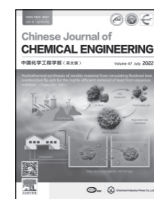




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Full Length Article

Conjugation of a zwitterionic polymer with dimethyl chains to lipase significantly increases the enzyme activity and stability

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ABSTRACT

Enzyme-polymer conjugates are complex molecules with great practical significance. This work was designed to develop a novel enzyme-polymer conjugate by covalently coupling a zwitterionic polymer with side dimethyl chains (pID) to *Candida rugosa* lipase (CRL) via the reaction between the anhydrides of polymer chains with the amino groups of the enzyme. The resulting two CRL-pID conjugates with different pID grafting densities were investigated in term of the catalytic activity, stability and structural changes. In comparison with native CRL, both the CRL conjugates displayed 2.2 times higher activity than the native enzyme, and showed an increase in the maximum reaction rate ($V_{\max}$) and a decrease in the Michaelis constant (K_m), thus resulting in about three-fold increases in the catalytic efficiency (k_{cat}/K_m). These are mainly attributed to the activation of lipase by the hydrophobic alkyl side chains. Moreover, the thermostability and pH tolerance of the lipase conjugates were significantly enhanced due to the stabilizing effect of the zwitterion moieties. For instance, a five-fold increase of the enzyme half-life at 50 °C for the high-pID conjugated CRL was observed. Spectroscopic studies reveal that the pID conjugation protected the enzyme in the changes in its microenvironment and conformation, well correlating with enhanced activity and stability of lipase conjugates. The findings indicate that enzyme conjugation to the zwitterionic polymer is promising for improving enzyme performance and deserves further development.

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1. Introduction

Enzyme stability is one of the key issues that restrict its industrial applications [1,2]. Chemical modification, especially polymer conjugation, is a promising strategy for improving the performance and increasing the application scope and value of enzymes [3–5]. To date, various polymers, such as polyethylene glycol (PEG) [6], dendrimers [7], polysaccharides (e.g., dextran and chitosan) [8,9], zwitterionic polymers (e.g., polycarboxybetaine [10]) and many other water-soluble macromolecular polymers with good biocompatibility, have been used as chemical modifiers of enzymes. Covalent conjugations of such polymers to enzymes can maintain the native conformation and improve the stability of enzymes, most notably thermostability [11,12]. It is generally considered that the stabilizing effect is mainly due to the shielding and protection of enzymes against denaturing conditions [13].

The attachment of polymers to enzyme molecules can be accomplished by either “grafting-to” or “grafting-from” approaches [14]. The “grafting-to” approach is conventional, which

couples a pre-synthesized polymer to an enzyme surface via the reactions between the end-groups or pendant groups of the polymer and the amino acid residues of enzymes. The most well-known representative is protein PEGylation by using the end-functionalized PEG to modify proteins, which have been proved to result in enhanced solubility and stability, prolonged *in vivo* circulation half-life and reduced immunogenicity [15,16]. In contrast to the well-defined “grafting-to” approach, the “grafting-from” strategy involves attachment of small molecule polymerization initiators or chain transfer agents to a biomolecule, with polymer chain growing *in situ* on the surface of the biomolecule, such as atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer (RAFT) [17,18]. The approach provides more available chances for construction of enzyme-polymer conjugates with enhanced enzyme functionality and stability [13,19,20]. However, challenges remain with respect to the covalent coupling of enzymes, particularly noted as the activity loss associated with the conjugation [21].

