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Purification and Molecular Analysis of a Monoamine Oxidase Isolated from *Narcissus tazetta*

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Semicarbazide-sensitive amine oxidase activity was detected in *Narcissus tazetta*. The enzyme was purified to homogeneity by the criterion of native polyacrylamide gel electrophoresis (PAGE) with DEAE-Sephacel, hydroxyapatite, and phenyl-Sepharose columns. The molecular mass of the enzyme, determined using a GS-520 HQ column, was estimated to be 135 kDa. SDS–PAGE yielded two bands of, 75 kDa and 65 kDa. The enzyme, which had catalytic activity for some aliphatic and aromatic monoamines, belongs to a class of monoamine oxidases (MAOs). The K_m value for *n*-propylamine was 5.9×10^{-5} M. A substrate analog, 2-bromoethylamine, inhibited enzyme activity. Redox-cycling staining detected a quinone in the MAO protein. By inductively coupled plasma mass analysis, it was determined that there were 2.44 moles of copper atoms per mole of the enzyme. Protein sequence analysis revealed that there was no identity between two N-terminal residues of the 75 kDa and 65 kDa proteins of narcissus MAO.

Key words: 2-bromoethylamine; copper; monoamine oxidase; *Narcissus tazetta*; quinoprotein

Amine oxidases (AOs) catalyze the oxidation of amines, monoamines, diamines, and/or polyamines to their corresponding aldehydes, hydrogen peroxide and ammonia. The superfamily of AOs isolated from mammals, higher plants, fungi, and bacteria are formed by a group of enzymes involved in the cellular and extracellular metabolism of amines. AOs have been divided into two categories depending on the cofactor, FAD or a copper-containing quinone. Enzymes containing FAD (EC.1.4.3.3) are further subdivided into monoamine oxidases (MAOs) and polyamine oxidases (PAOs), depending on substrate specificity. In the second category, AOs (EC 1.4.3.6) are characterized by the presence of topaquinone (TPQ) or lysyl tyrosylquinone,[1)] cupric Cu-containing quinone cofactors. The copper AOs include diamine oxidase (DAO) and semicarbazide-sensitive, mammalian MAO. In plants, there are two well-established types of AOs: copper-containing DAOs that act at the primary amine of many amine compounds, and flavin-containing PAOs that function at the secondary amine of polyamines.[2,3)] Plant DAO is one of the best known enzymes involved in amine catabolism. Spermidine and spermine are specific substrates for PAO. Additionally, MAO activity has been reported in several plants.[4–7)] In the plant species reported, Tsushida and Takeo used tea leaves to purify an AO that catalyzes monoamines including some alkylamines, ethanolamine, and benzylamine, although this last was not classified as an MAO.[7)] The cofactor in the tea-derived AO was not determined. Recently we reported a semicarbazide-insensitive MAO from *Avena sativa*.[8)] This FAD-containing enzyme had high substrate specificity for benzylamine and 2-phenethylamine, which are oxidized to benzaldehyde and 2-phenylactaldehyde. Many aromatic and flavor compounds containing fatty acid derivatives, benzenoids, and isoprenoids are found in many parts of the plant.[9)] The flavor volatiles 2-phenylacetaldehyde and 2-phenylethanol are presumably produced *in vivo* from 2-phenethylamine by an AO, dehydrogenase, or transaminase in tomatoes, although the plant genes responsible for the synthesis of the benzenoids have not yet been identified.[10)] AO activity has yet to be detected in the fragrant flowers of plant species examined in our laboratory, but it has been reported that AO activity was detected in the flowers of *Narcissus tazetta* (Y. Suzuki, personal communication). In this study, we purified an AO from *Narcissus tazetta* and examined its properties to determine whether it is involved in the flower-scent-producing pathway.

Materials and Methods

Plant materials. Flowering shoots of narcissus (*Narcissus tazetta* L. var. chinensis) were perchased from Kansaikako (Osaka, Japan). The shoots were used for enzyme purification.

