

Preparation and Monolayer Films of Cellobiose Alkyl Esters

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Cellobiose octa (decanoate) and cellobiose octa (octadecanoate) were prepared and used for studies on formation and fine structure of monolayers at the air-water interface. The surface pressure (π)-area (A) isotherms of monolayers of these cellobiose esters are similar to those of the corresponding cellulose esters previously reported, when A is expressed in nm² per alkyl chain instead of per glucose unit. Under electron microscopic examinations of the monolayers transferred from the water surface, the monolayers of the two cellobiose esters were found inhomogeneous. This makes a sharp contrast to the fact that the cellulose esters, considered as the polymers of these cellobiose esters, form homogeneous monolayers. These results obtained were discussed in comparison with that of stearic acid.

KEY WORDS: Cellobiose Esters/ Monolayer/ Surface Pressure - Area Isotherm/
Dark-Field Electron Micrograph/

INTRODUCTION

In the course of our structural studies of monolayer films, we have prepared as sample materials cellulose derivatives of several kinds and examined their monolayers formed at the air - water interface by means of surface pressure (π) - area (A) isotherm measurements and of electron microscopy¹⁾. These studies are based on the feasibility that cellulose and cellulose derivatives may be converted into amphiphilic compounds with varying the hydrophile-lipophile balance. This can be accomplished by substituting hydrophilic with hydrophobic groups of different chemical structure at different degrees of substitution.

It is well known that some representative amphiphilic molecules such as stearic and arachidic acids form monolayers at the air - water interface. Indeed, their monolayers seem homogeneous when judged from their π - A isotherms observed, but an electron microscope study has revealed that these monolayer films transferred on carbon surface are actually inhomogeneous, including densely scattered small holes²⁾. This unexpected finding let us prepare and use cellulose derivatives of several kinds for monolayer film studies.

As has been reported³⁾, two cellulose esters fully substituted with n-decanoyl and n-octadecanoyl groups have been found to form monolayer films homogeneous

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under examinations of electron microscope. To the best of our knowledge, this is the first report of successful formation of homogeneous monolayer films and, to be noted, the materials used are polymeric instead of ordinary low-molecular-weight compounds. A question now arises whether or not the sample materials should be macromolecules in order to form homogeneous monolayers.

The smallest unit of cellulose may be considered cellobiose provided that the mode of linkage, β -(1 $\rightarrow$ 4), of glucopyranose in cellulose is kept unchanged. So we prepared two corresponding cellobiose esters fully substituted with the same alkanoyl groups as used to cellulose and carried out similar experiments. Here, we describe the results obtained with these cellobiose esters.

EXPERIMENTAL

Synthesis of Cellobiose Esters

D(+)-cellobiose (guaranteed grade; Nacalai Tesque Inc., Kyoto) was used as received, but other chemicals were dried and distilled just before use according to the standard methods. Cellobiose esters (Cello-OCO(CH₂)_{n-2}CH₃) were prepared after Malm et al.⁴⁾ To the suspension of the cellobiose (3×10^{-3} mol) in 20 mL of 1,4-dioxane, pyridine (3.4×10^{-2} mol) and an acid chloride (2.9×10^{-2} mol), either n-decanoyl or n-octadecanoyl, were added dropwise. This esterification reaction was run under reflux over 18 h. The reaction mixture was poured into a methanol-water (9 : 1 by volume) mixture; the white precipitate was then separated by filtration and dried. This raw product was dissolved in tetrahydrofuran (THF) and the THF solution was centrifuged in order to sediment insoluble materials. The supernatant solution obtained was poured into hot methanol, the desired cellobiose ester fully substituted being precipitated. These procedures were repeated to purify the cellobiose esters. The sample codes of C10BS and C18BS will be used for cellobiose octa(decanoate) and cellobiose octa(octadecanoate), respectively.

