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Article

Comparison of the salt-induced activation of *Pseudomonas cepacia* lipase in organic media by phosphate buffer and sodium sulfate

JIN Qianru, JIA Guoqing, WANG Xiuli, LI Can *

State Key Laboratory of Catalysis, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, Liaoning, China

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ABSTRACT

The effects of a kosmotropic phosphate buffer (PB) or sodium sulfate (SS) on the enzymatic activity of crude *Pseudomonas cepacia* lipase (PCL) in organic media were explored. Previously, it was believed that SS acts as an effective enzyme activator, and PB was only used to adjust the solution pH. The present work showed PB-induced activation of PCL in the transesterification activity, where an increase of nearly 10-fold was achieved by carefully controlling the lyophilization condition. This activation, which gave about one-half of the native catalytic efficiency of PCL in aqueous media, was even more effective than the SS-induced activation. Thermogravimetric analysis of PCL preparations showed that different local environments surrounded PCL due to the presence of PB or SS. The local polarity of PCL preparations suspended in *n*-hexane was investigated by fluorescence spectroscopy using 2-(4'-amino-2'-hydroxy phenyl)benzoxazole as the probe. SS-incorporated PCL showed a much stronger local polarity than PB-incorporated and salt-free PCL. A correlation was found between the transesterification activity and local polarity of the enzyme preparation as a function of salt content. The data confirmed that the salt-induced activation of PCL in organic media can be attributed in part to the polar environment constituted by the salt-bonded water surrounding the PCL.

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1. Introduction

Nonaqueous enzymology dates back to at least 1984 in the work of Zaks and Klivanov [1–3], which greatly broadened the utility of enzymes in organic synthesis. However, enzyme reaction rates in organic media are generally several orders of magnitude lower than those in aqueous solution due to restricted substrate accessibility, structural changes, transition state stabilization, and reduced protein flexibility [4]. To overcome these problems, various methods have been developed to increase enzyme activity in organic media [5–10]. One of the most important methods is the incorporation of a lyoprotectant into the pure enzyme during the lyophilization process [6,11–14]. Massive lyoprotectants, e.g., dextrin, organic buffer,

surfactant, and inorganic salt, were found to dramatically increase the catalytic activity of the dry enzyme powder in organic media. Especially, the addition of combinatorial salts (1:1 KAc:CsCl) led to lyophilized penicillin amidase with 35000 times higher transesterification activity in hexane than the salt-free preparation [13].

This salt-induced activation of the enzyme in organic media was correlated with salt kosmotropicity through the Jones-Dole *B* coefficient, lyotropic number, surface tension increment and polarizability [15,16]. Kosmotropic ions with positive values of the *B* coefficient are considered to be more effective in activating the enzyme than chaotropic ions with negative values of the *B* coefficient because a kosmotropic ion has a strong affinity for water and would increase the water content in the local envi-

*Corresponding author. Tel: +86-411-84379070; Fax: +86-411-84694447; E-mail: canli@dicp.ac.cn

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ronment of the lyophilized enzyme. Eppler et al. [17] used ^2H NMR relaxation methods to study the water dynamics of salt-incorporated subtilisin Carlsberg and showed that the salt-induced activation in organic media was mainly due to the increase of enzyme flexibility. However, Bilanicova et al. [16] pointed out that the direct interaction of ions with the enzyme surface, rather than water structure mediation, better explained the effect of salt kosmotropicity on enzymatic activity in organic media. Moreover, the kosmotropic salt was speculated to stabilize the transition state and decrease the activation energy, thus increasing enzymatic activity. Although there is no consensus on the exact mechanism of salt-induced activation, the significant activating effect of a kosmotropic salt is generally accepted. Until now, the effect of a kosmotropic buffer on enzyme activity in organic media has been seldom studied. Salis et al. [18] reported that a buffer, similar to a strong electrolyte, has a specific effect on enzyme activity in aqueous media in the Hofmeister series. However, the phosphate buffer, which is one of the most kosmotropic salt (B coefficient > 0.34), is considered not to influence catalytic activation in organic media [11].

The salt-induced activation of the enzyme powder is unique in nonaqueous enzymology. In order to fully understand the salt-activation mechanism, enzyme structures in various organic media were characterized by X-ray crystallography [19], Fourier transform infrared (FTIR) spectroscopy [20], and electron spin resonance (ESR) [21]. Information derived from these suggested that the organic solvent has no appreciable effect on the secondary structure of the suspended protein. Enzyme deactivation in organic media is mainly attributed to the decrease of enzyme flexibility and active site polarity caused by the deprivation of water [4,22]. Although the co-lyophilization of the enzyme with the salt can increase water content, active site content, and enzyme flexibility, there is still a lack of evidence for that salt-induced activation is associated with the polarity of the biocatalyst. The characterization of the polarity of the lyophilized enzyme powder in organic media is still needed.

