UDP-D-Xylose: Flavonol 3-O-Xylosyltransferase from Young Leaves of *Euonymus alatus* f. *ciliato-dentatus*

Nariyuki Ishikura and Zhi-qing Yang

Department of Biological Science, Faculty of Science, Kumamoto University, Kumamoto 860, Japan

Z. Naturforsch. **46c,** 1003–1010 (1991); received February 28/June 28, 1991

Celastraceae, *Euonymus,* Flavonol, O-Xylosyltransferase, Characterization

From the young leaves of *Euonymus alatus* f. *ciliato-dentatus,* a novel enzyme, UDP-D-xylose: flavonol 3-O-xylosyltransferase (F3XT), catalyzing the transfer of D-xylose from UDP-D-xylose to the 3 position of 3,5,7,4′-tetrahydroxyflavone (kaempferol), was detected and purified about 16-fold by precipitation with ammonium sulfate and DEAE-cellulose CC, by which F3XT was separated from two coexisting flavonol O-glucosyltransferases (FGT). Thus, F3XT was isolated as a soluble enzyme with a pH optimum of 7.0 in Tris-HCl buffer. The molecular weight of F3XT, which had an isoelectric point at pH 6.1, was estimated by elution from a column of Sephadex G-100 to be about 48 kDa. The activity of F3XT was stimulated by 14 mM 2-ME and strongly inhibited by 1 mM Cu^{2+}, 1 mM Zn^{2+}, and various reagents that react with sulfhydryl groups. Among the substrates tested for F3XT, kaempferol was the best. The K_m values for kaempferol and UDP-xylose were determined to be 0.83 μM and 25 μM, respectively. F3XT mediated the transfer of xylose exclusively to the 3-hydroxyl group of kaempferol. Isorhamnetin, quercetin and fisetin also can function as xylosyl acceptor though less efficiently, but neither the 7-O-glucosides nor the 3-O-glucosides of kaempferol and quercetin were able to accept D-xylose. Dihydroflavonols were not xylosylated.

Introduction

A number of flavonoid O-glycosyltransferases have been studied in various plant tissues [1], and it has been reported that flavonoid-specific glycosyltransferases show a strict specificity for the position of the hydroxyl group, to which the sugar is transferred. Sutter and Grisebach [2] have shown by using UDP-D-glucose as the sugar donor that a glucosyltransferase specific for the 3-position of flavonol is separable from a 7-glucosylating enzyme in parsley cell cultures. As another flavonol 3-position-specific glycosyltransferase the enzyme using UDP-L-rhamnose as the sugar donor has been reported from the leaves of *Leucaena glauca* [3]. In a previous paper [4], we reported that the leaves of *Euonymus alatus* f. *ciliato-dentatus* contain the 3-O-xyloside, the 3-O-rhamnosylxyloside (euonymin) and the 3-O-rhamnosylxyloside-7-O-glucoside of kaempferol which have not been described from other plants. In addition, quercetin 3-O-xyloside-7-O-glucoside has been found in *Euonymus maackii* and *E. lanceifolia* [5]. Thus, it is of interest to ascertain whether xylosylation of the 3 position of kaempferol is catalyzed by a preparation of enzyme from leaves of *E. alatus* f. *ciliato-dentatus,* and nothing is known about the enzyme catalyzing this reaction. Therefore, in the present study, a preparation of enzyme from young leaves of this plant was assayed for UDP-D-xylose: flavonol 3-O-xylosyltransferase (F3XT) and also UDP-D-glucose: flavonol O-glucosyltransferase (FGT), although the latter was separated into at least two components. In particular attention was paid to the separation and characterization of F3XT. This enzyme is unable to catalyze xylosylation of flavonol glycosides at sugar hydroxyls. Therefore, it differs from the enzyme such as GT-III (UDP-xylose: flavonol 3-glycoside xylosyltransferase) which was isolated from anthers of *Tulipa* [6].

Abbreviations: DIECA, diethylammonium diethyldithiocarbamate; DMSO, dimethyl sulfoxide; FGT, UDP-D-glucose: flavonol O-glucosyltransferase; F3XT, UDP-D-xylose: flavonol 3-O-xylosyltransferase; 2-ME, 2-mercaptoethanol; TLC, thin-layer chromatography.

Reprint requests to Prof. N. Ishikura.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0939–5075/91/1100–1003 $ 01.30/0

Materials and Methods

Plant materials

Fresh young leaves of *Euonymus alatus* (Thunb.) Sieb. f. *ciliato-dentatus* (Fr. et Sav.) Hiyama were collected from a single tree in early

Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung") beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition "no derivative works"). This is to allow reuse in the area of future scientific usage.

