Purification, Cloning and Functional Characterization of Fructan: Fructan 1-Fructosyltransferase from Edible Burdock (Arctium lappa L.)

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Abstract: A fructan:fructan 1-fructosyltransferase (1-FFT) was purified from edible burdock for the first time, and a cDNA encoding 1-FFT was isolated from the plant. Two 1-FFTs named 1-FFTa and 1-FFTb were purified from extract of edible burdock by ammonium sulfate precipitation, followed by chromatographies on DEAE-Sepharose CL-6B, Toyopearl HW-55S and Sephadex G-100 columns. Inulin-type fructan such as nystose and fructooligosaccharides with higher DP were produced from 1-kestose by purified 1-FFTs. The general properties of both purified enzymes were very similar to each other. The purified 1-FFTa and 1-FFTb showed a single band by native PAGE and two bands, relative molecular masses of about 46,000 and 17,500 for 1-FFTa and 46,000 and 17,000 for 1-FFTb, by SDS-PAGE, respectively. The N-terminal sequences of the 46,000 peptides of both enzymes were the same, and those of the 17,500 and the 17,000 peptides were also identical. Based on the sequences, the 1-FFTa cDNA named alft1 was cloned. The alft1 encoded a polypeptide of 617 amino acids. The relative molecular mass and pl of the mature protein region of deduced polypeptide were calculated to be 60,213 and 4.89, respectively. The characteristics of recombinant protein produced by Pichia pastoris closely resembled those of the native 1-FFTs purified from edible burdock. In this study, we demonstrated that alft1 encoding edible burdock 1-FFT was involved in the elongation of fructosyl chains of fructooligosaccharides in edible burdock.

Key words: fructan, fructooligosaccharides, fructosyltransferase, edible burdock

Fructans (polyfructosylsucrose) are important storage carbohydrates in plants such as Poaceae (e.g., wheat and barley), Asteraceae (e.g., chicory and Jerusalem artichoke) and Liliaceae (e.g., onion and asparagus). Inulin-type fructan [1\(^{\beta}\)-(1→2)-link fructose] is a β-1,2 linked fructose-oligomer or -polymer terminated by glucose, which is mainly accumulated in Asteraceae plants. The saccharide is synthesized by sucrose:fructan 1-fructosyltransferase (1-SST, EC 2.4.1.99) and fructan:fructan 1-fructosyltransferase (1-FFT, EC 2.4.1.100). 1-SST synthesizes 1-kestose (1→(1→β)-link fructofuranosylsucrose, 1-kestotriose), an inulin-type trisaccharide, from two molecules of sucrose by fructosyltransfer. 1-FFT elongates fructose chains of inulin-type fructans by fructosyltransfer from 1-kestose to another 1-kestose or fructan.

We have already studied fructooligosaccharides and fructan metabolizing enzymes in asparagus, chicory and Jerusalem artichoke, and onion. Although three enzymes, 1-SST, 1-FFT and 6F-FFT (fructan:fructan 6\(^{\beta}\)-fructosyltransferase, EC 2.4.1.243), were involved in the synthesis of fructooligosaccharides in asparagus, a two-enzyme system, 1-SST and 6F-FFT coupled with 1-FFT activity, was revealed in onion bulbs. In our preliminary study, we found 1-FFT activity in the root of edible burdock (Arctium lappa L.), an Asteraceae plant which accumulates inulin-type fructan, and is eaten as a root vegetable rich in dietary fiber in Japan. Although fructooligosaccharide is accumulated in edible burdock, information on the fructan metabolism in the edible burdock is very limited. The synthesis of inulin and fructooligosaccharides in edible burdock is not clear. In this study, we have reported the purification, characterization and cloning and functional analysis of a 1-FFT involved in the synthesis of inulin type fructan from edible burdock.

MATERIALS AND METHODS

Plant materials. Edible burdock roots (A. lappa L.) had been freshly harvested on November from an experimental field of Makubetsu Agricultural Co-operative, Hokkaido, Japan and then stored in soil 1 m deep underground to May. After being washed with water the roots of edible burdock were stored at ~40 or ~80°C until use.

