Headgroup-Ordered Monolayers of Uncharged Glycolipids Exhibit Selective Interactions with Ions

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ABSTRACT: Selective interactions of ions with charge-neutral saccharides can have far-reaching consequences in biological and wet-technological contexts but have so far been observed only indirectly. Here, we directly quantify by total-reflection X-ray fluorescence the preferential accumulation of ions near uncharged saccharide surfaces in the form of glycolipid Langmuir monolayers at air/water interfaces exhibiting different levels of structural ordering. Selective interactions with ions from the aqueous subphase are observed for monolayers featuring crystalline ordering of the saccharide headgroups, as determined by grazing-incidence X-ray diffraction. The attracted ion species depend on the structural motifs displayed by the ordered saccharide layer. Our results may constitute a basis to understand the salt-specific swelling of wood materials and various phenomena in membrane biophysics.
molecular area, A = 2A0/cos(t) = 43.4 Å², allows the galactose moiety of MGDG-sat to orient parallel to the interface (the in-plane area of the sugar headgroup is reported to be 35.4 Å²),12,13 in agreement with previously reported data.14 Interestingly, two additional Bragg peaks are seen in the midangle region, i.e., at lower Qxy (Figure 1B,C). These peaks indicate an ordering of weakly hydrated galactose moieties,15 in good agreement with previous SAXS and WAXS data.16,17 A supercell indicating the ordering of entire molecules is identified (Figure 1E and Table S3). It is induced by strong intermolecular hydrogen bonds between the sugar headgroups, similar to the previously reported monolayer structure of a GPI fragment,18–20 although the existence of a superlattice in principle does not require crystalline ordering of the entire headgroups. This supercell (green parallelogram), reminiscent of subgel phases in bulk,21–23 is commensurate with the hydrocarbon chain lattice (a' = 2 × achain, b' = 2 × bchain, γ = 110.1°) and, with an area of 86.8 Å², contains two MGDG-sat molecules. The rigid network of hydrogen bonds between galactose headgroups dictates the packing order of the chains (no change upon lateral compression). The full width at half-maximum (fwhm) of the Bragg rods (SI) agrees well with the length of an extended C18 alkyl chain in all-trans conformation, confirming that the interfacial layer is a monolayer at all investigated surface pressures.24,25

MGDG-unsat does not form ordered monolayers. Gel phases in 3D systems have been found only at extremely low temperatures (−30 °C).26 Obviously, the highly ordered structure in MGDG-sat monolayers is a synergetic result of concomitant headgroup and chain interactions.

DGDG-sat does not exhibit diffraction peaks in the mid-to-wide-angle region, indicating the absence of headgroup order. Only the three diffraction peaks defining the alkyl chain lattice are observed in the wide-angle region (Figure 1G). The bulky

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**Figure 1.** (A,F) Chemical structures of MGDG-sat and DGDG-sat. (B,G) GIXD contour plots displaying the scattered intensity versus the in-plane and the out-of-plane components of the scattering vector, Qxy and Qz, respectively, obtained for MGDG-sat (Π = 30 mN/m) and DGDG-sat (Π = 10 and 30 mN/m) monolayers on 1 mM CsBr. (C) Bragg peaks obtained for MGDG-sat. (D,H) Schematic side-view of MGDG-sat and DGDG-sat monolayers, respectively, on the water surface. (E) Schematic top-view representation of the lattice formed by MGDG-sat. The positions of chains and headgroups are indicated with black dots and blue stars, respectively. Red, black, and blue triangles indicate the repeating unit cell of the alkyl chains. The unit cell of the molecule lattice is indicated with a green parallelogram. Violet line: delimitation of the molecules. (I) Schematic top-view representation of the lattice formed by the alkyl chains of DGDG-sat.
digalactose moiety of DGDG-sat seems to disturb the packing of the headgroups, presumably due to a higher hydration degree, and offers higher flexibility to the molecules (noticeable decrease of the tilt angle during compression). The larger area requirement mismatch between the headgroup and chains leads to a higher tilt angle of the chains (Figure 1H and Tables S7 and S9). The lack of strong H-bonded and structured headgroups of DGDG-sat is in agreement with previous reports showing that a disaccharide sugar headgroup in glycerol-based glycolipids dramatically lowers the phase transition temperature compared to the corresponding molecule with a monosaccharide headgroup.15,27,28 Because the DGDG-sat in-plane molecular area is only slightly larger than that of MGDG-sat, the two sugar moieties cannot arrange parallel to the interface but more likely adopt a perpendicular or tilted arrangement. This is in agreement with previous infrared reflection–absorption spectroscopy (IRRAS) studies reporting a tilt angle of the digalactosyl headgroups of \(\approx 40^\circ\) (ref 14). GIXD data recorded for monolayers of DGDG-unsat display no diffraction peaks at all, indicating disordered chains and headgroups.