Phenyl Cellobiose Derivatives

Any unreacted OH groups in the cellobiose esters prepared may be quantitatively replaced with phenyl groups. To check the presence of such unreacted OH groups, this reaction was carried out by treating a part of each cellobiose ester sample in pyridine with phenyl isocyanate at 373 K over 2 h.⁵⁾

General Analyses

Infrared (IR) spectra of the film cast on NaCl disks from each chloroform solution were recorded on a JASCO FT/IR-8000 spectrophotometer at room temperature. Elemental analyses were carried out with regard to C and H. The absorbance of phenyl groups in the cellobiose phenyl derivatives was measured in their chloroform solutions on a Shimadzu UV-200S spectrometer at 240 nm.

π -A Isotherm Measurements

The cellobiose ester monolayers were spread on the surface of water in the Teflon-coated rectangular trough (of the dimensions, $200 \times 500 \times 3$ mm³) after careful

sweeping of it with a Teflon-coated barrier. Solutions for spreading monolayers were prepared by dissolving 1 to 2 mg of each sample in 5 mL of benzene (spectrograde). The amounts, taken with a micropipette, of the dilute benzene solution were adjusted in such a way that an occupied area per glucose unit be 2 to 2.5 nm². The benzene molecules evaporate completely in 30 min at the subphase temperature of 293±0.3 K. The monolayer was compressed at a constant speed of 12 cm² min⁻¹. During compression of the monolayer, a surface pressure was measured by the Wilhelmy method. In addition, monolayers of stearic acid (purity 99.5%, Wako Pure Chemical Ind., Osaka) were also spread in a similar manner, where an occupied area per alkyl group was adjusted to that for the cellobiose esters. The π -A isotherm of stearic acid was recorded for reference.

Observation of Surface Structure of Transferred (LB) Films

Small patches of a monolayer were transferred, at several sequential stages during compression, from the water surface to specimen grids covered with thin carbon supporting film. This transfer was carried out by making the carbon film horizontally touched with the monolayer from air side and then lifting it. By this operation, the air side, i.e., hydrophobic surface of the monolayer adheres to the hydrophobic carbon film. The surface structure of the films transferred was observed on a JEOL JEM 200-CX electron microscope in the dark-field imaging mode at a direct magnification of 2,800 times. The minimum dose system was used to reduce the radiation damage to the sample film.

RESULTS

Sample Characterization

A cellobiose molecule carries eight OH groups, and the cellobiose esters prepared are expected to have alkyl degree of substitution (DS) of 8.0, if the reaction is completed. In order to confirm this expected value of DS, three methods of IR and UV (ultraviolet) spectroscopies and elemental analysis were employed. IR spectra of the cellobiose esters prepared and of cellobiose itself are presented in Fig. 1. The absorption bands around 3,500, 2,900 and 1,750 cm⁻¹ are due to OH, CH and C=O stretching vibrations, respectively. The CH and the C=O absorptions are significantly large in both cellobiose esters, whereas the strong OH absorption observed for cellobiose is hardly seen in the spectra of the cellobiose esters. The almost absence of hydroxyl absorption indicates the nearly complete substitution with alkyl groups.

The results of elemental analyses were in good accord with those calculated for the corresponding cellobiose esters with the assumed DS value of 8.0. This complete replacement of hydroxy groups with the alkyl esters were checked by means of UV measurements on the cellobiose phenyl derivative at 240 nm. The phenyl degree of substitution can be estimated by using the calibration curve of absorbance established for phenyl isocyanate. The results were 0.0₁ and 0.0₃ for C18BS and C10BS, respectively, the full substitution of hydroxy groups being confirmed.

