Characterization and Malathion Degradability of Carboxylesterase in Wheat Kernels

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Malathion residue in wheat kernels is enzymatically degraded into malathion monocarboxylic acids during sample preparation for pesticide residue analysis by the Japanese official method. To investigate whether the hydrolyzing enzyme is identical to carboxylesterase (CE), we compared the effects of various inhibitors against CE activity using *p*-nitrophenyl acetate as substrate with that against malathion-hydrolyzation activity. Although neither CE nor malathion-hydrolyzation activities were affected by EDTA, they were irreversibly suppressed by several serine esterase inhibitors and reversibly inhibited by cholinesterase inhibitor or sulfhydryl compounds. These inhibitions suggested that characteristics of both enzymes in wheat kernels were close to that of cholinesterase, though the enzymes were not able to be exactly classified by a classification method for mammals. When native polyacrylamide gel electrophoresis (PAGE) with esterase-zymography was performed with the addition of eserine sulfate into the sample and deoxycholic acid into the cathode buffer to prevent aggregation of CE isozymes, three clear bands of the isozymes were observed. These isozymes were confirmed by hydrophobic interaction chromatography (HIC). When malathion was reacted with each isozyme partially purified by chromatography techniques and native PAGE, malathion α-monocarboxylic acid as a major metabolite and malathion β-monocarboxylic acid as a minor metabolite were produced. These results suggested that all isozymes in wheat kernels responsible for the degradation of residual malathion in the kernels, though there are differences among malathion degradability by the isozymes.

Key words —— wheat kernel carboxylesterase, malathion degradation, esterase inhibitors, polyacrylamide gel electrophoresis, pesticide residue

INTRODUCTION

Malathion is widely used as an insecticide because of its low toxicity to mammals. The application of the pesticide is widespread, such as in post harvest treatment to wheat kernels,[1] pest control in farm fields[2] and kitchen gardens,[3] and extermination of mosquitoes as a malaria vector.[4] Malathion residue in wheat kernels is measured by the Japanese official method,[5,6] in which the wheat sample is pretreated by adding water so that it is swollen before acetone extraction. However, we previously showed that malathion residue could not be accurately determined by the official method, because of enzymatic degradation of the pesticide by the pretreatment.[7] Additionally, the supernatant of this wheat kernel homogenate did not decompose only malathion but other thion organophosphorus pesticides with carboxylester and *p*-nitrophenyl acetate as a typical substrate for carboxylesterase (3.1.1.1, CE).[8] Since the enzymatic hydrolysis of *p*-nitrophenyl acetate in the supernatant was inhibited by addition of malathion, the involvement of CE was suspected to cause the malathion degradation in the supernatant.[8] Malathion α-monocarboxylic acid and malathion β-monocarboxylic acid were identified as degradation products of malathion in the supernatant. The products also suggested that the degradation was due to CE.[9,10]

CE hydrolyzes carboxylesters and belongs to

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the esterase family.[11] 1-Naphthyl acetate and *p*-nitrophenyl acetate are generally recognized as typical substrates of CE. However, these substrates are also hydrolyzed by cholinesterase (EC3.1.1.7 or EC3.1.1.8) and arylesterase (EC3.1.1.2) which are also affiliated with esterases.[11] These esterases can be distinguished from CE by differences of their sensitivities to some inhibitors. According to the classification method for mammalian esterases using inhibitors,[12] isozymes of human blood esterase were discriminated into CE, cholinesterase and arylesterase.[13, 14]

CE exists in kernels of Chinese Spring Wheat that is one of the wheat cultivars, and the CE consists of several isozymes.[15] Rat also has isozymes of CE in its liver, and there are different specificities against various substrates among their isozymes.[16] However, it is unknown whether wheat kernel CE isozymes specifically hydrolyze malathion and whether there are differences on the malathion degradability among the CE isozymes. To solve these issues, wheat kernel CE isozyme should be separated and examined for its malathion degradability. As common separation methods for enzymes, salting out by ammonium sulfate, liquid chromatography (LC)[17] and polyacrylamide gel electrophoresis (PAGE)[18] were used. Even if the purification of CE is partially completed, the specific enzyme bands will be able to be found when the enzyme purified by LC is re-purified by native PAGE with zymography staining the active bands by 1-naphthyl acetate.