April, and they were assigned stage II by their weight and color as described elsewhere [7]. Leaves collected were weighed, frozen in liquid nitrogen and stored at −80 °C.

Labeled compounds

UDP-D-(U-^{14}C)xylose (9.6 GBq/mmol) and UDP-D-(U-^{14}C)galactose (9.9 GBq/mmol) were obtained from Du Pont Company, Biotechnology Systems. Wilmington, DE 19898 U.S.A. UDP-D-(U-^{14}C)glucose (10.9 GBq/mmol) was obtained from Amersham International plc, Buckinghamshire, England.

Buffers

The following buffers were used: A, 0.2 M Tris-HCl, pH 7.5, containing 14 mM 2-ME, 5 mM EDTA and 10 mM DIECA; B, 20 mM Tris-HCl, pH 7.5, containing 14 mM 2-ME and 10% glycerol; C, 50 mM Tris-HCl, pH 7.5, containing 14 mM 2-ME and 10% glycerol; and D, 20 mM Tris-HCl, pH 7.5, containing 10% glycerol.

Extraction and partial purification of enzymatic activities

Unless stated otherwise, all procedures were carried out at 2 to 4 °C. About 40 g of *Euonymus* leaves, frozen in liquid N_2, were mixed with Polyclar AT (8.0 g), homogenized in a mortar with about 140 ml of buffer A and filtered through nylon mesh. The filtrate was centrifuged at 15,000 × *g* for 15 min. The supernatant was stirred for 20 min with Dowex 1X2 (20%, w/v) which had previously been equilibrated with buffer A, then filtered through glass wool. The filtrate was fractionated by precipitation with solid ammonium sulfate, and the protein fraction that precipitated between 45 and 75% saturation was collected by centrifugation at 20,000 × *g* for 20 min and resuspended in a minimum volume (9 ml) of buffer B. 3 ml aliquot of the solution was desalted by passage through a column of Sephadex G-25 (18 mm i.d. × 180 mm) which had previously been equilibrated with buffer D. The desalted solution of enzyme was assayed for activity. 6 ml aliquot of the solution obtained above was applied to a column of DEAE-cellulose (20 mm i.d. × 255 mm) which had previously been equilibrated with buffer C. Proteins were eluted with a linear gradient of 0 to 300 mM KCl in buffer C, and fractions of 5.0 ml were collected and assayed for enzymatic activity.

Assay of enzymatic activity

The standard assay mixture consisted of 1.95 nmol of the flavonoid substrate (in 10 µl of 50% DMSO), 24.0 nmol of UDP-(U-^{14}C)xylose (containing 92.5 Bq in 10 µl of H_2O), UDP-(U-^{14}C)galactose (420.1 Bq in 10 µl of H_2O) or UDP-(U-^{14}C)glucose (462.5 Bq in 10 µl of H_2O), 1.3 µmol of $MgCl_2$, 1.8 µmol of 2-ME and 10 to 20 µg of enzyme protein in buffer B (or C in the case of the enzyme eluted from the column of Sephadex G-25) in a total volume of 130 µl. The reaction was started by the addition of flavonoid substrate, and the mixture was incubated for 40 min at 30 °C. The reaction was stopped by addition of 20 µl of 6 N HCl, and the reaction products were extracted with 400 µl of ethyl acetate. 200 µl aliquots of the organic phase were transferred to a scintillation vial and counted for radioactivity in a toluene-based scintillation fluid.

Chromatographic identification of reaction products

For identification of the products of reactions, the ethyl acetate extracts of several assays were pooled, evaporated to dryness, and then chromatographed on TLC plates. Ascending TLC on Avicel SF cellulose plates (Funakoshi Pharmaceutical Co. Ltd., Tokyo) were used for assays of flavonol glucosides. The solvent systems were 30% acetic acid (solvent I), 15% acetic acid (solvent II) and *n*-butanol-acetic acid-H_2O (4:1:5, v/v; solvent III). Identity of the [^{14}C]glycosylated products was confirmed by cochromatography with authentic kaempferol 3-O-xyloside, 3-O-glucoside, 7-O-glucoside and 3-O-rhamnosylxyloside (euonymin), examination under UV light (254 nm) with or without ammonia vapor, and autoradiography using Hyperfilm-β max (Amersham International plc). The reaction product was furthermore identified by cochromatography with authentic samples on an HPLC column (Finepak SIL C_{18}-5, 4.6 × 150 mm) in a Tri Rotar-V (Japan Spectroscopic Co. Ltd.) using the isocratic solvent system $CH_3CN-H_2O-H_3PO_4$ (30:70:0.2) at a flow rate of 1.0 ml·min^{-1}.

