Design of glycosyltransferase UGT for thermostability and efficient biocatalytic synthesis of Rebaudioside A

Kwang-Hyun Park¹,², Chandana S. Talwar², Young-Hoon Lee², Ju-Eun Kang², Salsabilla I. Nurheibah², Seong-Ryeong Go² and Eui-Jeon Woo¹*

Disease Target Structure Research Center, Korea Research Institute of Bioscience & Biotechnology, Daejeon 34141, Republic of Korea.

Critical Diseases Diagnostics Convergence Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, 34141, Republic of Korea

Keywords: glycosyltransferase, thermostability, rosetta, protein design

Diterpene glycosyltransferases UGT76G1 from Stevia rebaudiana Bertoni is a key enzyme in the targeted biosynthesis of noncaloric sweeteners with high-intensity sweetness, rebaudioside A. Enzymatic synthesis of rebaudioside A (RebA) from Stevioside (ST) can increase the ratio of RebA to ST in steviol glycoside, providing a conceivable strategy to improve the organoleptic properties of steviol glycoside. Current efforts focused on directly modifying the active site of UGT76G1 to extend the applications of the improved enzymes with tailored properties. Herein, we devised a computational sequence design strategy to improve the thermostability and glycosylation efficiency of a UGT76G1. Starting with mesophilic UGT76G1, the design of variants bearing ~50 mutations was generated using Rosetta. The designed type1 UGT76G1s exhibits a ~10°C increase in apparent Tm and a strikingly enhanced conversion activity of stevioside to RebA over 3 fold at ~60 °C. Furthermore, Designed type2 UGT76G1s could catalyze a selectively increased rebaudioside A production with diminished side product of rebaudioside I. Taken together, these results provide significant insights into the computational sequence design strategy of diterpenoid glycosyltransferases with improved thermostability and activities.

Figure 1 The Synthesis of Rebaudioside A and Structure of UGT76G1