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Catalytic Performance of a Recombinant Organophosphate-Hydrolyzing Phosphotriesterase from *Brevundimonas diminuta* in the Presence of Surfactants

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Abstract: Phosphotriestease (PTE), also known as parathion hydrolase, has the ability to hydrolyze the triester linkage of organophosphate (OP) pesticides and chemical warfare nerve agents, making it highly suitable for environment remediation. Here, we studied the effects of various surfactants and commercial detergents on the esterase activity of a recombinant PTE (His_6-tagged *Bd*PTE) from *Brevundimonas diminuta*. Enzymatic assays indicated that His_6-tagged *Bd*PTE was severely inactivated by SDS even at lower concentrations and, conversely, the other three surfactants (Triton X-100, Tween 20, and Tween 80) had a stimulatory effect on the activity, especially at a pre-incubating temperature of 40 °C. The enzyme exhibited a good compatibility with several commercial detergents, such as Dr. Formula® and Sugar Bubble®. The evolution results of pyrene fluorescence spectroscopy showed that the enzyme molecules participated in the formation of SDS micelles but did not alter the property of SDS micelles above the critical micelle concentration (CMC). Structural analyses revealed a significant change in the enzyme's secondary structure in the presence of SDS. Through the use of the intentionally fenthion-contaminated Chinese cabbage leaves as the model experiment, enzyme–Joy® washer solution could remove the pesticide from the contaminated sample more efficiently than detergent alone. Overall, our data promote a better understanding of the links between the esterase activity of His_6-tagged *Bd*PTE and surfactants, and they offer valuable information about its potential applications in liquid detergent formulations.

Keywords: phosphotriestease; *Brevundimonas diminuta*; esterase activity; pesticide remediation; surfactant; detergent

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1. Introduction

Enzymes are macromolecular biocatalysts that accelerate chemical reactions by providing an alternative reaction pathway with lower activation energy. Certain enzymes are of great interest and are being used as catalysts in various biological processes for the large-scale production of industrially important products [1,2]. Enzymes are widely employed in the detergent industry [3], but a key drawback for the use of enzymes in detergent formulations is their stability [4]. The prime ingredients of detergents include surfactants, bleaching agents, builders, foam regulators, corrosion inhibitors, optical brighteners, and other minor additives (e.g., enzymes, perfumes, and fabric tensors) [3–6]. Surfactants, an essential component in most cleaning products are surface-active compounds that have been extensively applied to a wide range of industrial domains, including detergents, cosmetics, fabric softeners, paints, and emulsions [7]. The molecular interactions between enzymes and surfactants can be beneficial to promoting the catalytic performance [8–11]

and reducing the aggregation propensity [12] of the biocatalysts, or harmful to the enzyme's three-dimensional structure [13]. The detrimental effect of surfactants has always been a particular problem in the detergent industry where active enzymes are absolutely necessary for effectively removing hardest and stubborn stains [3].

Previously, the impact of surfactants on protein stability and folding kinetics has been thoroughly reviewed by Otzen and co-workers [6,14,15]. Since surfactants and proteins are all amphiphilic molecules, some intermolecular interactions, particularly hydrophobic and electrostatic, can occur [16,17]. Protein–surfactant interactions at the hydrophobic interfaces have been shown to play a critical role in protein folding by enhancing the compactness of protein's unique three-dimensional shape [18]. The importance of electrostatic interactions can also be seen by studying the effect of pH on protein–surfactant interactions [19]. With regard to SDS, these interactions are much stronger than those of other denaturants (e.g., urea and GdnHCl), which have been proved to act through weak interactions with the backbone of proteins [20]. Surfactant-mediated denaturation of a specific protein is initiated from the surface-accessible regions and eventually leads to the changes in its secondary and tertiary structures [21]. In general, a protein subjected to surfactant-mediated denaturation can recover its native state upon removal of the denaturant, although the refolding yield varies substantially from protein to protein [22–24]. A research study on β-lactoglobulin, lysozyme, and bovine serum albumin has shown that the surfactant-mediated unfolding and refolding of these globular proteins are extremely complex processes with conformational rearrangements occurring on time scales from sub-milliseconds to minutes [24].

Phosphotriesterase (PTE; EC 3.1.8.1) is a membrane-associated metalloenzyme that hydrolyzes the phosphate-triester linkages of a variety of organophosphorus (OP) compounds [25,26]. Although the natural substrate for PTE is still a mystery now [27], the enzymes from different source organisms have convergently evolved its ability to hydrolyze OP pesticides and chemical warfare nerve agents [28,29]. Further details on the three-dimensional structure of *Brevundimonas diminuta* PTE (*Bd*PTE) have revealed that the overall fold of this enzyme consists of an α/β-barrel with eight strands of parallel β-pleated sheet [30,31], and the active site is situated at the C-terminal region of the β-barrel with three binding pockets for substrate accommodation [32]. Moreover, the enzyme's active site contains two metal ions separated by 3.7 Å and coordinated to the side-chains of His 55, His 57, His 201, His 230, and Asp 301, and connected together through a hydroxide and a carbamate functional group formed by the reaction of carbon dioxide with the ε-amino group of Lys 169 [32].