Enzyme activity. MAO activity at the purification steps and in the other experiments was assayed by spectrophotometric detection of the H_2O_2 produced in a reaction mixture by the modification of the quinoneimine dye method.[11)] The standard assay mixture contained 0.67 mM propylamine, 36 mM K-Pi buffer (pH 7.0), 0.33 mM *N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3-methylamine (Dojin Laboratories, Kumamoto, Japan), 0.33 mM 4-aminoantipyrine, and 3.3 purpurogallin units of horseradish-peroxidase (Wako Pure Chemical, Osaka, Japan) and enzyme solution in a total volume of 3 mL. The reaction was initiated by the addition of the amine. The mixture was incubated at

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Abbreviations: AO, amine oxidase; DAO, diamine oxidase; ICP-MS, inductive coupled plasma-mass; MAO, monoamine oxidase; NTB, nitroblue tetrazolium; PAGE, polyacrylamide gel electrophoresis; PAO, polyamine oxidase; TPQ, topaquinone

37 °C, and enzyme activity was determined by the linear increase in the absorbance at 555 nm for 5 min from initiation of the reaction.

The optimum pH of the assay mixture was determined at 37 °C using acetate-NaOH buffer (pH 4.0, 4.5, 5.0, and 5.5), phosphate-KOH buffer (pH 6.0, 6.5, 7.0, and 7.5), and Tris–HCl buffer (pH 8.0, 8.5, and 9.0). Enzyme activity was expressed in katal (kat, mol s^{-1}).

Enzyme purification. All procedures were carried out at 4 °C. Shoots of the narcissus plant (100 g) were ground in a Waring blender (MX-151S, Panasonic, Osaka) with 5 volumes of 100 mM K-Pi buffer (pH 7.0). The homogenate was filtered through four layers of gauze, and the filtrate was centrifuged at 10,000 × *g* for 25 min. The supernatant (500 mL) was supplemented with solid $(NH_4)_2SO_4$ to 65% saturation and incubated for 30 min. The precipitate obtained by centrifugation of the supernatant at 10,000 × *g* for 25 min was dissolved in 75 mL of 20 mM K-Pi buffer (pH 7.0), yielding buffer A, and centrifuged again at 10,000 × *g* for 25 min. The supernatant was applied to a Sephadex G-25 column (3.5 × 35 cm) equilibrated with buffer A, and then eluted with the same buffer to remove $(NH_4)_2SO_4$. The eluted protein fraction (70 mL) was heated at 60 °C for 5 min in plastic tubes using a metal-block heater. The heated solution was centrifuged at 12,000 × *g* for 10 min, and the supernatant was applied to a DEAE-Sephacel (GE Healthcare, Little Chalfont, UK) column (2.5 × 25 cm) equilibrated with buffer A and eluted with the same buffer. The active fractions were collected and applied to a hydroxyapatite (Nacalai Tesque, Kyoto, Japan) column (2.0 × 15 cm) equilibrated with buffer A and washed with the same buffer. The bound enzyme was eluted with 100 mL of a linear gradient of buffer A to 0.8 M K-Pi buffer (pH 7.0). The active fractions were pooled (20 mL) and concentrated to 4 mL using an Ultracel-100K centrifugal filter (Millipore Billerica, MA). The concentrated fraction was applied to a phenyl-Sepharose (GE Healthcare) column (1.5 × 5 cm) equilibrated with 0.8 M $(NH_4)_2SO_4$ in buffer A and then washed with 0.4 M $(NH_4)_2SO_4$ in buffer A. The bound enzyme was eluted stepwise with 100 mM and then with 50 mM $(NH_4)_2SO_4$ in buffer A. The active fractions eluted were through buffer A Ultracel-100K centrifugal filter. The flowthrough fraction from the Ultracel-100K filter was applied to an Amicon Ultracel-30K centrifugal filter. The concentrated fraction was washed with buffer A on the Ultracel-30K filter by centrifugation. The purified MAO preparation was stored at −20 °C in 25% (v/v) glycerol in buffer A.

The protein concentration of the pooled fraction from each purification step was estimated by the micro-assay method of Bradford[12] with bovine serum albumin as the standard.

Polyacrylamide gel electrophoresis. Electrophoresis was performed under native and denatured conditions in an electrophoresis unit. Native polyacrylamide gel electrophoresis (PAGE) was carried out a 7.5% (w/v) gel without SDS by the method described by Davis.[13] Propylamine-active staining on the native PAGE was used with the assay solution described above in "Materials and Methods" SDS–PAGE was done using a 15% (w/v) gel by the method described by Laemmli.[14] Full-Range Rainbow Molecular Weight Markers (GE Healthcare) were used as protein standards.

