ENZYMATIC SYNTHESIS OF GLUCAN DENDRIMER FOR PHARMACEUTICAL APPLICATIONS

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Bio-macromolecules (e.g. protein, peptide, antibody DNA or RNA) present in our bodies are now widely utilized in pharmaceuticals. Carbohydrates, on the other hand, have been receiving increasing attention as drug candidates, but the pharmaceutical usage of carbohydrates is limited to few glycosaminoglycans (e.g. heparin, hyaluronan or chondroitin sulfate), which are all endogenous polysaccharides. Glycogen is another polysaccharide present in our bodies as energy reserves, and its application for pharmaceuticals is not evident either.

Glycogen is a highly branched polysaccharide of glucose with a very attractive structure and characteristics. It is a single molecular nano-sized spherical particle with dendritic architecture where numerous non-reducing ends constitute the surface of the molecule. Dendrimers and nano-sized particles have received interest as carriers for drug delivery system because such carriers can enhance the performance and efficacy of drug molecules. From this context, we consider glycogen as potentially a promising polysaccharide as drug carriers; therefore we are currently attempting to develop synthetic glucan dendrimers suitable for drug carriers. Such attempts have been scarce in the recent research of drug candidates.

We have developed an enzymatic system to produce glucan dendrimer (GD) from sucrose by combined action of sucrose phosphorylase (EC: 2.4.1.7), glucan phosphorylase (EC: 2.4.1.1) and branching enzyme (EC: 2.4.1.18). This system enables us to produce GDs with strictly controlled molecular size (Mw/Mn value less than 1.1) and particle size ranging from 10 nm to 40 nm. Furthermore, we have developed non-reducing end specific glycosylation technology of GD by using glucan phosphorylase and its substrate analogs. Glucan phosphorylase from *Aquifex aeolicus* can use not only its original substrate, glucose 1-phosphate, but also other hexose 1-phosphates as substrate and transfer these hexoses moieties (glucuronic acid, glucosamine, N-acetyl glucosamine, galactose and mannose) to the non-reducing end of glucan. Using this enzymatic reaction, we can produce GDs whose surface is modified with these hexose residues. GDs having glucuronic acid and/or glucosamine residues are especially useful since they can be covalently conjugated with functional substances such as sugar chains, peptides, nucleotides and others. It is possible to control the conjugate ratio of the functional substance on the surface of GD. Surface engineered GD is a novel and versatile platform for carbohydrate drugs and drug carriers, and its application will be described.