Estimation of protein

Protein concentrations were determined by the method of Bradford [8] with bovine serum albumin as reference protein.

Determinations of molecular weight

The molecular weights of O-glycosyltransferases were determined as described earlier [9].

Dependence of reactions on pH

The pH optima of the O-glycosyltransferase activities eluted from the column of DEAE-cellulose were determined as described earlier [9].

Isoelectric focusing

The enzyme (1.8 mg protein in 9 ml) from a column of Sephadex G-25, which had been equilibrated with buffer B, was diluted to an appropriate concentration (see below) and loaded onto the Rotofor isoelectric focusing system (Bio-Rad Laboratories Co. Ltd., California, U.S.A.), according to the method of Egen *et al.* [10]. Running conditions were as follows: 1.8 mg protein in 40 ml of buffer B containing 1.25% Bio-lyte (pH 4–6); anode electrolyte, 0.1 M H_3PO_4; cathode electrolyte, 0.1 M NaOH. Power conditions: run for 1.5 h at 12 W; starting voltage, 500 V; final voltage, 1000 V. After running, aliquots of the protein fraction from each cell were used for the measurement of the F3XT and FGT activities and the estimation of protein.

Results

Partial purification of enzyme F3XT

Crude preparations of enzyme from the young leaves of *Euonymus alatus* f. *ciliato-dentatus* catalyzed the xylosylation of the 3-position of kaempferol in the presence of UDP-D-(U-^{14}C)xylose as xylosyl donor (Fig. 1) and also the glucosylation of kaempferol in the presence of UDP-D-(U-^{14}C)-glucose as glucosyl donor. The reaction product of xylosylation was identified as kaempferol 3-O-xyloside by TLC and autoradiography (Fig. 2). On TLC, the reaction product as well as kaempferol 3-O-xyloside appeared black in UV and turned yellow in UV with NH_3. This is quite different from kaempferol 7-O-glycosides, *e.g.* kaempferol 7-O-glucoside and 7-O-rhamnoside, appearing bright yellow in UV without NH_3, and according to ref. [11], flavonols with a free 3-hydroxyl group and with or without a free 5-hydroxyl group ap-

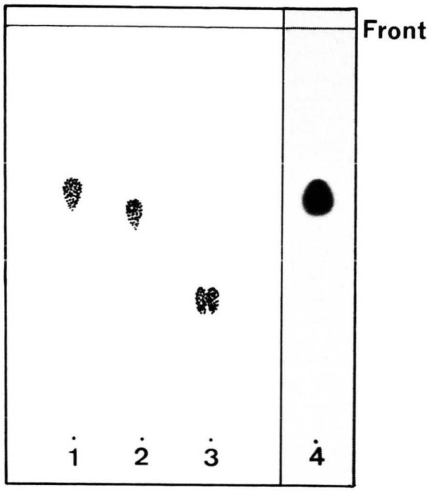

Fig. 2. Photograph of an autoradiogram after chromatography of the product (4, inset) of reaction catalyzed by F3XT with kaempferol and UDP-D-(U-^{14}C)xylose as substrates. Chromatography was performed with 30% acetic acid as the mobile phase on TLC plate of Avicel SF cellulose. Authentic samples of kaempferol 3-O-xyloside (1), kaempferol 3-O-glucoside (2) and kaempferol 7-O-glucoside (3) were cochromatographed with the reaction mixture and each is marked with black dots on the chromatogram.

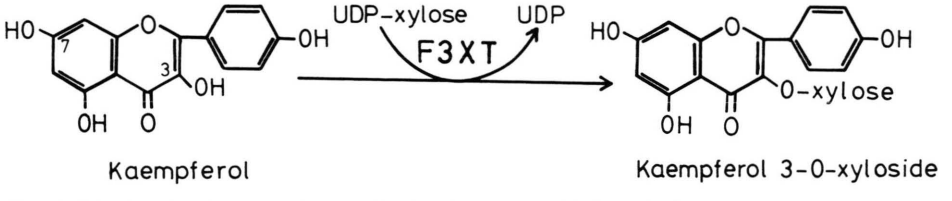

Fig. 1. The O-xylosylation of kaempferol to kaempferol 3-O-xyloside.

pear yellow-green or blue-green in UV without NH_3. TLC R_F values of the reaction product were 0.60 (in solvent system I), 0.36 (II) and 0.67 (III), and they coincided with those of authentic kaempferol 3-O-xyloside. HPLC t_R value of the reaction product also was 12.61 corresponding to that of authentic kaempferol 3-O-xyloside. Further confirmation of the reaction product was referred to [4, 12].