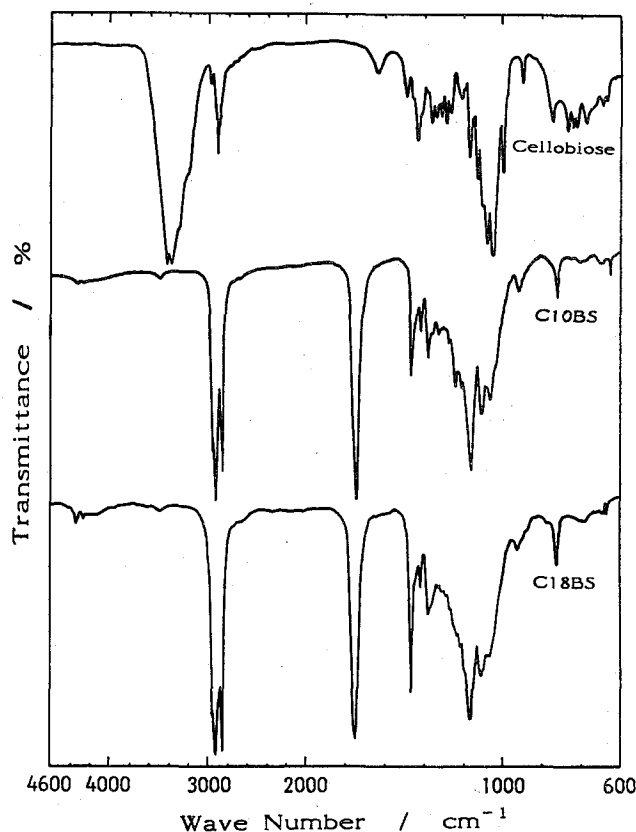


Fig. 1. Infrared transmission spectra of cellobiose and of the two cellobiose esters prepared. C10BS: cellobiose octa(decanoate), and C18BS: cellobiose octa(octadecanoate).

π -A Isotherms

Figure 2a and b show the π -A isotherms observed on both samples of the cellobiose esters. The area of monolayer A is expressed in nm² per glucose unit instead of per molecule for later convenience. From these figures one can see the followings.

The isotherm of C10BS is rather complex. It begins to rise, rather early, at about 1.60 nm² per glucose unit, then changes the slope to some one seventh of that of the initial rise and keeps it over a wider range from 1.35 to 0.5 nm² per glucose unit. Finally it shows the steep rise in surface pressure up to about 40 mN m⁻¹. These features are similar to those of the isotherm of myristic acid⁶⁾. That is, this isotherm apparently represents a transition from an expanded to a condensed film.

To the contrary, the isotherm of C18BS begins to rise at about 1.30 nm² per glucose unit, followed by a rather rapid increase in pressure. At about 50 mN m⁻¹, the isotherm terminates due to the collapse of the film. These features are

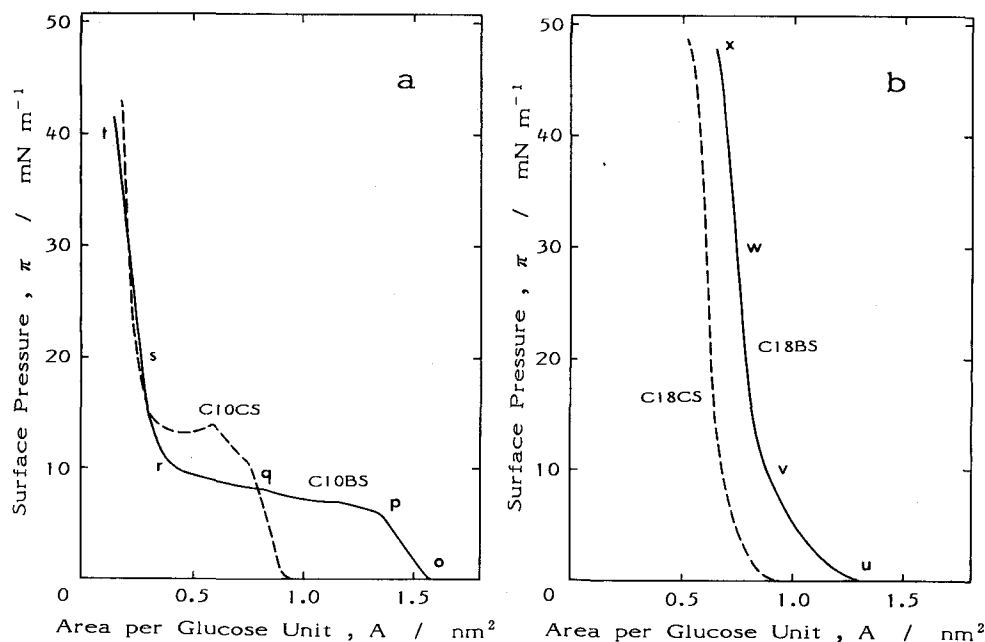


Fig. 2. Surface pressure (π)-area (A) isotherms of the cellobiose ester monolayers (solid line) spread from benzene solution at 293 K. Broken line: those of the corresponding cellulose esters. C10CS: cellulose tri(decanoate), and C18CS: cellulose tri(octadecanoate).

