482f Construction of Extracellular Matrix Mimics and Their Effect on the Kinetics and Thermodynamics of Receptor-Ligand Binding on Supported Lipid Bilayers

Jessica M. Tucker, Todd M. Przybycien, and Robert D. Tilton

A key to information flow and recognition in cellular processes is receptor-ligand binding. The response of cells to hormones such as insulin, for example, is dictated by the binding of the protein to receptors on the cell surface(1). Protein and polysaccharide molecules excreted by cells compose the extracellular matrix (ECM) that helps form tissues. The ECM can extend on the order of 100 nm or further from the surface of the cell(2). When we consider a large ligand such as immunoglobulin G, an antibody with approximate dimensions of 16 x 11 nm, the ECM likely provides a diffusive barrier for approaching the cell surface. The role of the ECM as a diffusive barrier for proteins approaching the cell surface is not well understood, however. Our goal is to quantify this potential barrier effect using supported lipid bilayer cell membrane mimics that include ECM mimics. Lipid vesicle adsorption was used to construct supported lipid bilayers. Vesicles of approximately 100 nm in diameter as confirmed by dynamic light scattering were formed by an extrusion method and consisted of egg phosphatidylcholine (egg PC), dinitrophenyl tagged phosphatidylethanolamine (dNP-PE), and, when necessary, biotinylated lipids. Adsorption to a silica surface was monitored using quartz crystal microgravimetry with dissipation (QCM-D). A bilayer configuration was confirmed using the Sauerbrey equation to calculate a surface area per molecule of approximately 53 $Å^2$, in close agreement with published data(3). The kinetics of vesicle adsorption were also confirmed: the merging and bursting of vesicles to create a bilayer on the surface can be monitored qualitatively using the dissipation factor. While there was negligible nonspecific adsorption of immunoglobulin G (IgG), specific adsorption of anti-dNP IgG did occur on this bilayer. Thus, an intact lipid bilayer was constructed, and some dNP is accessible for antibody binding. To investigate transport effects, an ECM mimic was developed. Two ECM mimics were anchored to the bilayer and evaluated: hydrophobically-modified hydroxyethyl cellulose (hm-HEC) and biotinylated hyaluronic acid (b-HA). In the case of b-HA, biotinylated lipids were present in the lipid bilayer. Avidin was then used to attach the b-HA to the bilayer. The effect of ionic strength on the swelling behavior and the viscoelastic properties of these mimics will be presented. Factors such as the relative concentration of avidin and b-HA were used to tune the thickness of the layer to more closely approximate conditions in the biological ECM. QCM-D and total internal reflection fluorescence (TIRF) were used to examine protein binding in the presence of these ECM mimics, and the kinetic and thermodynamic impact of the ECM mimic will be discussed.

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