Determination of propionaldehyde. HPLC was performed to measure the propionaldehyde produced in the reacted mixture. The mixture contained 0.67 mM propylamine, 33 mM K-Pi buffer (pH 7.0), and the purified MAO solution in a total volume of 3 mL. An aliquot (100 µL) of the reaction mixture was added to 1.0 mL of 0.25 mM 4-(*N*,*N*-dimethylaminosulfonyl)-7-hydrazino-2,1,3-benzoxadiazole (DBD-H; Tokyo Chemical Industry, Tokyo) in 70% acetonitrile and 0.05% trifluoroacetic acid (TFA). After 2 h, the DBD-H solution was centrifuged at 10,000 × *g* for 10 min, and the supernatant was injected into an HPLC sampler. Reversephase HPLC on a system composed of a pump (LC-10AD), a spectrofluorometer (RF-535, excitation 450 nm and emission 565 nm, Shimadzu Co., Kyoto, Japan), and a column of COSMOSIL C_{18}-PAQ (4.6 mm i.d. 9.25 cm; Nakarai Tesque, Kyoto, Japan) was used. The mobile phase was 70% acetonitrile and 0.05% TFA at a flow rate of 1 mL min^{-1}. The column temperature was maintained at 30 °C.

Determination of molecular mass using a GS-520 column. GS-520 HQ column (Shodex Asahipak, 7.8 × 300 mm) was used to determine the molecular mass of the narcissus MAO. The column was calibrated by the following protein standards: ovalbumin, α-glucosidase (yeast), lactate dehydrogenase (yeast), glucose oxidase (*Aspergillus nigar*), and ferritin type I (horse spleen). The purified enzyme and protein standards were applied to a column equilibrated with 0.1 M K-Pi buffer (pH 7.0) containing 0.3 M NaCl and then eluted with the same buffer.

Substrate specificity and inhibitor efficacy. Monoamines, diamines, and polyamines were used to determine the substrate specificity of the enzyme using a standard assay mixture containing 0.66 mM of the substrate. The inhibitors of human MAOs, pargyline and clorgyline, and those of Cu-AOs, o-phenanthroline, 2,2′-bipyridyl, 2-bromoethylamine, and semicarbazide, were employed using the standard assay to assess their effects on the catalytic function of the narcissus MAO. The enzyme was pre-incubated with 1.0 or 0.1 mM of each inhibitor for 5 min before activity was measured. The K_m values of some monoamines were determined from double reciprocal plots.

Cofactor identification. To determine copper and manganese content by atomic absorption analysis, an X5 inductive coupled plasma-mass spectrometer (ICP-MS, Bruker, Billerica, MA) was used. The spectrophotometric nitroblue tetrazolium (NBT)/glycinate test and quinone staining on PVDF membrane after SDS–PAGE were conducted as described by Paz *et al.*[15]

Protein sequence analysis. Amino acid sequencing of the narcissus MAO was carried out by the Edman degradation method by the Protein Sequence System (ABI Procise 491 HT, Praha, Czech) using an Immobilon 0.45 µm PVDF membrane (Millipore, Bedford, MA). The protein sample was separated by SDS–PAGE, and the protein bands were transferred to the membrane by a semi-dry electroblotting method. Two areas, corresponding to 65 kDa and 75 kDa, were excised from the membrane and analyzed individually by the Protein Sequence System.

