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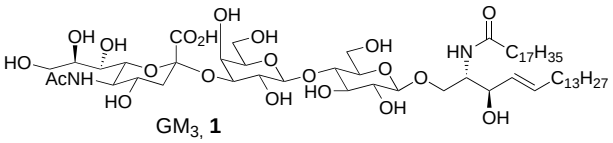
Carbohydrate–Carbohydrate Interactions

Probing Specificity in Carbohydrate–Carbohydrate Interactions with Micelles and Langmuir Monolayers**

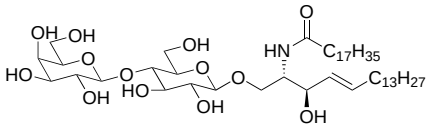
*Paul V. Santacroce and Amit Basu**

Carbohydrate–carbohydrate interactions (CCIs) between cell surface glycolipids are important mediators of cell adhesion during development, metastasis, and signal transduction.[1] CCIs are also important for the higher-order structure of polysaccharides, compaction of the myelin sheath, sperm–egg cell adhesion, and proteoglycan-mediated sponge cell aggregation.[2] The interaction has been studied by a variety of techniques including vesicle adhesion,[3a,b] surface plasmon resonance (SPR) spectroscopy,[3c,d] atomic force microscopy (AFM),[3e] nuclear magnetic resonance (NMR) spectroscopy,[3f,g] mass spectrometry (MS),[3h,i] infrared (IR) spectroscopy,[3j] surface force measurements,[,3k] quartz crystal microbalance (QCM) measurements,[3l] and Langmuir monolayer compression isotherms.[3m] These interactions, which are generally calcium ion dependent, require multivalent carbohydrate-–carbohydrate contacts, and the oligomeric nature of the association has hampered efforts to structurally characterize the carbohydrate aggregates at high resolution. In this communication we report a new method for detecting CCIs by monitoring the interactions of glycolipid micelles with a glycolipid monolayer. We show that small changes in carbohydrate structure significantly affect glycolipid association.

The adhesion of a melanoma cell to an endothelial cell is mediated by carbohydrate–carbohydrate recognition between the melanoma cell surface ganglioside sialosyllactosylceramide (GM_3, **1**) and the glycosphingolipid lactosylcera-



GM_3, **1**



LacCer, **2**

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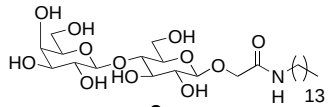
mide (LacCer, **2**) found on endothelial cells.[4] The GM_3–LacCer interaction activates signal transduction in B16 melanoma cells. The metastatic potential of the melanoma is correlated with levels of GM_3 expression in cells.[5] Multivalent lactose glycoconjugates inhibit melanoma metastasis, and a better understanding of the carbohydrate recognition event on a molecular level may aid in the design of better anti-melanoma agents.[5,6]

We sought a method for detecting this interaction which would be amenable for screening a variety of mutant glycolipids and would also allow us to modulate the membrane environment. We probed the specificity of CCIs with single-tailed glycolipids, since they readily self-assemble into polyvalent clusters in the form of micelles. Membrane morphology can be readily adjusted in Langmuir monolayers, which have been used extensively to study binding interactions at membrane interfaces.[3l,m,7] Glycolipid monolayers prepared over solutions of glycosylated polystyrenes show expanded pressure versus area isotherms and have been used to probe CCIs.[3l,m] The structure of the monolayer can be monitored readily by fluorescence or Brewster angle microscopy; the monolayers can also be transferred to solid supports for AFM, SPR, and other spectroscopic measurements.[8]

Although native glycolipid–glycolipid interactions occur in the context of a membrane environment, a ceramide tail is not required for the association, and CCI-mediated aggregation has been observed with oligosaccharides presented on a variety of multivalent platforms.[3c,d,f,l,m,9] We therefore prepared the simplified synthetic glycolipid **3** as a LacCer



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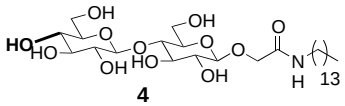

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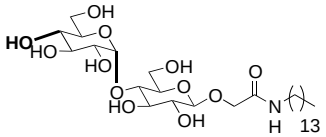

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analogue.[10] The critical micelle concentration (cmc) value for **3** was determined by monitoring the encapsulation of the fluorescent probe 1,6-diphenylhexatriene (DPH) and was found to be approximately 8 μM in 1 mM $CaCl_2$.[11]

