Surface Balance Studies of Bile Acid Monolayers

I. Cholanic and Glycocholanic Acid Monolayers

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A continuously recording surface balance has been employed to study monomolecular layers of cholanic and glycocholanic acids spread on acid substrates of pH 2. Both acids form monolayers of the condensed type on compression. Already at the point 0, where the surface pressure begins to increase, the monolayer molecules are densely packed and oriented more or less vertical to the substrate surface with the alkyl chain and its terminal carboxyl group projecting into the surface. The differences between bile acid and cholesterol monolayers are discussed.

The cholanic acid monolayer is liquid at 20°C; the surface layer solidifies only after the collapse point has been passed. At temperatures below 14°C the glycocholanic acid monolayer is solid already at point 0 and remains so on further compression. Between 15°C and 22°C a transition from a liquid to solid state occurs on compression; above 26°C the surface layer solidifies only after its collapse. The phase transformation in the monolayer is accompanied by a decrease in the internal energy amounting to 1 000 to 2 000 cal/mole. An increase in the sodium chloride content of the substrate has the same effect on the transformation as a moderate decrease in temperature.

There appears to be only one investigation that has dealt with the monolayer properties of bile acids. Adam, Askew and Danielli 1 studied the properties of apocholic and β-apochocholic acid monolayers which they seem to have compressed to an area of about 100 Å² per molecule and to a surface pressure of 9 dynes/cm. They found that both acids form gaseous monolayers in which the longitudinal axes of the molecules apparently lie parallel to the surface owing to the fact that the molecules contain three hydrophilic groups that are situated fairly far apart.

In connection with investigations of micelle formation in bile acid salt solutions in progress at the Institute of Physical Chemistry of the Åbo Aka-

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demi 2-7,15, we considered it of interest to study the monolayer properties of the bile acids 8. We present the results of a surface balance study of the monolayer properties of cholic and glycocholic acids on acid substrates in this paper.

MATERIALS AND METHODS

The bile acids were synthesized by one of us (A. N.) at the Department of Physiological Chemistry of the Lund University. The methods of synthesis and the physical properties of the acids have been described in earlier publications 9.

The cholesterol which was included as a reference compound was from Hoffmann-La Roche AG, Basel; it melted at 148.5°C.

Owing to the low solubilities of the bile acids in benzene, chloroform was employed as solvent. The chloroform solutions of the bile acids contained $2 \times 10^{18}$ molecules per ml. (It was found that cholic acid and cholesterol monolayers yield slightly smaller area values (by 0.8 Å² per molecule) when chloroform than when benzene is the solvent. The surface pressures were not affected by the solvent. The observed difference may be due to the fact that the solubility of chloroform in water is ten times greater than that of benzene.)

The chloroform solutions of the bile acids were added to the surface of the aqueous substrate in a Langmuir trough from an Agla micrometer syringe. The surface pressure-area curves of the monolayers were recorded with an automatic, self-recording surface balance of the Wilhelmy-Dervichian type (as improved by Andersson-Groth, Ställberg-Stenhagen and Stenhagen 14). Compression of the monolayer was begun 2 min after the substance had spread on the substrate surface. The rate of compression was 13-14 cm/min; the area values were found to be completely, and the pressure areas practically, independent of the rate of compression in this range. (When much lower rates of compression were employed, this was no longer the case; the area values tended to decrease and the pressure values to increase as the rate of compression was diminished. When the rate of compression was appreciably higher, from 25-50 cm/min, the pressure-area curves were no longer reproducible.)

The substrate was water or a 3 M sodium chloride solution; in both cases the pH was lowered to 1.9-2.0 with hydrochloric acid.

The viscosities of the monolayers were examined by a modified talcum powder method. If the powder did not move when a weak current of air was blown on the layer, the monolayer was judged to be solid.

EXPERIMENTAL RESULTS

Typical pressure-area curves recorded for the cholic and glycocholic acid monolayers are shown in Figs. 1 and 2. Curves for cholesterol monolayers recorded under identical conditions have also been drawn in the figures. The notations used to designate various characteristic points on the pressure-area curves in these figures will be employed for reference in the following.

Characteristic data for bile acid and cholesterol monolayers are given in Table 1. The thicknesses of the monolayers at the collapse point, $K_{c}$ or $K_{max}$, have been calculated by assuming that the densities of all the monolayer substances are 1.067 g/ml, i.e., that of crystalline cholesterol. (In the literature we have found only the density value for norcholic acid, $d = 1.13$).