To tackle the problem of sugar specificity for headgroup interactions, LacCer-sat is investigated. As DGDG-sat, LacCer-sat features two sugar moieties but of a different nature (galactose and glucose units) and forms condensed monolayers at room temperature on the surface of 1 mM aqueous salt solutions. Yet, the GIXD data revealed a much more complex diffraction pattern with a multitude of peaks (Figure 2B,C). Contrary to MGDG and DGDG, a stronger influence of the subphase (Figure S6) and the formation of different polymorphs are observed. On a 1 mM CsBr subphase, the three intense diffraction peaks in the wide-angle region can be attributed to the alkyl chain order, while the five additional weaker peaks in the mid-to-wide-angle region indicate headgroup ordering. The existence of a headgroup order in monolayers and in bulk crystals of synthetic glycolipids...
bearing lactose units has already been reported. Thus, it seems that lactose headgroups are more prone to be engaged in intermolecular interactions and layer structuring than the digalactosyl headgroups. The supercell (Figure 2E, green parallelogram) defines an area corresponding to four LacCer-sat molecules (Tables S10−S12). The H-bonding for LacCer-sat seems to be more complex due to the possible additional contribution of amide−sugar interactions. Different polymorphs (Tables S13−S16 and Figure S3) have been observed but will not be discussed here in detail. Literature agrees well with the tendency of LacCer-sat in forming different polymorphs in 3D systems. The Debye−Scherrer rings, seen only in the differentiation pattern at high surface pressures (Π = 30 mN/m, SI Figure S3), indicate the formation of 3D crystals coexisting with a monolayer at the air/water interface. Such strong lactose−lactose interactions could be responsible for the formation of LacCer-enriched microdomains in biological systems (cell surface plasma membranes of mouse neutrophils, microdomains presenting a high specificity for antibodies).

Monolayers of Trihexo-sat, which features the bulkiest headgroup, are found to be characterized by ordered alkyl chains and nonordered headgroups. Interestingly, this compound exhibits the lowest alkyl chain tilt, t ≈ 20° at 10 mN/m, which further decreases to t $\lesssim$ 15° at 30 mN/m, depending on the subphase (Tables S17−22 and Figure S6). The role played by the glucose unit in headgroup interactions is investigated in Glu-sitosterol monolayers (monoglucose-based glycolipids with saturated alkyl chains are not commercially available). The GIXD data reveal a monolayer structure defined by five Bragg peaks (Figure 2G,H). Three of them describe the order of the cholesterol moieties, and the additional Bragg peaks indicate headgroup order. The observed superstructure with an area of 156.3 Å² corresponds to four Glu-sitosterol molecules (Table S25). The cross-sectional area per molecule of 38.4 Å² is in good agreement with values of 37.7 Å² obtained for pure cholesterol. This in-plane area allows the sugar headgroup to adopt a parallel orientation to the interface. Complex GIXD patterns have been often described in the literature and even for Langmuir monolayers of cholesterol, attributed to the formation of multilayers. In the present case, we refute such a scenario based on stable compression isotherms (Figure S5) and on the fwhm value of the Bragg rods (Table S23) corresponding to a monolayer. Similar to LacCer-sat, the Glu-

![Figure 3](image3.png)

**Figure 3.** Variation of the tilt angle with the lateral pressure of the structured glycolipid monolayers on 1 mM CaBr$_2$.