The enzyme tentatively designated F3XT was precipitated with ammonium sulfate and partially purified by chromatography on DEAE-cellulose (Fig. 3). The purification of F3XT resulted in increases in specific activity of 15.7-fold as compared with activity of the crude extract, when kaempferol was used as substrate (Table I). Activity of FGT with kaempferol as substrate was recovered in two different fractions after chromatography on DEAE-cellulose, but F3XT and FGT activities were recovered within the same peak of protein when the protein fraction obtained by precipitation in 45–75% saturated ammonium sulfate was chromatographed on a column of Sephadex G-100 (Fig. 4).

Effect of pH

The pH optima for F3XT activity, as determined in different buffers, were found to be 7.0 and 7.5 in Tris-HCl buffer (pH 6.5–8.5) and imidazole-

Table I. Partial purification of the O-xylosyltransferase F3XT from *Euonymus* leaves.

Purification step	Total protein [mg]	Specific activity [nkat · mg protein^{-1}]	Purification [fold]	Recovery [%]
Crude extract	28.9	0.12	–	100
Dowex 1 × 2	25.5	0.08	0.7	59
Sephadex G-25 after 45–75% $(NH_4)_2SO_4$ fractionation	1.8	0.41	3.4	21
DEAE-cellulose	0.2	1.88	15.7	11

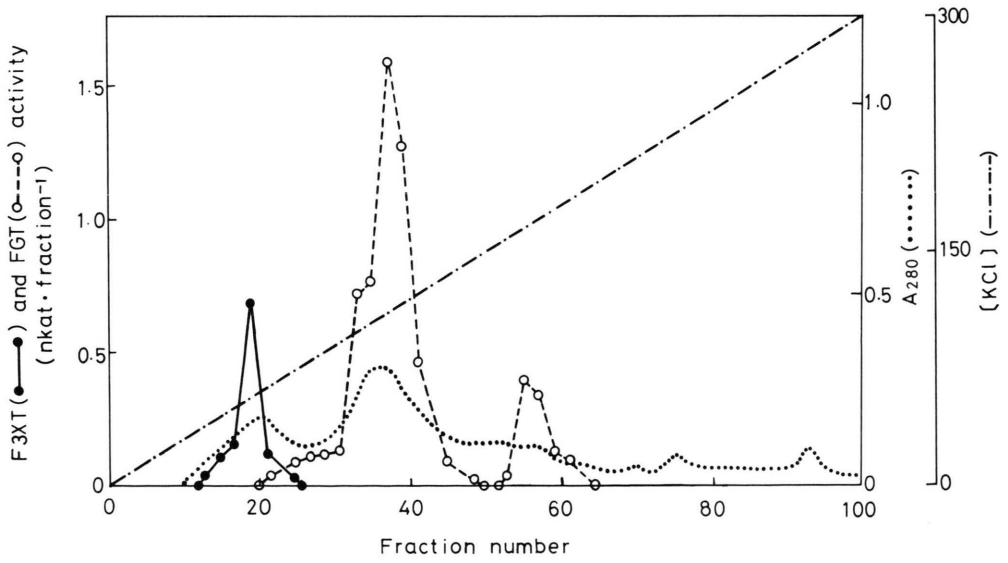

Fig. 3. Profile of elution of the activities of O-xylosyltransferase (F3XT) and O-glucosyltransferase (FGT) from a column of DEAE-cellulose. The enzyme from a column of Sephadex G-25 were applied to a column which had previously been equilibrated with buffer C and they were eluted with a linear gradient of 0 to 300 mM KCl in the same buffer. Fractions of 5.0 ml were collected. The fractions were assayed, with kaempferol, for both F3XT and FGT.