Results and Discussion

Purification of the narcissus MAO

AO activity was detected in all organs, specifically the leaves, stems, and flowers in the shoots (data not shown). The enzyme was purified from whole shoots by $(NH_4)_2SO_4$ fractionation, heat treatment, DEAE-Sephacel, hydroxyapatite, and phenyl-Sepharose column chromatography. Table 1 summarizes the steps involved in the purification procedures for the enzyme and the purification results when the process begins with approximately 100 g fresh weight of shoots. Minimal enzymatic activity was detected in the supernatant after the first centrifugation after the purification procedures. We postulated that small molecular material in the supernatant would disturb the enzyme assay system. In fact, when the supernatant was dialyzed against 20 mM K-Pi buffer (pH 7.0), activity was detectable. The activity of the crude extract displayed in Table 1 represents the use of the dialyzed enzyme solution in the assay method. The enzymatic activity eluted from the G-25 column was up to 10 min at 60 °C, and approximately 67% of the protein within the desalted fraction was denatured by the heat treatment. The ensuing purification steps were accomplished using DEAE-Sephacel, hydroxyapatite, and phenyl-Sepharose columns. As Table 1 shows, 500 mL of homogenate, which was purified to 20% yield from the crude ground extract, contained 37.8 nkat of total MAO activity. The specific activity of the purified MAO was 81.85 nkat mg^{-1} protein, and a purification factor of 1,544-fold was obtained. The purified enzyme was applied to a gel filtration column (GS-520 HQ), and this

Table 1. Summary of the Purification of Monoamine Oxidase from Narcissus Shoots

Purification step	Total volume (mL)	Total protein (mg)	Total activity (nkat)	Specific activity (nkat mg^{-1} protein)	Purification (fold)	Yield (%)
Crude extract	500	718	37.80	0.053	1	100
$(NH_4)_2SO_4$ precipitate	70	728	25.87	0.036	0.68	68
Heat treatment	70	242	23.52	0.097	1.83	62
DEAE-Sephacel	60	30.3	19.15	0.632	11.9	51
Hydroxyapatite	20	0.54	11.08	20.52	387	29
Phenyl-Sepaharose	4	0.092	7.53	81.85	1544	20

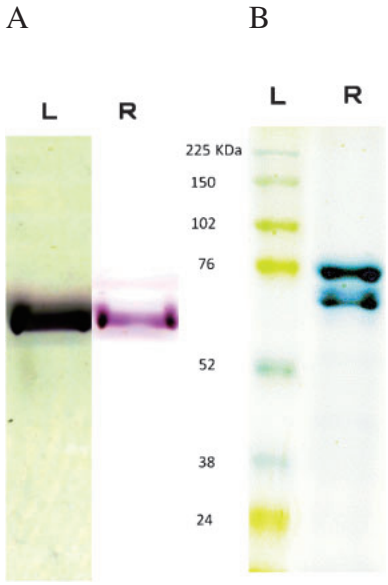

Fig. 1. Polyacrylamide Gel Electrophoresis of Narcissus Monoamine Oxidase.

A, SDS-free gel of narcissus monoamine oxidase. L, Coomassie-stained SDS-free gel; R, *N*-propylamine-positive spot on SDS-free gel. B, SDS-gel of narcissus monoamine oxidase. L, Marker; R, Coomassie-stained SDS-gel. For other details, see "Materials and Methods."

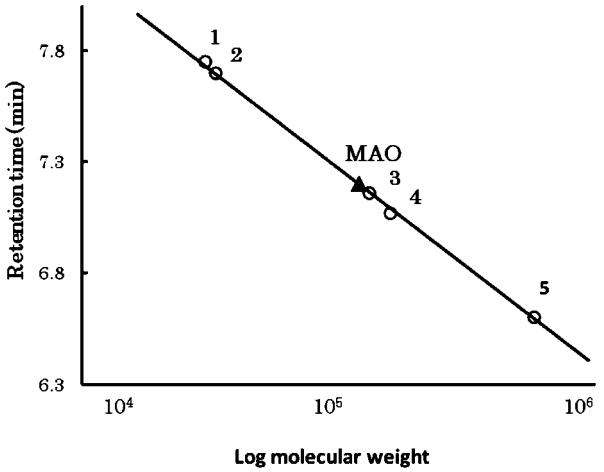

Fig. 2. Determination of Molecular Mass of Narcissus Monoamine Oxidase.

Gel filtration (GS-520 HQ, HPLC) column; 1, ovalbumin (45 kDa); 2, α-glucosidase from yeast (52 kDa); 3, lactate dehydrogenase from yeast (140 kDa); 4, glucose oxidase from *Aspergillus nigar* (186 kDa); 5, ferritin type I from horse spleen (440 kDa). ▲ narcissus monoamine oxidase (135 kDa). For other details, see "Materials and Methods."

resulted in a single peak as observed in the column-elution profile (data not shown). The enzyme was subjected to native PAGE analysis. The gel was divided into two portions after electrophoresis. One portion was stained with Coomassie Brilliant Blue G-250, and the other was stained at 37 °C in enzyme-active staining (see "Materials and Methods"). A single band migrated to the same site in both areas of the gel (Fig. 1A), indicating that the enzyme was purified to homogeneity and that the active-stained band is an MAO protein.

