Cross-linked enzyme-polymer conjugates with excellent stability and detergent-enhanced activity for efficient organophosphate degradation

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Abstract

Background: Enzymatic biodegradation of organophosphate pesticides (OPs) is a promising technology to remove these toxic compounds. However, its application in industrial washing was restricted by the lack of efficient immobilized enzymes that can work at high temperatures and high pHs in the presence of various detergents. Therefore, it is necessary to develop a simple method to prepare a robust immobilized enzyme for efficient degradation of OPs.

Results: An organophosphate hydrolase (OPH), *Po*OPH$_{M9}$, was conjugated and immobilized with a commercially available polymer, Pluronic F127. The prepared cross-linked enzyme-polymer conjugate (CLEPC) displayed higher pH stability in the range from 7.0 to 11.0 and a higher optimal temperature (50 °C) than that of free *Po*OPH$_{M9}$ (30 °C). Its half-life and apparent k_{cat}/K_M reached 12.8 h at 50 °C and 390.3 ± 7.8 mM^{-1} s^{-1}, respectively, which were even better than that of the traditional cross-linked enzyme aggregates (CLEA, 7.2 h and 10.9 ± 1.7 mM^{-1} s^{-1}). The activity of *Po*OPH$_{M9}$ CLEPC was further enhanced up to 2.5-fold by the anionic, nonionic and biocompatible detergents, which was first observed. 0.15 mM Malathion was degraded completely by *Po*OPH$_{M9}$ CLEPC after activation within 10 min in the presence of 0.1% (w/w) detergents of all types at pH 9.0 and 25 °C, demonstrating its capability in degrading OPs at practically relevant conditions.

Conclusion: The conjugation of Pluronic F127 in enzyme immobilization could effectively reduce the activity loss of immobilized enzymes and enhance their stability and activity at high temperatures and high pHs. In addition, the activity of CLEPC can be even enhanced in the presence of various detergents. This technology can be extended easily to produce other immobilized polymer-enzyme conjugates due to its simplicity.

Keywords: Organophosphate, Organophosphate hydrolase, Immobilization, Biodegradation, Enzyme-polymer conjugates, Pluronic F127

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Background

The accumulation of toxic organophosphate pesticides (OPs) in food and environment has caused an increasing threat to public health (Mostafalou and Abdollahi 2017; Cycon et al. 2017; Hernandez et al. 2017). OPH based biodegradation, which can efficiently hydrolyze OPs to benign molecules, is one of the most attractive technologies for removing OPs (Schenk et al. 2016; Kim et al. 2014a, b; Ramalho et al. 2016; Theriot and Grunden 2011). Many naturally occurring or engineered OPHs have been developed so far (Cherny et al. 2013; Khare et al. 2012; Luo et al. 2014; Abe et al. 2014; Bigley et al. 2016; Chen et al. 2015; Jackson et al. 2009). However, in spite of high activity, free OPHs are difficult to be recovered from aqueous solution, and are often denatured quickly under harsh environments such as high temperatures and high pHs that are usually used in industrial washing and water treatment, hindering its usage in practical applications (Giudice et al. 2016; Bai et al. 2017). Therefore, it is essential to develop a simple and cost-effective method for fully exploiting the potential of OPHs in biodegradation.

Currently, various enzyme immobilization methods to improve the stability and recyclability of OPHs have been extensively explored, including the immobilization of enzymes on nanoparticles (Breger et al. 2015; Hondred et al. 2017), mesoporous silica (Singh et al. 1999), membranes (Yan et al. 2015), textiles (Gao et al. 2014), and encapsulation or complexation of enzymes with amyloid fibrils (Raynes et al. 2011), metal–organic frameworks (Li et al. 2016), polyurethane foam (LeJeune et al. 1997) and polymers (Suthiwangcharoen and Nagarajan 2014; Wei et al. 2013). Carrier-free method, such as cross-linked enzyme aggregate (Zheng et al. 2011; Pan et al. 2011), has been used in the production of value-added chemicals in large quantities (Pan et al. 2014). Although enzyme immobilization gives rise to a better thermostability, it is usually achieved at the expense of the decreased catalytic activity of an enzyme. In addition, the immobilization method should face challenges from practical applications. For example, the immobilized OPHs need to be stable and active at very harsh conditions such as high temperatures and alkaline pH in industrial washing of OP contaminated food. Particularly, they should bear various detergents that can easily denature proteins. Therefore, this special application requirement makes the immobilization of OPHs an extremely difficult task, compared with other immobilized enzymes for traditional biocatalysis.