In recent years, zwitterionic polymers have shown great potential in maintaining the bioactivity and structural stability of proteins by either directly coupling to enzymes or as carrier mod-

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ifiers to immobilize enzymes [10,22–24]. In our previous work, a novel zwitterionic polymer (pID) was synthesized via the reaction between poly(isobutylene-*alt*-maleic anhydride) (PIMA) and *N,N*-dimethylethylenediamine, and was then grafted onto silica nanoparticles (SNPs) for lipase immobilization [25]. Owing to lipase activation by the alky side chains and the protein structure stabilization by the zwitterion moieties, *Candida rugosa* lipase (CRL) immobilized on the pID-grafted SNPs showed enhanced stability and catalytic efficiency. However, the performance of immobilized enzyme was affected by many factors including support materials and immobilization methods, and the existence of solid supports made it difficult to systematically characterize the structural changes of the enzyme before and after immobilization. In contrast, a soluble enzyme-polymer conjugate is not only conducive to investigate the microstructure alterations of enzyme protein caused by polymer modification, but also can minimize mass transfer resistance of the substrate into the active site of the enzyme and promote the catalytic reaction. Therefore, this work is to develop a novel enzyme-polymer conjugate by attaching pID to CRL molecules. In view of the low reactivity of the carboxyl groups of pID with the amino-groups of enzymes, it was designed to use the reaction between the cyclic anhydride groups in pID and the amino groups of the enzyme for the conjugation under a mild condition without using any other chemical reagents. To this end, the zwitterionic polymer with residual anhydride groups was synthesized by controlling the reaction degree of the anhydride groups in PIMA chains to DMEA and was then conjugated to CRL using the residual anhydride groups (Fig. 1). Two CRL-pID conjugates with different CRL loads were prepared and the as-prepared CRL-pID conjugates were characterized in terms of the catalytic activity, enzymatic kinetics, stability and conformational transitions for correlation with the changes in the enzymatic properties.

2. Materials and Methods

2.1. Materials

Lipase from *Candida rugosa* (CRL, type VII), *N,N*-dimethylethylenediamine (DMEA) and poly(isobutylene-*alt*-maleic anhydride) (PIMA, average Mw ~6000 Da, 39 repeat units) were obtained from Sigma-Aldrich (St. Louis, MO, USA). BCA protein assay kit was obtained from Dingguo Biotech (Tianjin, China). *p*-Nitrophenyl acetate (*p*-NPA) was from Aladdin (Shanghai, China).

Sodium dihydrogen phosphate, dibasic sodium phosphate and tris (hydroxymethyl) aminomethane (Tris) were of analytical grade from Sangon Biotech (Shanghai, China). Citric acid and trisodium citrate dihydrate were of analytical grade from Kewei (Tianjin, China). *N,N*-dimethylformamide (DMF) and other reagents were purchased from Jiangtian Chemical Technology (Tianjin, China).

2.2. Synthesis of zwitterionic polymer

The zwitterionic polymer of pID with a small number of residual anhydride groups (denoted as pID-*n*) was prepared by controlling ring-opening reaction of the anhydride groups (with less addition of DMEA than the anhydride groups in the feed), as shown in Fig. 1. Briefly, 1 g of PIMA (6.5 mmol monomer unit) was dissolved in 5 mL DMF, followed by adding 0.44 mL of DMEA (3.9 mmol) in the mixture. The ring-opening reaction was performed by magnetic stirring at room temperature for 12 h. The resulting polymer was precipitated by adding excess acetone, and recovered by centrifugation and repeated washing with acetone. The product was finally lyophilized. The ring-opening degree of the anhydride groups in PIMA was analyzed by ¹H NMR spectroscopy with a Varian Inova 500 MHz NMR spectrometer (Varian, California, USA) and the resulting zwitterionic polymer was referred to as pID-*n* (*n* represents the zwitterionization degree of PIMA).

2.3. CRL modification

The conjugation of pID to lipase is also shown in Fig. 1. A definite amount of CRL was dissolved in 20 mmol·L⁻¹ phosphate buffer (pH 7.0) to obtain an enzyme solution of 0.25 mg·mL⁻¹ protein content determined by the BCA method [26]. The above polymer was added to 10 mL of the lipase solution to make the molar ratio of the polymer to CRL at 2:1 and 8:1, respectively. After incubating the mixture at 4 °C for 12 h, the unreacted polymer was removed by ultrafiltration (a molecular weight cutoff of 30000). The final products (CRL-pID conjugates) were obtained by lyophilization and denoted as CRL-pID-2 and CRL-pID-8 according to the pID-*n* feed ratio.

