Kinetic studies of protein-carbohydrate interactions at the bilayer interface of catanionic vesicles

Mohammad R Islam
Faculty: Douglas English

Department of Chemistry

Abstract. In this paper, we describe our work on surface functionalization and modification of catanionic vesicles with an aim toward increasing their range of applications. Conventional vesicles are formed from double-tailed phospholipids and have important roles in biology. Unlike conventional phospholipids vesicles, aqueous mixtures of cationic and anionic single-tailed surfactants can spontaneously form unilamellar vesicles without sonication or extrusion. These vesicles dubbed "catanionic vesicles" in the literature are extremely stable with respect to salt and pH and are composed of inexpensive components. They are promising candidates for a variety of biotechnological applications including drug delivery and vaccine development. Our studies report that the ability to control the distribution of glycoconjugates in the vesicle bilayer surface provides a method to study protein-carbohydrate multivalent binding kinetics in a biomimetic environment. In this work, the exterior of catanionic vesicles was controllably functionalized by insertion of the hydrocarbon chain of the glycoconjugate n-dodecyl-β-D-glucopyranoside (C_{12}-glucose) at varying concentrations. We demonstrate how this platform consisting of a carbohydrate functionalized bilayer can be use to evaluate binding inhibitors for the lectin ConA.

1. Introduction

Protein-Carbohydrate interactions are prevalent throughout biology and play important roles in cell-cell recognition including the infectivity of pathogens, immune response and reproduction [1]. Interactions between carbohydrates and proteins are typically weak. To provide interaction strength and specificity, it is possible for proteins to interact with carbohydrates in a multivalent fashion where more than one binding event occurs simultaneously. In biological systems, multivalent binding is facilitated by imbedded a protein or carbohydrate in cell membrane and the affinity depends on both ligand density and spatial arrangements. Double-tailed phospholipids vesicles have been extensively used as model membrane systems to study interactions at the bilayer interface. In this paper we use a novel type of vesicle developed in our lab which consists of inexpensive single-tailed surfactants and has the advantages of superior stability and easier preparation compared to conventional carbohydrate functionalized vesicles or bilayers. We use a mixture of cationic and anionic single-tailed surfactants that can form unilamellar vesicle and add varying amounts of a glucose-based glycol-lipid. These new vesicles and our ability to vary carbohydrate concentrations over a wide range should increase their potential in biotechnological applications including targeted drug delivery and as new tools in the area of glycomics.

2. Experiment, Results, Discussion, and Significance

SDBS-rich catanionic vesicles were prepared simply by dissolving 3 to 1 mole ratio mixture of surfactants sodium dodecylbenzenesulfonate (SDBS) and cetyltrimethylammonium tosylate (CTAT) in water maintaining total surfactant concentration 1 wt%. Vesicle surface were decorated by hydrophobic insertion of the 12 carbons alkyl chain of the glycoconjugate n-dodecyl-β-D-glucopyranoside (C12-glucose). Various C12-glucose mole fractions in the bilayer vesicle surface were obtained in the range of 0.0 to 0.05 in a controllable fashion.

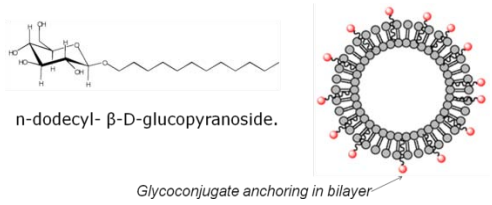

Fig 1: Glycoconjugate structure and cartoon depiction of functionalized vesicle surface.

Aggregation of this C12-glucose modified SDBS-rich vesicle was observed upon addition of the lectin Concanavalin A (ConA). ConA is a tetrameric protein and has four binding sites. Strong binding between ConA and glucose occurs in a multivalent fashion when more than one simultaneous binding

event is possible. Our ability to control C12-glucose densities in the bilayer enables us to find the C-12 glucose densities where multivalent binding is enabled. This method provides us a way to determine the effective binding site separation distance of ConA and glucose [3].

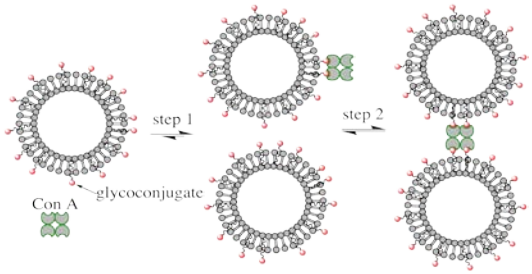

Fig 2: Cartoon depiction of ConA induced aggregation of functionalized vesicle.

ConA induced aggregation was monitored by turbidity change in a stopped-flow instrument after rapid mixing with functionalized vesicles. Kinetic assay of aggregation was obtained from turbidity at 450nm as a function of time. Binding kinetics were monitored by evaluating the initial binding rate from the first half second of a kinetic plot. A linear regression was applied to acquire the initial rate of ConA-glucose multivalent binding.

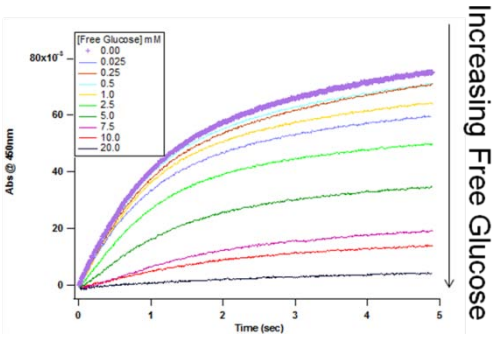

Fig 3. Inhibition binding assay of ConA and C12-glucose imbedded in catanionic vesicle bilayer by addition of free water soluble glucose in buffer.

To evaluate the role that the bilayer plays in facilitating multivalent binding, competitive inhibition experiments were conducted using free glucose in solutions. In this experiment, free glucose was added in the vesicle sample prior to rapid mixing with ConA in the stop flow instrument and inhibition binding assay was obtained. Figure 3 shows that binding rate decreases uniformly with the increasing concentration of free glucose inhibitor. Results shows that 100% inhibition occurs at free glucose concentration 20.0 mM when concentration of bilayer c12-glucose is about 0.035 mM. The result shows that roughly 600 times more inhibitor free glucose is required to completely inhibit multivalent binding of ConA and glucose illustrating how important the bilayer is in facilitating multivalent binding.

3. Conclusions

Catanionic vesicle of CTAT-SDBS system has been functionalized in a controlled fashion for kinetic studies of ConA-glucose multivalent binding. This method can be a model platform for investigating multivalent binding. Also this system can be a useful tool in evaluating competitive inhibitors of multivalent binding.

4. Acknowledgements

This work was supported in part by a grant from the NSF (CTS-0608906) and by the Wichita State University Foundation.

[1] Taylor, M. E.; Drickamer, K. Introduction to Glycobiology, 2nd ed.,Oxford University Press: New York, 2006.
[2] E. W. Kaler, A. K. Murthy, B. E. Rodriguez and J. A. N. Zasadzinski, Spontaneous Vesicle Formation in Aqueous Mixtures of Single-Tailed Surfactants, Science, Vol. 245, p. 1371-1374, 1989.
[3] G. B. Thomas, L. H. Rader, J. Park, L. Abezgauz, D. Danino, P.DeShong and D. S. English, Carbohydrate Modified CatanionicVesicles: Probing Multivalent Binding at the Bilayer Interface, J. Amer. Chem. Soc., Vol. 131, p. 5471-5477, (2009)