Recently, the functional expression and physiochemical characterization of His_6-tagged *Bd*PTE have been performed [33]. Given the fact that the recombinant enzyme can efficiently degrade ethion and fenthion (two commonly used organophosphate pesticides in Taiwan), it surely has the potential application as an additive in surfactant-based formulations for the removal of pesticide residues from fruits and vegetables. In spite of advances made in the biochemical and structural properties of *Bd*PTE [27,32], the effects of surfactants and commercial detergents on enzyme function are poorly explored. Therefore, the overall goal of this investigation was to evaluate the influence of several surfactants on the catalytic performance of His_6-tagged *Bd*PTE. The experimental data may provide valuable insights into the practical use of this enzyme in liquid detergent formulations.

2. Results and Discussion

2.1. Impacts of Surfactants and Commercial Detergents on the Esterase Activity

To understand the impacts of SDS, Triton X-100, Tween 20, and Tween 80 on the esterase activity of His_6-tagged *Bd*PTE, the enzyme was pre-incubated with these surfactants at the indicated temperatures for 1 h, and the residual activity was immediately determined as described below. The experimental results were recorded as the percentage of residual activity calculated with reference to the activity controls that were running in parallel in the absence of surfactants. As shown in Figure 1, the esterase activity of His_6-tagged *Bd*PTE was severely inhibited by SDS even at a lower concentration of 0.5%.

Earlier, there are also some other studies dealing with how SDS acts as an enzyme inactivator to reduce the catalytic performance of fatty acid synthase [34], α-glucosidase [35], zinc-dependent aminoacylase [36], lactate dehydrogenase [37], hyperthermophilic inorganic pyrophosphatase [38], and protein tyrosine phosphatase [39]. Although it has been reported that SDS can activate *Sulfolobus soltataricus* PTE-like lactonase (*Sso*Pox) and its engineered variant [40–42], the catalytic activity and stability of a recombinant organophosphorus hydrolase (*Po*OPH) from *Pseudomonas oleovorans* are seriously impacted by this surfactant [43]. Possible explanations for the catalytic enhancement of *Sso*Pox by SDS have been proposed to be detergent-induced dissociation and conformational change of the enzyme homodimer, but all these possibilities are finally ruled out by analyses of the control and treated samples with size-exclusion chromatography, dynamic light scattering, and tryptophan fluorescence [41]. SDS consists of a 12-carbon tail and a sulfate head group, which endow it amphiphilic properties. The peculiar structural characteristics usually allow SDS to interact with proteins to form a negatively charged complex, thus disrupting the non-covalent interactions of protein chains and eventually causing a protein to lose its native structure [22]. It is currently unknown whether this phenomenon occurred during the treatment of His_6-tagged *Bd*PTE with SDS. This can be verified by examining the interaction mechanism of SDS to the enzyme. In contrary to SDS, the water-soluble non-ionic surfactants (Triton X-100, Tween 20, and Tween 80) had a stimulatory effect on the esterase activity of His_6-tagged *Bd*PTE, especially at 40 °C (Figure 1). The enhancement of catalytic activity by non-ionic surfactants has already been demonstrated in *Sso*Pox [43], *Flavobacterium* sp. PTE (*Fs*PTE) [44], and other hydrolytic enzymes [8,9,45–47]. Moreover, the immobilization of *Po*OPH as a cross-linked enzyme–polymer conjugate has also proved to greatly enhance its organophosphate-hydrolyzing ability in the presence of non-ionic surfactants [48]. In the case studies of the enzymatic hydrolysis of cellulose and lignocellulose, non-ionic surfactants can make substrates more accessible for the enzyme [49], increase enzyme adsorption sites on the substrate's reducing ends [50], stabilize the enzyme molecules during hydrolysis [51], reduce the non-productive adsorption of enzymes onto substrates [52], and avoid enzyme deactivation caused by shear force and the air–liquid interface [47]. Although we do not have enough data to propose a mechanism for the activity stimulation of His_6-tagged *Bd*PTE by these three non-ionic surfactants, this finding definitely provides promising information to future applications of the enzyme. It is also worth mentioning that a significant reduction in the esterase activity was observed after one-hour incubation of the enzyme at 60 °C for 1 h in the presence and absence of non-ionic surfactants (Figure 1). A sharp decrease in activity could be due to the thermal denaturation of His_6-tagged *Bd*PTE at the elevated temperatures.

Nowadays, more than half of detergents contain hydrolytic enzymes, making the detergent industry a dominant market for commercial enzymes [3,53]. In power detergents, enzymes are usually prepared in the form of dust-free granulates that are subsequently fabricated with hydrophobic coatings to protect them against damage by detergent components. Upon the release of enzymes from the dust-free granulates, they should still able to carry out the reaction in the presence of anionic and non-ionic detergents. As documented by Bjerre et al. [54], the major challenge for enzyme stability in powder detergents is bleach, which in combination with humidity can oxidize the biocatalyst. The stability of enzymes is also a fairly serious drawback in liquid detergent formulations so that several efforts, including protein engineering, proper balancing of chelators, and enzyme–polymer conjugation, have been devoted to overcome this problem [55–57]. Except for the above-mentioned efforts, the discovery and development of microbial enzymes with special characteristics can offer a promising alternative to help address enzyme instability in liquid detergents [3,58]. To determine whether His_6-tagged *Bd*PTE can be considered as a good detergent additive, the affinity-purified enzyme was added to different concentrations of commercial detergents, and its residual activity was subsequently assayed after one-hour incubation. As shown in Figure 2, His_6-tagged *Bd*PTE was very stable in two brands of detergents, Dr's Formula® and Sugar Bubble®. That may be because of soft surfactants are

used by these two detergents as the essential ingredients. For most detergents tested, the enzyme was relatively active under a working concentration of less than 1.5%. Nevertheless, as compared to other commercial detergents, His_6-tagged *Bd*PTE exhibited less activity in the presence of greater than 1.5% Persil®. A logical explanation for this observation is that Persil® formulates with a certain amount of sodium laureth sulfate, which is an anionic surfactant used frequently as a cleaning agent in the commercial detergents. Giving that His_6-tagged *Bd*PTE is compatible with a variety of commercial detergents, this should provide ample space for its future application in liquid detergent formulations.

