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Structural basis of production differences in naringenin α-glycosides catalyzed by amylosucrases

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Naringenin, a flavanone abundant in herbs and fruits, has antioxidant, anti-inflammatory, immunomodulatory, and antibacterial activities, but its poor solubility and stability limit applications. Amylosucrases (ASases, E.C. 2.4.1.4) catalyze transglycosylation, attaching glucose moieties to acceptor molecules and thereby enhancing solubility. ASases from *Deinococcus geothermalis*, *Deinococcus planocera*, and *Deinococcus wulumuqiensis* (DgAS, DplAS, and DwAS) transfer glucose units to the 4′-hydroxyl group of naringenin to yield naringenin-4′-O-α-glucoside (NaG) and naringenin-4′-O-α-maltoside (NaM). DgAS and DwAS predominantly produce NaG, whereas DplAS predominantly yields NaM. To elucidate this difference, we generated ensemble structures of glycosyl-enzyme intermediates via molecular dynamics simulations, docked naringenin and NaG, and compared the resulting complexes. The DplAS–NaG docking complexes exhibited more plausible binding conformations than DgAS-NaG and DwAS-NaG. Although most active-site residues are conserved, residues 295–297 differ, with Thr–Asp–Cys in DgAS and DwAS and Gly–Ala–Ser in DplAS. This substitution in DplAS widens the entrance to the active pocket, facilitating the accommodation of larger acceptors and altering production specificity. These findings provide structural insights into ASase-mediated flavonoid glycosylation and highlight potential targets for enzyme engineering to improve flavonoid solubility and bioavailability.