Substrates and measurement of enzyme activity. Saccharides as substrates were prepared as follows. Crystalline 1-kestose [3a: 1\(^{\beta}\)-(1→β)-link fructofuranosylsucrose, 1-kestotriose] and nystose [4a: 1\(^{\beta}\)-(1→β)-link fructofuranosylsucrose, 1, 1-kestotetraose] were prepared from sucrose using Scopulariopsis brevicaulis \(\beta\)-fructofuranosidase. The standards 5a, 6a, 7a and 8a [1\(^{\beta}\)-(1→β)-link fructofuranosylsucrose, m= 3, 4, 5, 6] were prepared from Jerusalem artichoke tubers in our laboratory. The reaction mixture, 25 µL of enzyme in 10 mM sodium phosphate buffer (pH 6.5), 50 µL of 200 mM 1-kestose in distilled water, 25 µL
of 100 mM sodium phosphate buffer (pH 6.5) and a small amount of toluene, was incubated at 30°C. The reaction was stopped by boiling for 3 min. One unit of 1-FFT activity was defined as the amount of enzyme which produced 1 mol of nystose per min under the above reaction conditions. For analysis of reaction products, high performance anion-exchange chromatography (HPAEC) was done on a DX300 chromatograph (Dionex Corp., Sunnyvale, USA) with a CarboPac PA-1 anion exchange column (Dionex Corp.) and a pulsed amperometric detector (PAD) as described previously.

In the experiment on optimum pH, Britton-Robinson buffer (pH 3.0–10.0) was used, and the reaction was stopped by addition of 900 µL of 150 mM sodium hydroxide. For pH-stability profiles, the reaction mixture, 10 µL of enzyme and 20 µL of Britton-Robinson buffer (pH 3.0–10.0), was incubated for 24 h at 4°C, and then the reaction mixture was adjusted to pH 6.5 and was incubated with 1-kestose at 30°C for 1 h. The reaction was stopped by boiling for 3 min. In the experiment on temperature-stability profiles, enzyme solutions were pre-incubated with 0.1 M sodium phosphate buffer (pH 6.5) for 15 min at 25, 30, 35, 40, 45, 50, 55 or 60°C, and then the pre-incubated solution was cooled to 0°C. After the pre-incubation, the mixtures were incubated with 1-kestose at 30°C for 30 min. To examine the effects of metal salts and chemicals on the activity, a mixture of enzyme (0.5 U/mL, 15 µL), 200 mM 1-kestose (50 µL) and 100 mM sodium phosphate buffer (pH 6.5, 25 µL) was incubated at 30°C in the presence of water, a metal salt or a chemical (1 mM; 0.1 mM p-CMB). After the reaction was stopped by heating at 100°C for 5 min, the mixture was subjected to HPAEC. All the experiments were done in duplicate.

**Quantitative determination of proteins.** Proteins were determined by measuring \( A_{280} \) with reference to \( E_{1cm}^{1cm} = 9.38 \) in aldolase.

**Purification of 1-FFT from edible burdock.** All operations throughout the purification were performed at 0–4°C. Roots of edible burdock (200 g each, total 2 kg) were chopped, and then homogenized in 10 mM sodium phosphate buffer (pH 6.5, 400 mL each) containing 2 mM dithiothreitol (DTT). The homogenate was filtered through cheesecloth and centrifuged for 30 min at 10,000 × g. The supernatant (4940 mL for 2 kg of edible burdock) was dialyzed overnight against 100 mM sodium phosphate buffer (pH 6.5) and a small amount of ammonium sulfate. The dialyzate was loaded onto a column (1.6 × 30 cm) of Toyopearl HW55S equilibrated with the same buffer. The elution was achieved with a linear gradient from 30 to 0% saturation of ammonium sulfate in the same buffer. Each active fraction was dia lyzed overnight against 50 mM sodium phosphate buffer (pH 6.5) containing 100 mM sodium chloride and 2 mM DTT. The dialyze was concentrated to 0.5 mL by ultrafiltration on VivaSpin concentrator cutting off at 10 kDa (VivaScience, UK). The concentrated solution was filtered on a column (1.6 × 70 cm) of Sephadex G-100 equilibrated with the same buffer.

**Native- and SDS-polyacrylamide gel electrophoresis (PAGE) and analysis of N-terminal amino-acid sequence.** Native-PAGE was performed with Phast Gel Gradient 10–15 by a Phast system (GE Healthcare, UK). SDS-PAGE for N-terminal amino-acid sequences was conducted according to the method of Laemmli. Proteins in the gel were stained with Coomassie Brilliant Blue R-250. After SDS-PAGE of the purified enzyme, the protein band was blotted onto a polyvinylidene difluoride (PVDF) membrane. The N-terminal amino-acid sequences were analyzed using a HP G1005A protein sequencing system (Hewlett-Packard Company, USA).