Recently, a triblock amphiphilic copolymer, poly (ethylene oxide-b-propylene oxide-b-ethylene oxide) F127 (known as Pluronic F127), has been demonstrated to enhance the stability and activity of free OPHs through the interaction between the hydrophobic segments of polymers and the hydrophobic regions of enzyme surface (Suthiwangcharoen and Nagarajan 2014; Kim et al. 2014b). In addition to OPHs, various enzymes can be modified by direct conjugation or covalent binding with Pluronic F127 (Wu et al. 2014, 2015; Zhang et al. 2013). Although advances in the conjugation of Pluronic F127 and free enzymes have been made so far, the application of Pluronic F127 in immobilized enzymes has not been achieved. One possible reason is that the current methods of preparing enzyme-polymer conjugates are performed in aqueous phase (Suthiwangcharoen and Nagarajan 2014; Kim et al. 2014b) and the carrier-immobilized enzymes are not soluble in aqueous solution. More importantly, the improving effect of Pluronic F127 in the catalytic properties of free OPH has not been demonstrated in immobilized OPH yet. Thus, it is of interest to develop a simple method of preparing immobilized OPH-Pluronic F127 conjugates with the expectation of maintaining the high stability and activity of OPH with Pluronic F127 simultaneously.

In the previous study, we engineered a newly discovered phosphotriesterase (*Po*OPH$_{M9}$, Luo et al. 2016) with improved catalytic activity and thermostability for efficient malathion degradation, and demonstrated its effectiveness in removing malathion using free *Po*OPH$_{M9}$ in the presence of various detergents (Bai et al. 2017), which prompted us to further explore the possibility of preparing immobilized enzymes for potential industrial applications. Herein, we reported a simple method to prepare cross-linked enzyme-polymer conjugates (CLEPC) by directly incorporating Pluronic F127 in the process of carrier-free *Po*OPH$_{M9}$ immobilization. The catalytic efficiency and stability of *Po*OPH$_{M9}$ CLEPC were systematically investigated, and its capability in degrading malathion in the presence of various detergents under practically relevant conditions were demonstrated.

Materials and method

Chemicals

All chemicals of reagent grade were purchased from Sigma (St. Louis, MO). Malathion (99% purity) was purchased from Shanghai Pesticide Research Center. The *P. pastoris* strain X33 (Mut$^+$His$^+$) bearing the plasmid of pPICZαA containing *Po*OPH$_{M9}$ gene was constructed in our lab. Detergents containing coconut oil derivatives (COD, Komi, Lot 6907974981509), Sodium dodecyl sulphate (SDS, Mama Lemon, Lot 6903624600158) and alkyl polyglycoside (APG, Diaopai, Lot 6910019001841) were purchased from a local supermarket without further treatment.

Preparation of *Po*OPH$_{M9}$ CLEPC

*Po*OPH$_{M9}$ was produced extracellularly by high-density fermentation of an engineered *Pichia pastoris* strain described previously (Bai et al. 2017). After fermentation, the broth was centrifuged at 8000 rpm for 20 min, and the supernatant was concentrated and freeze-dried to form lyophilized enzyme powders. The lyophilized enzyme powders were dissolved in a Tris–HCl buffer (pH 7.0) to 20 mg/mL, to which ammonium sulfate was slowly added to 0.5 g/mL. The solution was then stirred at 4 °C for 30 min to precipitate enzymes. Subsequently, Pluronic F127 was slowly added into the suspension to 80 mg/mL and the mixture was continuously stirred at 4 °C for 30 min. Glutaraldehyde of 40 mM was added to cross-link the aggregates and the suspension was stirred at 4 °C for 3 h. Then, the suspension was centrifuged at 12,000 rpm for 3 min, and the precipitate was washed three times and re-suspended in 0.5 mL Tris–HCl buffer (pH 7.0).

Activity assays of *Po*OPH$_{M9}$ CLEPC

The catalytic activity of *Po*OPH$_{M9}$ CLEPC toward malathion was monitored by determining the increase in absorbance at 412 nm caused by the reaction between the hydrolysis product, mercaptan, and the Ellman reagent, 5,5′-dithiobis-(2-nitrobenzoic acid) (Bai et al. 2017). Each reaction was performed in a 1 mL Tris–HCl buffer (45 mM, pH 9.0) containing 1.8 mM DNTB, 0.5 mM malathion and appropriate amount of the immobilized enzyme. Absorbance readings were taken every min at 30 °C using a PowerWave XS2 spectrophotometer (BioTek, USA). The kinetic parameters were determined by fitting the Michaelis–Menten equation with the substrate concentrations from $0.02 \times K_m$ to $10 \times K_m$. The activity recovery of the CLEPC and CLEA was calculated according to Eq. (1).

