Specific and Non-specific Interaction between Double Perfluoroalkyl-chain Steroidal Glycoside Monolayers and Cellulase at the Air–Water Interfaces

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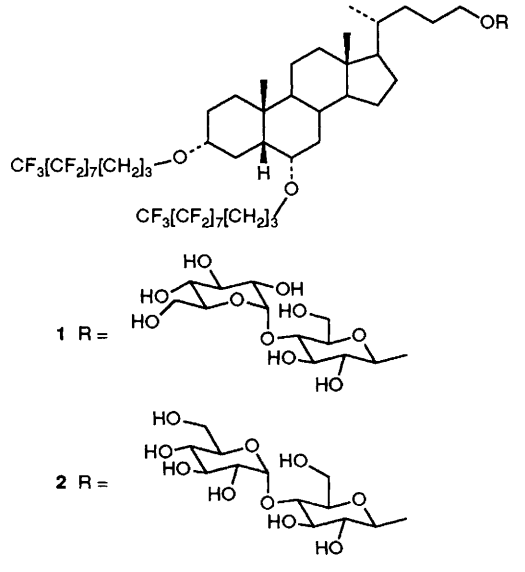
The monolayers of double perfluoroalkyl-chain steroidal amphiphiles bearing cellulose or maltose head groups show pressure-area behaviour relating to specific or non-specific interactions with cellulase at air–water interfaces.

Molecular recognition between an enzyme and a substrate is regarded as the primary process of enzyme-catalysed specific reactions, leading initially to enzyme–substrate complexation with attachment at a specific recognition site.[1] Long-chain amphiphile monolayers on water surface provide an example of a membrane-mimetic 2D assembly corresponding formally to one half of the biolipid membrane of a cell. Monolayer behaviour, characterized mainly by surface pressure–area (π–A) isotherm measurements, depends directly on the structure of the membrane-forming molecules and the interaction between the molecules and subphase species.[2] Therefore, it should be possible to detect recognition between monolayer molecules and subphase species on the macroscopic level by, for example, π–A diagrams. Recently, double perfluoroalkyl chain steroidal amphiphiles bearing cellubiose (**1**) or maltose (**2**) as hydrophilic head group have been synthesized and characterized.[3] These amphiphiles are assumed to form stable monolayers on the surface of water. Since there are various applications of monolayers in mimicking biomembranes,[2,4] it would be interesting to study monolayer behaviour in molecular recognition processes between an enzyme and its substrate at the air–water interface. It is well known that cellulase specifically interacts with cellubiose and cellulose, and facilitates their hydrolysis.[1] This enzyme, however, interacts non-specifically with maltose. Thus, the amphiphiles **1** and **2** would be expected to show typical specific and non-specific interaction with cellulase. We have now used the amphiphiles **1** and **2** to form monolayers at the air–water interface and examined the interaction between these monolayers and the water-soluble enzyme, cellulase.



The samples of **1** and **2** were obtained as reported previously.[3] The activity of the cellulase used in this experiment (Yakult Honsha Co., Ltd., Japan) was 7.2 IU mg^{-1} Pr and the enzyme sample proved to be inactive to maltose. Water was doubly distilled after passing through an ion-exchange resin column. The amphiphiles **1** and **2** were dissolved (*ca.* 3.5×10^{-3} mol l^{-1}) in a 4:1 (v:v) mixture of chloroform and methanol, and were spread on the water surface evenly from solution. For the monolayer experiments with a cellulase subphase, the enzyme was injected under the monolayers prior to compression and the compression was started after incubation for appropriate periods (*ca.* 30 min). A computer-controlled film balance system described previously was employed for measuring surface pressure as a function of molecular area.[5] The trough size was 200 mm × 500 mm. The π–A isotherms were recorded at a compression rate of 20 mm min^{-1} and the temperature of the aqueous subphase was maintained at 20 ± 1 °C. Each π–A isotherm was reproducible.

Fig. 1 compares the π–A behaviour of monolayers of **1** on various subphases. The amphiphile **1** forms a stable monolayer on pure water. Its molecular area was estimated from the

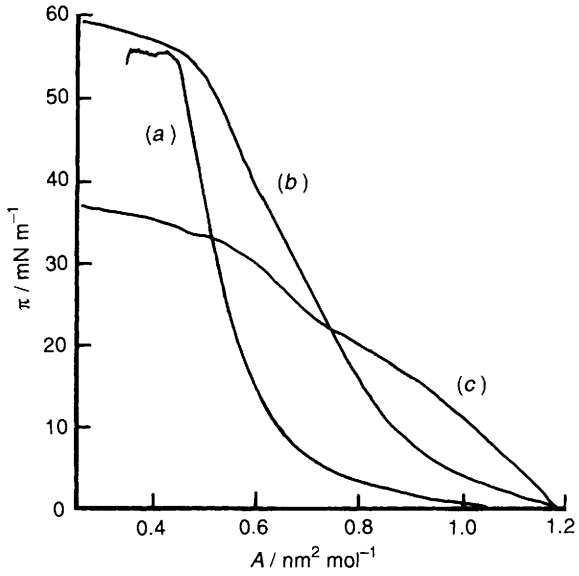


Fig. 1 π–A isotherms of monolayers of **1**: (*a*) on pure water; (*b*) on a subphase containing active cellulase; (*c*) on a subphase containing inactivated (cellubiose-saturated) cellulase