**Molecular cloning of 1-FFT cDNA from edible burdock.** From 1.0 g of edible burdock root powder, which was ground in liquid nitrogen, total RNA was prepared using an RNeasy Plant Mini Kit (Qiagen K.K., Tokyo, Japan). The first-stranded cDNA was synthesized, using a SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen Corporation, Carlsbad, USA). The cDNA was used for degenerate PCR as a template. Degenerate PCR was done using a primer set of p52Fw and p21Rv, which were designed according to amino-acid sequences of the purified enzymes (Table 1). The PCR consisted of an initial 2-min denaturation step (94°C) followed by 35 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 1 min and then a final step at 72°C for 7 min. The PCR was done using Blend Taq polymerase (Toyobo Co., Ltd., Osaka, Japan). The PCR product was subcloned in pGEM-T plasmid, selected in Escherichia coli DH5α cells (Toyobo Co., Ltd.). The plasmid was purified by a GenElute Plasmid Purification Kit (Sigma-Aldrich, USA).

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**Table 1.** PCR primers for cDNA cloning and construction of expression vector.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>DNA sequence (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p52Fw</td>
<td>ACGCCTTTCCATYTTCCACRC*</td>
</tr>
<tr>
<td>p21Rv</td>
<td>CANGCRTARTCRTCRTTNGC*</td>
</tr>
<tr>
<td>Gene Racer 5’ primer</td>
<td>CGACTGGAAGCAGACGGACTAGA</td>
</tr>
<tr>
<td>5’ GFP</td>
<td>CACGCGCTGACCCCATGACATTTGCC</td>
</tr>
<tr>
<td>Gene Racer 3’ primer</td>
<td>GCTGTCACAGTACGCTAGTAAACG</td>
</tr>
<tr>
<td>3’ GFP</td>
<td>CCCATCTACTCAATGCGCGGTGAGG</td>
</tr>
<tr>
<td>FFT_Fw</td>
<td>CTGAACCTACTTACCTTATT</td>
</tr>
<tr>
<td>FFT_Rv</td>
<td>GCCACATTTCTTCTAACAAGATC</td>
</tr>
<tr>
<td>V78FwPvd1</td>
<td>AAGGCTGCGTGGACGCGTGCTGCTGCTG**</td>
</tr>
<tr>
<td>F618RvKpn1</td>
<td>GAGGTACCGAAGAAGGATAAGGTCGGA-TAA G**</td>
</tr>
</tbody>
</table>

*R=(A, C), N=(A, T, G, C), Y=(C, T). Bold characters indicate the sequence of restriction sites.
mid Mini-Prep Kit (Sigma Aldrich Inc., USA), and its insert DNA was sequenced. The insert was denoted as DegeFFT.

On the resulting DNA sequence of DegeFFT, gene-specific primers (5’ GSP and 3’ GSP) were designed, and used for 5’ and 3’ RACEs to amplify the 5’- and 3’-flanking regions of DegeFFT. RACE was done using Gene Racer Kit (Invitrogen Corporation). A template cDNA was synthesized from the total RNA prepared from roots of edible burdock described above. The 5’ and 3’ RACEs consisted of an initial 2-min denaturation step (94°C) followed by 35 cycles (40 cycles in the case of 3’ RACE) of 94°C for 15 s, 63°C for 30 s and 68°C for 1.5 min and then a final step at 68°C for 10 min. The 5’ and 3’ RACE were done using KOD plus (Toyobo Co., Ltd.). After adenine residue was attached to RACE products by an A-addition kit (Qiagen K.K.), these products were cloned into pGEM-T vector, and then its insert DNA sequence by the same procedures mentioned above.

On the resulting DNA sequence of the 5’ and 3’ RACEs products, gene-specific primers (FFT_Fw and FFT_Rv) were designed, and used for PCR to amplify the DNA consistent with the open reading frame of the target gene. The PCR was done using KOD plus and 1st strand cDNA prepared from the total RNA described above. The PCR consisted of an initial 2-min denaturation step (94°C) followed by 35 cycles of 94°C for 15 s, 50°C for 30 s and 68°C for 1.5 min and then a final step at 68°C for 10 min. The amplified DNA fragments named FFTm were sequenced by the same procedures mentioned above. Full length cDNA was compiled by overlapping the sequences of FFTm and RACE PCR products (Fig. 1).