$$\text{Activity recovery (\%)} = \frac{\text{Activity of immobilized enzyme } (U)}{\text{Activity of the initial free enzyme for prep.}(U)} \times 100 \quad (1)$$

Enzymatic characterization of *Po*OPH$_{M9}$ CLEPC

*Po*OPH$_{M9}$ CLEPC and free *Po*OPH$_{M9}$ were incubated separately at 10–80 °C for 15 min, respectively, and then their activities were measured to obtain the temperature profile. Similarly, the optimal pH was obtained by incubating the enzymes between pH 7.0 and 11.0 for 15 min before activity assay. The activity of the *Po*OPH$_{M9}$ CLEPC and free *Po*OPH$_{M9}$ relative to their optimal activity was calculated according to Eq. (2).

$$\text{Relative activity (\%)} = \frac{\text{Activity detected under the certain condition } (U)}{\text{Activity detected under the optimal condtion } (U)} \times 100 \quad (2)$$

To determine the thermostability, *Po*OPH$_{M9}$ CLEPC, *Po*OPH$_{M9}$ CLEA and free *Po*OPH$_{M9}$ were incubated at 50 °C for 7 h. The samples of the enzyme solutions were taken at different time intervals for activity assay. The residual activity of the samples at different time was calculated according to Eq. (3).

$$\text{Residual activity (\%)} = \frac{\text{Activity detected at time t } (U)}{\text{Activity detected at time 0}(U)} \times 100 \quad (3)$$

The relative activity of immobilized and free enzymes in the presence of various detergents at different time was calculated according to Eq. (3). To determine the reusability, the reaction mixture was centrifuged at 12,000 rpm for 3 min to recover the immobilized enzymes. The collected enzymes were washed for 3 times and the relative activity of *Po*OPH$_{M9}$ CLEPC after each round was measured and calculated against the initial activity detected in the first batch.

Degradation of malathion by *Po*OPH$_{M9}$ CLEPC in the presence of detergents

Typically, 980 μL of 50 mM Tris–HCl buffer (pH 9.0) containing 0.3 mM DNTB and 0.1% (w/w) of COD, APG and SDS was mixed with 10 μL of 15 mM malathion and 10 μL of 420 U/mL *Po*OPH$_{M9}$ CLEPC. The mixture was stirred at 25 °C or 50 °C for 30 min and the absorbance readings were taken every minute. The hydrolysis percentage was calculated by comparing the absorbance at different times with that of the completely hydrolyzed product.

Results and discussion

Preparation of *Po*OPH$_{M9}$ CLEPC

Previous study demonstrated that the hydrophobic PPO blocks of Pluronic F127 can interact with hydrophobic amino acids such as Phenylalanine (Phe) on the surface of OPH, and physically associate with OPH to form a non-covalent binding enzyme-polymer complex (Kim et al. 2014b). *Po*OPH$_{M9}$ (PDB: 4O98) is an engineered OPH belonging to the superfamily of phosphotriesterase (Luo et al. 2016), and 10 Phe residues (22 in total) among other hydrophobic residues are located on the surface (Additional file 1: Figure S1). Thus, we modified the process of preparing CLEA and added Pluronic F127 into the reaction mixture after protein precipitation for enzyme-polymer conjugation (Fig. 1). Particularly, the ratio of mass concentration (g/L) of *Po*OPH$_{M9}$ to Pluronic F127 was adjusted from 1:0 to 1:6, affording the highest activity

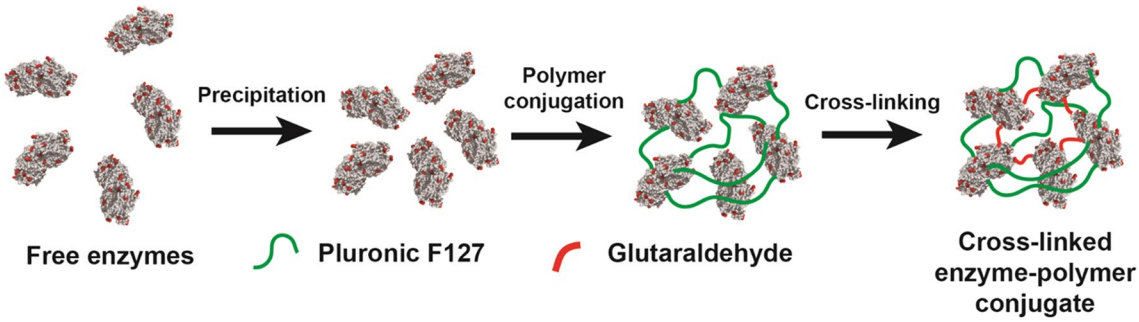

Fig. 1 Schematic diagram of preparing CLEPC. The red dots on the surface of the free enzyme *Po*OPH$_{M9}$ represent the lysine residues that are cross-linked by glutaraldehyde

