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Enzymatic Transformation of Physiologically Active Compounds

----Glycosylation and Phosphatidylation----

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In plants, most of the compounds having unstability, toxicity, and hydrophobicity or volatility, occur in glycosylated forms.

It suggests that glycosylation is an excellent biotransformation method for preparing physiologically active compounds with a high stability, non-toxicity and water-solubility and so on. In contrast to enzymatic glycosylation(solubilization in water), enzymatic phosphatidylation(solubilization in organic solvents) may be a suitable method to improve the physical properties and modify its biological activities of physiologically active compounds, because the phosphatidyl residue is well characterized as a non-toxic carrier moiety that has a high affinity for cell membranes and gives stability to hydroxy compounds in water systems by forming lipid-microsphere-like bodies. The present paper describes our recent works on the enzymatic glycosylation and phophatidylation of physiologically active compounds.

I. Glycosylation of vitamins.

1. Thiamin glycosides.

Glycosylated thiamin has not been reported to be synthesized chemically or biologically. Three new derivatives of thiamin(thiamin β-galactoside, thiamin α-glucoside, and thiamin β-N-acetylglucosaminide) were enzymatically synthesized

from nitrophenyl β-glycosides(or dextrin) and thiamin by several glycosidases and cyclomaltodextrin glucanotransferase(CGTase), respectively. All these glycosides were odorless, mildly sweet with no stimulative tongue-pricking taste, and were more stable than thiamin · HCl in aqueous solutions at pHs 7.0 and 9.3. Thiamin β-galactoside and thiamin α-glucoside showed about 50% and 80% biological activities of the equivalent moles of thiamin · HCl, respectively, against thiamin deficient male rats.

2. Pyridoxine glycoside.

Vitamin B_6 occurs naturally in plants mainly in conjugated forms of glucose and pyridoxine(PN). A remarkable accumulation of 5′-O-(β-D-glucopyranosyl) pyridoxine(PN 5′-β-glucoside) was observed in soybean seedlings and its suspension cells cultured in a PN solution. Many other PN-glycosides, such as two PN α-glucosides(4′- and 5′-), two PN β-galactosides, PN 4′-β-glucoside, PN 5′-β-fructoside, two PN α-mannoside, PN 5′-α-galactoside, and two PN β-N-acetylglucosaminides were synthesized enzymatically and biologically. Comparative studies on bioavailability of PN and PN glycosides by B_6-deficient rats revealed that PN α-glucosides served as well as PN, as a B_6-nutrient.

II. Glycosylation of flavonoids and phenolic compounds.

B. Stearothermophilus CGTase regioselectively transferred the glucosyl residues from dextrin not only to the OH groups at the C-4 positions of glucose moieties of rutin and hesperidin, but also to the OH groups at the C-3 positions of glucose moieties of both naringin and naringin dihydrochalcone, and the resulting 4^G-α-D-glucopyranosyl-rutin, 4^G-α-D-glucopyranosyl-hesperidin, 3^G-α-D-glucopyranosyl-naringin and 3^G-α-D-glucopyranosyl-naringin dihydrochalcone were obtained. The solubilities of two glucosides of rutin and hesperidin in water were about 30×10^3 and 10^5 times higher than those of rutin and hesperidin, respectively. Moreover, the CGTase catalyzed the transglycosylation reactions from dextrin to CH_2OH groups of thiamin, PN, riboflavin, and also to OH groups of phenolic compounds such as phenol, pyrocatechol, pyrogallol. gallic acid, protocatechuic acid and sesamol. All glucosylated phenolic antioxidants were much more stable than aglycones against the oxidation by peroxidase in the presence of hydrogen peroxide.

III. Glycosylation of ginsenosides.

CGTase were observed by Y. H. Kim et al. to have a higher transglycosylation activity to ginsenosides Rb_1, Rc, Re and Rg_1, and several α-glucosides of these compounds were obtained by the combined actions with glucoamylase. Recently, they have reported rice α-glucosidase and rat small intestine homogenate catalyze the transglycosylation reaction from maltose to ginsenosides Rg_1 at a considerable efficiency. Six α-glucosides of ginsenoside Rg_1 were isolated and identified. Moreover, two β-xylosides of ginsenoside Re were enzymatically synthesized by S. R. Ko et al. from p-nitrophenyl β-xyloside or phenyl β-xyloside and ginsenoside Re by crude fungal glycosidases. Also, jack bean α-mannosidase catalyzed the transformannosylation reaaction from methyl α-mannoside to ginsenosides.

IV. Phosphatidylation of physiologically active compounds.

Streptomyces sp. phospholipase D(PLD) was observed to be highly active in transferring the dipalmitoyl-phosphatidyl(DPP) residue from 1,2-dipalmitoyl-3-sn-phosphatidylcholine(DPPC) to the CH_2OH group in the acceptors such as vitamins(B_1, B_2, PN, pantothenic acid, and B_1 disulfide-related compounds), arbutin, kojic acid, genipin, and dihydroxyacetone in biphasic system. DPP-arbutin and DPP-kojic acid showed the same inhibitory activity to tyrosinase as arbutin and kojic acid, respectively. DPP-genipin showed 6-52 times stronger cytotoxicity than genipin to HeLa, HEL, and MT-4 cells. DPP-genipin was found to react with L-phenylalanine in organic solvents to give a clear blue solution having a similar color to an aqueous solution of the natural blue pigment "gardenia blue". This is the first example for the preparation of hydrophobic pigment from a phosphatidyl derivative of water-soluble compounds. Moreover, the PLD was first found to bring about the transfer of DPP-residue from DPPC to aromatic hydroxy group of acceptors such as various phenols, naphthol, and 5-hydroxyindole in a biphasic system of an organic solvent with low water solubility and buffer.