Transformation and cultivation of P. pastoris were performed according to the instructions of the manufacturer with minor modification. P. pastoris X-33 was transformed with 20 µg of the Pme1-linearized vectors by electroporation, and transformants were selected on YPDS (yeast extract pepton dextrose sorbitol)-Zeocin agar plates. A freshly prepared single colony was inoculated in 5 mL of BMGY (buffered glycerol-complex medium, pH 6.0), and cells were grown at 29°C in a shaking incubator at 200 rpm for 24 h. The cells were collected by centrifugation, resuspended as a cell pellet to an A600 of 1.0 in 15 mL of induction medium (buffered methanol-complex medium, BMMY, pH 6.0, containing 2% methanol) and incubated at 29°C for 72 h under aerobic conditions, adding 300 µL of methanol to the culture at intervals of 24 h. The culture was centrifuged and the supernatant was obtained.

The supernatant was concentrated to 1.0 mL. After it desalted with 15 mL of 10 mM sodium phosphate buffer (pH 6.5), the desalted solution was concentrated by ultrafiltration on a VivaSpin concentrator cutting off at 10 kDa (VivaScience). The concentrate was filled up to 1.0 mL with the same buffer and was used as an enzyme solution. All the experiments were done in duplicate.

RESULTS AND DISCUSSION

Purification of 1-FFT from edible burdock.

The enzyme was purified from edible burdock by successive chromatographies using DEAE-Sepharose CL-6B, Toyopearl HW55S and Sephadex G-100 columns. The chromatogram of Toyopearl HW55S is shown in Fig. 2. Edible burdock 1-FFT was separated to two fractions by hydrophobic chromatography, using a Toyopearl HW55S column. Each active fraction was separately chromatographed on a column of Sephadex G-100. The active fractions were named 1-FFTa and 1-FFTb and regarded as
purified enzymes.

Purification procedures of 1-FFTa and 1-FFTb are summarized in Table 2. Specific activities of 1-FFTa and 1-FFTb were 4.57 and 8.19 U/µg of protein. Although each protein showed a single band by native PAGE, two bands were detected by SDS-PAGE (Fig. 3). The bands of large peptides from both enzymes were detected in nearly the same position, and relative molecular masses (Mr) of the large peptides were estimated to be about 46,000. On the other hand, the small peptides from both enzymes were detected in slightly differential positions, and the Mr of the small peptides from 1-FFTa and 1-FFTb were estimated to be about 17,500 and 17,000, respectively. The two bands of about 46,000 from 1-FFTa and 1-FFTb were named AL46 and BL46, respectively. In the same manner, the two bands of 17,500 and 17,000 from 1-FFTa and 1-FFTb were named AS17.5 and BS17, respectively. The N-terminal sequences of the AL46 and BL46 peptides were the same (VQPSAAERLTWERTAFHFQP), and those of AS17.5 and BS17 peptides were also the same (ANDEYACTTSSGAERGSFG).

General properties of the enzymes.

The enzymatic properties of 1-FFTa and 1-FFTb were investigated. Optimum pH of both 1-FFTa and 1-FFTb was 6.5 (Table 3). Both of the enzymes were stable from pH 4.5 to 7.5. These enzymes were stable up to 50°C, and were completely inactivated at 60°C.

Effects of metal salts and chemicals on the activity of 1-FFTa and 1-FFTb were examined (Table 4). The activities were inhibited by Zn²⁺, Hg²⁺, CuSO₄ and AgNO₃.

Formation of tetrasaccharides and higher saccharides was determined by HPAEC (Fig. 4). When 1-kestose was incubated with the enzymes, nystose and sucrose were the initial products, and were produced proportionally up to 24 h. Production of 5a was also found with an initial lag phase. Furthermore, 6a, 7a and 8a were gradually produced (Fig. 5).

Enzymatic properties of the two purified enzymes were very similar to each other except that the specific activities of the two purified enzymes differ by a factor of two.

Molecular cloning of edible burdock 1-FFT cDNA.

Edible burdock 1-FFT cDNA was obtained by PCR with degenerate primers designed on the basis of amino-
Table 4. Effect of metal salts and chemicals on the activity of native and recombinant 1-FFT.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (nM)</th>
<th>Relative activity (%)</th>
<th>Native</th>
<th>1-FFTa</th>
<th>Recombinant</th>
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</thead>
<tbody>
<tr>
<td>None</td>
<td>1</td>
<td>100.0</td>
<td>100.0</td>
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<tr>
<td>CaCl2</td>
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<td>97.2</td>
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<td>ZnCl2</td>
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<td>51.3</td>
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<td>MgCl2</td>
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<td>AgNO3</td>
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<tr>
<td>SnCl2</td>
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<td>97.5</td>
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<td>99.5</td>
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</table>

Fig. 4. HPAEC analysis of saccharides produced from 1-kestose by 1-FFTa or 1-FFTb purified from edible burdock.