recovery of 60% at 1:4, which was used in the following experiments (Fig. 2a). Interestingly, the order of adding Pluronic F127 in the preparation procedure affected the activity recovery of the immobilized enzyme. The polymers can be added into the enzyme solution before protein precipitation (PL–AS–GL, simply for the order of Pluronic F127/ammonium sulfate/glutaraldehyde), after the formation of enzyme aggregates (AS–PL–GL) and after the formation of CLEA (AS–GL–PL). As shown in Fig. 2b, the activity recovery of AS–PL–GL (83%) was higher than that of PL–AS–GL (55%), AS–GL–PL (37%) and CLEA (32%). Notably, all enzyme-polymer conjugates incorporating Pluronic F127 showed higher activity recoveries than that of CLEA, demonstrating Pluronic F127 indeed stabilized enzymes during immobilization. The concentrations of $(NH_4)_2SO_4$, *Po*OPH$_{M9}$ and glutaraldehyde were also optimized to improve the activity recovery (Fig. 2c–e). The prepared catalysts can be easily distributed in aqueous phase during the degradation reaction and recovered by centrifugation for recycle (Fig. 2f). The particles of *Po*OPH$_{M9}$ CLEPC were observed in TEM analysis with a mean diameter of 0.66 ± 0.12 μm (Fig. 2g).

*Po*OPH$_{M9}$ CLEPC displayed a higher apparent k_{cat} (49.1 ± 1.2 s^{-1}) than that of *Po*OPH$_{M9}$ CLEA (1.1 ± 0.1 s^{-1}, Table 1). Although *Po*OPH$_{M9}$ CLEPC had a slightly increased K_M (125.7 ± 7.7 μM) compared to that of *Po*OPH$_{M9}$ CLEA (103.8 ± 1.7 μM) because of the polymers, its catalytic efficiency (k_{cat}/K_M, 390.3 ± 7.8 mM^{-1} s^{-1}) was much higher that of *Po*OPH$_{M9}$ CLEA (10.9 ± 1.7 mM^{-1} s^{-1}) and also a little bit higher than that of the free enzyme (265.4 ± 2.0 mM^{-1} s^{-1}). This result suggested that the addition of Pluronic F127 during immobilization can effectively increase the catalytic efficiency of immobilized enzyme, which was consistent with the effect of Pluronic F127 on free enzymes (Kim et al. 2014a, b).

Enzymatic properties of *Po*OPH$_{M9}$ CLEPC

As shown in Fig. 3a, *Po*OPH$_{M9}$ CLEPC had the same optimal pH of 9.0 as the free *Po*OPH$_{M9}$, but it exhibited a better stability in strong alkaline solutions. At pH 8.0, 10.0 and 11.0, it can maintain over 80% of the maximum activity. However, the relative activity of the free enzyme dropped to 32%, 66% and 40%, respectively. In addition, the optimal temperature of CLEPC was 50 °C which was higher than that of *Po*OPH$_{M9}$ (30 °C, Fig. 3b). In particular, the immobilized enzyme still maintained 80% activity at 60 °C, but the free enzyme *Po*OPH$_{M9}$ was inactivated completely at this temperature. The thermostability of *Po*OPH$_{M9}$ CLEPC, *Po*OPH$_{M9}$ CLEA and *Po*OPH$_{M9}$ was tested and compared at 50 °C in Fig. 3c. *Po*OPH$_{M9}$ quickly lost its activity with a half-life of 0.5 h. *Po*OPH$_{M9}$ CLEA maintained 68.3% activity after 5 h, yielding a half-life of 7.2 h at 50 °C. Notably, *Po*OPH$_{M9}$ CLEPC displayed the best thermostability with a half-life of 12.8 h at 50 °C. This result suggested that Pluronic F127 incorporated in CLEPCs could significantly improve the thermostability of enzymes. The high thermal tolerance of CLEPCs is a prominent advantage for industrial applications because high temperatures facilitate the removal of OPs during washing.

