

Tuanta Getsang 2007: Expression of a Recombinant Mulberry Leaf Lectin in Bacterial Host and Computational Structure Analysis. Master of Science (Biochemistry), Major Field: Biochemistry, Department of Biochemistry. Thesis Advisor: Assistant Professor Amornrat Promboon, Ph.D. 92 pages.

N-glycolylneuraminic acid (Neu5Gc) specific lectins, named MLL1 were previously purified, characterized and full-length cDNA were successfully cloned using RACEs strategy. In this thesis, the coding sequence of the mature MLL1 were subcloned, sequenced and expressed in *Escherichia coli* BL21 (DE3). Moreover, this study is focused on homology modeling and the interaction between MLL1 and Neu5Gc was studied by molecular docking simulation using AutoDock 3.0.5 program. The resulting, in order to obtain the high amount of soluble recombinant protein, the recombinant cells were induced with 0.5 mM IPTG and 0.4% glucose at 25 °C. The recombinant MLL showed an apparent molecular mass of about 20 kDa in Tricine-SDS-PAGE analysis, while calculated mass by computing program is 25 kDa. Purified MLL was obtained from DEAE-ion exchange and Ni<sup>2+</sup>-NTA affinity column chromatography. This protein did not show any hemagglutination activity. The result from computational analysis showed that MLL preferred to bind with Neu5Gc a sialic acid derivative better than GalNac and Gal, respectively. The result is in good agreement with carbohydrate-binding specificity of the mulberry leaf lectins. Serine and Isoleucine in binding site played an important role in sugar binding specific binding. In molecular docking, Gly-6 forms strong H-bond with glycolyl group of Neu5Gc. The Ile- 84 and Trp-128 contributed hydrophobic interactions to the sugar. Serine in binding site of MLL may be played an important role in the recognition of the sialic acid carboxylate. Additionally, the electrostatic distribution on molecular surface of MLL is neutral nearly positive charge. These reasons may be the important recognition processes for the strong affinity and specificity of MLL for acidic sugar as Neu5Gc a sialic acid derivative.

Tuanta Getsang  
Student's signature

Amornrat Promboon  
Thesis Advisor's signature

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