quite similar to those of the typical isotherm of a condensed film reported²⁰ and actually observed (see below) on stearic acid. As described above, so dissimilar are the two isotherms of the cellobiose esters with alkyl groups different in chain length, when examined at a fixed temperature of 293 K.

Fine Structure of the Transferred (LB) Films

Figure 3 shows a series of electron micrographs of the C10BS monolayer transferred at six stages (Points o to t in Fig. 2a) of π - A isotherm. At Point o (A/nm^2 glucose unit⁻¹, $\pi/\text{mN m}^{-1}$) = (1.60, 0), where the isotherm is about to rise, no structures having sufficient contrast can be seen, and the monolayer LB film of C10BS molecules seems to be more or less homogeneous. However, this occupied area of 1.6 nm^2 per glucose unit is an intermediate value; in the two extreme cases where the present molecules have the substituents vertical or parallel to the water surface, the occupied area is estimated to be 0.8 or 2.5 nm^2 per glucose unit, respectively. Accordingly, this cannot be a homogeneous monolayer film in a precise sense. In the plateau region (Points p to r), the molecules are seen to form large structure like skin and seeds. On further compression, as judged from the last two electron micrographs, this strange structure seems to grow with increasing surface pressure. Obviously, the C10BS LB films were inhomogeneous up to maximally possible surface pressure.

Similar electron micrographs are shown, in Fig. 4, for the C18BS monolayer

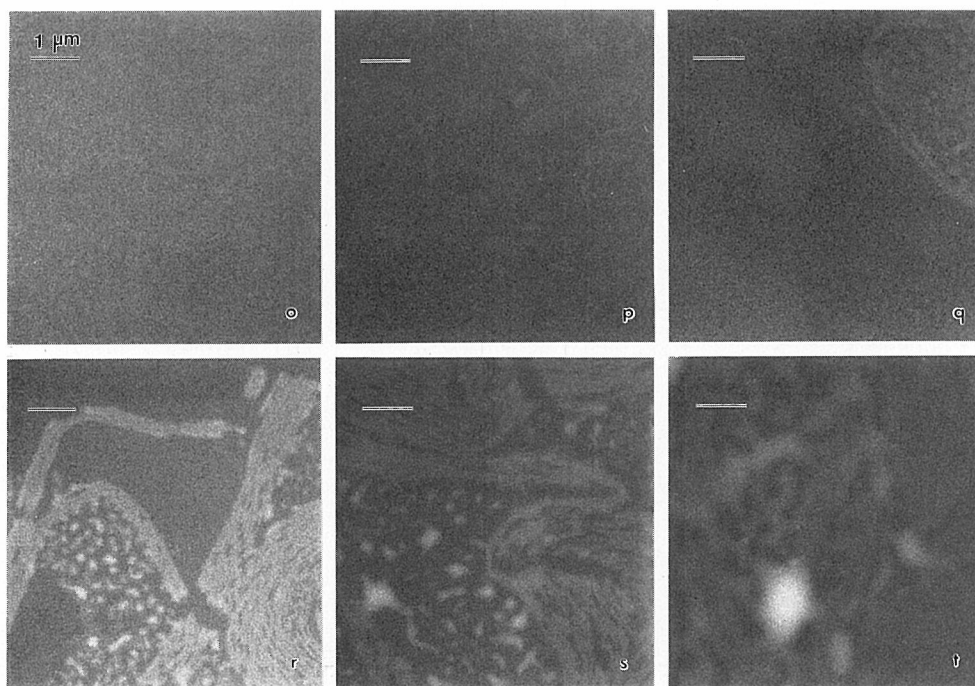


Fig. 3. Dark field images of cellobiose octa(decanoate) monolayers transferred at different stages of the π -A isotherm as indicated in Fig. 2a. o: ($\text{Å}/\text{nm}^2$ glucose unit $^{-1}$, $\pi/\text{mN m}^{-1}$) = (1.60, 0) p: (1.35, 6), q: (0.83, 8), r: (0.42, 10), s: (0.27, 20), and t: (0.16, 40).