Effect of detergents on the activity of *Po*OPH$_{M9}$ CLEPC

Previously, we found that various commercially available detergents can influence the activity of *Po*OPH$_{M9}$ (Bai et al. 2017). For example, the anionic and non-ionic detergents, such as sodium dodecyl sulphate (SDS) and alkyl polyglycoside (APG), decreased the enzyme activity while the biocompatible detergent, coconut oil derivatives (COD), showed a less negative impact on the enzyme activity. In this work, we compared the catalytic activity of *Po*OPH$_{M9}$ CLEPC and free *Po*OPH$_{M9}$ incubated in the presence of 0.1% (w/w) detergents at 25 °C (Fig. 3d). As observed previously, the activity of

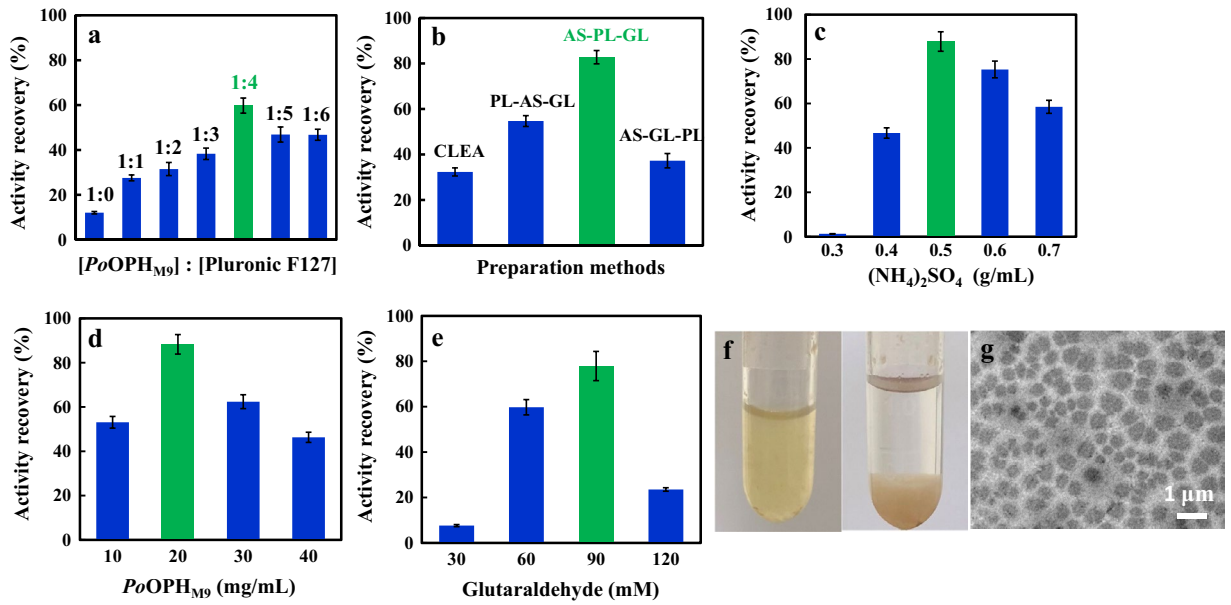

Fig. 2 The mass ratio of *Po*OPH$_{M9}$ to Pluronic F127 (**a**), the different order of adding Pluronic F127 (**b**) and the concentrations of ammonium sulfate (**c**), the free enzyme (**d**) and glutaraldehyde (**e**) were optimized for preparing the immobilized catalyst CLEPC. The immobilized enzymes were distributed in aqueous phase during the enzymatic reaction (**f**, left) and easily recovered by centrifugation (**f**, right). **g** The transmission electron microscopy of CLEPC showed the aggregation of immobilized enzymes with the mean diameter of 0.66 ± 0.12 μm

Table 1 Kinetic parameters of the free and immobilized enzymes toward malathion

Enzymes	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M ($mM^{-1}\ s^{-1}$)	Refs
Free *Po*OPH$_{M9}$	24.2 ± 0.3	91.2 ± 5.3	265.4 ± 2.0	Luo et al. (2016)
*Po*OPH$_{M9}$ CLEPC	49.1 ± 1.2	125.7 ± 7.7	390.3 ± 7.8	This work
*Po*OPH$_{M9}$ CLEA	1.1 ± 0.1	103.8 ± 1.7	10.9 ± 1.7	This work