On a salt-free substrate the bile acid monolayers must be compressed to an area of 44 Å² per molecule before the surface pressure begins to increase. With cholic acid the pressure then increases steeply up to the collapse point. (The peak at the collapse point disappears when a lower rate of compression is employed.) In the pressure-area curve for glycocholic acid a curvature

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Fig. 1. Structural formulae of cholic acid (1), glycocholic acid (2) and cholesterol (3). Typical monolayer pressure-area curves. Substrate: water of pH 2, 20°C. 1. Cholic acid; 2. Glycocholic acid; 3. Cholesterol. Notation: 0, the point where the pressure begins to increase on compression. $K_1$, $K_{extr}$, $K_{max}$, points where collapse occurs. $i_1$, $i_2$, inflexion points. tr, transition point.

showing that a transition takes place is seen at a pressure of 6.0 dynes/cm; on both sides of the transition region, the curves have a large slope. The pressure required to effect collapse of the glycocholic acid monolayer is almost 50% greater than that required for the cholic acid monolayer. The area per molecule at the collapse point is 40 Å² for cholic acid and 39 Å² for glycocholic acid.

The area and pressure values recorded on a 3 M sodium chloride substrate are generally slightly higher than those recorded with pure water as substrate. An exception is the area value at point 0 for glycocholic acid which is 1.4 Å² per molecule lower. This is due to the fact that the transition range and the

Fig. 2. Typical monolayer pressure-area curves of bile acids and cholesterol on 3M sodium chloride of pH 2, 20°C. 1. Cholic acid; 2. Glycocholic acid; 3. Cholesterol.

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Table 1. Characteristic monolayer data for cholic acid, glycocholic acid and cholesterol at 20 °C, pH 2.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Substrate</th>
<th>Areas in Å² per molecule</th>
<th>Surface pressures in dynes/cm</th>
<th>Thickness of monolayer at the collapse point (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$A_0$</td>
<td>$A_{tr}$</td>
<td>$A_{K_1}$</td>
</tr>
<tr>
<td>Cholic acid</td>
<td>Water</td>
<td>44.2</td>
<td>—</td>
<td>40.2</td>
</tr>
<tr>
<td></td>
<td>3M NaCl</td>
<td>45.8</td>
<td>—</td>
<td>40.6</td>
</tr>
<tr>
<td>Glycocholic acid</td>
<td>Water</td>
<td>44.5</td>
<td>41.6</td>
<td>38.8</td>
</tr>
<tr>
<td></td>
<td>3M NaCl</td>
<td>43.1</td>
<td>—</td>
<td>39.3</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Water</td>
<td>37.4</td>
<td>—</td>
<td>35.5</td>
</tr>
<tr>
<td></td>
<td>3M NaCl</td>
<td>40.0</td>
<td>—</td>
<td>35.7</td>
</tr>
</tbody>
</table>

lower section in the pressure-area curve of glycocholic acid do not become evident when the sodium chloride content of the substrate is high enough; this is shown by the curves in Fig. 3 recorded with increasing salt concentration.

In order to determine the effect of temperature on the transition that occurs in the glycocholic acid monolayer, pressure-area curves were recorded at different temperatures (Fig. 4). It was found that at temperatures below 14 °C the monolayer is solid already at point 0 and remains so on further

*Fig. 3. Effect of substrate salt content on the glycocholic acid monolayer. Range 0—3 M NaCl. pH 2. 20°C.*

*Fig. 4. Effect of temperature on the glycocholic acid monolayer. Water of pH 2.*

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compression. At temperatures where the transition is observed, the monolayer is liquid up to the transition point (the point "tr"), but is solid above the latter, at least up to 22 °C. At temperatures above 26 °C, however, the surface layer becomes solid only after the monolayer has collapsed. A transition from a liquid to a solid phase thus occurs at the point tr, at least at temperatures between 15° and 22 °C.

DISCUSSION

In contrast to apocholic acid, cholanic and glycocholanic acids form monolayers of the condensed type (Figs. 1—4). This is obviously due to the presence of only one hydrophilic group in their molecules, i.e., the carboxyl group at the end of the alkyl chain.

On the basis of information reported in the literature on the configurations of the bile acids and cholesterol ¹¹ and with the aid of molecular models constructed from Stuart atomic calottes, we have attempted to compute the smallest areas that the molecules can take up in densely packed monomolecular layers (Table 2).