2.4. Characterization of polymers, CRL and CRL-pID conjugates

The molecular structures of PIMA, pID-*n*, CRL-pID-2, CRL-pID-8 and CRL were characterized by a Nexus Fourier transform infrared spectroscopy (FTIR) (ThermoNicolet, Madison, WI, USA). CRL-pID

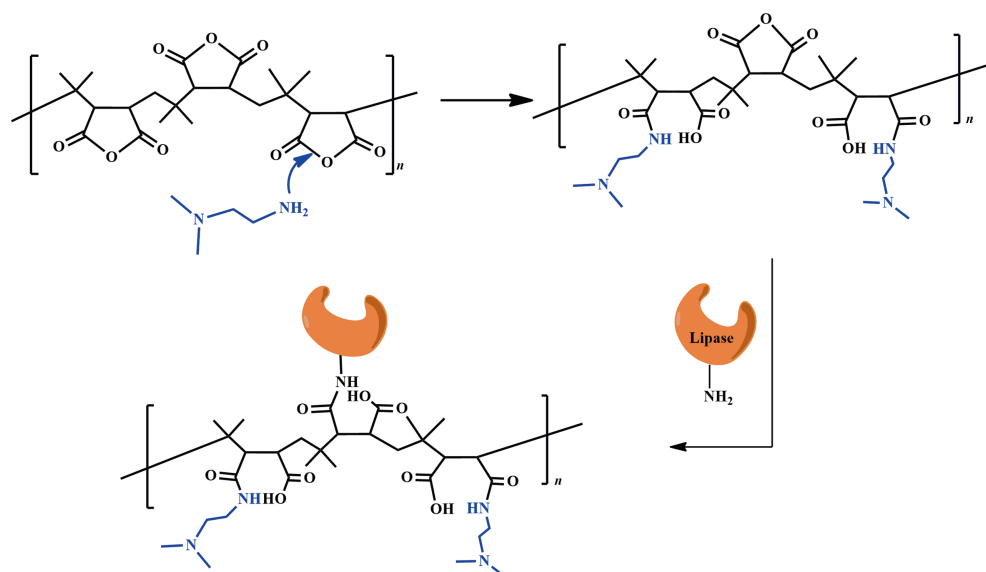


Fig. 1. Schematic diagram for the preparation of pID with residual anhydride groups and the following synthesis of CRL-pID conjugates.

conjugates were analyzed by gel filtration chromatography (GFC) using an Agilent 1100 (Agilent Technologies, USA) equipped with a TSK-GEL G4000 PWxl column (7.8 mm I.D. $\times$ 30 cm, TOSOH, Japan) detected at 280 nm. Phosphate buffer (20 mmol·L⁻¹, pH 7.0) was used as the mobile phase at 0.5 ml·min⁻¹. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out to analyze molecular weight change using 5% concentration gel and 12% of separation gel followed by a Coomassie brilliant blue staining [27].

2.5. Activity assay and determination of kinetic parameters

Lipase activity was determined using *p*-NPA as a substrate [28]. In assay, 0.1 mL of native or modified CRL sample was added to the substrate solution formed by mixing 2.4 mL of 20 mmol·L⁻¹ phosphate buffer (pH 7.0) and 0.5 mL of 10 mmol·L⁻¹ *p*-NPA in acetonitrile at 37 °C with stirring. The enzymatic reaction was continued for 10 min, followed by immediate measurement of the absorbance of the mixture at 400 nm. One unit of enzyme activity (U) was defined as the amount of lipase required to liberate 1 μ mol *p*-nitrophenol per minute under the assay condition.

The specific activity of enzyme conjugate was calculated from the ratio of enzyme conjugate activity to its protein. Then, the relative specific activity (RSA) was defined as the ratio of the specific activity of enzyme conjugate to that of native one.