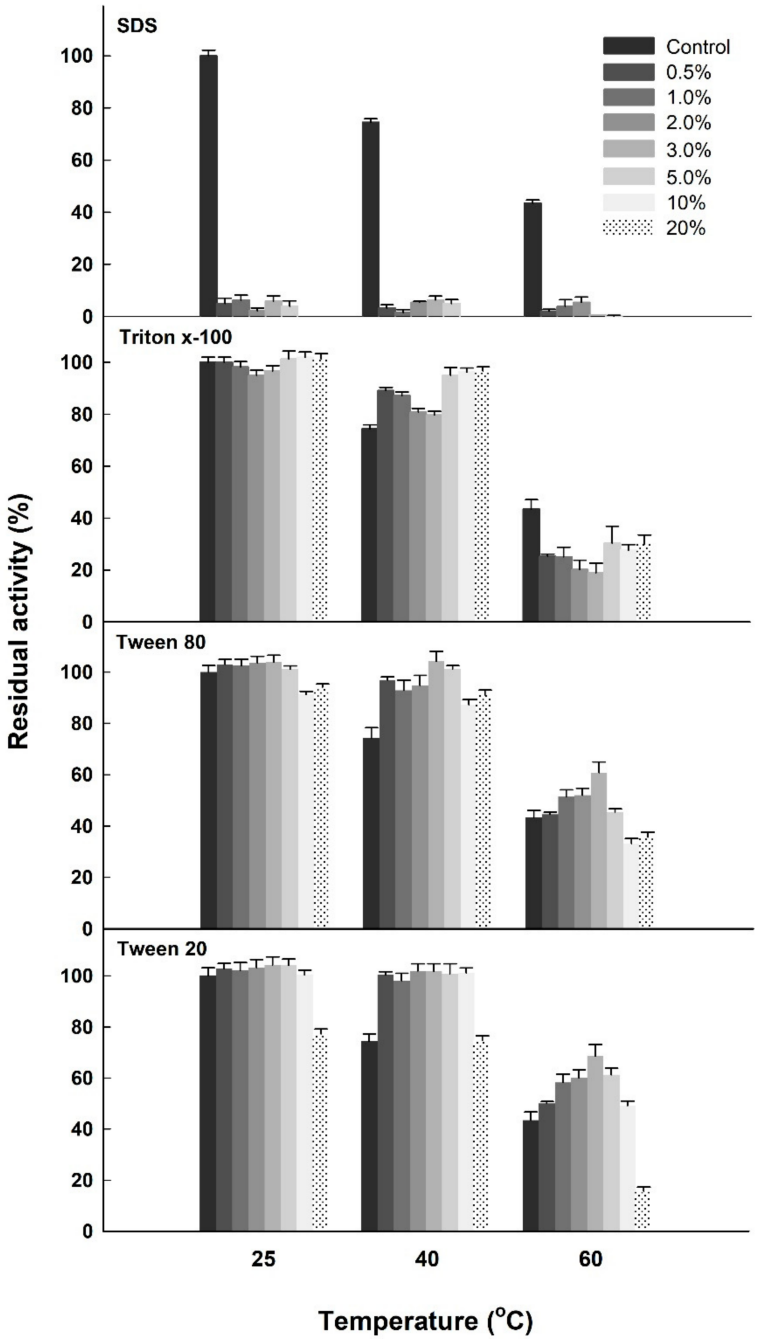

Figure 1. Effects of four different surfactants on the esterase activity of His_6-tagged *Bd*PTE. The enzyme samples were pre-incubated at the indicated temperatures for 1 h before activity assays. The esterase activity in the absence of surfactants was used as a control (100%).