Table 2. Lengths and smallest cross-sectional areas of bile acids. Calculations based on molecular models.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Length of molecule ( \text{Å} )</th>
<th>Molecular area perpendicular to major axis ( \text{Å}^2 )</th>
<th>Smallest projection area with the molecule inclined 12° from the vertical ( \text{Å}^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholanic acid</td>
<td>18.5</td>
<td>45.5</td>
<td>44</td>
</tr>
<tr>
<td>Glycocholanic acid</td>
<td>22.0</td>
<td>45.5</td>
<td>44</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>21.8</td>
<td>35</td>
<td>35</td>
</tr>
</tbody>
</table>

The experimentally found areas per molecule at point 0 \( (\Lambda_0) \) are of the same magnitude as the computed areas. This shows that the bile acid molecules are densely packed already at this point. A further compression can occur only if the molecules are fitted into each other. Between the points 0 and K, the molecules are oriented almost perpendicular to the substrate surface with the alkyl chain and its terminal carboxyl group projecting into the surface. Lacking exact values for the densities of the acids, only approximate values can be calculated for the thicknesses of the monomolecular layers. They are somewhat lower than the calculated maximum lengths of the molecules, apparently because the monolayer molecules at the collapse point are inclined about 12° from the vertical and because the molecules are somewhat curved. The experimental value for cholanic acid agrees fairly well with the value for desoxycholic acid (13.5 Å) calculated from data obtained in X-ray investigations of choleic acid crystals ¹².

It is interesting to compare the monolayers of the bile acids with that of cholesterol. From molecular models, Harkins, Ries and Carman ¹³ have estimated the smallest cross-sectional area of a cholesterol molecule (assumed

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to be a parallelepiped) to be 37 Å². Our calculations gave for the minimum projection area of cholesterol the value 35 Å². The former value is in fairly good agreement with our experimentally determined area requirement at point 0, A₀ = 37.4 Å², and the latter with the experimental value at the collapse point, A_{\text{extr}} = 35.4 Å². The molecules in the cholesterol monolayer are thus densely packed and nearly perpendicular to the surface at the point 0. The carbon atom 3 bearing the hydrophilic hydroxyl group and the carbon atom 17 which carries the alkyl chain with its terminal carboxyl group are at opposite ends of the molecule. As a consequence the arrangement of the cholesterol and the bile acid molecules is different in compressed monolayers. The former stand with the hydrophenanthrene skeleton pointing downwards, whereas the latter stand with their alkyl chains projecting into the substrate and the ring skeleton pointing upwards. It should further be noted that the alkyl chain of the bile acids is shorter than that of cholesterol and that the rings A and B are in the cis positions in the bile acid molecule but in the trans positions in the cholesterol molecule. There is further a double bond in the 5, 6 position in cholesterol. In a study of sterol monolayers, Adam, Askew and Danielli ¹ found that the introduction of a double bound in the 5, 6 position increases the area requirement by approximately 1.8 Å² and that the change in the positions of the rings A and B from trans to cis configuration increases the molecular area by approximately 2.8 Å² at a surface pressure of 20 dynes/cm. From this it may be concluded that the area requirement of the bile acid ring skeleton is larger than that of cholesterol by about 1 Å². The observed difference of 3—5 Å² between the molecular areas in bile acid and cholesterol monolayers (Table 1) is thus for the most part due to the different orientations of the molecules in the monolayers. The appreciably lower collapse pressures found for the bile acid monolayers are apparently due to the same factor. The arrangement with the alkyl chain pointing downwards and the hydrophenanthrene ring system upwards apparently results in a less dense packing and a weaker cohesion than the opposite arrangement.

Since the only difference between cholic anhyd and glycocholic acids is that the latter contains a conjugated glycine molecule, this must be responsible for the curvature observed in the pressure-area curve of the latter acid at temperatures between 16° and 26°C. At the lowest temperature studied (6—7°C), the solid glycocholic acid monolayer gives a smooth pressure-area curve (Fig. 4) and the area can be reduced to about 37 Å² per molecule before a gradual collapse occurs. The A₀ values are 45—46 Å² per molecule, and the compressibilities calculated from the slopes of the curves at the inflexion points are rather high (—κ = 5—6 × 10⁻³). When, however, the temperature rises above 10°C, the compressibility diminishes gradually, the curvature at the collapse point becomes sharper, and A_{\text{extr}} increases to a slightly higher value. The values of the surface pressures, areas per molecule and the compressibilities at different temperatures are given in Figs. 5, 6 and 7. In the temperature range 14°—26° where the transformation is observed, the properties of the liquid phase at low pressures (Phase 1), and those of the solid phase at high pressures (Phase 2) are well defined; the compressibilities of the two phases remain constant (—\frac{\partial A}{\partial \sigma} = —κ = 3—6 × 10⁻³ and 1.8 × 10⁻³ cm/dyne).