The enzymatic kinetics with native or modified CRL was determined by measuring the initial catalytic reaction rate at varying substrate concentration under the activity assay conditions described above. In the reaction, protein concentration of the enzyme sample was kept at 3 μ g·mL⁻¹ (5×10^{-5} mmol·L⁻¹ calculated from the molecular weight of CRL, 60000 [29]). Michaelis constant (K_m) and the maximum reaction rate (V_{max}) in the following Michaelis-Menten equation were obtained by the Lineweaver-Burk plot method [24],

$$v = \frac{V_{max}[S]}{K_m + [S]} \quad (1)$$

where v is the reaction rate (mmol·L⁻¹s⁻¹), $[S]$ is the substrate concentration (mmol·L⁻¹), V_{max} is the maximum rate (mmol·L⁻¹s⁻¹), and K_m is the Michaelis constant (mmol·L⁻¹).

2.6. Stability investigations

To investigate thermal stabilities of native CRL and the CRL conjugates, the enzyme samples were incubated in 20 mmol·L⁻¹ phosphate buffer (pH 7.0) at 50 °C for 10 h. The aliquots of the enzyme samples were withdrawn at different time intervals to determine the residual activity.

pH tolerance of the enzyme preparations was determined by incubating the native or modified CRLs in buffer solutions with different pH values (50 mmol·L⁻¹ citrate buffers for pH 4.0 and 5.0; 50 mmol·L⁻¹ phosphate buffers for pH 6.0, 7.0, and 8.0; 50 mmol·L⁻¹ Tris-HCl buffer for pH 9.0) at 25 °C for 4 h. The residual activity was assayed as described above.

In the above assays, the enzyme activity before treatment was defined as 100% to calculate the relative activity to determine the stability.

2.7. Fluorescence spectroscopy and circular dichroism (CD) spectroscopy

A certain amount of CRL and CRL-pID conjugates were dissolved in 20 mmol·L⁻¹ phosphate buffer (pH 7.0) to prepare an enzyme solution of 0.2 mg·mL⁻¹ protein concentration determined by BCA method, followed by measuring the fluorescence spectroscopy

of the enzyme preparations. The excitation wavelength was set at 270 nm and the emission wavelength was recorded in the range of 280–450 nm with a scanning speed of 200 nm·min⁻¹. The excitation and emission slit widths were fixed at 12.0 nm and 5.0 nm, respectively. Every sample was assayed four times and the average value with its standard deviation is reported.

The circular dichroism (CD) spectra of the CRL preparations (0.2 mg·mL⁻¹ protein) were recorded in the wavelength range from 250 to 190 nm at a scanning speed of 100 nm·min⁻¹ on a JASCO J-810 spectrophotometer (JASCO, Tokyo, Japan). A 1.0 mm circular cell was used for measurement at room temperature and each sample was scanned three times. The changes in secondary structure of lipase before and after polymer modification were analyzed by the online BeStSel algorithm (<http://bestsel.elte.hu/>).

3. Results and Discussion

3.1. Characteristics of polymers and CRL-pID conjugates

Fig. S1 (Supplementary Material) presents the NMR spectrum of the polymer. The zwitterionization degree of PIMA was determined by comparing the integration ratio of the peaks at $\sim$ 2.77 and 0.9, assigned to the methyl protons held by a nitrogen atom and the methyl protons of the PIMA backbone, respectively. The ratio of 0.71 indicates that about 70% of the anhydride groups in the polymer chain of PIMA reacted with DMEA to form zwitterionic groups (Fig. 1), and thus the resulting polymer is named pID-0.7.

Fig. S2 shows the FTIR spectra of PIMA, pID-0.7, CRL-pID-2, CRL-pID-8 and CRL. The spectrum of PIMA presents a strong peak at 1778 cm⁻¹ and a shoulder peak at 1855 cm⁻¹ (Fig. S2(a)), which is characteristic of the anhydride [30]. For pID-0.7, significant decreases of these peaks are observed (Fig. S2(b)), indicating that most of the anhydride rings had reacted with DMEA to form zwitterionic polymer pID-0.7. With further reaction between the residual anhydrides and the amino groups of CRL, these peaks diminished (Figs. S2(c) and S2(d)), implying that pID-0.7 was coupled to enzyme molecules.