Chromatograms (a) and (b) show saccharides produced from 1-kestose by 1-FFTa and 1-FFTb, respectively. Reaction mixture consisted of enzyme (25 µL), 200 mM 1-kestose (50 µL, 100 mM final concentration), 100 mM sodium phosphate buffer (pH 6.5, 25 µL) and toluene (a trace amount), and was incubated at 30°C. The reaction was stopped by heating in a boiling water bath for 3 min. The reaction mixture was diluted 100 times with distilled water and filtered and an aliquot (25 µL) was applied to HPAEC. G, glucose; F, fructose; S, sucrose; 1-K, 1-kestose; Nys, nystose; 5a, 6a, 7a and 8a, [1'β(1-β-D-fructofuranosyl)ₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖₖ maritime plant. The full length cDNA named alft1 consisted of 2151 bp and contained an open reading frame (ORF) of 1854 bp and a poly (A) sequence at the 3' end. The ORF encoded a polypeptide of 617 amino acids (Fig. 6). The deduced polypeptide was denoted as AlFT1. The large (AL46 or BL46) and small (AS17.5 or BS17) peptides encoded by alft1 containing an ORF since the N-terminal sequences of these peptides were identical to AlFT1. Similar results were reported in soluble invertase from carrot (Daucus carota) and FFT from chicory (Cichorium intybus) and globe thistle (Echinops ritro). Generally, fructosyltransferases seem to consist of heterodimers containing a large subunit and a small subunit although the reason for the cleavage of purified enzymes is not clear.

The Mr and pI of the mature protein region of AlFT1 were calculated to be 60,213 and 4.89, respectively. The Mr of the mature protein region of AlFT1 was different from the sum of the Mr of AL46 (BL46) and AS17.5 (BS17). The difference in the Mr might be caused by a post-translational modification such as glycosylation. Indeed, the deduced amino acid sequence contains four potential N-glycosylation sites (N-X-S/T). The separation of 1-FFTa and 1-FFTb by hydrophobic chromatography using Toyopearl HW55S column might be affected by glycosylation.
The N-terminal amino acids of AL46 and BL46 are identical to 78th Val in AlFT1. The first 77 amino acids in AlFT1 probably contain the vacuolar targeting signal because the synthesis of fructan in plants is known to locate in a vacuole,22,23) and a cleavage site was predicted between the 54th and 55th (or 63th and 64th) amino acids in AlFT1.24) In the screening of 1FFT gene, we could not find other genes encoding a 1FFT-like protein except for aleh1. Therefore, 1FFTa and 1FFTb were encoded by aleh1 although we need the analysis of internal amino acid sequences of both enzymes or further screening of 1FFT genes. 

AlFT1 belongs to glycoside hydrolase family 32 (GH32), which includes invertases and fructosyltransferases from plant and invertases, inulinases and levanases from bacteria and fungi.25) AlFT1 contains three conserved amino acid sequences, such as NDPN (βfructosidase motif), RDP (RDP motif) and EC (catalytic site), in various fructosyltransferases and invertases (Fig. 6). The Asp (D) and the Glu (E) residues in βfructosidase and the catalytic site from yeast invertase were identified as a nu-

![Fig. 6. Comparison of deduced amino-acid sequence of alft1 with those of other Asteraceous 1-FFT.](image-url)

The N-terminal amino acids of AL46 and BL46 are identical to 78th Val in AlFT1. The first 77 amino acids in AlFT1 probably contain the vacuolar targeting signal because the synthesis of fructan in plants is known to locate in a vacuole,22,23) and a cleavage site was predicted between the 54th and 55th (or 63th and 64th) amino acids in AlFT1 by signal P server.26) 

In the screening of 1-FFT gene, we could not find other genes encoding a 1-FFT-like protein except for aleh1. Therefore, 1-FFTa and 1-FFTb were encoded by aleh1 although we need the analysis of internal amino-acid sequences of both enzymes or further screening of 1-FFT genes.

