PURIFICATION & CHARACTERIZATION OF GLYCANS OF UROMODULIN FROM ANEMIA PREGNANT WOMEN

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ABSTRACT

Tamm-Horsfall protein (THP) and Uromodulin (UM) are glycoproteins produced and excreted from kidney to urine. The molecular size of THP and UM is about 80kDa. The carbohydrate moiety of THP and UM is 30% of the total molecular weight. THP and UM contained both O-glycans and N-glycans. THP and UM have same amino acid sequence but different in glycosylation part. UM is representing THP in pregnancy. The main role of THP and UM is immunosuppressive effect on T-cell. UM have 13 fold more effective in immunosuppressive effect than THP due to the glycosylation different. The glycosylation of UM in pregnant women associated to anemia is studied in this paper. UM of normal pregnant and THP of female anemia and male anemia were act as a control. In this study, the UM was isolated from anemia and normal pregnant women urine in second trimester and third trimester of pregnancy period. The percentage of protein contain of each samples was measured by Bradford method. It found that the percentage of protein content of fifth month pregnancy is highest in anemia pregnant, while third month pregnancy contain the highest amount of protein in normal pregnant. The averages of protein content in non-pregnant and male anemia patients were higher than anemia and normal pregnant. The age of donor, pH and volume of urine have no correlations with percentage of protein content. The efficiency of three purification methods i.e. sephadex G100 gel filtration, sepharose 6B gel filtration and 10% SDS PAGE were compared. The Sephadex G100 gel filtration was found as the most suitable method due to the white colour cotton-like protein pellets containing desired glycoproteins was obtained. The biological characteristic of UM’s glycans and THP’s glycans were analyzed by using antibacterial screening. It found that all the N-glycans samples from anemia pregnant, normal pregnant, female anemia and male anemia groups have generated inhibition zone against E.coli O157:H7 agar culture. The average size of inhibition zone is the highest in the female anemia and male anemia groups (3.11 cm), and lowest in normal pregnant group (2.61 cm).