transferred at four stages of π -A isotherm. At Point u (1.30, 0) the C18BS LB films are seen to form a continuous film holding irregular vacancies. At Point v (0.88, 10), the film still holds many holes irregular in size and shape. Even at a point of higher pressure, w (0.83, 30), there exist holes and one can recognize some streaks, which must be images of shrinks of the monolayer. And at Point x (0.76, 50), shrinks become obvious, and the monolayer film is seen to be about to collapse.

DISCUSSION

The two cellobiose esters gave quite different π -A isotherms, but both were found not to form homogeneous monolayers. This makes a sharp contrast to the fact that the corresponding cellulose esters have been found to form homogeneous monolayers³⁾. So it is interesting to compare the isotherms of the cellobiose esters with those of the cellulose esters, cellulose tri(octadecanoate) (C18CS) and cellulose tri(decanoate) (C10CS).

In Fig. 2, the isotherms observed on the cellulose esters are reproduced in broken lines. Their features are similar to those of the corresponding cellobiose esters, but different in the absolute value of area at a given surface pressure. This is because the area is counted per glucose unit. A glucose molecule in chair form occupies different cross sectional area, depending on the materials examined:

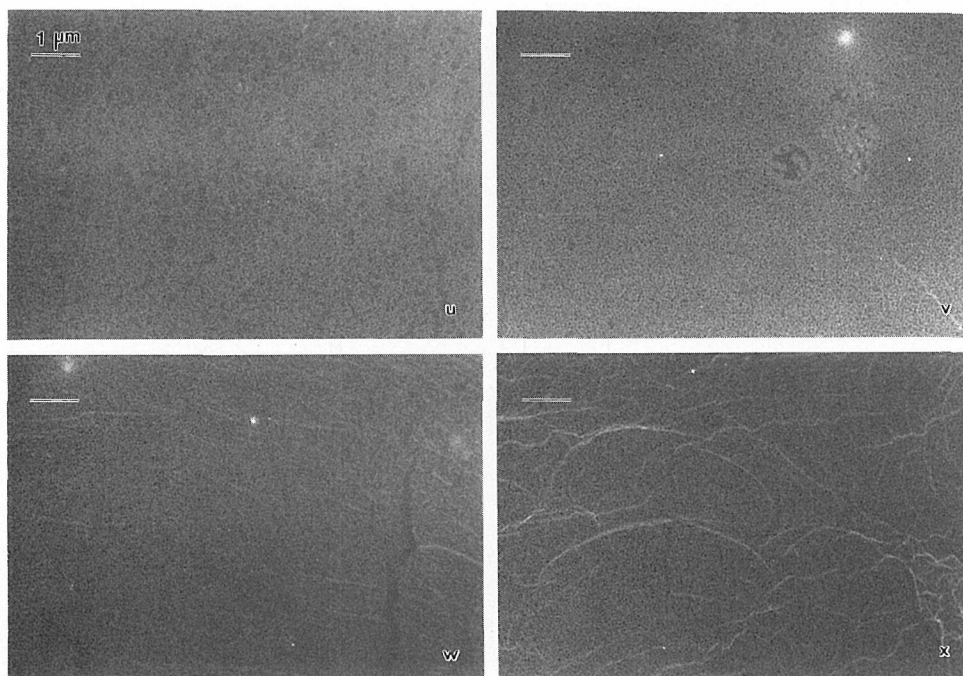


Fig. 4. Dark field images of cellobiose octa(octadecanoate) monolayers transferred at different stages of the π -A isotherm as indicated in Fig. 2b. u: (1.30, 0), v: (0.88, 10), w: (0.83, 30), and x: (0.76, 50).