We studied the CCI between **3** and GM_3 by preparing a Langmuir monolayer containing 10% GM_3, then injecting a micellar solution of **3** into the aqueous subphase under the monolayer.[12] After injection the change in the surface pressure ($\Delta\pi$) over time was monitored. A representative set of data showing the interaction of **3** with the GM_3 monolayer is given in Figure 1. When the concentration of **3** is well below the cmc, the values of $\Delta\pi$ decrease over time (Figure 1b). The magnitude of this decrease is similar to that observed in a "holding" experiment, in which no micelles have been injected into the subphase (Figure 1a).[13] However, at concentrations close to or above the cmc, the $\Delta\pi$ values increase over time, usually plateauing within an hour (Figure 1c–f). This increase is consistent with glycolipid insertion into the monolayer. Glycolipid **3** exhibits a concentration-dependent increase in "final" $\Delta\pi$ values ($\Delta\pi$ at 167 min), as shown in the uppermost plot in Figure 2. Additionally, injection of a methyl lactoside solution (100 μM) under the GM_3 monolayer also failed to result in any change in $\Delta\pi$ different from the "holding" experiment. The concentration dependence of $\Delta\pi$ also demonstrates that polyvalent presentation of carbohydrates is required. These

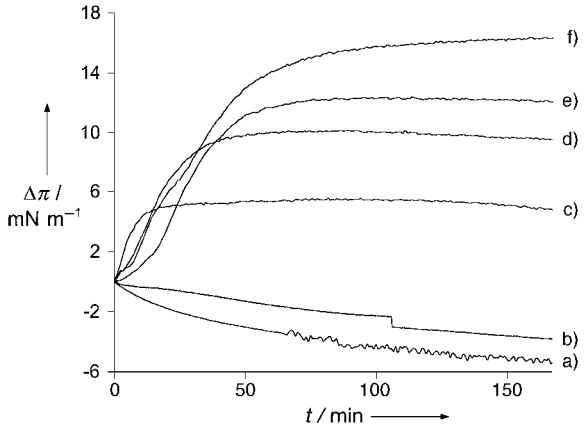

Figure 1. Plots of the change in surface pressure ($\Delta\pi$) over time for the binding of **3** to a DPPC/GM_3 (9:1) monolayer (initial pressure = 30 mN m^{-1}) at the following concentrations of **3**: a) 0 μM; b) 1 μM; c) 5 μM; d) 10 μM; e) 15 μM; f) 25 μM. DPPC = dipalmitoylphosphatidylcholine.

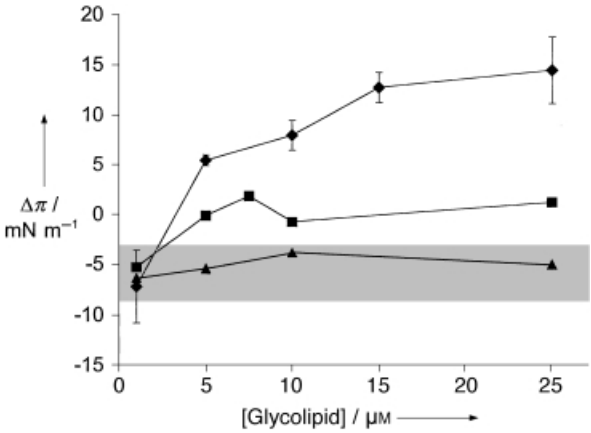

Figure 2. Final $\Delta\pi$ values (t = 167 min) as a function of the identity and concentration of the subphase glycolipid. The monolayer is DPPC/GM_3 (9:1); initial π = 30 mN m^{-1}). ◆ = **3**, ▲ = **4**, ■ = **5**. Each point represents the average of two or more measurements. The shaded region corresponds to the $\Delta\pi$ values observed in the absence of any micelles in the subphase (i.e. a "holding" experiment). Errors generally range from 5 to 20%, and a single representative set for measurements with glycolipid **3** is shown for clarity.

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results establish that CCIs can be detected with Langmuir monolayers and micelles as models for biomembranes.