The aim of this study was to verify the following two issues. The first was whether the malathion degradable enzyme in wheat kernels is CE as defined by the classification method of mammalian esterases. To resolve the issue, the influence of inhibitors against both hydrolyzing activities of malathion and *p*-nitrophenyl acetate in the supernatant of wheat kernel homogenate was examined. The second matter was whether the hydrolyzing enzyme of malathion in wheat kernels is same as the CE enzyme. Therefore, the influence of inhibitors on those activities was compared. In addition, CE separated from wheat kernels by LC and PAGE was examined for its malathion degradability.

MATERIALS AND METHODS

Chemicals and Chromatographic Materials —— All reagents and solvents were of the highest quality available. Malathion standard, mercaptoethanol (ME), dithiothreitol (DTT) and reduced glutathione (GSH) were purchased from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan). Parathion as an internal standard for Gas Chromatography Mass Spectrometric (GC/MS) analysis was from Riedel-de Haën Sigma-Aldrich Laborchemikalien GmbH (Seelze, Germany). Tetrabutylammonium acetate (TBAA) was from Fluka Chemie AG (Buchs SG, Switzerland). Paraoxon, diisopropyl fluorophosphates (DFP) and phenyl methyl sulfonylfluoride (PMSF) were from Sigma-Aldrich Corporation (St. Louis, MO, U.S.A.). *p*-Nitrophenyl acetate, 1-naphtyl acetate and dibuthylamine acetate (DBAA) were from Tokyo Kasei Chemical Industries Co, Ltd. (Tokyo, Japan). TSKgel DEAE-650S packing material and TSKgel Phenyl-5PW column (7.5 × 75 mm) were purchased from Tosoh Corporation (Tokyo, Japan). A hydroxyapatite column, Bioscale CHT2-I column (10 μm particle size, 7 × 52 mm), was from Bio-Rad Laboratories, Inc. (Münich, Germany). Vivaspin centrifugal concentrators were from Sartorius AG (Goettingen, Germany), and the NAP 10 gel filtration column was from Amersham Biosciences AB (Uppsala, Sweden).

Preparing Wheat Kernel Extract —— The wheat kernel extract was prepared as follows: Five g of Canadian wheat (Canada Western Red Spring wheat, *Triticum aestivum*) kernels was homogenized in 10 ml of 30 mM sodium phosphate buffer (pH 7.6) by IKA Ultra Turrax homogenizer (Staufen, Germany). The homogenate was centrifuged at 6000 × *g* for 10 min. The obtained supernatant was adjusted to liberating of 660 nmol *p*-nitrophenyl acetate per min per ml and the diluted solution was defined as the "wheat kernel extract."

CE Activity with *p*-Nitrophenyl Acetate —— CE activity using *p*-nitrophenyl acetate as substrate was determined by a microplate reader in 30 mM sodium phosphate buffer (pH 7.6) at 36°C. Ten microliters of wheat kernel extract or an LC fraction was mixed with 190 μl of 30 mM sodium phosphate buffer, and the mixture was preincubated at 36°C for 2 min. Ten microliters of 25 mM *p*-nitrophenyl acetate in acetone was added to the mixture to initiate the reaction. The liberation of *p*-nitrophenol was measured for 10 min at 402 nm by a Tecan Sunrise

Rainbow Thermo microplate reader (Maennedorf, Switzerland). Two negative controls were included: no wheat kernel extract, and no substrate. The CE activity of sample solution was shown as the amount of *p*-nitrophenol liberated per 1 min per ml of the solution.