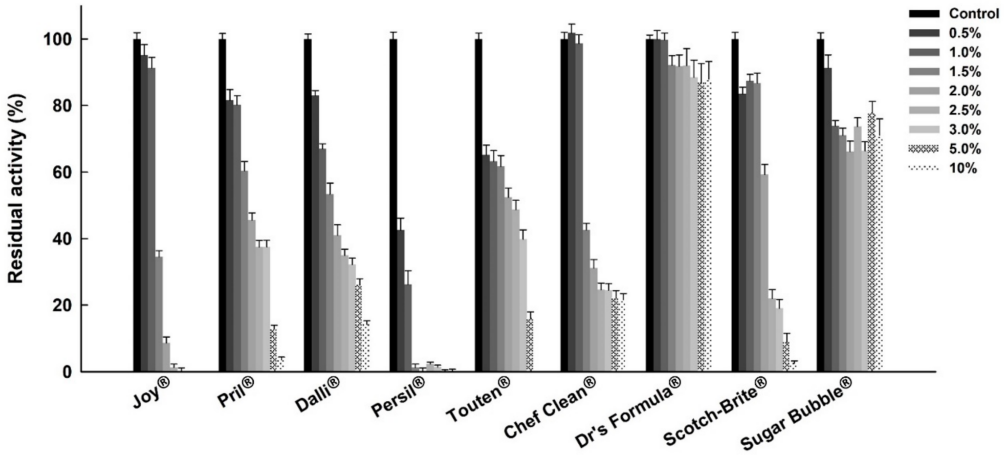

Figure 2. Effects of some brands of liquid detergents on the esterase activity of His_6-tagged *Bd*PTE. The enzyme samples were pre-incubated at ambient temperature for 1 h before activity assays. The esterase activity in the absence of detergents was used as a control (100%).

2.2. Fluorescence Spectra of the His-Tagged Enzyme in the Presence of Surfactants

To determine the effects of SDS, Tween 20, and Tween 80 on the tertiary structure of His_6-tagged *Bd*PTE, the intrinsic fluorescence spectra of the enzyme in the presence of 3% surfactants were collected. Considering the benzene ring in Triton X-100 has fluorescent and UV absorption properties that overlap with those of proteins, this surfactant was excluded from the fluorescence experiment. The spectral results showed that His_6-tagged *Bd*PTE had a maximum emission wavelength (λ_{max}) of 336.2 nm and its λ_{max} was red-shifted by 1.0 and 1.4 nm, respectively, when the incubation temperature was increased to 40 and 60 °C (Figure 3). It is noteworthy that the maximum fluorescence intensity (I_{max}) had been changed by SDS and its alteration was more profound at 60 °C. Interestingly, no significant shift in λ_{max} coupled with a slight reduction in I_{max} was observed at 60 °C in the presence of Tween 20 (Figure 3). However, both λ_{max} and I_{max} of His_6-tagged *Bd*PTE were significantly altered upon the addition of Tween 80. Tryptophan residue usually has a λ_{max} at 348 nm after being exposed to solvents [59]. Since the λ_{max} of untreated His_6-tagged *Bd*PTE is 336.3 nm, the tryptophan residues should largely be buried in the hydrophobic interior of the enzyme. The λ_{max} was redshifted about 4.4 nm along with the increase of I_{max} after pre-incubating His_6-tagged *Bd*PTE with 3% Tween 80 at 60 °C for 1 h, suggesting an induced exposure of the enzyme's tryptophan residues into a relatively hydrophilic microenvironment. This conformational change appeared not to greatly affect the catalytic performance of His_6-tagged *Bd*PTE (Figure 1). Thus, further study is warranted to elucidate the real reason for this observation.

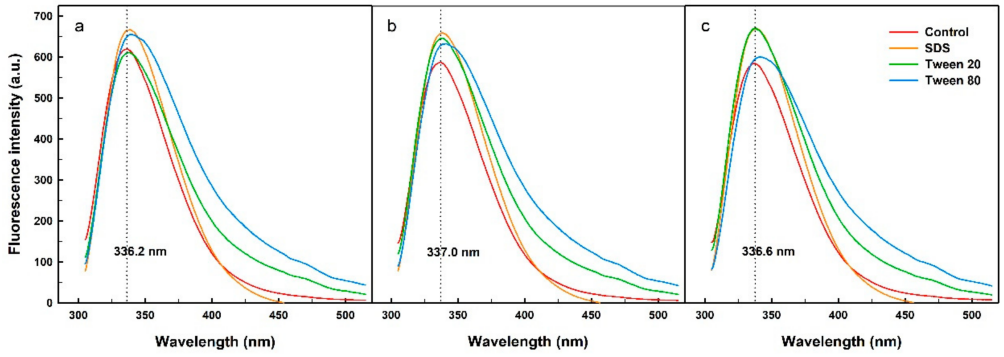

Figure 3. Fluorescence spectra of His_6-tagged *Bd*PTE in the absence and presence of 3% (*v*/*v*) surfactants. Spectral analyses were carried out after one-hour incubation of the enzyme samples at ambient temperature (**a**), 40 °C (**b**), and 60 °C (**c**), respectively, for 1 h.

2.3. Pyrene Fluorescence Spectra of SDS and Tween 20 in the Absence and Presence of the His-Tagged Enzyme

Critical micelle concentration (CMC) is an important parameter for surfactant concentration optimization and therefore needs to be determined in the application and development of surfactants. The determination of CMC using pyrene fluorophore is based on its change in the intensity ratio (I_3/I_1) of two emission peaks at 372.5 (I_1) and 383.5 (I_3) nm with surfactant concentration [60]. In the absence of enzyme (Figure 4a,b), this ratio changed from 0.63 to 0.91 upon the partition of pyrene fluorophore into SDS micelles, indicating that SDS micelles are formed in the concentration range of 3.9–8.7 mM. In presence of 0.3 μM His_6-tagged *Bd*PTE, pyrene apparently experienced a more hydrophobic environment than water in the concentration range of 2.0–7.9 mM SDS. This may be due to the formation of SDS aggregates on the surface of enzyme molecule, into which pyrene is able to partition. It is noteworthy that approximately 69% of the control activity was retained in the presence of 1 mM SDS, and the enzyme was nearly completely inactivated when the surfactant concentration was increased to 4 mM (data not shown). This result indicated that a complete loss of the esterase activity occurs when the SDS concentration reached the CMC. The same experiment was also carried out with Tween 20 as the surfactant (Figure 4c,d). In the absence of His_6-tagged *Bd*PTE, the I_3/I_1 ratio changed very quickly over a concentration range of 0.1 to 1.0 mM. A large-scale evolution of I_3/I_1 ratio indicated that the formation of Tween 20 micelles is a relatively fast process as compared with SDS. As shown in Figure 4d, the evolution of I_3/I_1 ratio remained unchanged in the presence of His_6-tagged *Bd*PTE, suggesting that the enzyme does not participate in the formation of Tween 20 micelles. This result may explain that Tween 20 has shown no detrimental effects on the esterase activity of His_6-tagged *Bd*PTE (Figure 1).