To probe the fine specificity of the GM_3–Lac interaction we modified the structure of the lactose disaccharide slightly. We prepared the cellobiosyl lipid **4** (cmc $\approx 4\ \mu M$) and studied the interaction of micelles of **4** with a monolayer containing GM_3. Remarkably, the concentration-dependent response seen with **3** is not observed when minor modifications are introduced into the carbohydrate portion of the glycolipid. The conversion of a single stereocenter in the disaccharide by epimerizing the axial OH group in **3** to the equatorial OH found in **4** completely abrogates the binding at all concentrations examined (Figure 2).[14] This loss in binding might arise from a weakened interaction with the metal ion, as calcium ions preferentially bind *cis* diols on sugars.[15,16]

An alternative explanation for the smaller $\Delta\pi$ value of **4** may lie in the low intrinsic surface activity of **4**.[17] However, subsequent studies with **5** (cmc $\approx 5\ \mu M$), which has very similar surface activity to **3**, indicated that **5** did not exhibit a concentration-dependent change in $\Delta\pi$, even at glycolipid concentrations well above the cmc (Figure 2).[18] Thus, the increase in $\Delta\pi$ for **3** cannot simply be a result of its intrinsic surface activity.[19] In summary, very subtle modifications, that is, epimerization, of the carbohydrate headgroup of a lactosyl lipid result in significant changes in the interaction of the synthetic glycolipids with a GM_3-containing monolayer. This indicates that the concentration-dependent increase in $\Delta\pi$ seen with **3** is a function of carbohydrate–carbohydrate recognition.

Since the observed positive values of $\Delta\pi$ most likely arise from insertion of **3** into the monolayer, we postulated that decreasing the initial pressure of the monolayer should facilitate insertion and result in larger $\Delta\pi$ values. This is exactly what was observed in binding studies with **3** and **5** when the initial pressure of the monolayer was varied from 5 to 55 $mN\,m^{-1}$ (Figure 3).[20] At each pressure at which binding was examined the $\Delta\pi$ value for **3** is consistently higher than that observed for **5**, again indicating that surface activity alone cannot explain the observations. Furthermore, **3** continues to have positive $\Delta\pi$ values at starting pressures as high as 50 $mN\,m^{-1}$, while **5** does not show any appreciable binding above 30 $mN\,m^{-1}$, consistent with GM_3–Lac interactions at the interface.

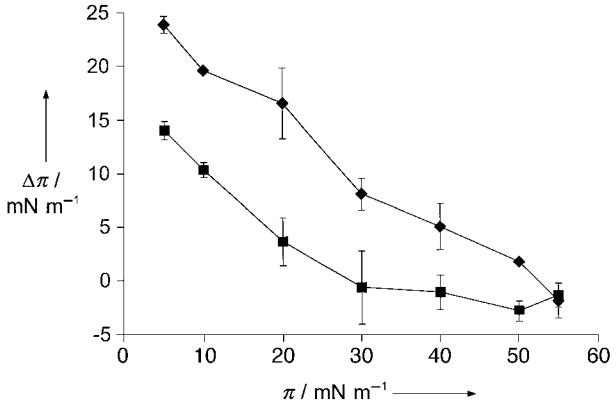

Figure 3. Plot of the final $\Delta\pi$ values as a function of initial monolayer pressure. Glycolipid concentrations of **3** and **5** are 10 μM. ◆ = **3**, ■ = **5**.

In conclusion, we have shown that CCIs can be studied by examining the interaction of glycolipid micelles with glycolipids embedded in a monolayer at the air–water interface. Furthermore, the GM_3–Lac interaction is highly specific for lactose, and very minor modifications to the structure of the carbohydrate headgroup have significant effects on binding. Our results indicate that CCIs involve very specific molecular recognition events between carbohydrates and does not arise simply from nonspecific interactions between two polyhydroxylated surfaces formed by membrane-bound glycolipids. In future studies directed towards elucidating the details of this molecular recognition phenomenon we will synthesize glycolipids that probe the role of calcium binding and examine the role of the formation of microdomains in the membrane in mediating CCIs.[4c]

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[10] The glycolipids were prepared from the corresponding peracetylated disaccharides by conversion into the glycosyl bromides with HBr followed by silver triflate mediated glycosylation with allyl alcohol. Subsequent ozonolysis, oxidation, and coupling to tetradecylamine followed by Zemplen deacylation provided the desired compounds. The synthetic glycolipids were characterized by ^{1}H and ^{13}C NMR spectroscopy and high-resolution mass spectrometry.

