expanded state.

2. μ_s covers all contributions of the normal dipole moments of the hydrocarbon chains, i.e., that of the terminal methyl groups (Vogel and Möbius, 1988) and the ester carbonyls (Paltauf et al., 1971).

This work was supported by grants from the Deutsche Forschungsgemeinschaft (Graduiertenkolleg "Magnetische Kernresonanz in vivo und in vitro für die biologische und medizinische Grundlagenforschung" and Project B7 of the Sonderforschungsbereich 176) and by the Fonds der Chemischen Industrie.

REFERENCES

Adam, N. K. 1938. The Physics and Chemistry of Surfaces. Oxford University Press, London.

Albrecht, O., H. Gruler, and E. Sackmann. 1978. Polymorphism of phospholipid monolayers. *J. Phys. (Paris)*. 39:301–313.

Andersen, O. S., A. Finkelstein, I. Katz, and A. Cass. 1976. Effect of phloretin on the permeability of thin lipid membranes. *J. Gen. Physiol.* 67:749–771.

Antonenko, Y. N., and A. A. Bulychev. 1991. Effect of phloretin on the carrier-mediated electrically silent ion fluxes through the bilayer lipid membrane: measurements of pH shifts near the membrane by pH microelectrode. *Biochim. Biophys. Acta*. 1070:474–480.

Bechinger, B., and J. Seelig. 1991. Interaction of electric dipoles with phospholipid headgroups. A ^{2}H and ^{31}P NMR study of phloretin analogues in phosphatidylcholine membranes. *Biochemistry*. 30: 3923–3929.

Blume, A. 1979. A comparative study of the phase transition of phospholipid bilayers and monolayers. *Biochim. Biophys. Acta*. 557:32–44.

Brockman, H. 1994. Dipole potential of lipid membranes. *Chem. Phys. Lipids*. 73:57–79.

Bürner, H., R. Benz, H. Gimmler, W. Hartung, and W. Stillwell. 1993. Abscisic acid-lipid interactions: a phospholipid monolayer study. *Biochim. Biophys. Acta*. 1150:165–172.

Bürner, H., M. Winterhalter, and R. Benz. 1994. Surface potential of lipid monolayers with grafted polyethylene glycols. *J. Colloid Interface Sci.* 168:183–189.

Coster, H. G. L., and J. R. Smith. 1974. The molecular organization of bimolecular lipid membranes. A study of low frequency Maxwell-Wagner impedance dispersion. *Biochim. Biophys. Acta*. 373:151–164.

Cousin, J. L., and R. Motais. 1978. Effect of phloretin on chloride permeability: a structure-activity study. *Biochim. Biophys. Acta*. 507: 531–538.

Cseh, R., and R. Benz. 1998. The adsorption of phloretin to lipid monolayers and bilayers cannot be explained by Langmuir adsorption isotherms alone. *Biophys. J.* 74:1399–1408.

De Jonge, P. C., T. Wieringa, J. P. M. van Putten, H. M. J. Krans, and K. van Dam. 1983. An uncoupler and an inhibitor of mitochondrial oxidative phosphorylation. *Biochim. Biophys. Acta*. 722:219–225.

De Levie, R., S. K. Rangarajan, P. F. Seelig, and O. S. Andersen. 1979. On the adsorption of phloretin onto a black lipid membrane. *Biophys. J.* 25:295–300.

Dill, K. A., and D. Stigter. 1988. Lateral interactions among phosphatidylcholine and phosphatidylethanolamine headgroups in phospholipid monolayers and bilayers. *Biochemistry*. 27:3446–3453.

Forman, S. A., A. S. Verkman, J. A. Dix, and A. K. Solomon. 1982. Interaction of phloretin with the anion transport protein of the red blood cell membrane. *Biochim. Biophys. Acta*. 689:531–538.

Franklin, J. C., and D. S. Cafiso. 1993. Internal electrostatic potentials in bilayers: measuring and controlling dipole potentials in lipid vesicles. *Biophys. J.* 65:289–299.

Fuhrmann, G. F., S. Dernedde, and G. Frenking. 1992. Phloretin keto-enol tautomerism and inhibition of glucose transport in human erythrocytes (including effects of phloretin on anion transport). *Biochim. Biophys. Acta*. 1110:105–111.

Gaines, G. L., 1966. Insoluble Monolayers at Liquid-Gas Interfaces. Interscience, New York.

Gawrisch, K., D. Ruston, J. Zimmerberg, V. A. Parsegian, R. P. Rand, and N. Fuller. 1992. Membrane dipole potentials, hydration forces, and the ordering of water at membrane surfaces. *Biophys. J.* 61:1213–1223.

Gunn, R. B., J. O. Wieth, and D. C. Tosteson. 1975. Some effects of low pH on chloride exchange in human red blood cells. *J. Gen. Physiol.* 65:731–749.