![Figure 4](image4.png)

**Figure 4.** Relative excess fluorescence intensities of K$^+$, I$^-$, Cs$^+$, Br$^-$, and Ca$^{2+}$ near various uncharged glycolipid monolayers on aqueous subphases containing 1 mM KI, CsBr, or CaBr$_2$. Data are averages over up to three measurements at Π = 10, 20, and 30 mN/m. Error bars represent one standard deviation. They are absent when only one data point was available. Horizontal dashed lines in panels (A) and (C) indicate the intensity level expected from a generic charge-neutralization effect (see the main text).
sitosterol monolayers exhibit stronger response to the subphase nature (Figure S6). Polymorphs of different thermodynamic stability, possibly induced by headgroup hydration/dehydration, are observed but again will not be discussed here in detail.

Overall, the investigated electrically neutral glycolipid monolayers can be divided into three classes: (i) highly structured monolayers, characterized by ordered headgroups and ordered alkyl chains (MGDG-sat, LacCer-sat, and Glu-sitosterol), (ii) structured monolayers with ordered alkyl chains but disordered headgroups (DGGD-sat, Trihexo-sat), and (iii) nonstructured monolayers with no headgroup and no chain order (MGDG-unsat and DGGD-unsat). Classes (i) and (ii) are distinguishable also by the variation of the tilt angle of the alkyl chains with the surface pressure (Figure 3). The highly structured monolayers in tendency exhibit less variation of the tilt angle due to the rigid headgroup lattice. Those are structured monolayers in tendency exhibit less variation of the headgroup hydration.38

TRXF experiments were carried out in order to quantify preferential interactions of ions with the glycolipid monolayers in terms of interfacial ion excesses per unit area. The method is highly sensitive to interfacial excesses because the X-ray beam is totally reflected and only illuminates the immediate vicinity of the interface with an evanescent wave.39–42 The excess of each ion type is then deduced from the intensity of its element-characteristic X-ray fluorescence. Importantly, in the presence of the 1 mM salt used in the TRXF experiments, the Debye length ($\kappa^{-1} \approx 10$ nm for KI and CsBr, $\kappa^{-1} = 6$ nm for CaBr$_2$) is comparable to the intensity decay length of the evanescent wave ($\Lambda \approx 7$ nm) for the used combination of incident angle ($\theta = 0.11$ or 0.07°) and beam energy ($E = 8.0$ or 15.0 keV). The fluorescence intensity of an ion species preferentially interacting with the surface is therefore higher than that of the corresponding counterion species, which approximately obeys a Gouy–Chapman distribution to achieve charge neutrality on the length scale of $\kappa^{-1}$ (ref 43) but does not reach a 1:1 ion stoichiometry within the illuminated volume. As a consequence, the measurements allow identification of the preferentially interacting ion species for each salt type.

Figure 4 shows the relative excess fluorescence intensities, $I_{ex}$ of K', I', Cs, Br, and Ca near monolayers of MGDG-sat, DGGD-sat, LacCer-sat, Glu-sitosterol, MGDG-unsat, and Trihexo-sat on aqueous subphases containing 1 mM KI, CsBr, or CaBr$_2$. Data are averages over up to three measurements at $\Pi = 10, 20$, and 30 mN/m. $I_{ex} = (I - I_0)/I_0$ is the relative change in the measured intensity $I$ with respect to the intensity $I_0$ expected in the absence of any preferential interactions, i.e., assuming bulk-like ion concentration up to the monolayer surface. $I_0$ in turn, is obtained by measuring the intensity from the bare aqueous subphase, $I_{bare}$, and taking into account the reduction of the illuminated aqueous volume in the presence of the monolayer in the form of a prefactor $f$, $I_0 = f \cdot I_{bare}$. This prefactor is given by the electron density profile of the monolayer and robust with respect to minor uncertainties in the characteristics of these profiles (SI). The ion distributions at the bare air/water interface are approximated as constant, neglecting minor deviations affecting $I_{bare}$ only by few percent.39 As shown in the SI, the employed methodology is consistent with an absolute intensity calibration using charged monolayers.