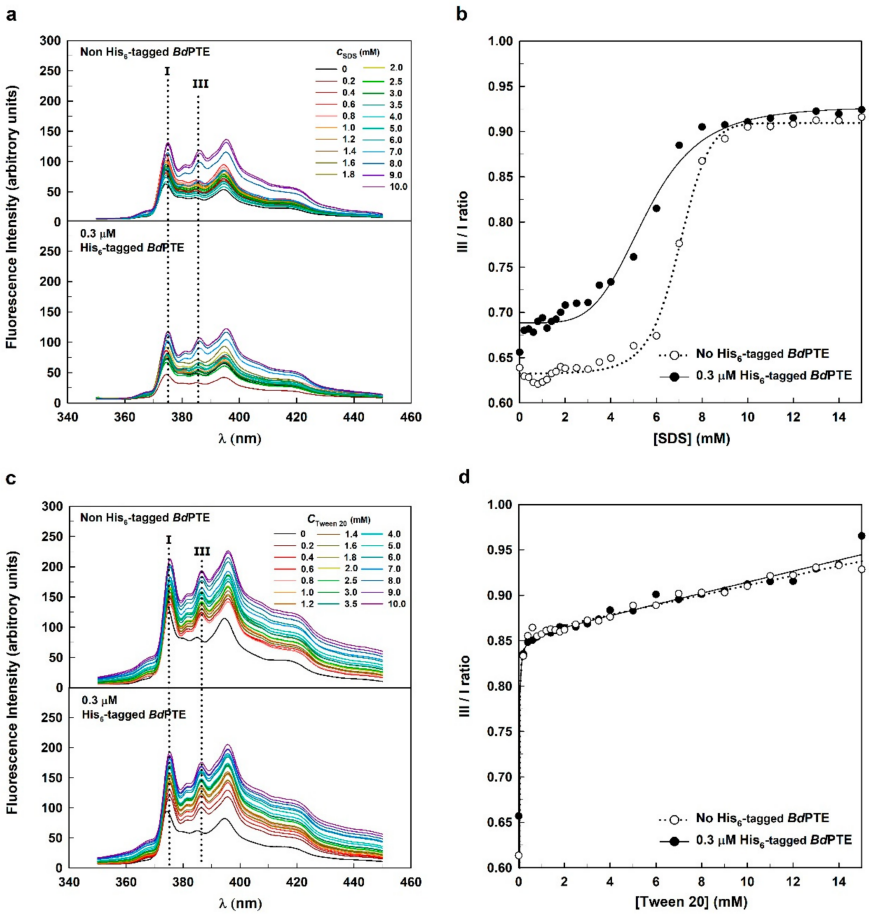

Figure 4. Fluorescence emission spectra of pyrene with a series of concentrations of SDS (**a**) and Tween 20 (**c**), and the relationship between surfactant concentration and the I_3/I_1 ratio of pyrene (**b**,**d**).

2.4. Far-UV CD Spectra of the His-Tagged Enzyme in the Presence of Surfactants

Since both Triton X-100 and Tween 80 had a very strong interference on the CD signal of His_6-tagged *Bd*PTE, we did not determine the enzyme's CD spectra in the presence of these two surfactants. To reveal the impact of SDS on the secondary structures of His_6-tagged *Bd*PTE, far-UV CD spectra of the enzyme in the presence of 0–10% SDS were collected. The CD spectral results showed that the molar ellipticity at 208 and 222 nm was dramatically increased by SDS in a temperature-independent manner (Figure 5), suggesting an induced formation of the enzyme's α-helical structure by this surfactant. Considering that *Bd*PTE is a membrane-bound enzyme [61], the amphiphilic properties of SDS may provide a more proper environment for its folding in comparison with aqueous solutions. However, it is also true that the esterase activity of His_6-tagged *Bd*PTE is sensitive to the presence of SDS (Figure 1). In the proposed mechanism of *Bd*PTE catalysis [32], a water molecule in the active site is initially activated by the bimetallic center to produce the hydroxide ion. When the OP substrates enter the active site, they can coordinate with the αZn^{2+} ion to form an enzyme–substrate complex. Then, the activated nucleophile attacks the phosphoryl center through an S_N2-like mechanism of which the anionic intermediate is stabilized by both Zn^{2+} ions. Once the bond between the phosphate and leaving group is broken, the subsequent progress toward expulsion of the phosphorus moiety allows for enzymatic regeneration via uptake of a catalytic water molecule from the bulk solution. In this respect, a reasonable explanation for the conflicting results is that the anionic group of SDS may allow it to interact with the divalent zinc center of the enzyme and consequently affects the binding of OP substrates. Moreover, the CD spectrum of His_6-tagged *Bd*PTE remained basically unchanged in the presence of Tween 20 unless the pre-incubation temperature was shifted to 60 °C (Figure 5). The changes in secondary structures together with a minor alteration in tertiary structure may be responsible for the loss of activity occurred at the pre-incubation temperature of 60 °C (Figure 1).