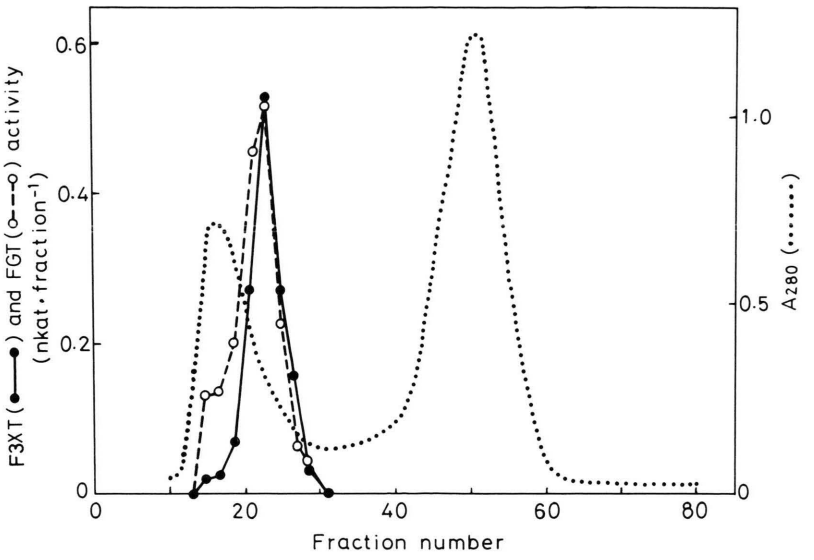

Fig. 4. Profile of elution of the activities of O-xylosyltransferase (F3XT) and O-glucosyltransferase (FGT) from a column of Sephadex G-100 (20 mm i.d. × 463 mm) with kaempferol as substrate. The protein fraction obtained by precipitation with 45–75% ammonium sulfate was applied to a column which had been equilibrated with buffer C, and 3 ml fractions were collected and assayed for both activities. The column was calibrated with reference proteins (*cf.* Fig. 6).

HCl buffer (pH 6.5–8.5), respectively (Fig. 5). Maximum activity of F3XT was observed with a rather broad peak in Tris-HCl buffer. Incidentally, the pH optima for FGT activity in Tris-HCl and imidazole-HCl buffers were found to be 7.5 and 8.0, respectively.

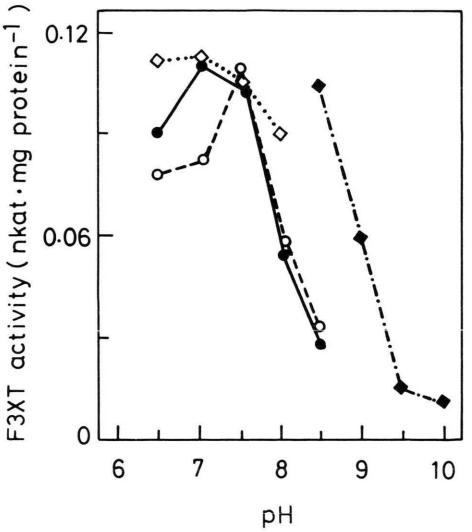

Fig. 5. The pH optima of F3XT activity with kaempferol as substrate. —●—, F3XT in Tris-HCl; – –○– –, in imidazole-HCl; ····◇····, in histidine-HCl; –·–◆–·–, in glycine-HCl buffers.

Linearity of reactions

At the optimum pH, the rates of reactions catalyzed by F3XT and FGT were linear for at least 40 min and 60 min, respectively, and also the rates were proportional to the amount of protein in the assay.

Stability of enzyme

Storage of the enzyme F3XT, which was eluted from a column of Sephadex G-25 after 45–75% ammonium sulfate fractionation, in buffer B at −20 °C resulted in a 11% loss of activity after two days. About 45% and 16% of the original activity remained after 8 and 40 days, respectively, at −20 °C. However, the enzyme stored in buffer D without 2-ME at −20 °C, was very unstable and lost the activity with in two or three days completely.

Substrate specificity

Of the various substituted flavonols and dihydroflavonols (Table II) tested for their ability of xylosyl acceptor, the best substrate for F3XT was kaempferol. The other flavonols, isorhamnetin, quercetin, rhamnetin and fisetin also were good xylosyl acceptors, whereas partially methylated kaempferols and dihydroflavonols were very poor. Regardless of the nature of substitution on the fla-

Table II. Acceptor specificity of the O-xylosyltransferase F3XT*.