0.29, 0.36 or 0.43 nm^2 for β -D-glucose itself⁷⁾, cellobiose⁸⁾, or cellulose⁹⁾, respectively. On the other hand, three alkyl groups occupies much wider area of 0.55 nm^2 when they are packed in orthorhombic shape¹⁰⁾. In the cellobiose esters, four alkyl chains are present per glucose unit. Accordingly, the comparison should be made in plots of surface pressure against the area of monolayer per alkyl chain unit, which are depicted in Fig. 5a and b.

As for the samples of C10BS and C10CS, the two π -A isotherms are dissimilar, but they share the essential features that each isotherm consists of three parts, the initial rise with moderate slope, the following nearly plateau part and the final steep rise. In the steep rise part, the occupied areas of these isotherms are seen to take much smaller values than those for other molecules shown in panel b. This fact may be considered to suggest that the monolayer film has already been transformed to multilayer film, definitely not homogeneously as has been seen in Fig. 3. When we recall the π -A isotherms for some lower aliphatic carboxylic acids¹¹⁾, it may be judged that C10BS molecules do not have the lateral hydrophobic interaction strong enough for the substituents to adhere to each other when located at the air-water interface. On the other hand, the π -A isotherms of C18BS and C18CS are seen to be very similar to each other and also to that of stearic acid. It is seen that the three kinds of molecules have substituents or portion hydrophobic enough to form condensed monolayers.

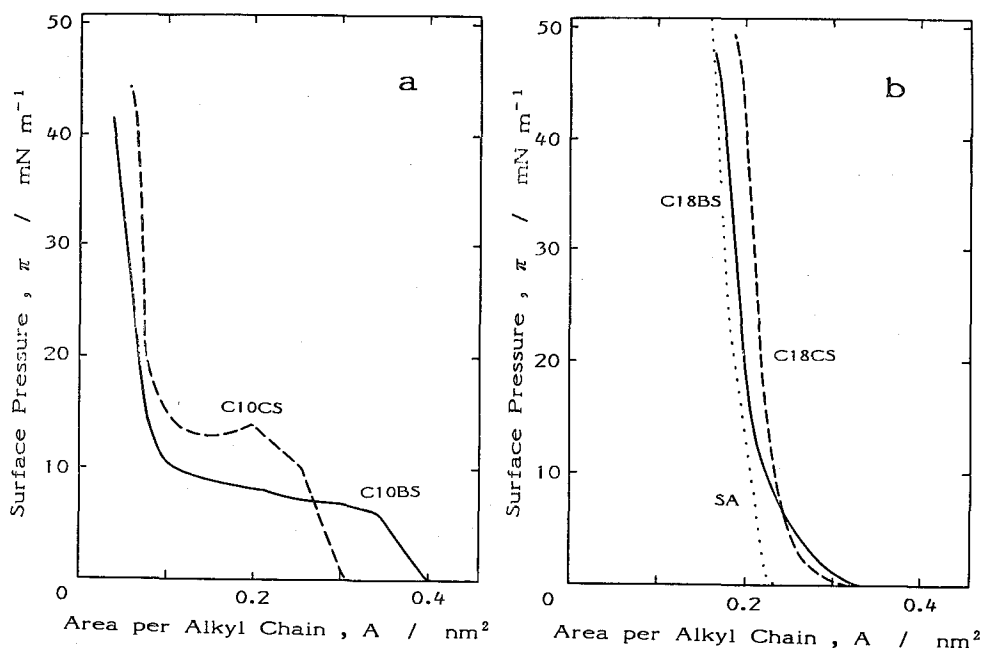


Fig. 5. Comparison between π -A (per alkyl chain) isotherms. Solid line: the cellobiose esters, broken line: the corresponding cellulose esters, and dotted line: stearic acid.