The results of GFC of the native and CRL-pID conjugates are shown in Fig. S3. The retention time decreased with the increase of the grafting amount, as shown by 19.4, 18.8 and 18.5 min of the retention times for native CRL, CRL-pID-2 and CRL-pID-8, respectively. The shorter retention times of the enzyme conjugates implies their higher molecular weights than CRL by coupling with pID-0.7, further demonstrating that the polymer pID-0.7 was successfully grafted onto CRL. According to modification procedure described above, CRL-pID-8 was modified with more pID than CRL-pID-2, so CRL-pID-8 was larger than CRL-pID-2 in molecular weight.

The SDS-PAGE patterns (Fig. S4) of the native and CRL-pID conjugates show that the electrophoretic band of CRL-pID-2 was close to that of CRL, while CRL-pID-8 presented a band with higher molecular weight. This is consistent with the GFC analysis (Fig. S3), further demonstrating more polymer-grafting onto CRL for CRL-pID-8.

3.2. Catalytic performance

The relative specific activities of the enzyme conjugates as compared to native CRL are summarized in Table 1. It can be seen that both the CRL-pID conjugates exhibited an activity over 2.2 times higher than the native one, which is consistent with the results reported in our previous work with immobilized CRL on pID-modified SNPs [25]. The enhanced activity may be due to the interfacial activation of lipase by the hydrophobic alkyl chains of pID [29], as well as the regulation of enzyme conformation by the zwitterionic groups of pID, which may increase the flexibility of the

Table 1

Relative specific activities of the enzyme conjugates as compared to native CRL.

Enzyme preparation	Feed molar ratio of polymer-to-enzyme protein	Relative specific activity (RSA)
Native CRL	–	1
CRL-pID-2	2:1	2.27 ± 0.13
CRL-pID-8	8:1	2.22 ± 0.10

enzyme conformation and favor the improvement of enzyme activity.

The kinetic parameters of native CRL and CRL-pID conjugates determined by the Lineweaver-Burk plot method (Fig. S5) are listed in Table 2. Compared with native CRL, the smaller K_m values and higher V_{max} values are observed for both the CRL-pID conjugates, so about three-time increases in catalytic efficiency (k_{cat}/K_m) were obtained. The results indicate the significant improvement of the enzyme-substrate affinity and catalytic activity of the pID-conjugated CRLs. The high catalytic efficiency of CRL-pID conjugates may be attributed to the high hydrophilicity of pID, which can drive water away from the hydrophobic region of the protein, resulting in the vicinity of the enzyme molecule more hydrophobic, and thus allowing the substrate to interact with the binding site more easily [10]. Another important reason may be the hydrophobic interactions between the enzyme and the dimethyl side chains of pID (see Fig. 1), which may shift the enzyme from an inactive state to active one, promoting the substrate to get access into the active sites of the modified lipase [29].

Ge et al. reported that lipase conjugated to hyperbranched aromatic polyamide (HBPA) presented an approximate K_m to the native lipase while V_{max} was increased by 20% [31]. In this work, K_m and V_{max} of the conjugated lipase decreased by 16%–35% and increased by 87%–125%, respectively, demonstrating great advantage of the zwitterionic polymer pID for improving catalytic efficiency of lipase.

3.3. Thermal stability and pH tolerance

Fig. 2 shows the thermal stabilities of native CRL and the CRL-pID conjugates at 50 °C. The activity losses for the three enzyme samples increased with increasing heat treatment, but the conjugates exhibited much better thermostability than native lipase (Fig. 2(a)). After 10 h of incubation, the native CRL remained 14% of its initial activity, while the relative specific activities of CRL-pID-2 and CRL-pID-8 held as high as 0.72 and 0.82, respectively. The results reflect the advantage of the conjugates for use at higher temperatures. The enhanced thermal stability can be attributed to the covalent attachment of pID onto the surface of the enzyme molecule, reinforcing the intramolecular forces of the enzyme conjugates and preventing protein unfolding at harsh conditions. On the other hand, pID around lipase also generates additional steric hindrance, which reduces the exposure of the conjugated lipase to the external environment, and provides a physical barrier for lipase [32].