Substrate**	Relative activity [%]
Kaempferol	100
Kaempferide (kaempferol 4′-monomethyl ether)	45
Kaempferol 3,4′-dimethyl ether	1
Kaempferol 5,7,4′-trimethyl ether	15
Quercetin	76
Isorhamnetin (5,7,4′-trihydroxy-3′-methoxyflavonol)	85
Quercetin 5-monomethyl ether	20
Rhamnetin (5,3′,4′-trihydroxy-7-methoxyflavonol)	67
Myricetin	48
7,4′-Dihydroxyflavonol	32
Fisetin	62
Astragalin (kaempferol 3-O-glucoside)	2
Kaempferol 7-O-glucoside	3
Kaempferol 3,4′-O-diglucoside	2
Euonymin (kaempferol 3-O-rhamnosyl-xyloside)	9
Euonymin + Kaempferol	128
Isoquercitrin (quercetin 3-O-glucoside)	3
Quercimeritrin (quercetin 7-O-glucoside)	5
Aromadendrin (dihydrokaempferol)	25
Taxifolin (dihydroquercetin)	5
Pinobanksin (3,5,7-trihydroxyflavanone)	19

* The enzyme (1.88 nkat·mg protein^{-1}) used was the fraction after chromatography on DEAE-cellulose in buffer C. Activities were compared to the reaction rate determined with kaempferol = 100%.

** Final concentration of all substrates in the reaction mixture is 15 μM.

vonoid ring system, flavonol 3-O-glucosides and 7-O-glucosides were not accepted for xylosylation. It is interesting to note that xylosylation of kaempferol was somewhat promoted by co-existence of euonymin in the reaction mixture. The specificity of F3XT for the sugar donor is quite distinct. Only UDP-D-xylose can function as sugar donor, and no activity was found with UDP-D-glucose and UDP-D-galactose. Investigations of the specificity of FGT for sugar acceptors and donors are in progress.

Kinetic data and molecular weight

The apparent K_m and V_{max} values were determined in the standard incubation according to Lineweaver and Burk [13]. The apparent K_m values of F3XT for kaempferol and quercetin as substrates were 0.83 and 1.19 μM, respectively, and also for the co-substrate UDP-xylose the apparent K_m value was 25 μM when kaempferol was used as xylosyl acceptor. The V_{max} value of F3XT for kaempferol was 0.94 μmol·mg protein^{-1}·min^{-1}, and the V_{max} value for quercetin was about 70% of that for kaempferol. The molecular weight of F3XT was estimated to be about 48 kDa by column chromatography on Sephadex G-100 (Fig. 6).

Effects of inorganic ions, EDTA and SH reagents

The results obtained are shown in Table III. EDTA at 1–10 mM concentration did not inhibit the F3XT activity which seems to indicate that the O-xylosylation at the 3-position of flavonol had no requirement for divalent cations. Mg^{2+}, Ca^{2+} and Mn^{2+}, and also K^+, showed an inhibitory effect on the activity at higher concentration (10 mM). Other cations, such as Co^{2+}, Cu^{2+} and Zn^{2+}, strongly inhibited xylosylation at 1 mM concentration. The activity of F3XT was strongly inhibited by SH group inhibitors such as 1 mM N-ethylmaleimide and 1 mM *p*-chloromercuribenzoate, and the addition of 14 mM 2-ME resulted in 100% recovery from inhibition in the presence of their inhibitors. 14 mM 2-ME itself stimulated the enzyme activity, even if 10 mM Mg^{2+} was present together.

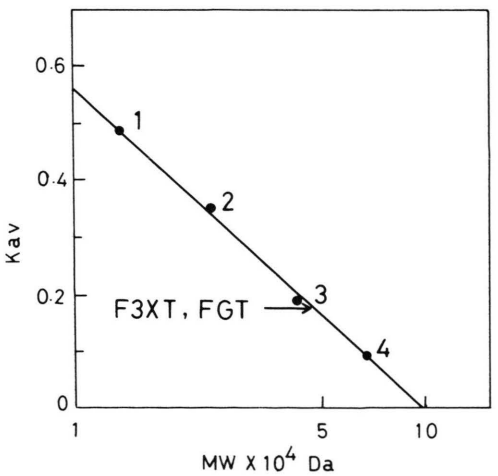

Fig. 6. Estimation of the molecular weight of the O-xylosyltransferase F3XT by elution from a Sephadex G-100 column which had previously been calibrated with the reference proteins indicated as 1 (ribonuclease A, 13.7 kDa), 2 (chymotrypsinogen A, 25 kDa), 3 (ovalbumin, 43 kDa), 4 (albumin, 67 kDa).

Table III. Effects of inorganic ions, EDTA, 2-ME and SH group inhibitors.