The molecular differences in the cellobiose esters and the corresponding cellulose esters are two-fold: the macromolecularity and the number of alkyl chains introduced per glucose unit. From a comparison between the results mentioned above, it is seen that the macromolecularity favors the formation of homogeneous monolayer films. Indeed, this conclusion is clear, but it must be accepted with reservations. Cellulose itself is a linear, extended, stereoregular macromolecule. Such a skeletal structure of the cellulose esters might be favorable for forming homogeneous monolayers. In addition to the present work, another comparative study on monolayers of cellulose esters and of amylose esters will afford us an answer to this question. It is because these polysaccharides are chemically similar but conformationally different. Here, we will remain prudent until such a study is completed¹²⁾. The latter difference is out of question, for the area of monolayer is counted per alkyl chain. So it could be sensible to revisit the π -A isotherms, presented in Fig. 5b, for C18BS and C18CS as well as for stearic acid.

These three isotherms are similar not only in shape but also in absolute values of π and A. For example, the A values at 30 mN m⁻¹ are read as 0.19 and 0.21 nm² per alkyl chain for C18BS and C18CS, respectively. It should be noted that the C18BS monolayer takes an A value similar to, or strictly speaking, 10% smaller than the C18CS monolayer does, whereas holes were found in the former but not in the latter. So these findings are quite unreasonable. For stearic acid, the A value at that pressure is read as 0.18 nm² per molecule, which is close to that of the C18BS monolayer.

It could be interesting to compare those A values with that of the occupied

area of polyethylene; it is, in orthorhombic crystal, for example, 0.18_3 nm^2 per molecule¹⁰⁾. This is quite close to those found for the monolayers of stearic acid and of C18BS molecules. This fact seems to imply that long alkyl chains of these molecules examined are nearly close-packed in each monolayer like in crystals. The occupied areas of these molecules seem to be not large enough to contain many small holes. No vacant area as large as observed could be accommodated in these monolayers. The monolayers of C18BS and of stearic acid should be homogeneous, so long as their A values are concerned and also if those are really monolayers. As these A values are read at a fixed surface pressure of 30 mN m^{-1} , it could be worth knowing how high the surface pressure is in an ordinary sense.

The surface pressure measured actually bears on the molecular area of the monolayer. When the molecular height of the C18BS monolayer is taken to be 2.4 nm , the surface pressure of 30 mN m^{-1} corresponds to 123 atmospheric pressure. This cannot be taken as a pressure high enough to appreciably reduce the occupied area of these molecules. So it could be reasonable for C18BS and stearic acid molecules to take A values close to 0.18_3 nm^2 for polyethylene. What is strange is, however, the finding that these monolayers are inhomogeneous, including many small holes. On the other hand, the A value of the C18CS monolayer was 10% larger than that of C18BS and of stearic acid, whereas the C18CS LB film was found homogeneous. This apparently conflicting results could be accepted if the resolution of electron photomicrograph was taken into account and if the structure of the film transferred was considered not to be identical to that of the film on the water surface.

Only holes larger than 50 nm in diameter could be seen experimentally. Otherwise it is impossible to detect them. So the results obtained might reflect differences in hole-size distribution between the three kinds of monolayers. Indeed, the degree of freedom, on an average, of octadecanoyl groups must depend on the molecules which those groups belong to. Their degree of freedom will increase in the order of C18CS, C18BS, and stearic acid, because those groups in the first compound are rooted to a long skeletal chain, those in the second are merely substituents of a low-molecular-weight compound, and those in the third are only portions of the molecules. At a given surface pressure, therefore, it could be natural for those compounds to occupy larger area in that order. Accordingly, the monolayer of C18CS might include vacancies smaller or narrower beyond the experimental resolution. This is only a speculation, because no experimental means to check it are available.

The most serious point to be recognized is, as has been raised above, that isotherms were measured on monolayers spread on the water surface, while electron micrographs were recorded on the monolayer films transferred on the carbon surface of a specimen grid. On this account, it is highly necessary, we should think, to examine the surface structure of monolayers without applying any operation of transferring them from the water surface.

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