Determination of molecular mass

SDS–PAGE of the phenyl-Sepharose column-purified enzyme revealed two protein bands (molecular masses of 75 kDa and 65 kDa) (Fig. 1B). Many studies indicate that Cu-DAOs in plants are typically homodimers.[16] Tea AO is also a homodimer, with a molecular mass of 80 kDa per subunit.[7] The product of GS-520 HQ column chromatography had an apparent molecular mass of 135 kDa (Fig. 2). The two bands visualized on SDS–PAGE, which represented narcissus MAO, indicated that the narcissus-derived enzyme is comprised of two different subunits, indicating a heterodimer. There is another possibility that a 75 kDa-homodimer of MAO was partially cleaved by an endogenous protease(s) during purification. However, despite the addition of protease inhibitors (0.5 mM phenylmethylsulfonyl fluoride and 1 mM EDTA) to the buffers used in enzyme purification, SDS–PAGE of MAO purified by the method with inhibitors gave the same results as Fig. 1B (data not shown).

Determination of other enzymatic parameters

When the enzyme solution was pre-incubated for 10 min at temperatures ranging from 50 to 80 °C to determine heat stability, the purified enzyme retained its activity at temperatures of 70 °C, but lost activity quickly after pre-incubation at temperatures of 75 °C (Fig. 3). These results indicate that purified MAO is stable at up to 70 °C, which differs from the heat stability of the MAO in desalted solution after $(NH_4)_2SO_4$ fractionation. The difference in heat stability as between MAO in the desalted fraction and purified MAO might have been due to the proteases in the fraction. For comparison, tea AO is stable at up to 60 °C,[7] not 70 °C as for the narcissus MAO. Additionally, the optimum pH of the narcissus MAO was 7.0 (Fig. 4), similar to that of other Cu-AOs.[3]

Substrate specificity and inhibitor efficacy

The substrate specificity of the enzyme is summarized in Table 2. The enzyme catalyzed the oxidation of aliphatic and aromatic short-chain monoamines. *N*-

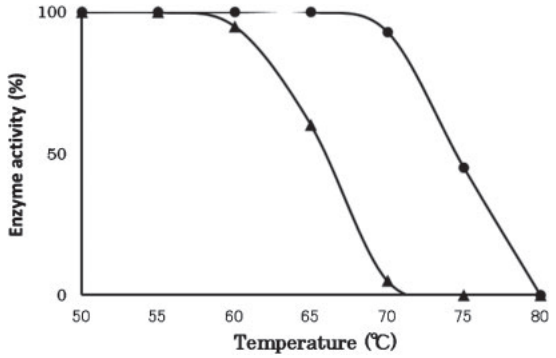

Fig. 3. Thermal Inactivation Curve for Narcissus Monoamine Oxidase.

Purified monoamine oxidase solution (●) or desalted solution after ammonium sulfate precipitation (▲) was incubated at various temperatures for 10 min and activity of each was measured. See "Materials and Methods."

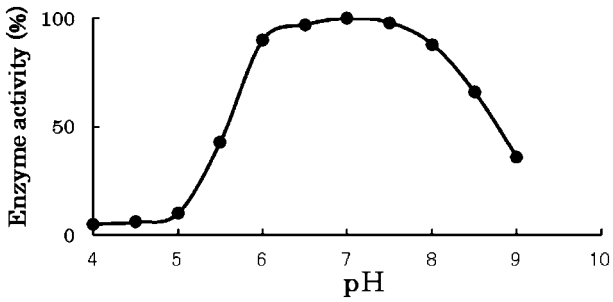

Fig. 4. Effects of pH on Narcissus Monoamine Oxidase Activity.