Hydrolyzing Activity of Malathion —— The sample solution of 125 μl wheat kernel extract or LC fraction was mixed with 392.5 μl of 30 mM sodium phosphate buffer (pH 7.6), and the mixture was preincubated at 36°C for 2 min; 7.5 μl of 3.0 mM malathion in acetone was then added to the mixture to initiate the reaction (the final concentration of malathion was 43.3 μM). An aliquot of 10 μl was sequentially taken and mixed with 90 μl acetonitrile including 4.5 μM parathion as internal standard. The mixture was centrifuged at $6000 \times g$ for 3 min, and malathion in the supernatant was measured by GC/MS and Liquid Chromatography/Electrospray Ionization-Mass Spectrometer (LC/ESI-MS) in the same way as in our previous report.[10)]

Effect of Esterase Inhibitors Against CE Activity —— One milliliter of wheat kernel extract was preincubated for 10 min with 5 ml of 30 mM sodium phosphate buffer (pH 7.6) and 1 ml of 25.2 mM inhibitor solutions at 36°C, an aliquot of the mixture (70 μl) was diluted with 130 μl of 30 mM sodium phosphate buffer (pH 7.6), and the mixture was preincubated at 36°C for 2 min. The activity was measured in a similar way to the "CE Activity with *p*-Nitrophenyl Acetate" (the final concentration of inhibitor was 1.2 mM). The positive controls, containing no inhibitors, were also included for comparison.

Effect of Esterase Inhibitors Against Hydrolyzing Activity of Malathion —— Five milliliter of wheat kernel extract was preincubated for 10 min with 1 ml of 25.2 mM inhibitors at 36°C, an aliquot of the mixture (150 μl) was diluted with 367.5 μl of 30 mM sodium phosphate buffer (pH 7.6), and the mixture was preincubated at 36°C for 2 min, 7.5 μl of 3.0 mM malathion in acetone was then added to the mixture to initiate the reaction. The malathion residue of the reaction mixture of 30 min after the initiation was measured in a similar way to the "Hydrolyzing Activity of Malathion" (the final concentration of inhibitor was 1.2 mM). The positive controls, containing no inhibitors, were also included for comparison.

Partial Purification of Wheat Carboxylesterase —— Chromatographic purification was performed by a Shimadzu Class LC-10VP series HPLC system including three LC10ADVP pumps, a SPD10AV UV detector (Kyoto, Japan) and a JASCO SF-212N fraction collector (Tokyo, Japan) with the column temperature of 5°C. Wheat kernels (Canada Western Red Spring wheat) were powdered by fiber mixer and put through a 35 mesh sieve. The sifted powder was rinsed with acetone to remove lipids. Ten grams of the wheat powder was homogenized in 20 ml buffer (pH 6.0) containing 5 mM bis Tris-HCl, 1.2 mM DTT and 1.2 mM eserine sulfate, and centrifuged. The supernatant was applied to a Tskgel DEAE-650S column (10×175 mm) equilibrated with 5 mM bis Tris-HCl buffer (pH 6.0, buffer A) and then eluted with 2 ml/min flow rate of buffer A containing 150 mM NaCl. The CE active fraction collected was mixed with 1.2 mM DTT and 1.2 mM eserine sulfate and concentrated with a Vivaspin centrifugal concentrator. The concentrate was replaced in buffer containing 5 mM potassium phosphate, 1.2 mM DTT and 1.2 mM eserine sulfate (pH 6.8) by NAP 10 column. The obtained solution was applied to a Bio-Rad Bioscale CHT2-I column (7×52 mm) equilibrated with the 5 mM potassium phosphate buffer (pH 6.8), and eluted with 36 mM potassium phosphate buffer (pH 6.8). The CE active fractions were concentrated with a Vivaspin and replaced in buffer (pH 7.6) containing 1.25 M potassium phosphate, 5 mM TBAA, 1.2 mM DTT and 1.2 mM eserine sulfate by NAP 10 column. The obtained solution was applied to a TSKgel phenyl-5PW column (7.5×75 mm) equilibrated with buffer containing 1.25 M potassium phosphate and 5 mM DBAA (pH 7.6, buffer B). Gradient elution of CE was carried out at a flow rate of 0.5 ml/min with buffer B and 5 mM potassium phosphate buffer with 5 mM DBAA (pH 7.6).