AlFT1 belongs to glycoside hydrolase family 32 (GH32), which includes invertases and fructosyltransferases from plant and invertases, inulinases and levanases from bacteria and fungi.25) AlFT1 contains three conserved amino-acid sequences, such as NDPN (β-fructosidase motif), RDP (RDP motif) and EC (catalytic site), in various fructosyltransferases and invertases (Fig. 6). The Asp (D) and the Glu (E) residues in β-fructosidase and the catalytic site from yeast invertase were identified as a nu-
cleophile and a proton donor, respectively.26,27 The Asp (D) in RDP motif in levansucrase from Bacillus subtilis was identified as a transition state stabilizer.28 The primary sequence of AIFT1 exhibits high identity with that of asteraceous 1-FFTs such as Cynara scolymus (88%), Cichorium intybus (82%), Helianthus tuberosus (79%) and Echinops ritro (76%).

**Heterologous expression of recombinant AIFT1 protein in Pichia pastoris.**

A heterologous expression system using *P. pastoris* is very suitable confirmation of the product translated by encoding the gene of plant fructosyltransferases and invertases.29 *P. pastoris* does not produce any fructosyltransferases or invertases, and background activities of the enzymes are not detected in culture medium from *P. pastoris*. A recombinant protein was obtained by expression of *alft1* in *P. pastoris*. From the enzyme assay with 1kestose and nystose as a substrate, 1-FFT activity was detected in the enzyme solution prepared from the culture supernatant of *P. pastoris*. By incubation of recombinant AIFT1 protein with 100 mM 1-kestose for 1, 48 and 168 h, nystose, 5a, 6a, 7a, 8a and other oligosaccharides with higher DP were detected (Fig. 7). Time course of formation of the products from 1-kestose by recombinant 1-FFT were similar to that by native enzymes (data not shown). The optimum pH of recombinant 1-FFT was 6.8. The enzyme was stable in the range of pH 3–8 at 4°C for 24 h. The enzyme was stable up to 45°C for 15 min of incubation. The activity of the recombinant 1-FFT was inhibited by Zn⁺², Hg⁺², CuSO₄, and AgNO₃. The relative residual 1-FFT activity of recombinant enzyme against AgNO₃ was 4.2%, whereas those of purified enzymes were about 43% (Table 4). The recombinant and purified 1-FFTs were not affected by the SH reagent. The 1-FFT from other plant such as Jerusalem artichoke and chicory were reported to be not inhibited by other SH reagents, 2-nitrobenzoic acid (DTNB) and iodoacetamide.30 Burdock 1-FFTs seem to be not SH enzyme. As for onion 6G-FFT, detergents such as Tween 80 and Triton X activate the activity.31 Burdock 1-FFT was not activated by the detergent.

Enzymatic properties of recombinant AIFT1 are summarized with those of native 1-FFTα and 1-FFTβ in Table 3. Several plant fructosyltransferases produced by *P. pastoris* are known to be monomeric proteins, whereas the native enzyme occurs as heterodimers.21 Furthermore, the difference of N-glycosylation between recombinant 1-FFT produced by *P. pastoris* and native 1-FFT was known.21 From the fact, the differential properties such as enzyme stability and effect of metal salts in native and recombinant 1-FFTs in this study might be affected by difference in sugar chain, monomeric structure or purity of recombinant 1-FFT.

When native and recombinant 1-FFTs were incubated with 100 mM 1-kestose, the formation of nystose, 5a, 6a, 7a and 8a was detected by HPAEC. Moreover, fructans with DP from 9 to 14 were detected by HPAEC (data not shown). Fructooligosaccharides with DP up to 30 were found in edible burdock (our preliminary data). The reason for the difference of maximum DP of fructooligosaccharides between in vivo and in vitro could be explain the accumulation of relatively high concentration of sucrose in the in vitro experiments. Plant 1-FFT was generally known to use sucrose as an acceptor but not to use it as a donor, and acceptor efficiency of sucrose is higher than that of 1-kestose or nystose.30 Therefore a high concentration of sucrose acts as a competitive inhibitor for fructan elongation.29,30

In this study, we demonstrated that *alft1* encoding edible burdock 1-FFT is involved in the elongation of fructosyl chains of fructooligosaccharides in edible burdock.

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**Fig. 7.** HPAEC analysis of saccharides produced from 1-kestose by recombinant 1-FFT.

Reaction mixture consisted of recombinant enzyme (25 μL), 200 mM 1-kestose (50 μL, 100 mM final concentration), 100 mM sodium phosphate buffer (pH 6.8, 25 μL) and toluene (a trace amount), and was incubated at 30°C. Preparation of HPAEC samples followed the same procedures as in Fig. 4. G, F, S, 1-K, Nys, 5a, 6a, 7a and 8a represent the same saccharides as listed in Fig. 4.