Reagent*	Concentration [mM]	Relative activity** [%]
Control	–	100
$MgCl_2$	1	63
	10	23
$MnCl_2$	1	36
	10	2
$CaCl_2$	1	59
	10	25
$CoCl_2$	1	4
$CuCl_2$	1	1
$ZnCl_2$	1	1
KCl	1	94
	10	73
EDTA	1	113
	10	90
2-ME	14	124
2-ME + $MgCl_2$	14 + 10	123
PCMB	1	1
PCMB + 2-ME	1 + 14	107
NEM	1	6
NEM + 2-ME	1 + 14	119
Phenylmercuriacetate	1	2
DTE	10	61
Iodoacetamide	1	56
Iodoacetate	1	90

* PCMB = *p*-chloromercuribenzoate, NEM = N-ethylmaleimide, DTE = dithioerythritol.

** The enzyme used was the fraction after chromatography on Sephadex G-25 in buffer D. Kaempferol was used as substrate for assay of F3XT activities, which amounted to 100 pkat·mg protein^{-1} in the control assays (= 100%).

Isoelectric points (pI) of F3XT and FGT

By loading onto the Rotofor isoelectric focusing system, the activity of F3XT was separated from that of FGT (Fig. 7). The peaks of both activities appeared in the range of pH 6.1 and 5.0, respectively, corresponding to each apparent pI of F3XT and FGT.

Discussion

A preparation of enzyme extracted from the young leaves of *Euonymus alatus* f. *ciliato-dentatus* catalyzed the xylosylation of kaempferol at the 3-position, using UDP-D-(U-^{14}C)xylose as xylosyl donor. This is the first report of the detection of an enzyme (F3XT) that catalyzes the 3-O-xylosylation of a flavonol. Another enzyme (FGT), catalyzing the glucosylation of kaempferol, has also been detected in the crude enzyme extract and separated from F3XT by chromatography on DEAE-cellulose. Both enzymes of F3XT and FGT have the same molecular weight of about 48 kDa, which ranges within the reported values of the flavonoid O-glucosyltransferases from other sources between 42 and 52 kDa [9, 14]. The leaves of *E. alatus* f. *ciliato-dentatus* contain kaempferol 3-O-rhamnosylxyloside-7-O-glucoside in addition to kaempferol 3-O-xyloside [4], so that the 7-O-glucosylation of kaempferol may be mediated by FGT which was detected in the present study. The

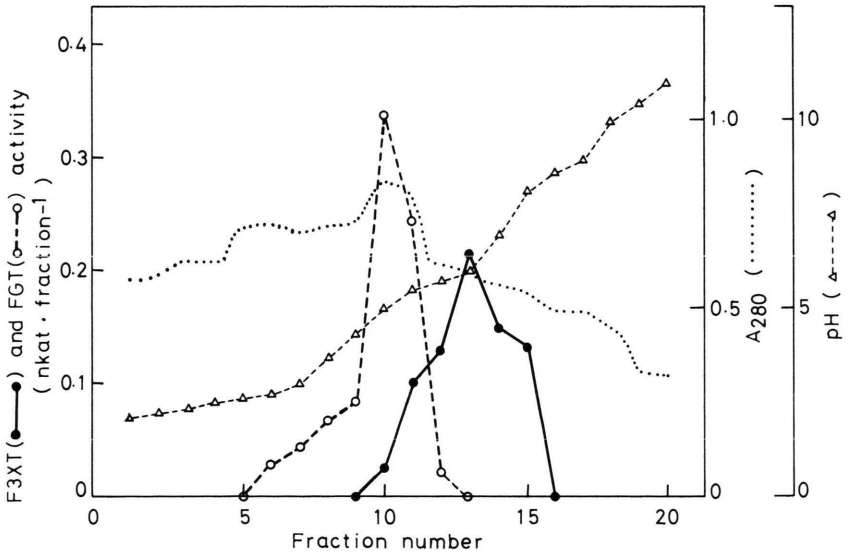

Fig. 7. Analysis of the fractions for pH, protein content and the activities of F3XT and FGT after application of the Rotofor isoelectric focusing. See Materials and Methods for the conditions.