Native Polyacrylamide Gel Electrophoresis with Zymography (Native PAGE-zymography) —— Sample loading buffer including 30% glycerin, 0.01% bromophenol blue, 0.05% DTT and 0.5% eserine in 0.125 M Tris-HCl (pH 6.8) was prepared. The sample was mixed with the same quantity of the sample loading buffer. The mixture was subjected to vertical electrophoresis apparatus under 20 mA with precasting gel PAG mini "DAIICHI" 4–15% gradient gel (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). The anode electrophoresis buffers (pH 8.3) included 25 mM Tris and 192 mM glycine, and the cathode electrophoresis buffer (pH 8.3) was the anode buffer with 0.1% deoxycholic acid. After the electrophoresis, the gel was stained for detection of esterase activity with 1-naphthyl acetate as the

substrate. The gel was rinsed with distilled water and soaked in 30 mM sodium phosphate buffer (pH 7.6). One ml of 20 mg/ml 1-naphthyl acetate in acetone was poured into the soaking buffer. The staining was carried out by adding 40 mg Fast Blue BB salt, and the stained gel was soaked in 7.5% acetic acid. For preparation of enzyme solutions for the active band, CE in the cutout of the band was eluted by Model 422 Electro-Eluter (Biorad Laboratories, Inc., Hercules, CA, U.S.A.).

RESULTS AND DISCUSSION

Effect of Esterase Inhibitors on Activities of CE and Hydrolyzing Enzyme for Malathion

To investigate whether enzymatic characterization of hydrolyzing enzyme for *p*-nitrophenyl acetate as CE in wheat kernels is similar to that of the hydrolyzing enzyme for malathion, we compared the extent of inhibition by various inhibitors against the two enzymatic activities. The inhibitors examined were paraoxon, DFP and PMSF (serine esterase inhibitors),[19–21] eserine (cholinesterase inhibitor),[22, 23] ME, DTT and GSH (sulfhydryl compounds)[24–27] or EDTA (chelating agent for bivalent metal ions).[28–30]

Figure 1 shows the inhibitory extent by esterase inhibitors against hydrolyzing activity of malathion or *p*-nitrophenyl acetate in wheat kernels after incubation of wheat kernel extract with 1.2 mM esterase inhibitor for 10 min and after removal of the inhibitor by a NAP10 gel filtration column.

An addition of EDTA did not influence the hydrolyzing activity against either substrate (Fig. 1A and B). Bacterial esterases are reported to require some bivalent cations like Co^{2+};[28] however, this result suggested that neither hydrolyzing activity in wheat kernel extract requires bivalent cation.

All of the serine esterase inhibitors suppressed CE activity and malathion hydrolase activity in the order of the inhibitory potency: paraoxon ≈ DFP > PMSF (Fig. 1C and D). These inhibitions were irreversible, since they were still present after the inhibitors were removed from the reaction mixtures by gel filtration. These results indicate that CE and the hydrolyzing enzymes of malathion are serine esterases.

Eserine sulfate as a typical cholinesterase inhibitor suppressed both hydrolyzing activities (Fig. 1E and F). The inhibition was reversible, because both activities were recovered by removal

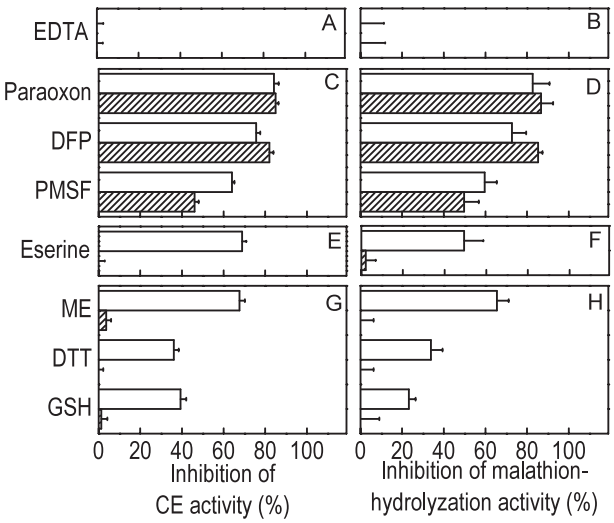

Fig. 1. Effect of Esterase Inhibitors on Malathion or *p*-Nitrophenyl Acetate-hydrolyzation Activities in Wheat Kernels