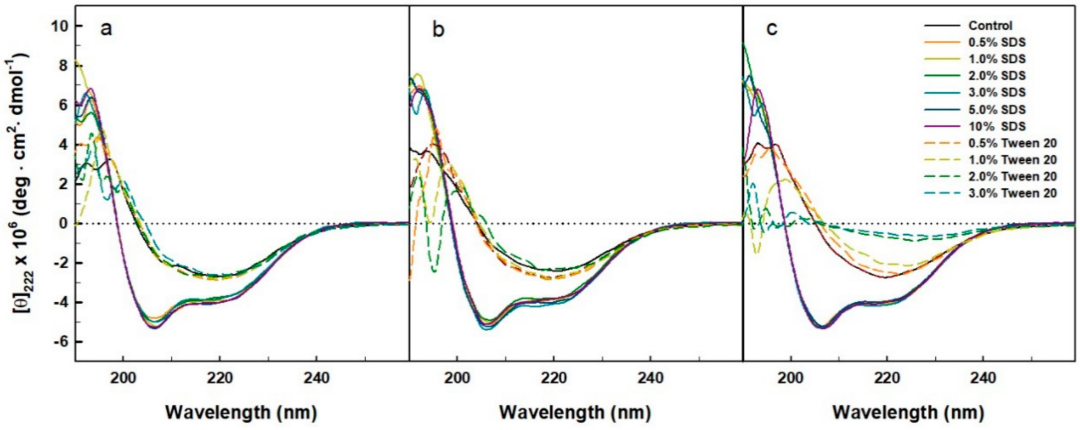

Figure 5. Far-UV CD spectra of His_6-tagged *Bd*PTE in the absence and presence of surfactants. Spectral analyses were carried out after one-hour incubation of the enzyme samples at ambient temperature (**a**), 40 °C (**b**), and 60 °C (**c**), respectively, for 1 h.

2.5. Removal of Fenthion from the Artificially Contaminated Sample

Fresh vegetables and fruits are the important part of a healthy diet due to the widespread presence of significant amounts of nutrients and minerals in them [62]. Nevertheless, these foods can sometimes turn out to be the source of toxic substances, particularly pesticides [63]. Over the last decades, pesticides have been intensively used in agriculture to increase the world food production in response to the growth of global population [64]. Among various pesticide classes, the OP pesticide group is the most popular class of agricultural pesticides [65]. This class of pesticides kills insects by targeting their nervous system so that they are also known to have neurotoxic effects in animals [66]. To evaluate the potential

application of His_6-tagged *Bd*PTE in liquid detergent formulations for the decontamination of OP pesticides, the intentionally fenthion-contaminated Chinese cabbage samples were individually washed with water, 0.5% (*v*/*v*) Joy® washer, and enzyme–Joy® washer solution, and the pesticide residues in these samples were quantified by HPLC (Figure 6). Technically, the use of 80% methanol along with 20% water as the mobile phase allowed us to identify a fenthion peak with a retention time of 7.3 min, which is in good agreement with a previous study [67]. As shown in Figure 6, a removal efficiency of 18.1% was obtained when the fenthion-contaminated Chinese cabbage leaves were washed with just water. The cleanup efficiency was improved a little bit upon washing with 5% Joy® washer, whereas about half (48.7%) of the added pesticide was eliminated by enzyme–Joy® washer solution. These observations suggest that the incorporation of His_6-tagged *Bd*PTE into the liquid detergent formulations for the pesticide removal seems to be a promising way to improve the overall performance of the compatible detergent.