Table 2. Substrate Specificities of Narcissus MAO

Substrate	Relative activity (%)	K_m (M)	κ (s^{-1})
n-Propylamine	100	5.9×10^{-5}	53.4
n-Butylamine	98	1.1×10^{-4}	
Ethylamine	84	1.3×10^{-4}	
Benzylamine	74	4.1×10^{-4}	
n-Pentylamine	56	5.0×10^{-4}	
Ethanolamine	50	2.0×10^{-3}	
Methylamine	34	1.1×10^{-4}	
Phenethylamine	**9**	1.4×10^{-3}	
Hexylamine	7		
Tryptamine	0		
Serotonin	0		
Dopamine	0		
Tyramine	0		
Histamine	0		
Putrescine	0		
Cadaverine	0		
Diaminodecane	0		
Spermidine	0		
Spermine	0		

Propylamine was found to be the best substrate for the amines. Other short-aliphatic monoamines, ethanolamine and benzylamine, were also good substrates for the enzyme. By HPLC, propionaldehyde was detected stoichiometrically, and the mixture produced hydrogen peroxide when propylamine was incubated with the enzyme (Fig. 5). We concluded from these results that the enzyme from *Narcissus tazetta* was an MAO. The K_m values for *n*-propylamine and benzylamine were 5.9×10^{-5} M and 4.1×10^{-4} M respectively. The enzyme did not catalyze the oxidation of hydroxylated benzylamines,

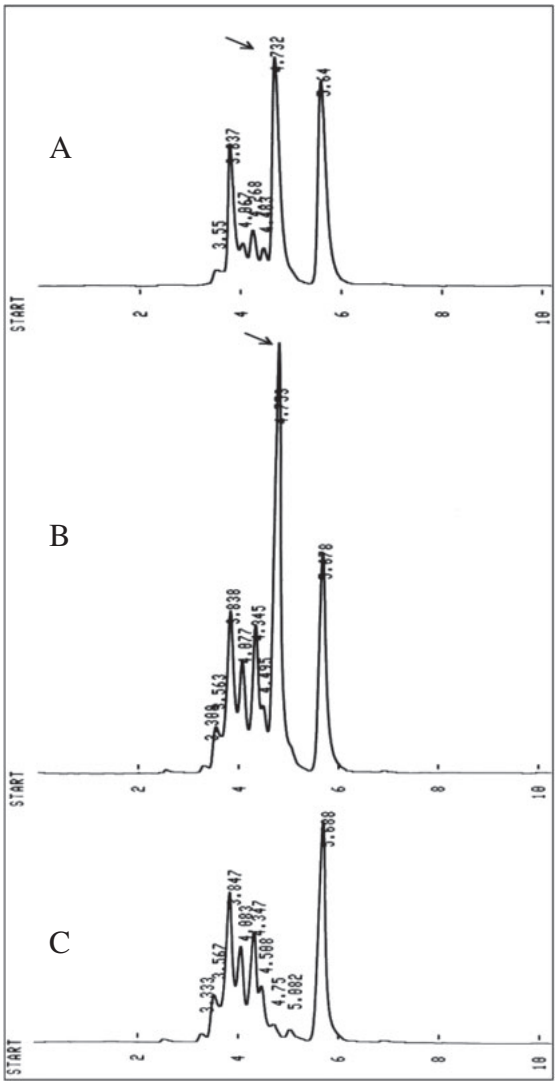

Fig. 5. Detection of Propionaldehyde as a DBD-H Derivative by HPLC.

A, authentic propionaldehyde solution; B, reaction mixture with *n*-propylamine as substrate; C, before treatment, 2-bromoethylamine (0.1 mM) was added to the mixture. Arrows indicate peaks of propionaldehyde-DBD-H. For details, see "Materials and Methods."

Table 3. Effects of Inhibitors and Manganese Chloride on Narcissus MAO Activity

Inhibitor	Concentrations (mM)	Inhibition (%)
Semicarbazide	0.01	100
	0.001	51
2-Bromoethylamine	0.01	100
	0.001	71
	0.0005	42
2,2′-Bipyridyl	1	50
o-Phenanthroline	1	4
Pargyline	1	2
Clorgyline	1	1
$MnCl_2$	1	90
	0.1	122
	0.05	105

diamines, or polyamines. It was not inhibited by 1 mM pargyline or clorgyline, both of which are potent selective inhibitors of MAOs in mammals (Table 3). Semicarbazide, an inhibitor of enzymes having a quinone cofactor, inhibited the narcissus-derived enzyme. The

75 kDa subunit	1	NPNPDHESETLLP	13	Current study
65 kDa subunit	1	ALPPRRAASVVQF	13	Current study
Barley predicted AO	132	ALPPRRAVAVVRF	144	Matumoto *et al.* 2011

Fig. 6. Comparison of Amino Acid Sequences of Two Subunits of Narcissus Monoamine Oxidase with the Homologous Sequence of a Predicted Barley Amine Oxidase.