Wheat kernel extract was incubated with EDTA as a chelating agent (A, B), paraoxon, DFP and PMSF as serine esterase inhibitors (C, D), eserine sulfate as a cholinesterase inhibitor (E, F) and ME, DTT and GSH as sulfhydryl compounds (G, H) at 1.2 mM as final concentration for 10 min. *p*-Nitrophenyl acetate hydrolyzation-activity (A, C, E, G) or malathion hydrolyzation-activities (B, D, F, H) were quantitatively measured before gel filtration (□) and after gel filtration to remove the inhibitors (▨). Percent of inhibition represents mean ± S.D. (n = 4).

of the inhibitor from the reaction mixture. These findings suggest that both CE and the hydrolyzing enzyme of malathion in wheat kernels may possess an active site similar to cholinesterase, as cholinesterase has not only serine residue but also a negative charge region in its active site.

All sulfhydryl compounds inhibited the hydrolysis activities for both substrates in the order of the inhibitory potency: ME > DTT > GSH (Fig. 1G and H). Since both activities recovered after the sulfhydryl compounds were removed from the reaction mixtures, the inhibition of the hydrolysis activities was reversible. The inhibitions by sulfhydryl compounds suggest that the wheat kernel hydrolases may possess disulfide linkages around their active sites. Indeed, the hydrolytic activities for both substrates were irreversibly inhibited by *N*-ethyl maleimide which is specific alkylating reagent for sulfhydryl group (data not shown). Furthermore, it is reported in bacterial esterases that the disulfide linkages around the active sites are reductively cleaved by sulfhydryl compounds and the enzymatic activities are inhibited.[26, 27]

Both enzyme activities were classified by Aldridge's method for mammalian esterases,[12] which divides esterases into the three classes, CE,

cholinesterase and arylesterase based on the influence profile of various inhibitors against the enzyme activities. In this classification method, neither CE nor cholinesterase is inhibited by sulfhydryl compounds or EDTA, but by serine-esterase inhibitors. While the former is not inhibited by eserine, the latter is inhibited. Arylesterase is not affected by serine esterase inhibitors or eserine sulfate, but is inhibited by EDTA and sulfhydryl compounds.

According to the classification, it is suggested that characteristics of both CE and hydrolyzing enzyme for malathion in wheat kernels are closely related to cholinesterase in respect to their inhibition by serine esterase inhibitors and eserine sulfate, though the enzymes are unable to be exactly classified.

Electrophoretic Profile by Native PAGE and Elution Pattern by Hydrophobic Interaction Chromatography for Wheat Kernel CE

For detection of wheat CE, wheat kernel extract was separated by common native PAGE following Davis' method,[31] and then CE-active bands were visualized by zymographic technique in which the hydrolyzate of α-naphthyl acetate as a typical substrate of CE was colored by diazo-coupling reaction. As shown in Lane A of Fig. 2, the zymogram consisted of a broad band of 140–440 kD and three sharp bands of 28, 30 and 33 kD. Barber *et al.* have reported that there are slow-moving esterases and fast-moving esterases in wheat kernels by native PAGE, and the latter contain isozymes.[32] The broad band and three sharp bands on Lane A in Fig. 2 probably correspond to the slow-moving and fast-moving esterases in Barber's report, respectively. We assumed that the enzymes in the broad band were aggregates of CE in sharp bands by hydrophobic and/or electrostatic interaction. Of those bands with CE activity, we focus on the three sharp bands.

