Thesis Title	Development of New Methods for Characterization of Sugar-Lectin Interaction
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ABSTRACT

Sugar specificity and the binding constant between a lectin and its specific sugar are basic characteristics of a lectin. In this study, two new methods were developed, one for identification of the sugar specificity and the other for measuring the binding constant. A homogeneous method was developed in this study for the determination of the sugar-binding constant (Ka) for a lectin. The method was based on enzymatic assay of free sugar in the presence of lectin-bound sugar using steric hindrance and kinetic assay principles. Using the new homogeneous method and the Hill equation, the binding constant (Ka) of Con A was measured to be $(4.9\pm0.8)\times10^{3}$

M^{-1} for mannose and (2.85±0.42)x10^3 M^{-1} for glucose. For the binding of galactose to *Artocarpus heterophyllus* or jackfruit lectin (JFL), the Ka value was determined by this method to be (3.66±0.88)x10^3 M^{-1}. In addition, the method showed that there were 4 carbohydrate-binding sites for Con A and 2 binding sites for JFL. The binding constant of mannose to Con A was found to increase in the presence of 1 mM Mn^{2+} or 1 mM Ca^{2+}. By comparison, the binding constant obtained by the homogeneous method was higher than that obtained by the equilibrium dialysis but not different from the published data.

Sugar-lectin binding assay (SLBA) was developed as a simple method which employed direct coating of microtiter plate with galactose-binding lectins. Biotin-galactose conjugate was synthesized and used to bind to the immobilized lectins. The bound conjugate was then detected using streptavidin-horseradish peroxidase. JFL was shown to be specific for α-D-galactopyranoside using SLBA in conjunction with various competing sugars. Comparison with the maximal inhibition and the concentration for half maximal inhibition values among 28 sugars tested, 8 were inhibitory in the following decreasing order: p-nitrophenyl-α-D-galactopyranoside, p-nitrophenyl-N-acetyl-α-D-galactosaminide, p-nitrophenyl-N-acetyl-β-D-galactosaminide, methyl-α-D-galactopyranoside, α-D-melibiose, p-nitrophenyl-β-D-galactopyranoside, D-galactose and N-acetyl-D-galactosamine. The di-, tri- and oligosaccharides of galactose were either weakly inhibitory or non-inhibitory. The results with JFL suggested that SLBA should be useful in identifying the sugar specificity of other lectins using appropriate sugar-biotin conjugates.