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Fig. 5. Variation of the surface pressure of glycocholanic acid monolayer with the temperature. Water of pH 2.

Fig. 6. Variation of molecular area with the temperature in the glycocholanic acid monolayer. Water of pH 2.

Fig. 7. Variation of compressibility at the inflexion point with the temperature in the glycocholanic acid monolayer. Water of pH 2.

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Fig. 8. The change in the molecular area at the transition point, $\Delta A_{tr}$, of glycocholanic acid monolayer.

Fig. 9. The change in molal heat content when transition takes place in the glycocholanic acid monolayer.

(Fig. 7) and the areas at the inflexion points decrease linearly with rising temperature. Above 22°C, however, the high pressure phase (Phase 2) becomes less well defined, and is no longer observed above 26°C. At temperatures above the latter the surface layer solidifies only after the collapse point has been passed.

On the basis of the two dimensional analogue of the Clausius-Clapeyron equation

$$\frac{d\pi_{tr}}{dT} = \frac{\Delta H_{tr}}{T \cdot \Delta A_{tr}}$$

we have calculated the changes in molar heat content, $\Delta H_{tr}$, accompanying the monolayer transformation in the range from 17° to 24°C. In this equation $\pi_{tr}$ is the equilibrium transition pressure of the monolayer at temperature $T$ and $\Delta A_{tr}$ is the net change in molar area. We have taken $\Delta A_{tr}$ equal to the difference between the tangents drawn to the curves at the inflexion points. The value of $\Delta A_{tr}$ decreases linearly with rising temperature between 17° and 24°C (Fig. 8).

The calculated $\Delta H_{tr}$ values vary from about $-1000$ to about $-2500$ cal/mole (Fig. 9). The expression $\frac{\partial \Delta H_{tr}}{\partial T}$ is negative up to 22°C, but positive above the latter temperature. Since $(\frac{\partial \Delta H_{tr}}{\partial T})_{\pi} = C_{\pi} - C_{\pi} = \Delta C_{\pi}$ (where $C_{\pi}$ is the molar heat content of the phase), the values of this expression suggest that whereas the degrees of kinetic freedom of phase 2 are restricted compared to those of phase 1 below 22°C, the opposite is true above this temperature. Our observations show that Phase 2 is solid and has a constant low compressibility up to the latter temperature, but that the properties of this phase are

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Fig. 10. Variation of the transition surface pressure with salt content of the substrate in the glycocholic acid monolayer. 20°C.

less well defined (the compressibility increases continuously) between 22°C and 24°C. Further conclusions cannot be drawn owing to the limited experimental information available at present.

For a transformation at constant pressure, the net change in molar heat content is

$$\Delta H_{tr} = \Delta E_{tr} + \pi_{tr} \Delta A_{tr}$$

(2)

where $\Delta E_{tr}$ is the net change in the molar internal energy of the monolayer system and $\pi_{tr} \Delta A_{tr}$ the work done by the system. At zero transition pressure, $\Delta H_{tr} = \Delta E_{tr}$. For the observed transition pressures, the values of $\Delta E_{tr}$ are only 10—25 cal/mole more positive than the $\Delta H_{tr}$ values; the temperature dependence of both is the same. The phase transformation is accompanied by a decrease in the internal energy, and the magnitude of $\Delta E_{tr}$ (—1000 to —2000 cal/mole) suggests that hydrogen bond formation takes place between the molecules in the monolayer. If this is true, it is probable that the hydrogen atom of the imino group is involved. In the range 14—22°C a liquid monolayer is thus first formed which on compression is transformed into a solid layer, obviously with the formation of hydrogen bonds. This transition does not occur above 26°C, and above this temperature the glycocholic acid monolayer is liquid up to the collapse point similarly as that of cholic acid.

An increase in the sodium chloride content of the substrate has the same effect as a moderate decrease in temperature. The transition pressure decreases and the liquid phase 1 disappears (Fig. 10). When the salt content of the substrate becomes sufficiently high, the monolayer is directly transformed on compression into phase 2 with a low compressibility.

REFERENCES


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