Native PAGE was performed with addition of 0.1% deoxycholic acid as a surfactant into the cathode buffer solution to prevent CE aggregation. However, the broad band was only shifted to the side of lower molecular weight, retaining its activity (Lane B in Fig. 2).

Wheat kernel CE is expected to have at least one anionic amino residue in its active center, because the activity is inhibited by a cationic regent, eserine sulfate. If the aggregation of CE was caused by electrostatic effect of the anionic residue, the aggregation can be prevented by formation of an ion pair with the cationic reagent. The broad band is not moved by adding eserine sulfate to the sample solution, while the density was attenuated (Lane C in Fig. 2).

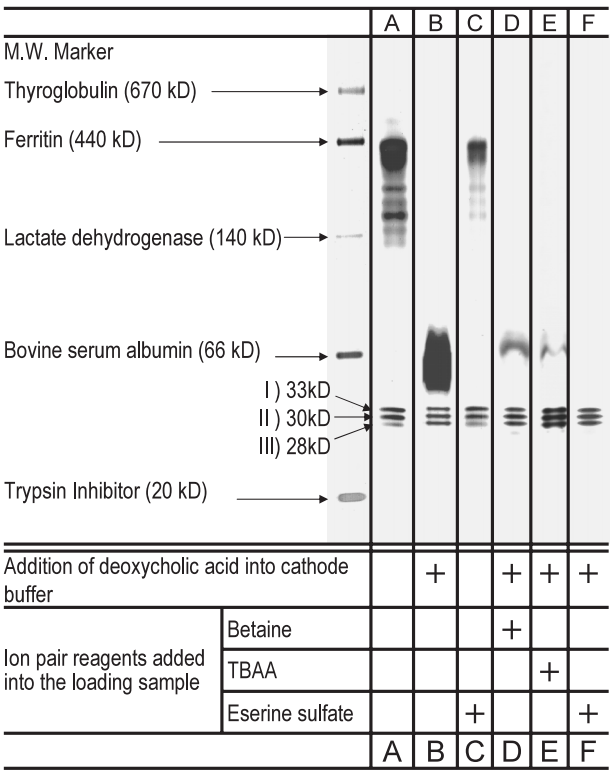

Fig. 2. Effect of Deoxycholic Acid and Ion Pair Reagents on Native PAGE-esterase Zymogram of Wheat Kernel Homogenate

Wheat kernel extract was subjected to native PAGE with addition of deoxycholic acid into cathode buffer (B, D, E and F) and an ion pair reagent into loading sample (C, D, E and F). The CE active bands were stained by zymographic technique.

Native PAGE was performed with a combination of deoxycholic acid and the cationic ion pair reagent: betaine, TBAA acetate or eserine sulfate. In the case of betaine (Lane D), the area of broad band was reduced more than the area in Lane B or C. The area of broad band in Lane E with addition of TBAA acetate was decreased compared with Lane D and almost disappeared. The addition of eserine sulfate completely eliminated the broad band, and only three bands (33, 30 and 28 kD) were consequently recognized as shown in Lane F.

When the extract from sharp bands was resubjected to native PAGE by conventional Davis' method, the broad band was apparent with the original sharp bands (data not shown). The reconstruction of the broad band suggests that wheat kernel CE in sharp bands tend to aggregate in the broad band CE under the conventional conditions of na-

tive PAGE, though there is a possibility that broad band CE was inactivated by dividing into subunits under modified conditions of native PAGE.

Although the three sharp bands with CE activity were observed by native PAGE-zymography of the wheat kernel extract, there is a possibility that these CE-active bands are due to artifacts. To test whether these three bands possessed CE activity, wheat kernel CE was partially purified by DEAE column and hydroxyl apatite column and then fractionated by hydrophobic interaction chromatography (HIC). CE activity and hydrolyzing activity for malathion in each fraction were measured and native PAGE-zymography of CE active fractions was performed.