free *Po*OPH$_{M9}$ decreased to various extent in the presence of COD, APG and SDS. In contrast, the activity of *Po*OPH$_{M9}$ CLEPC were all enhanced by 2- to 2.5-fold in the presence of the three detergents after 2 h incubation. It has been reported that SDS can activate phosphotriesterase (PTE) and enhance its activity (Giudice et al. 2016), but inactivate *Po*OPH$_{M9}$ (Bai et al. 2017). However, the phenomena that all kinds of detergents can enhance the activity of immobilized enzyme-polymer conjugates was first observed. To confirm this, we compared the activity of *Po*OPH$_{M9}$ CLEPC in the absence of detergents under the same condition (red symbols, Fig. 3d). The result showed that the activity of *Po*OPH$_{M9}$ CLEPC increased to approximately 154% after 2 h, which was lower than that of CLEPC in the presence of detergents (2- and 2.5-fold). The result suggested that detergents indeed activated and further enhanced the catalytic activity of *Po*OPH$_{M9}$ CLEPC.

Effect of Pluronic F127 on the activity of *Po*OPH$_{M9}$ CLEPC

First, we prepared and incubated *Po*OPH$_{M9}$ CLEA in the absence or presence of detergents. However, the activity in all cases decreased (Additional file 1: Figure S2), indicating that only CLEA cannot enhance the activity of *Po*OPH$_{M9}$. Compared with the result in Fig. 3d, it can be concluded that Pluronic F127 is essential in improving the activity of immobilized enzymes. Second, we prepared the conjugate *Po*OPH$_{M9}$-PF127 formed between the free *Po*OPH$_{M9}$ and Pluronic F127 and measured their activity in the absence of detergents after 2 h incubation (Additional file 1: Figure S3a). The activity of *Po*OPH$_{M9}$-PF127 was slightly increased to 105% by 1% and 10% (w/w) Pluronic F127 after 2 h incubation (Additional file 1: Figure S3a). Interestingly, when 0.1% (w/w) detergents were added, the activity of *Po*OPH$_{M9}$-PF127 conjugates was all enhanced to approximately 120% relative to that of the free enzyme (Additional file 1: Figure S3b). This result suggested that the activity improvement was achieved by the cooperation between Pluronic F127

