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Food Biochemical Study on Fructans and Related Synthesis Enzymes

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There are more than 500 kinds of oligosaccharide occurring in nature, and all of these are synthesized by chemical and enzymatic reactions. Various studies have recently identified physiological and physical applications of such oligosaccharides in agricultural chemistry, nutrition and medicine. New technologies for the production of oligosaccharides from natural resources have been developed in the food industry and several oligosaccharides are now produced on a large scale as ingredients in animal feed. We have investigated the isolation of fructo-oligosaccharides, as well as the enzymatic synthesis of oligosaccharides that have functional activities as tertiary functional ingredients in food. We have studied the purification and characterization of several fructosyltransferases from asparagus roots and onion bulbs, including sucrose: sucrose 1-fructosyltransferase (1-SST), fructan: fructan 1-fructosyltransferase (1-FFT), and the novel enzyme fructan:fructan 6^G-fructosyltransferase (6G-FFT). We have also reported that asparagus 1-FFT synthesized new functional oligosaccharides elongated with one or two additional fructose units by fructosyltransfer from 1-kestose to 4^G-β-D-galactosylsucrose, and this compound selectively stimulates the growth of *Bifidobacteria*. For industrial applications, we attempted the isolation and expression of cDNAs encoding 6G-FFT, 1-FFT and 1-SST from asparagus plants. The cDNAs encoding 6G-FFT, 1-FFT and 1-SST were isolated from a cDNA library of asparagus leaves or roots, and the isolated cDNAs were designated *aoft1*, *aoft2* and *aoft3*, respectively. The deduced amino acid sequences of these cDNAs showed high homology with those of plant fructosyltransferases. Expression of these cDNAs was performed using *Pichia pastoris*. The recombinant protein from *Pichia* transformed with *aoft1* produced 1^F,6^G-di-β-D-fructofuranosylsucrose, neokestose and sucrose from 1-kestose, while the transformant with an empty vector produced no saccharides. These

results confirmed that 6G-FFT was expressed in *P. pastoris*. Similarly, the recombinant protein from *Pichia* transformed with *aoft2* produced nystose from 1-kestose and the recombinant protein with *aoft3* produced 1-kestose from sucrose. These results confirmed that 1-FFT and 1-SST were expressed in *P. pastoris*, and that the recombinant proteins had enzymatic properties similar to those of 6G-FFT, 1-FFT and 1-SST from asparagus roots. We then examined the conversion of substrate specificity from 6G-FFT to 1-FFT by point mutations in the β-fructosidase motif. The asparagine in this motif in *aoft1* was changed to serine, and the mutant recombinant protein was characterized. We found that this amino acid substitution in wild-type *aoft1* changed the substrate specificity from 6G-FFT to 1-FFT. Finally, we studied the activities of the main enzymes involved in the synthesis and hydrolysis of fructo-oligosaccharides during the post-harvest life of onion bulbs and asparagus spears, and discuss the mechanisms triggering these enzyme activities, as well as the mechanisms by which fructo-oligosaccharides contribute to the quality and perishability of the vegetables.

Key words: fructan, fructo-oligosaccharide, fructosyltransferase, post-harvest, *Pichia pastris*

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