CE activity was detected from the 28th to 34th fractions of HIC (Fig. 3A). Hydrolyzing activity of malathion was also detected in CE active fractions (Fig. 3B). When the CE active fractions were subjected to native PAGE by the conditions of Lane F in Fig. 2, three bands of 28, 30 and 33 kD were detected and CE isozymes of those bands were eluted through the HIC column from low molecular weight CE (Fig. 4). These results indicate that the three bands are not due to artifacts and that there are three CE isozymes in wheat kernels. Thus, CE and the hydrolyzing enzyme for malathion in CE-active fractions are probably identical.

Zymographic techniques of CE isozymes have been used for cultivar discrimination of wheats;[33,34] however, our result indicates that these isozymes easily aggregate. Thus, the electrophoretic conditions of the reports seemed to be improper to use for the discrimination. If the discrimination is carried out, our conditions of Lane F in Fig. 2 may bring a more accurate result.

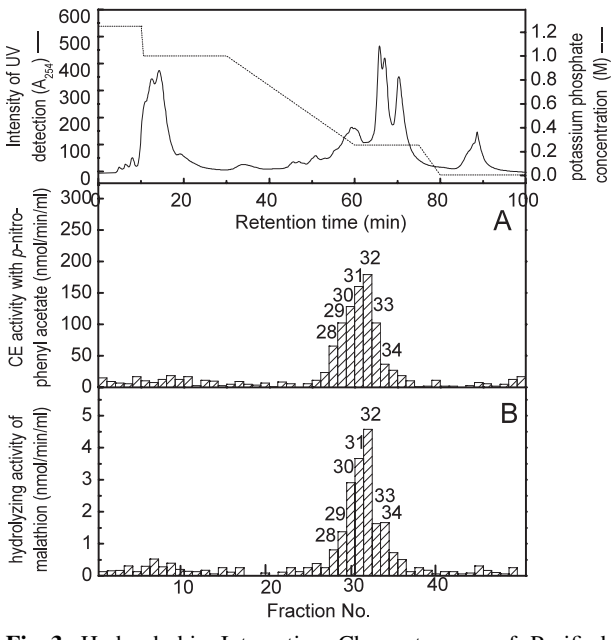

Fig. 3. Hydrophobic Interaction Chromatogram of Purified Wheat Kernel Extract and *p*-nitrophenyl Acetate and Malathion-hydrolyzation Activities of Their Fractions

Wheat kernel CE was partially purified from wheat kernel extract by DEAE column and hydroxyapatite column. The CE active fractions were collected and then applied to HIC. The collected CE active-fractions were measured using *p*-nitrophenyl acetate (A) and malathion (B).

Malathion Degradability by CE Isozymes

To identify CE with hydrolyzing activity for malathion, we investigated malathion degradability by CE isozymes that were extracted from the three bands of native PAGE with the conditions of Lane F in Fig. 2. Each CE isozyme of the active bands was cut out from the gel in Fig. 4, and extracted by an electro eluter. The extracts were diluted to adjust them all to the same CE activity. The degradability of malathion in the extracts was estimated by GC/MS and LC/ESI-MS.

All CE isozyme solutions from I to III bands degraded malathion into malathion α-monocarboxylic acid and malathion β-monocarboxylic acid as shown in Fig. 4. In all the hydrolysis reactions, the production amount of malathion α-monocarboxylic acid was larger than that of 4 hr after the reaction. Such predominant production of malathion α-monocarboxylic acid has also been found in plants such as rice[35] and beans[36] as well as mammals.[37–39] These report suggest that malathion α-monocarboxylic acid may be a universally primer hydrolytic metabolite of malathion. The reason that malathion α-monocarboxylic acid is predominantly produced might be explained by the electronic theory. The electron accepting group thion (S = P)

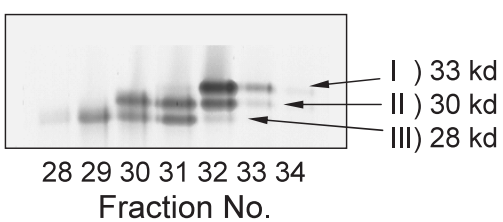

Fig. 4. Native PAGE Esterase Zymograms of CE-active Fractions Separated by HIC

Each CE-active fraction separated by HIC as shown in Fig. 3 was subjected to native PAGE with addition of deoxycholic acid into cathode buffer and eserine sulfate into loading sample as shown in Lane F of Fig. 2. The CE active bands were stained by zymographic technique.