The thermal inactivation process was described by a two-step series model [33,34],



where k_1 and k_2 are inactivation rate coefficients, E and E_1 represent the initial and intermediate states of an enzyme, α_i represents the ratio of specific activity of E_i to E . By assuming a non-zero specific activity for the final state (E_2), the weighted-average activity (a) can be expressed as:

Table 2Kinetic parameters of the native CRL and enzyme-pID conjugates ^①.

Kinetic parameters	Native CRL	CRL-pID-2	CRL-pID-8
$V_{max} \times 10^4/\text{mmol} \cdot \text{L}^{-1} \cdot \text{s}^{-1}$	4.04 ± 0.17	9.11 ± 0.67	7.56 ± 0.11
$K_m/\text{mmol} \cdot \text{L}^{-1}$	13.0 ± 1.29	10.9 ± 2.58	8.50 ± 1.38
k_{cat}/s^{-1}	8.07 ± 0.34	18.2 ± 1.35	15.1 ± 0.22
$(k_{cat}/K_m)/\text{s}^{-1} \cdot \text{mmol}^{-1} \cdot \text{L}$	0.62 ± 0.04	1.72 ± 0.32	1.81 ± 0.31

^① Data represent average values with standard deviations.

$$a = \left(1 + \frac{\alpha_1 k_1 - \alpha_2 k_2}{k_2 - k_1}\right) \exp(-k_1 t) - \left(\frac{k_1}{k_2 - k_1}\right) (\alpha_1 - \alpha_2) \exp(-k_2 t) + \alpha_2 \quad (3)$$

Fig. 2(b) shows thermal inactivation of the native and modified lipases. It can be observed that the scatters were well distributed on the calculated curves, verifying the validity of the selected model. The deactivation parameters obtained by fitting the experimental data to the model with Matlab software are listed in Table 3. The decreases in k_1 and k_2 values are observed for both the CRL conjugates, especially CRL-pID-8, indicating a reduction in the change rate of the protein conformation by coupling pID. In addition, the increases in α_1 and α_2 indicate the higher activity of E_1 and E_2 for the conjugates than native lipase. Moreover, the half-life ($t_{1/2}$) increased by 3.77-fold for CRL-pID-2 and 5.01-fold for CRL-pID-8. Compared with lipase-HBPA conjugate which had a half-life 3.27-fold higher than native lipase [31], the zwitterionic polymer pID exhibits more significant stabilizing effect on lipase.

Fig. 3 shows pH tolerances of native CRL and the CRL-pID conjugates. In spite of the similar trends in the pH range of 4–9, both the CRL-pID conjugates presented better pH tolerance than native lipase. It can be found that the activity of the native CRL declined rapidly under more acidic or alkali conditions, while CRL-pID-2 and CRL-pID-8 exhibited less sensitivity to the pH changes. The phenomenon is considered due to the zwitterionic groups of pID, which, as buffering groups, protect the enzyme structures from acidity and alkalinity to some extent.

3.4. Spectroscopic analysis

Fluorescence spectroscopy provides information on the enzyme-polymer interaction and the conformational changes of proteins [35]. Fig. 4 shows the fluorescence spectra of native CRL and enzyme conjugates, and their maximum fluorescence emission wavelength are recorded in Table S1. It is observed that the fluorescence emission maximum experienced a blue-shift from 340 nm for native CRL to 339 nm for both the lipase conjugates, indicating the structural changes of CRL induced by pID. The shift indicates that the structural changes resulted in a more hydrophobic environment around tryptophan residues of CRL and a more compact conformation, implying the presence of hydrophobic interactions between lipase and pID [36,37]. However, the red shifts of the emission maximum are observed for the three lipase samples after incubation at 50 °C for 2 h (Fig. 4), indicating the protein unfolding at this temperature, corresponding to the reduced enzyme activity (Fig. 2) [38]. It is obvious from Table S1 that the CRL conjugates exhibited less shift than native CRL, indicating less changes in the protein structure and higher resistance to heat stress, in consistency with the results shown in Fig. 2.

