Supaporn Seetaha 2010: Expression of Mulberry Leaf Lectin in Bacterial and Insect Cells and Docking Studies between Lectin and Sugars in Computer. Master of Science (Biochemistry), Major Field: Biochemistry, Department of Biochemistry. Thesis Advisor: Assistant Professor Amornrat Promboon, Ph.D. 215 pages.

Mulberry leaf lectin1 (MLL1) is a lectin that is found in Mullberry leaf (*Morus rotunbiloba Koidz.*) that bind specifically to a sialic acid. The sialic acid is a sugar that is found on the surface of pathogenic bacteria and cancer cells. In our research we tried to express MLL1 protein in large scale and increase specificity of lectin to the sialic acid. We cloned and expressed the lectin protein in bacteria and insect cell, we expressed the protein from bacteria cells system by cloning the MLL1 gene into pET21a in 3 forms. That is fMLL, nsMLL and LMLL. However our result showed no expression of pET-fMLL. Furthermore, the pET-nsMLL showed the expression of major protein at 13 kDa and minor one at 9 kDa. Only pET-LMLL showed the expression major protein at 16 kDa and minor one at 13 kDa. However, when all bacterial expressed proteins were checked for aggregation of rabbit red blood cells, none of them has an activity at all. Next, we further expressed the protein from Insect cells system by cloning the MLL1 gene into pFastBac HT-A. in 2 forms. That is fMLL and nsMLL, our result showed no expression of both pFast-fMLL and pFast-nsMLL in the insect cells was observed. After that, we turned to use the computational method to understand molecular interaction with sialic acid. The homology modeling of LMLL was created by using PDB access code 1JOT as a template. The 3D model LMLL was docked with GalNAc, Galβ1,3GalNAc and Neu5Gc and with AutoDock 3.0.5 and visualized by Discovery Studio 2.1. We found that LMLL can bind to Neu5Gc with highest interaction energy as shown in the previous experiment. The interaction was further investigated by molecular dynamics simulation to understand the forces for the sugar binding. To find the mutant that bind to Neu5Gc stronger, we further did *in silico* site direct mutagenesis one by one amino acid in the binding site. All of mutants model were docked with Neu5Gc and GalNAc. We found that if we changed Phenylalanine at position 47 to Asparagine (F47N), the different between the binding energy of Neu5Gc and GalNAc was most increased. Therefore, our knowledge can be use for further modify the lectin to get the protein in the medical application in the future.

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