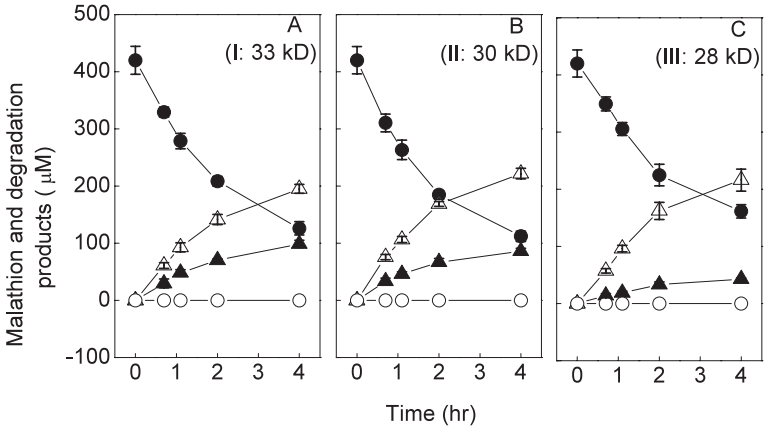

Fig. 5. Malathion Decomposition and Its Products by CE Isozymes Partially Purified by LC and Modified Native PAGE

Malathion was reacted in the isozyme solutions extracted from the electrophoretic band of 33 kD(A), 30 kD(B) and 28 kD(C) shown in Fig. 4. Malathion (●), malathion α-monocarboxylic acid (△), malathion β-monocarboxylic acid (▲) and malathion dicarboxylic acid (○) in the reaction mixture were measured by GC/MS and LC/ESI-MS. Values are means ± S.D. of 4 samples.

in malathion molecule predominantly withdraws an electron of the α-carboxylester, and thus the ester is predominantly hydrolyzed. The generation ratios of malathion α-monocarboxylic acid to malathion β-monocarboxylic acid after the reaction for 4 hr by the isozymes of the bands I (33 kD), II (30 kD) and III (28 kD) were 1.9, 2.6 and 5.0, respectively. The isozyme III (28 kD) as the lowest molecular weight CE tended to produce a relatively small amount of malathion β-monocarboxylic acid, though the predominant amount of malathion α-monocarboxylic acid was similar to that of other isozymes. Such variation of the generation ratios seems to be due to the enzymic active center-specificity against the β-ester structure of malathion, since predominant production of the metabolites is considered to be caused by the electro-withdrawing effect of S = P.

In conclusion, all CE isozymes in wheat kernels correspond to degradation of malathion residue in the kernels, though there are some differences among the isozymes. According to the classification, it is suggested that characteristics of wheat kernel CE are close to cholinesterase in respect to the inhibition by serine esterase inhibitors and eserine sulfate, though wheat kernel CE in wheat kernels cannot be precisely classified. This enzyme contains three isozymes with the molecular weights of 28, 30 and 33 kD, and these isozymes tended to aggregate with each other. We have already reported that most residual malathion in wheat kernels degraded when the pesticide was measured following the official Japanese method.[7] However, most of this residual malathion seemed hardly hydrolyzed, probably because most of the postharvest malathion locates on the surface of wheat kernels. When the residual malathion is measured by the official method, swelling of wheat kernels by water causes hydrolyzation of malathion residue. From the results of this study, therefore, the solvent extraction without water-swelling[7] or the addition of serine esterase inhibitors to inactivate CE may be effective for accurate analysis of determining the residual malathion in wheat kernels.

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