Haydon, D. A., and V. B. Myers. 1973. Surface charge, surface potential and membrane conductance. *Biochim. Biophys. Acta*. 307:429–443.

Heckl, W. M., B. N. Zaba, and H. Möhwald. 1987. Interactions of cytochromes b_5 and c with phospholipid monolayers. *Biochim. Biophys. Acta*. 903:166–176.

Hsieh, C.-H., S.-C. Sue, P.-C. Lyu, and W.-G. Wu. 1997. Membrane packing geometry of diphytanoylphosphatidylcholine is highly sensitive to hydration: phospholipid polymorphism induced by molecular rearrangement in the headgroup region. *Biophys. J.* 73:870–877.

Ibdah, J. A., and M. C. Phillips. 1988. Effects of lipid composition and packing on the adsorption of apolipoprotein A-I to lipid monolayers. *Biochemistry*. 27:7155–7162.

Jendrasiak, G. L., R. L. Smith, and T. J. McIntosh. 1997. The effect of phloretin on the hydration of egg phosphatidylcholine multilayers. *Biochim. Biophys. Acta*. 1329:159–168.

Jennings, M. L., and A. K. Solomon. 1976. Interaction between phloretin and the red cell membrane. *J. Gen. Physiol.* 67:381–397.

Krupka, R. M. 1985. Asymmetrical binding of phloretin to the glucose transport system of human erythrocytes. *J. Membr. Biol*. 83:71–80.

LeFevre, P. G., and J. K. Marshall. 1959. The attachment of phloretin and analogs to human erythrocytes in connection with inhibition of sugar transport. *J. Biol. Chem.* 234:3022–3026.

Lindsey, H., N. O. Petersen, and S. I. Chan. 1979. Physicochemical characterization of 1,2-diphytanoyl-sn-glycero-3-phosphocholine in model membrane systems. *Biochim. Biophys. Acta*. 555:147–167.

Luckham, P., J. Wood, S. Froggatt, and R. Swart. 1993. The surface properties of gangliosides. *J. Colloid Interface Sci.* 156:164–172.

Macey, R. J., and R. E. L. Farmer. 1970. Inhibition of water and solute permeability in human red cells. *Biochim. Biophys. Acta*. 211:104–106.

Melnik, E., R. Latorre, J. E. Hall, and D. C. Tosteson. 1977. Phloretin-induced changes in ion transport across lipid bilayer membranes. *J. Gen. Physiol.* 69:243–247.

Owen, J. D. 1974. The effect of phloretin on the potassium conductance in *Aplysia* giant neurons. *J. Membr. Biol*. 16:65–78.

Paltauf, F., H. Hauser, and M. C. Phillips. 1971. Monolayer characteristics of some 1,2-diacyl, 1-alkyl-2-acyl and 1,2 dialkyl phospholipids at the air-water interfaces. *Biochim. Biophys. Acta*. 249:539–547.

Phillips, M. C., and D. Chapman. 1968. Monolayer characteristics of saturated 1,2-diacylphosphatidyl-cholines (lecithins) and phosphatidylethanolamines at the air-water interface. *Biochim. Biophys. Acta*. 163:301–313.

Pickar, A. D., and R. Benz. 1978. Transport of oppositely charged lipophilic probe ions in lipid bilayer membranes having various structures. *J. Membr. Biol.* 44:353–376.

Pickard, W. F., K. C. Sehgal, and C. M. Jackson. 1979. Measurement of phospholipid monolayer surface potentials at a hydrocarbon-electrolyte interface. *Biochim. Biophys. Acta*. 552:1–10.

Pohl, P., T. I. Rokitskaya, E. E. Pohl, and S. M. Saparov. 1997. Permeation of phloretin across bilayer lipid membranes monitored by dipole potential and microelectrode measurements. *Biochim. Biophys. Acta*. 1323: 163–172.

Reyes, J., F. Greco, R. Motais, and R. Latorre. 1983. Phloretin and phloretin analogs: mode of action in planar lipid bilayers and monolayers. *J. Membr. Biol.* 72:93–103.

Toon, M. R., and A. K. Solomon. 1987. Modulation of water and urea transport in human red cells: effects of pH and phloretin. *J. Membr. Biol.* 99:157–164.

Verkman, A. S., and A. K. Solomon. 1982. A stepwise mechanism for the permeation of phloretin through a lipid bilayer. *J. Gen. Physiol.* 80: 557–581.

Vilallonga, F. 1968. Surface chemistry of L-α-dipalmitoyl lecithin at the air-water interface. *Biochim. Biophys. Acta*. 163:290–300.

Vogel, V., and D. Möbius. 1988. Local surface potentials and electric dipole moments of lipid monolayers: contribution of the water/lipid and the lipid/air interfaces. *J. Colloid Interface Sci.* 126:408–420.

Yamins, H. G., and W. A. Zisman. 1933. A new method of studying the electrical properties of monomolecular films on liquids. *J. Chem. Phys.* 1:656–661.