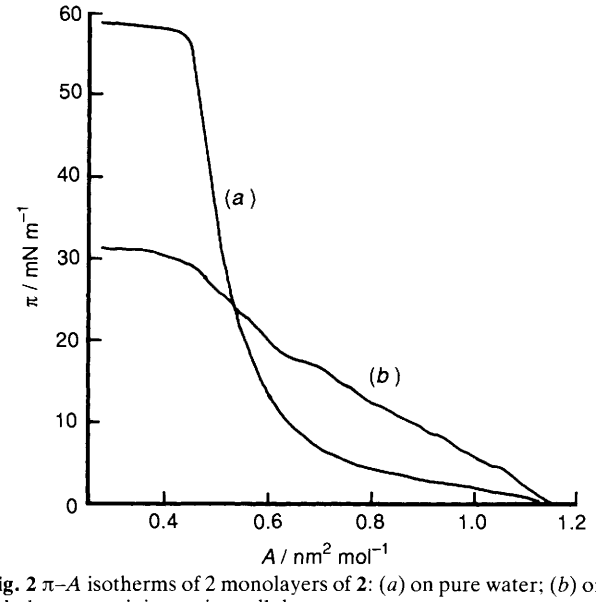


Fig. 2 π–A isotherms of 2 monolayers of **2**: (*a*) on pure water; (*b*) on a subphase containing active cellulase

π–A curve by extrapolating the condensed region to zero pressure: $A_0 = 0.59$ nm^2. This value is a little larger than twice the cross section, 0.25 nm^2, of a single fluorocarbon chain.[6] This may arise from the separation between the two tail chains in molecules of **1**. On an aqueous subphase containing cellulase, **1** also gave a fairly stable monolayer, but the π–A isotherm is clearly shifted with respect to that on pure water. Since the head group of **1**, cellubiose, is known to interact specifically with cellulase,[2] this shift is presumably ascribable to the specific interaction between the monolayer and the enzyme. However, account has to be taken of the fact that cellulase not only interacts specifically with the membrane-bound cellubiose but also undergoes non-specific adsorption at interfaces. In order to examine this non-specific interaction, we inactivated cellulase by saturating its specific recognition sites with cellubiose and injected this inactivated enzyme under a monolayer of **1**. As shown in Fig. 1(*c*), unlike what happens in the case of the specific interaction mentioned above, the monolayer on the inactivated cellulase is much expanded; the surface pressure increases slowly with decreasing area, giving a break point at *ca.* 33 mN m^{-1}. This π–A feature is undoubtedly a result of the non-specific interaction

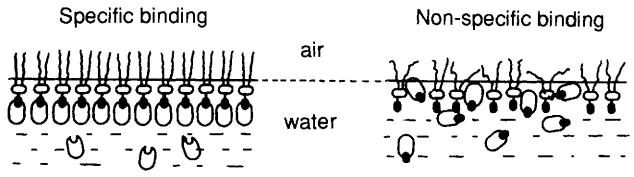


Fig. 3 Schematic drawing of the specific and non-specific interaction of a monolayer of **1** or **2** with cellulase at the air–water interface

between the monolayer and the inactivated cellulase because the enzyme has no free binding sites for specific interaction with the membrane-bound cellubiose. Hence, the behaviour of the monolayer of **1** changes owing to recognition of cellulase binding to the cellubiose head group of the amphiphile. These findings indicate that the specific and non-specific interactions can be distinguished by π–A isotherms at the air–water interfacial monolayer.

The effect of the spacer chain between the head group to be recognized and the membrane-forming long chain was noted in the biotin-lipid–streptavidin recognition system, which resulted in identical π–A behaviour for specific and non-specific interactions.[4] The amphiphile **1** does not have such a spacer chain, and a spacer effect is certainly not operative in the interaction between cellulase and monolayers of **1**, as observed in this experiment.

The π–A behaviour of a monolayer of **2** on a cellulase subphase also provides evidence for a non-specific interaction at the air–water interface. The structure of **2** is similar to that of **1**, bearing a maltose head group which is different from cellubiose only in the configuration about the glycoside bond. Its monolayer on pure water displays a π–A isotherm identical with that of **1** (see Fig. 2) and its molecular area is estimated to be the same as that of **1**. Unlike **1**, the monolayer of **2** is expanded when cellulase is added to the subphase [Fig. 2(*b*)], giving a pattern identical with that obtained for a monolayer of **1** on an inactivated cellulase subphase, indicating a non-specific interaction. Expanded monolayers arise from such non-specific interactions.

These results show that in the present system non-specific interactions cause a much larger change in π–A plots than specific interactions, and are therefore stronger. This contrasts with the streptavidin–biotin-lipid monolayer systems, from which it was deduced that specific interactions are stronger than non-interactions.[7] As schematically shown in Fig. 3, in the specific interaction the amphiphile **1** is attached to cellulase at the recognition site, forming a monolayer with good orientation order. However, in the non-specific interaction the cellulase interacts strongly with the monolayer, attaching itself to the amphiphilic molecules not only at the recognition site making the monolayer more water-soluble and disordered.

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