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[12] The Langmuir monolayer experiments were carried out on a KSV minitrough equipped with a Wilhelmy plate and balance. A chloroform/methanol solution of a 10% GM_3/90% DPPC mixture was deposited with a microsyringe on a subphase of 1 mM $CaCl_2$. The solvent was allowed to evaporate over 10 min, and the monolayer was compressed at a rate of 3 mm min^{-1} until the desired initial monolayer pressure for the binding experiment was reached. Once the monolayer was at the target pressure the barriers were stopped, and the subphase was stirred gently outside the barriers. The binding experiment was initiated by injection of 1.5 mL of a concentrated glycolipid solution into the subphase outside the Teflon barriers.

[13] Negative $\Delta\pi$ values are indicative of either lipid loss from the monolayers owing to the high solubility of the glycolipids and/or rearrangements within the monolayer after compression has ceased. See a) J. Esnault, J.-M. Mallet, Y. Zhang, P. Sinäy, T. LeBouar, F. Pincet, E. Perez, *Eur. J. Org. Chem.* **2001**, 253; b) G. L. Gaines, *Insoluble Monolayers at Liquid–Gas Interfaces*, Interscience Publishers, New York, **1966**, p. 143.

[14] Single-site modifications can have measurable effects on CCIs. For examples in the context of glycopolymer recognition, see references [3c,l,m].

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[16] Another observation consistent with this hypothesis is that the cmc value of **3** is higher in 1 mM $CaCl_2$ than in deionized water. This is in contrast to **4**, **5**, and Tween-80,[18] all of which show decreases in cmc values on going from water to 1 mM $CaCl_2$, as might be expected in a solution of greater ionic strength.

[17] The surface activities or equilibrium surface pressures for **3**, **4**, and **5** are: 26, 6, and 22 mN m^{-1}, respectively.

[18] Binding studies of the GM_3 monolayer at 30 mN m^{-1} with Tween-80, a nonionic hydroxy-terminated detergent, which has an equilibrium surface pressure of 28 mN m^{-1} and a cmc of 3 μM, also failed to show a concentration-dependent increase in $\Delta\pi$.

[19] When GM_3 was omitted from the monolayer, injection of 25 μM **3** or **5** afforded a $\Delta\pi$ of 8 mN m^{-1} (30 mN m^{-1} starting pressure). The rate of increase in π was slower in the absence of GM_3 which suggests that CCIs facilitate insertion by targeting the glycolipids to the interface. These observations indicate that in the absence of CCIs intrinsic surface activity becomes the primary contributor to $\Delta\pi$.

[20] Glycolipid **4** had a $\Delta\pi$ of 2.7 mN m^{-1} at an initial pressure of 10 mN m^{-1}. At all other pressures examined the $\Delta\pi$ value was indistinguishable from the values observed in a "holding" experiment.

Synthesis of Pyrroles and Furans

1,2-Migration of the Thio Group in Allenyl Sulfides: Efficient Synthesis of 3-Thio-Substituted Furans and Pyrroles**

*Joseph T. Kim, Alexander V. Kel'in, and Vladimir Gevorgyan**

The 1,2-migration of the thio group is an important chemical transformation that is extensively used in carbohydrate chemistry for stereoselective Mitsunobu-type substitution at the anomeric center [Eq. (1)].[1] There are also reports on employment of a 1,2-shift of the thio group in the synthesis of heterocycles [Eq. (2)][1a,b] Known 1,2-migrations of the thio group can be classified as one of two types: 1) An S_N2-type attack of the lone pair of electrons of the sulfur atom at the adjacent sp^3 center in **A** produces the thiiranium intermediate **B**, which after subsequent nucleophile-assisted ring opening affords **C**, a product of 1,2-migration of the thio group [Eq. (1)].[1] 2) The migration is triggered by attack of the sulfur atom at the sp^2 carbon atom of the iminium[2a,b] or imine[2c] moiety of **D** to form the thiiranium species **E**. The latter either produces sulfide **F** through nucleophilic attack[2c] or gives the thioenamine **G** as a result of a deprotonation/ring-opening process [$R^1 = H$, Eq. (2)].[2a,b] In all cases the migrations of the thio group proceeded from an sp^3 center to either another sp^3 [Eq. (1)][1] or to an sp^2 [Eq. (2)][2] carbon center. To the best of our knowledge, there are no reports of 1,2-migration of the thio group from an olefinic carbon atom.

Herein we wish to report a novel 1,2-migration of the thio group from an sp^2 carbon atom in allenyl sulfides. This unprecedented migration allowed the development of an

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

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