properties of FGT will be reported in detail later. Flavonoid 3-O-glycosyltransferases from many plant sources have usually been shown to glycosylate flavonoid with a broad substrate specificity [1]. However, F3XT is highly specific to catalyze the transfer of xylose exclusively to the 3-hydroxyl group of kaempferol. The position specificity of flavonoid glycosyltransferases has been demonstrated by separating a glucosyltransferase specific for the 3-position of flavonoids from a 7-O-glucosylating enzyme in parsley cell cultures [2]. No activity in the F3XT preparation was found with UDP-glucose and UDP-galactose other than UDP-xylose. F3XT had very low K_m value of 0.83 μM for kaempferol, indicative of a high affinity for this substrate. Among the substrates tested, the 3-O-glucosides and the 7-O-glucosides of flavonols, as well as some of partially methylated kaempferols and dihydroflavonols, could not function as xylosyl acceptors. Therefore, it suggests that the 3-O-xylosylation of flavonol occurs prior to the 7-O-glucosylation, and also that F3XT is unable to catalyze glycosylation of sugar hydroxyls of flavonol glycosides other than phenolic hydroxyls of their aglycones. Xylosylation of flavonol 3-O-glucosides at sugar hydroxyls has been reported to be catalyzed by an enzyme which was isolated from anthers of *Tulipa* [6]. It is interesting to note that xylosylation of kaempferol was promoted by co-occurrence of euonymin in the incubation mixture, but the reason is unknown. F3XT also catalyzed xylosylation of isorhamnetin, quercetin and fisetin though less efficiently. It is noticeable that isorhamnetin (4,5,7-trihydroxy-3′-methoxyflavonol) has the same hydroxylation pattern as that of kaempferol, except the presence of a methoxyl group at the 3′-position, and that fisetin lacks a hydroxyl group at the 5-position.

Some properties of F3XT are similar to those of the other reported flavonol O-glucosyltransferases [9, 15]. Divalent cations, such as Co^{2+}, Cu^{2+} and Zn^{2+}, and SH group inhibitors, such as N-ethylmaleimide and *p*-chloromercuribenzoate, strongly inhibited the F3XT activity. The effects on the F3XT activity of these agents suggest the presence of SH groups at the active site of the enzyme. 2-ME was very effective on the activity, but Mg^{2+}, Ca^{2+}, Mn^{2+} and K^+ at high concentration (10 mM) showed an inhibitory effect on it. The enzyme stored without 2-ME was very unstable and became completely inactive during only two or three days. The apparent isoelectric points (pI) of F3XT and FGT activity were determined by the Rotofor isoelectric focusing of the enzyme preparation. Both activities gave two different peaks in the fractions corresponding to pI of 6.1 and 5.0, respectively. pI of F3XT at pH 6.1 is very characteristic, and such a high pI has not been reported previously for the other flavonoid O-glycosyltransferases, which show their pI in the pH range of 5.0–5.6 [14–17]. A further attempt to purify F3XT is in progress.

Acknowledgement

The authors are grateful to Prof. N. Morita (Toyama Medical and Pharmaceutical University) for providing the authentic samples of some flavonols.

[1] W. Hösel, in: The Biochemistry of Plants (E. E. Conn, ed.), **Vol. 7,** p. 725, Academic Press, New York 1981.
[2] A. Sutter and H. Grisebach, Biochim. Biophys. Acta **309,** 289 (1973).
[3] G. A. Barber and M. T. Y. Chang, Phytochemistry **7,** 35 (1968).
[4] N. Ishikura and S. Sato, Bot. Mag. Tokyo **90,** 83 (1977).
[5] M. Nogradi, L. Farkas, and V. Olechnowiez-Stepien, Chem. Ber. **104,** 3618 (1971).
[6] G. Kleinehollenhorst, H. Behrens, G. Pegels, N. Srunk, and R. Wiermann, Z. Naturforsch. **37c,** 587 (1982).
[7] N. Ishikura, Bot. Mag. Tokyo **89,** 251 (1976).
[8] M. M. Bradford, Anal. Biochem. **72,** 248 (1976).
[9] N. Ishikura and K. Yamamoto, Plant Cell Physiol. **31,** 1109 (1990).
[10] N. B. Egen, W. Thormann, G. E. Twitty, and U. S. Bier, in: Electrophoresis '83 (H. Hirai, ed.), p. 547, W. de Gruyter, Berlin 1984.
[11] K. R. Markham, Techniques of Flavonoid Identification, Academic Press, London 1982.
[12] N. Ishikura, S. Sato, and K. Kurosawa, Phytochemistry **15,** 1183 (1976).
[13] I. H. Segel, Biochemical Calculations, John Wiley & Sons, Inc., New York 1971.
[14] G. Hrazdina, Biochim. Biophys. Acta **955,** 301 (1988).
[15] K. L. Bajaj, V. DeLuca, H. Khouri, and R. K. Ibrahim, Plant Physiol. **72,** 891 (1983).
[16] A. Sutter, R. Ortmann, and H. Grisebach, Biochim. Biophys. Acta **258,** 71 (1972).
[17] L. M. V. Jonsson, M. E. G. Aarsman, J. Bastiaannet, W. E. Donker-Koopman, A. G. M. Gerats, and A. W. Schram, Z. Naturforsch. **39c,** 559 (1984).