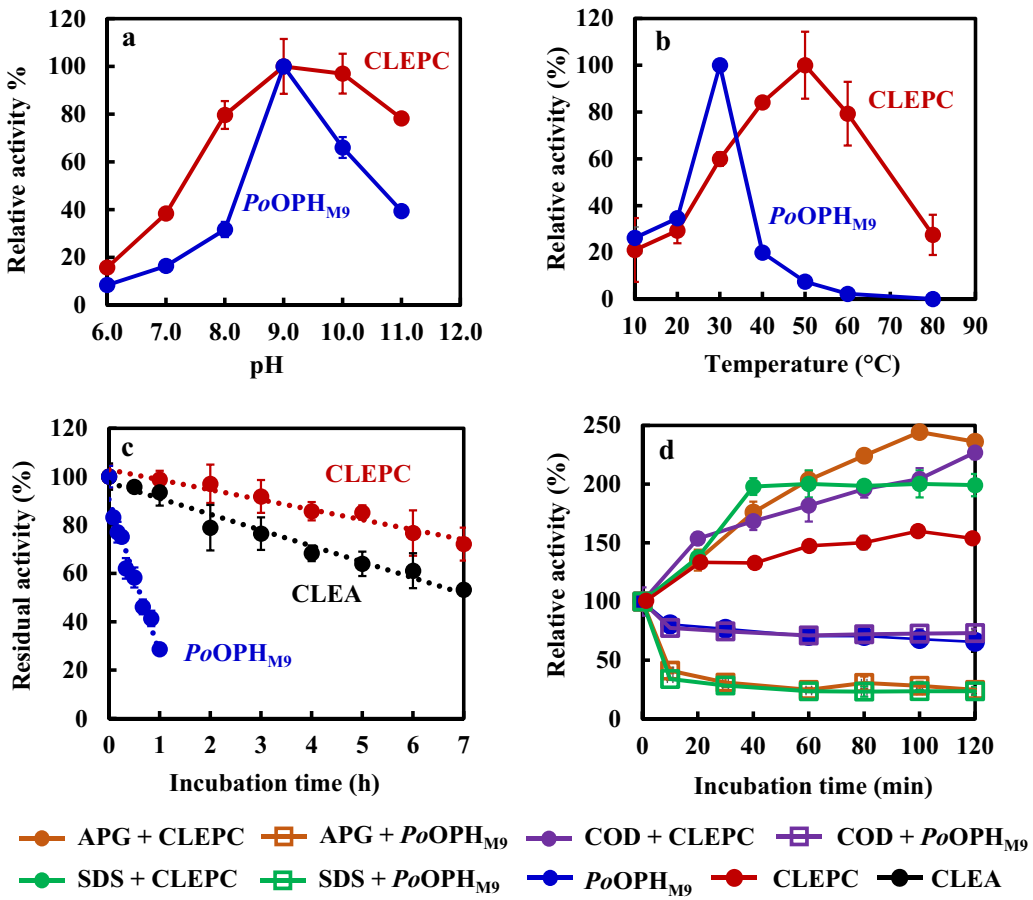

Fig. 3 Enzymatic assay of *Po*OPH$_{M9}$ CLEPC. The optimal pH (**a**) and temperature (**b**) of CLEPC and free enzymes were determined. Thermostability (**c**) was investigated by measuring the residual activity of different catalysts incubated at 50 °C. **d** The relative activity of CLEPC and *Po*OPH$_{M9}$ incubated in the presence of 0.1% (w/w) detergents were measured at 25 °C. The relative activity of *Po*OPH$_{M9}$ CLEPC in the absence of detergents was also measured (red dots)

and detergents. However, this activity improvement was lower than that of *Po*OPH$_{M9}$ CLEPC incubated with detergents (2- and 2.5-fold), indicating that the enzyme aggregation and cross-linkage in *Po*OPH$_{M9}$ CLEPC are very important in the activity enhancement (Akbar et al. 2007; Perzon et al. 2017; Wang et al. 2011). In summary, the significant enhancement in the activity of *Po*OPH$_{M9}$ CLEPC was caused by the synergic effect of Pluronic F127, enzyme immobilization and detergents.

Degradation of malathion by *Po*OPH$_{M9}$ CLEPC

Finally, we tested the effect of malathion degradation by CLEPC in a pH 9.0 solution containing 0.15 mM malathion, 4.2 U *Po*OPH$_{M9}$ CLEPC and 0.1% (w/w) detergents (Fig. 4). As shown in Fig. 4a, *Po*OPH$_{M9}$ CLEPC degraded 100% malathion after 30 min in the presence of COD, and 92% malathion was removed by CLEPC in the presence of APG and SDS at 25 °C. Next, we increased the temperature to 50 °C and found that 100% malathion was removed within 10 min in the presence of COD, which was faster than that at 25 °C (Fig. 4b). For APG, the washing effect was also enhanced as malathion was completely removed within 15 min, but the effect was almost unchanged in the presence of SDS. As mentioned above, the activity of *Po*OPH$_{M9}$ CLEPC can be activated and enhanced after incubation with detergents. Therefore, we tested the degradation effect by incubating *Po*OPH$_{M9}$ CLEPC with each of the three detergents (COD, APG and SDS) for 2 h in prior to adding malathion in the buffer at 25 °C (Fig. 4c). To our delight, malathion was totally degraded within 10 min in the presence of the three detergents, which was the best degrading effect compared with the results in Fig. 4a. Finally, the reusability test of CLEPC showed that it still maintained 85.4% activity after 10 cycles (Fig. 4d), demonstrating its potential for practical applications.