The secondary structure changes of CRLs were evaluated by CD spectroscopy. Fig. S6 shows CD spectra of native CRL and the enzyme-pID conjugates. The negative bands around 208 nm and 220 nm appears in all the CD spectra, representing α -helical structures [39]. However, the intensity of the negative bands decreased after the conjugation of pID, implying the alteration in the secondary structure of CRL. Table S2 lists the secondary structures

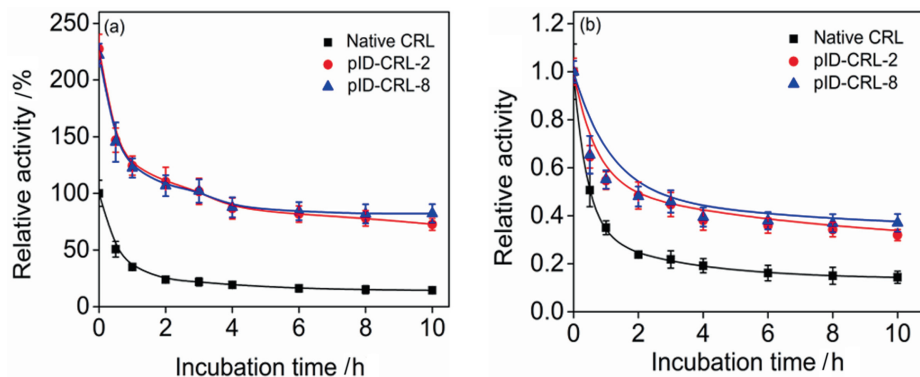


Fig. 2. Thermal stabilities of the native CRL and enzyme-pID conjugates in phosphate buffer (pH7.0) at 50 °C. (a) The relative activity was compared to the initial enzyme activity of native CRL that is defined as 100%. (b) The initial activity of every enzyme sample was defined as 1 to represent the relative activity and its change with time. The solid lines are calculated from the proposed kinetic deactivation scheme (Eq. (2)).

Table 3

Lipase deactivation parameters at 50 °C obtained from the kinetic deactivation scheme.

Parameters	Native CRL	CRL-pID-2	CRL-pID-8
k_1	2.375	1.440	0.887
k_2	0.326	0.130	0.123
α_1	0.311	0.506	0.470
α_2	0.135	0.267	0.328
$t_{1/2}/h$	0.514	1.940	2.576
$t_{1/2}/t_{1/2,0}$ ^①	1	3.774	5.012

^① $t_{1/2,0}$ represents the half-life of native CRL.

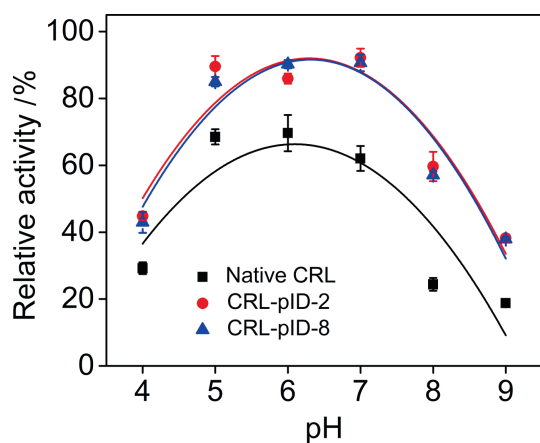


Fig. 3. pH tolerances of native CRL and the enzyme-pID conjugates. The initial activity of every enzyme sample was defined as 100% to calculate the relative activity and estimate the pH tolerances of CRLs.

of the CRLs preparations calculated by the online BeStSel algorithm (<http://bestsel.elte.hu/>). It can be seen that CRL-pID-2 and CRL-pID-8 exhibited an increase in α -helix and β -sheet contents with respect to native CRL, which might be responsible for the enhanced CRL activity (Table 1) [40].