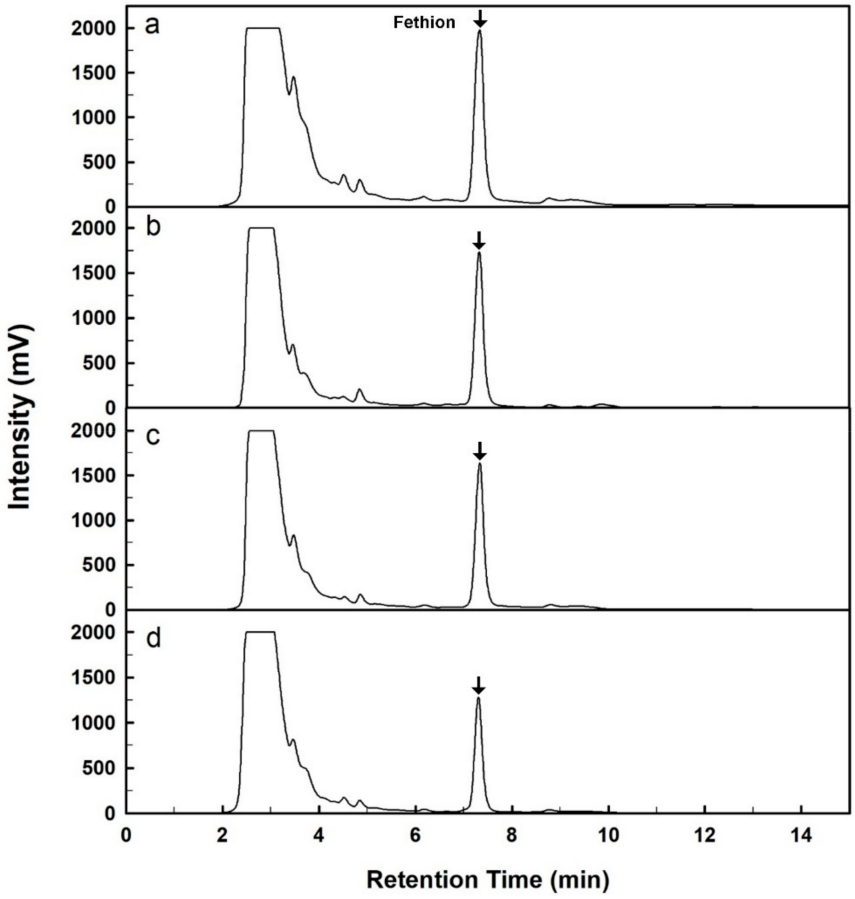

Figure 6. HPLC chromatographs of the intentionally fenthion-contaminated samples. (**a**) The pesticide-contaminated sample without washing steps was used as a control; (**b**) The pesticide-contaminated sample was washed with water before HPLC analysis; (**c**) The pesticide-contaminated sample was washed with Joy® washer before HPLC analysis; (**d**) The pesticide-contaminated sample was washed with enzyme–Joy® washer solution before HPLC analysis.

3. Materials and Methods

3.1. Chemicals and Reagents

Luria–Bertani (LB) media for the cultivation of recombinant *Escherichia coli* M15 cells were purchased from Difco Laboratories Inc. (Detroit, MI, USA). Ni-NTA superflow agarose resin for His-tagged enzyme purification was acquired from Qiagen (QIAGEN Taiwan Company Ltd., Taipei, Taiwan). Paraoxon-ethyl, *p*-nitrophenol (*p*-NA), sodium dodecyl sul-

fate (SDS), *t*-octylphenoxypolyethoxyethanol (Triton X-100), poly(oxyethylene)$_{20}$ sorbitan monolaurate (Tween 20), poly(oxyethylene)$_{80}$ sorbitan monolaurate (Tween 80), and fenthion reference standards CAS# 55-38-9 were obtained from Sigma-Aldrich Fine Chemicals (St. Louis, MO, USA). Commercially available detergents, including Joy®, Pril®, Dalli®, Persil®, Touten®, Chief Clean®, Dr's Formula®, Scotch-Brite®, and Sugar Bubble® were all brought from a local retailer (Carrefour, Taiwan). Protein Assay Dye Reagent Concentrate (5 ×) was purchased from Bio-Rad Laboratories, Taiwan Branch (Taipei, Taiwan). Other chemicals labeled with analytical or biological grade were supplied by local vendors and were used as received without any purification.

3.2. Enzyme Preparation and Esterase Activity Assay

The growth of *E. coli* M15 cells harboring pQE-*Bd*PTE, and IPTG-induced protein expression were essentially carried out as described previously [33]. The cell-free extract of *E. coli* M15 (pQE-*Bd*PTE) was subjected to affinity chromatography using the Ni-NTA Superflow Cartridge (Qiagen) according to the supplier's instructions, and SDS-12% polyacrylamide gel was used to check the purity of His-tagged enzyme. Protein concentration was routinely determined by reference to a calibration curve established with a series of bovine serum albumin (BSA) standards.

Esterase activity was assayed with a colorimetric method described by Rochu and coworkers [68]. Briefly, the reaction mixture (0.5 mL) comprised of 0.48 mM paraoxon-ethyl in 25% (*v*/*v*) methanol, 1.0 mM Co^{2+}, 10 μL of appropriately diluted enzyme solution, and 25 mM Tris-HCl buffer (pH 8.0). Following the incubation at 60 °C for 10 min, the release of *p*-NP was measured directly by absorbance at 405 nm with an ultraviolet-visible spectrophotometer (Great Tide Instrument Co., Ltd., Taipei, Taiwan). All enzyme assays were performed in triplicate, and the mean absorbance value was taken. A calibration curve for the action of His$_6$-tagged *Bd*PTE was simultaneously established by adding various concentrations of *p*-NP chromophore instead of paraoxon-ethyl into the reaction mixture. One unit of PTE activity is defined as the amount of enzymes required to release 1 μmol of *p*-NP/min from paraoxon-ethyl.

3.3. Effects of Surfactants and Commercial Detergents on the Esterase Activity

Effects of ionic (SDS) and non-ionic (Triton X-100, Tween 20, and Tween 80) surfactants on the esterase activity of His$_6$-tagged *Bd*PTE were evaluated by individually incubating 300 μL of enzyme sample (≈0.6 μg/mL) with an equal volume of different concentrations of above-mentioned surfactants at ambient temperature, 40, and 60 °C for 1 h. Following the incubations, their residual activities were measured under the standard assay conditions. All the experiments were performed in triplicate, and data were shown as the mean ± SD.