Two N-terminal subunits of the narcissus MAO were sequenced by the Edman degradation method. The NCBI GenBank accession number for barley is BAJ85075. Amino acids of barley AO that are identical the 65 kDa subunit of the narcissus MAO are shaded in gray. For details, see "Materials and Methods."

enzyme was also inhibited by a metal-chelating reagent, 2,2′-bipyridyl. In the inhibition experiments, diethyldithiocarbamate and phenylhydrazine were not available for use owing to their ability to inhibit peroxidase activity in the enzyme assay system. These results for substrate specificity and inhibitory effects indicate that narcissus MAO is comparable to tea AO,[7] and is similar to semicarbazide-sensitive AOs in mammals. Although the cofactor of the tea AO was not identified, it appears to be a copper- and quinone-containing enzyme based on the effects of inhibitors. The narcissus MAO was irreversibly inhibited by 2-bromoethylamine with an inhibition constant (K_i) of 0.65 μM. Medda *et al.* indicated that 2-bromoethylamine (an irreversible inhibitor of lentil DAO) is a poor substrate and combines directly with TPQ in the enzyme.[17] This strong, specific inhibitor may be useful as a probe in searching for physiological roles of narcissus MAO, especially for any involvement related to fragrant aldehydes in the narcissus flower.

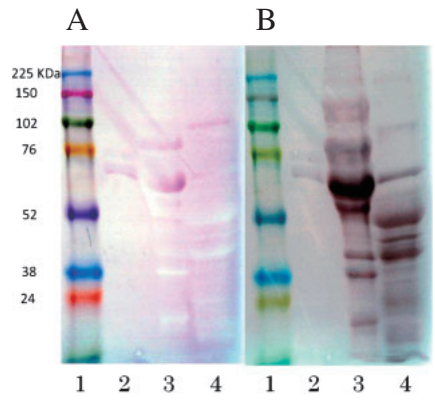

Fig. 7. NBT/Glycinate Staining of Quinoproteins on an SDS-Polyacrylamide Gel Electrophoresis Electroblot.

A and B: lane 1, marker; lane 2, purified narcissus monoamine oxidase; lane 3, bovine serum amine oxidase; lane 4, pig kidney diamine oxidase. The electroblot was first stained with NBT/glycinate (A) and then counterstained with Ponceau (B). For details, see "Materials and Methods."

Cofactor identification

The copper and manganese contents of a purified MAO from *Narcissus tazetta* were measured by an ICP-MS. The concentrations of copper and manganese in the purified enzyme were estimated to be 54.37 ppb (Cu) and 2.61 ppb (Mn)/0.3419 μM of the protein. Then, the narcissus MAO was found to be a copper-containing protein having 2.45 atoms of copper per mole of protein. AOs have been divided into two categories depending on their cofactor, FAD or Cu plus quinone. The narcissus MAO, the latter type of AO, appears to contain two copper atoms in a narcissus MAO molecule. In addition, the Mn content of the protein was calculated to be 0.14 atom of manganese per mole of protein. In general, Cu-AOs contain one copper atom per subunit.[15] Fenugreek Cu-AO contains Mn, and its contents are 0.2 moles per mole of native enzyme as determined by atomic absorption spectrometry.[18] When manganese ions were added to the reaction mixture for MAO, enzyme activity accelerated to 122% (Table 3), but the catalytic importance of manganese ions is obscure.