Positive values of $I_{ex}$ in Figure 4 correspond to an accumulation of the respective ion species at the respective monolayer, and negative values correspond to a depletion. It is seen that significant ion accumulation occurs only for certain monolayer/salt combinations. Strong accumulation is observed for K’ near monolayers of MGDG-sat, which implies preferential interactions of K’ with the monolayer surface. The moderate excess of the counterion I’, on the other hand, must be interpreted as a secondary effect of charge neutralization. The horizontal dashed line in Figure 4 indicates the intensity level expected from this secondary effect within a Poisson–Boltzmann model described in the SI. The measured I’ excess is somewhat below this estimate, suggesting even slightly unfavorable interactions of I’ with the interface. The magnitude of the K’ excess, as deduced from $I_{ex}$ within the model, is $\Gamma_{K'} \approx 0.02$ nm$^{-2}$, corresponding to an area per adsorbed ion of $A_k = 1/\Gamma_{K'} \approx 50$ nm$^2$ or 1 ion per approximately 100 lipids. This excess is roughly 2 orders of magnitude smaller than previously measured ion excesses compensating a certain number of charges per lipid in charged lipid monolayers.41,42 Nonetheless, the preferential interaction of K’ with the charge-neutral monolayer is significant. At this point, it should be noted that neither charged impurities nor monolayer ionization by X-ray irradiation can be the cause for the observed accumulation because other cations including the divalent Ca$^{2+}$ ions do not accumulate significantly at the same surface under the same conditions (Figure 4A). The same reasoning holds for other monolayer/salt combinations. A similar extent of ion accumulation is found for KI near monolayers of LacCer-sat (Figure 4C). Interestingly, the situation is reversed: I’ instead of K’ ions exhibit preferential interactions with this interface, with an ion excess of $\Gamma_{I'} \approx 0.014$ nm$^{-2}$. The horizontal dashed line again indicates the intensity level expected from a generic charge-neutralization effect. Pronounced ion accumulation is also found for Ca$^{2+}$ near Glu-sitosterol monolayers (Figure 4D), with $\Gamma_{Ca} \approx 0.012$ nm$^{-2}$. The depth of the ion-adsorbing potential, $\Delta G$, can be estimated from $\Gamma$ via Boltzmann inversion, $\Delta G = -k_B T \ln(\Gamma/\Gamma_0)$, where $k_B$ is the Boltzmann constant and $d$ the width of the ion-adsorbing region. For a reasonable d range ($2 < d < 7$ Å), we obtain $\Delta G = -11.2 \pm 2$ kJ/mol (K’ at MGDG-sat), $\Delta G = -10.3 \pm 2$ kJ/mol (I’ at LacCer-sat), and $\Delta G = -9.7 \pm 2$ kJ/mol (Ca$^{2+}$ at Glu-sitosterol). These numbers roughly correspond to 1/3 of the free energy per hydrogen bond in water.45

All monolayers exhibiting pronounced preferential interactions with at least one ion species belong to monolayer class (i) featuring headgroup order. On the other hand, monolayers of classes (ii) or (iii), without headgroup order, do not seem to exhibit any clear trends. This notion suggests that the defined structural motifs displayed by headgroup-ordered surfaces are responsible for the pronounced ion selectivity. It further provides a possible explanation for the observation that different headgroup chemistries, which lead to different structural motifs, exhibit selectivity for different ions. The selectivity likely arises due to a match between the hydrogen bond configurations inside of the defined saccharide ‘pockets’ and those of the ions’ hydration shells, which are known to be species-dependent. With that, the observed phenomenon appears to be related to the remarkable ion selectivity of
crown ethers owing to their characteristic polyether cavity. Indeed, crown ethers such as dibenzo-18-crown-6 (18C6), an 18-atom heterocyclic containing 6 oxygen atoms, selectively capture K+, while other polyether rings like 15-crown-5 (15C5) or 21-crown-7 (21C7) selectively capture Na+ (Ca2+) and Cs+ ions, respectively. Our results demonstrate impressively that uncharged hydrophilic surfaces in general and saccharide surfaces in particular can selectively attract ions, the species being dependent on the structural motifs displayed by the surfaces. At first glance, the determined ion excesses appear to be weak. Note, however, that the bulk ion concentrations in the present study were chosen to be very low. The excess increases with the bulk concentration (albeit underproportionally) and therefore can be expected to reach considerable levels at biologically or technologically relevant concentrations. Selectivity to only one ion species in a salt solution inevitably leads to a charge separation that, in turn, results in electrostatic interactions between Lewis X-related trisaccharides studied by NMR measurements of residual dipolar couplings. J. Am. Chem. Soc. 2007, 129 (29), 9080–9085.

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