In summary, CRL conjugated to pID exhibited significantly improved catalytic activity and enhanced stabilities, endowing the enzyme with more extensive application prospects. Apart from CRL, many lipases from other sources are also available for bio-transformation, such as lipase from *Thermomyces lanuginosus* (TLL), *Rhizomucor miehei* (RML), *Candida antarctica* (CAL) and *Geotrichum candidum* (GCL). Table S3 lists the structural characteristic parameters of some lipases. Lipases from different sources consist of amino acids ranging from 270 to 641 with molecular weights

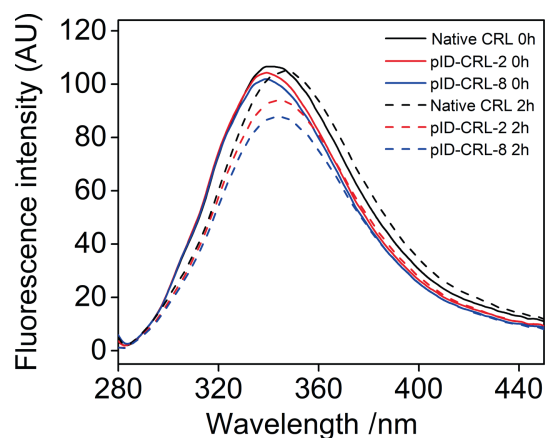


Fig. 4. Fluorescence spectra of native CRL and the enzyme conjugates. The protein concentrations were kept at 0.2 mg·mL⁻¹ in phosphate buffer (pH 7.0). The solid lines represent the spectra of the samples before heat treatment and the dash lines represents these after incubation at 50 °C for 2 h.

from 29 kDa to 100 kDa. The amino acid sequences of the lipase proteins may be quite different; however, their active centers are relatively conservative and possess similar or identical structure, i.e. catalytic triad formed by Ser-His-Asp/Glu [41,42]. Moreover, there is a common property of these lipases, which is the active site covered by a polypeptide chain called “lid”, relevant with interfacial activation of the enzymes. Therefore, it is possible that the lipases from other sources such as TLL and RML conjugated to pID might exhibit favorable properties similar to the CRL-pID conjugate.

4. Conclusions

In this work, the zwitterionic polymer (pID-0.7) with residual anhydrides was synthesized by ring-opening zwitterionization and conjugated to CRL via the reaction of anhydrides with the amino groups of the enzyme to prepare two CRL-pID conjugates (CRL-pID-2 and CRL-pID-8) with different grafting densities. Both the CRL-pID conjugates exhibited 2.2 times higher activity than the native lipase, mainly due to the activation of lipase by the hydrophobic dimethyl chains of pID. The increased V_{max} and decreased K_m further demonstrated the conjugation of pID to CRL resulted in enhancement of enzymatic efficiency. Moreover, the lipase conjugates showed the enhanced thermostability and pH tolerance. The half-lives of CRL-pID-2 and CRL-pID-8 was about

3.8-fold and 5.0-fold higher than that of native CRL, respectively. Fluorescence analysis indicated that the conjugates possessed more stable protein conformation, demonstrated by the blue-shifts relative to the fluorescence emission maximum of native CRL and less red-shifts after heat treatment. CD studies suggested the conjugation resulted in the alteration in the secondary structures of CRL, which may be related to the enhanced performance of CRL-pID conjugates. These results indicate that the zwitterionic polymer containing hydrophobic chains have a positive effect on improving lipase performance, and deserve further development for efficient and stable biocatalysts.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary Material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cjche.2021.04.023>.

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