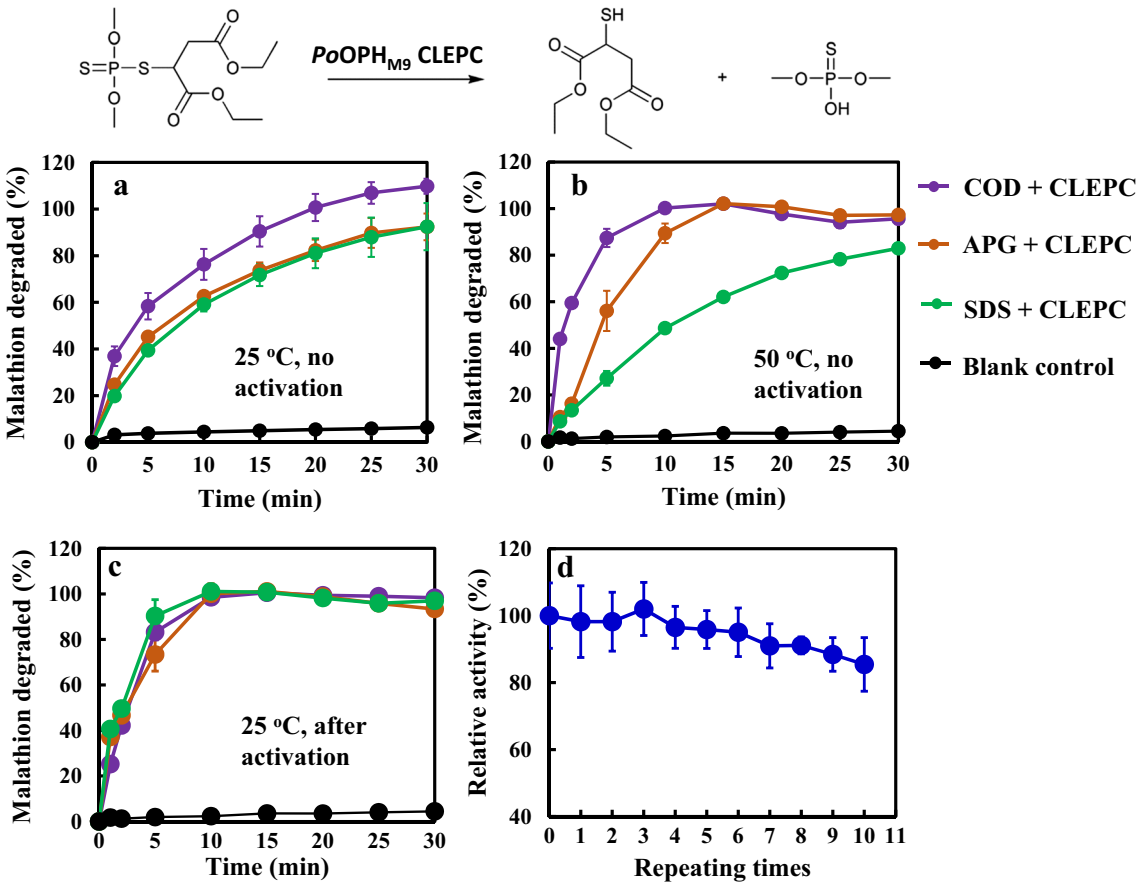

Fig. 4 Degradation of 0.15 mM malathion by *Po*OPH$_{M9}$ CLEPC in the presence of 0.1% (v/v) COD, APG and SDS. The experiments were performed immediately after the addition of detergents at 25 °C (**a**) and 50 °C (**b**). **c** The degradation was performed at 25 °C using *Po*OPH$_{M9}$ CLEPC that was incubated with detergents for 2 h in advance. **d** Recycling CLEPC for malathion degradation. The relative activity of *Po*OPH$_{M9}$ CLEPC was measured at 25 °C after each cycle for 10 times

Conclusion

In conclusion, we developed a simple method to prepare an immobilized organophosphate hydrolase (*Po*OPH$_{M9}$) conjugated with Pluronic F127 for efficient malathion degradation. Compared to the traditional *Po*OPH$_{M9}$-Pluronic F127 CLEA, *Po*OPH$_{M9}$-Pluronic F127 CLEPC has a 2.2 times higher activity recovery. It features excellent thermostability with a half-life of 12.8 h at 50 °C, which is 25.6 and 1.7 times higher than that of the free *Po*OPH$_{M9}$ and *Po*OPH$_{M9}$-Pluronic F127 CLEA CLEA. Particularly, *Po*OPH$_{M9}$ CLEPC was not deactivated by detergents; instead, its activity can even be further enhanced by up to 2.5-fold in the presence of detergents such as coconut oil derivatives, sodium dodecyl sulphate and alkyl polyglycoside. Degradation of 0.15 mM malathion with *Po*OPH$_{M9}$ CLEPC at 50 °C or 25 °C in the presence of detergents was demonstrated, showing the potential of this new immobilized OPH in practical industrial applications. We believe this technology can be extended easily to produce other immobilized polymer-enzyme conjugates, owing to the simplicity of this technology.

Additional file

Additional file 1. Supplementary information.

Abbreviations

OP: organophosphate pesticide; OPH: organophosphate hydrolase; CLEPC: cross-linked enzyme-polymer conjugate; CLEA: cross-linked enzyme aggregate; SDS: sodium dodecyl sulphate; APG: alkyl polyglycoside; COD: coconut oil derivative.

Authors' contributions

XYZ, JG and YPB designed the experiments; HC, YLZ and XJL performed the research experiments; DSX, QD and XC helped in the experiments. XYZ, JG and YPB analyzed the data; JHX helped in manuscript preparation; HC, XYZ, JG and YPB wrote the manuscript. All authors read and approved the final manuscript.

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Not applicable.

Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
All data generated or analyzed during this study are included in this published article and its supplementary information files.

Consent for publication
All of the authors have read and approved to submit it to bioresources and bioprocessing.

Ethics approval and consent to participate
Not applicable.

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