Effects of commercial detergents, including Joy®, Pril®, Dall®, Persil®, Touten®, Chief Clean®, Dr's Formula®, Scotch-Brite®, and Sugar Bubble®, on the esterase activity of His$_6$-tagged *Bd*PTE were also determined toward *p*-NA assay. The enzyme sample (300 μL) with a protein concentration of ≈0.6 μg/mL was incubated with an equal volume of different concentrations of the aforementioned detergents at ambient temperature for 1 h, and their residual activities were monitored under the standard assay conditions. All the experiments were performed in triplicate and data are shown as the mean ± SD.

3.4. Fluorescence Studies

All fluorescence experiments were carried out on a JASCO Spectrofluorometer FP-6500 (JASCO International Co., Ltd., Tokyo, Japan) operated in the ratio mode. Fluorescence spectra of His$_6$-tagged *Bd*PTE at a final concentration of 0.3 μg/mL were measured at 25 °C (unless otherwise stated) in the presence or absence of surfactants. After the addition of surfactants, the enzyme samples were allowed to equilibrate for 1 h before spectral analysis. The intrinsic fluorescence of His$_6$-tagged *Bd*PTE was excited at 295 nm and the emission spectra were recorded within the wavelength range of 305–500 nm at a scanning rate of 240 nm/min. Final spectra were the average of three corrected spectra.

The critical micelle concentration (CMC) of surfactants was determined as described previously [69]. A pyrene ethanol stock solution (0.5 mM) was prepared and added to the samples up to a final concentration of 1 μM. Excitation at 335 nm was carried out with excitation and emission slit widths of 3.5 nm. The ratio of the intensity of the emission at 372.5 nm to the intensity of the emission at 383.5 nm was used for further analysis.

3.5. Circular Dichorism (CD) Studies

CD measurements were recorded on the JASCO J-815 spectropolarimeter (JASCO Corporation, Tokyo, Japan) using a 0.1-cm path length quartz cuvette under constant nitrogen flux at 25 °C. All scans were taken in the ultraviolet region (190–250 nm) under a protein concentration of 0.2 μg/mL. A scanning speed of 20 nm/min was carried out with an averaging time of 4 s and a wavelength step of 0.2 nm. At least 10 scans were accumulated and averaged for each spectrum, and the control signal was taken simultaneously and subtracted from the correspondence analysis to avoid the generation of a false signal.

The CD data were expressed as mean residue ellipticity (MRE) in deg cm^2/dmol according to Equation (1) [70]:

$$\text{MRE} = \frac{\text{observed CD (m deg)}}{C_\text{p} nl \times 10}. \quad (1)$$

where c_p represents the protein concentration (mol/L) of the tested sample, n is the number of amino acid residues of the enzyme, and l denotes the path length of the quartz cell (cm).

3.6. Enzyme Treatment of the Fenthion-Contaminated Chinese Cabbage Leaves

The effectiveness of enzyme treatment to reduce the pesticide residue in the intentionally fenthion-contaminated Chinese cabbage leaves was evaluated in the presence and absence of Joy®, a popular vegetable and fruit washer. The sliced Chinese cabbage leaves (≈5 g) was firstly washed with a suitable amount of water, wiped dry with paper towel, and then immersed into 20 mL of the fenthion solution (0.5 mg /mL) for 10 min. Thereafter, the fenthion-treated cabbage leaves were placed at a chemical hood for air dry and individually added to 50 mL of water, Joy® washer (0.5%, *v*/*v*), and enzyme (30 μg/mL)–0.5% Joy® washer solution. After 1 h of incubation at ambient temperature, each sample was homogenized and thoroughly mixed with 50 mL acetonitrile. The filtrated extract was collected with a serum bottle containing 5 g NaCl. Following the vigorous shaking at ambient temperature for 1 min, the acetonitrile faction was recovered and dried at 40 °C. The dried samples were re-constituted in 1 mL of methanol and were kept in −20 °C until used.

HPLC analyses of the afore-prepared samples were carried out on Hitachi L2130 pump system (Utech Scientific Co., Ltd., New Taipei, Taiwan) equipped with a Mightsil RP-18 GP column (250 mm × 4.6 mm; particle size, 5 μm). The analyte was eluted at a flow rate of 1 mL/min under isocratic mode with 80% (*v*/*v*) methanol as the mobile phase. Fenthion was detected using the diode array detector at a fixed wavelength of 254 nm. The data generated were analyzed using D-2000 Elite software.

4. Conclusions

To date, the enzymatic remediation of pesticides appears to be a promising approach to reduce the harmful impact of OP pesticides on the environment. Our previous report has shown that His_6-tagged *Bd*PTE confers the ability to degrade two OP pesticides commonly used in Taiwan and exhibits some degree of tolerance to a variety of organic co-solvents. In this study, we further observed that the enzyme is very active in the presence of water-soluble Tween and Triton surfactants, and it shows a good compatibility with a number of commercial detergents. Based on these findings, His_6-tagged *Bd*PTE may be applicable in liquid detergent formulations for the effective remediation of the OP-contaminated fruits and vegetables.

Author Contributions: M.-C.C., T.-Y.L., and M.-G.L. performed the experiments, analyzed the data, and provided feedbacks on the interpretation of results. L.-L.L. participated in the design of the study and wrote the original draft. T.-F.W. acted as the project administrator to supervise the study and revised the draft. All authors have read and agreed to the published version of the manuscript.

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