The NBT/glycinate method was used as a specific stain to detect quinoproteins. Narcissus MAO protein after SDS–PAGE was electroblotted onto a PVDF membrane. Figure 7, panel A displays staining of the mammalian quinoprotein, the purified narcissus MAO (lane 2), bovine serum AO (lane 3), and pig kidney AO (lane 4). Panel B in Fig. 7 shows the electroblot seen in panel A counterstained red for protein with Ponceau S. The MAO consists of a heterodimer containing 75-kDa and 65-kDa subunits (Fig. 1B). As panel B (lane 2) shows two bands were stained by NTB/glycinate in the same 65 kDa and 75 kDa positions. These results indicate that the 65 kDa and 75 kDa subunits were quinoproteins. Based on the other copper AOs, the quinone in the narcissus MAO may be TPQ.[19] The oxidized form of the AOs has a distinctive pink color at about 470–490 nm due to the presence of TPQ. The absorbance at 490 nm was 0.29 in lentil DAO solution (6.9×10^{-5} M enzyme c)[16] and 0.05 in tea MAO solution (8.6×10^{-6} M).[7] The spectrum of pink was shifted to that of yellow at 346, 432, and 462 after the addition of putrescine.[16] No obvious spectrum at about 470–490 nm was detectable in the narcissus enzyme solution (6.8×10^{-7} M). The amount of 0.092 mg of the purified enzyme must have been too little for detection of absorption spectrum. In the future, large amounts of enzyme will be necessary to examine electric charge of copper by electron paramagnetic resonance.

Amino acid sequencing of narcissus MAO

The N-terminal amino acid sequence of the two subunits of the narcissus MAO was determined by the Edman degradation method. Two identified 13 residues from each subunit of narcissus MAO were neither identical to partial sequences of any protein reported elsewhere nor similar to any amino acid sequence of leguminous DAOs or gramineous PAOs. While the N-terminal 13 residues of the 65 kDa subunit of the MAO showed fair homology (77% identity) to a predicted protein of AO from barley,[20] the 75 kDa subunit had no homology anywhere with the barley

AO (Fig. 6). A comparison of the quinone-containing sequences with the gene-encoded sequences indicated that the precursor of the cofactor was a specific Tyr residue occurring in a highly conserved sequence: Asn-Tyr(TPQ)-Asp/Glu-Tyr.[21] Researchers should examine hereafter the sequencing of the two monomers to determine whether narcissus MAO has two different monomers containing TPQ.

In mammals, three main functions have been postulated concerning the biological importance of Cu-AOs: to remove biologically active molecules such as primary amines; to form corresponding aldehydes that in some cases are known to affect cell differentiation, proliferation, and survival dramatically; and to produce hydrogen peroxide as a signal molecule rather than a harmful side product on-site.[22] In prokaryotes and fungi, AOs allow for the use of primary amines as sole sources of nitrogen for growth. In *Aspergillus niger*, two AOs were induced in cells in a growth medium containing benzylamine as nitrogen source.[23] In plants, enzymes containing DAOs are important to the synthesis of alkaloids, cell-wall formation, and wound healing,[24] but no physiological role of plant MAOs has yet been clearly defined. In a previous paper, a FAD-containing MAO (EC.1.4.3.4) purified from oat seedlings was suggested to have a catabolic role in the oxidation of phenethylamine, resulting in the decarboxylation of phenylalanine, which results in the hydrolysis of storage proteins in oat grain during germination.[8] The narcissus MAO has broad substrate specificity for short-chained aliphatic and aromatic monoamines and exerts no activity on any diamines or polyamines. MAO, a key enzyme in the rice *sl*-mutant, oxidizes tryptamine, which is biosynthesized from tryptophan by tryptophan decarboxylase.[25] Tryptamine-induced leaf lesions were significantly suppressed in the presence of semicarbazide, but narcissus MAO, despite having similar inhibitory properties, did not oxidize tryptamine (Table 2). Tea AO, similar to narcissus AO, is thought to have a role in the catabolism of ethylamine as a main component of the leaves,[26] but there is no report on monoamines in the narcissus species. The MAO may also be involved in the catabolism of short-chained aliphatic and aromatic monoamines in the shoots. Many volatile compounds containing some benzenoids have been isolated from a fragrant flower of the genus *Narcissus*,[27] but the narcissus MAO does not appear to be involved in the synthesis of the flavor benzenoids 2-phenylactaldehyde and 2-phenylethanol, because it was a lower affinity for phenethylamine (Table 2). We intend to conduct further studies of endogenous substrates of MAOs in plant species.

Acknowledgment

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