In the present work, *Pseudomonas cepacia* lipase (PCL) was selected as the target enzyme because PCL is one of the most widely used biocatalysts in organic synthesis and it is commonly used for the kinetic resolution of secondary alcohols [23]. From a biotechnological perspective, enhancing the enzymatic activity of PCL in nonaqueous media is important. Here, we further activated crude PCL by the addition of phosphate buffer (PB) and sodium sulfate (SS), which resulted in a preparation with about one-half of the native catalytic efficiency of PCL in aqueous media. The effects of phosphate buffer or sodium sulfate on the local environment and catalytic activity of the lyophilized PCL were studied using thermogravimetric analysis and fluorescence spectroscopy. The data confirmed that the phosphate buffer has a specific activation effect on enzymatic activity that is similar to sodium sulfate. The correlation between catalytic activity and local polarity of the salt-incorporated enzyme powder suggested that salt-induced activation of PCL preparations was due in part to the polar environment constituted by the salt-bonded water surrounding

the PCL.

2. Experimental

2.1. Materials

Amano lipase PS from *Pseudomonas cepacia* (>30000 unit/g) was purchased from Sigma-Aldrich. 2-Aminophenol, 4-aminosalicylic acid, and triacetin were obtained from Alfa Aesar. DL-1-phenylethanol was purchased from Avocado Research Chemicals Ltd. Vinyl acetate, *n*-hexane, NaH_2PO_4 , Na_2HPO_4 , and Na_2SO_4 were obtained from Shanghai Chemical Reagent Company of the Chinese Medicine Group. DL-1-phenylethanol, vinyl acetate, and *n*-hexane were dried over 4A molecular sieve for 24 h or longer before use.

2.2. Enzyme preparation

Enzyme powder was used directly after purchase without further purification. For lyophilized enzyme samples, 100 mg of crude PCL was dissolved in 5 ml of phosphate buffer solution ($\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ at pH 8.0), and the concentration of phosphate buffer solution ranges from 0 to 200 mmol/L. The enzyme solution was lyophilized on a 2.5 L Labconco Freeze Dry at a condenser temperature of -49°C and a pressure of 20 Pa. The resultant enzyme preparation was termed PCL-PB. PCL-PB preparations with different water contents were obtained by altering the lyophilization time and pressure. For crude PCL incorporated with Na_2SO_4 (termed as PCL-SS), 100 mg of crude PCL was dissolved in 5 ml of phosphate buffer solution (50 mmol/L) with different amounts of Na_2SO_4 . The enzyme solutions were lyophilized in the same manner as above. After preparation, the sample was stored at -20°C under a nitrogen atmosphere. The transesterification and hydrolytic activity of the PCL preparation were assayed using the procedure described in a previous paper [24].

2.3. Characterization

The morphology of the enzyme powder was characterized using a FEI Quanta 200F scanning electron microscope using an acceleration voltage of 20 kV. The surface area of the enzyme preparation was measured on a Quantachrome NOVA 4200e N_2 adsorption apparatus at -196°C using the multipoint Brunauer-Emmett-Teller (BET) method. Thermogravimetric analysis (TGA) was performed on a Perkin-Elmer Pyris Diamond TG instrument with a heating rate of $5^\circ\text{C}/\text{min}$ under a nitrogen atmosphere. Fluorescence spectra were recorded on a FLS 920 fluorescence spectrometer with 340 nm filter (Edinburgh Instruments, UK). The formation of product and enantiomer excess were measured using a gas chromatograph (6890, Agilent Instruments) equipped with a HP-Chiral-20B 250 μm capillary column (30 m length and 0.25 μm inside diameter). The oven temperature was kept at 110°C for 50 min. The injector (split 1:50) temperature was 250°C and the detector FID temperature was 280°C . The flow rates of the carrier gases argon, hydrogen, and air were 10, 40, and 400 ml/min, respectively. ^1H

NMR spectrum was recorded on a Bruker DRX 400 using tetramethylsilane (TMS) as the internal standard and dimethylsulfoxide- d_6 as the solvent at room temperature.

2.4. Fluorescence measurement

2-(4'-Amino-2'-hydroxyphenyl)benzoxazole (4-AHBO) was synthesized according to a published procedure [25,26]. The structure and purity of 4-AHBO were confirmed by ^1H NMR (Figure not shown). During the fluorescence measurement, the enzyme preparation (10 mg) was suspended in 2 ml of 4-AHBO solution (5.65 mg in 1 L of *n*-hexane). The fluorescence emission spectra were collected using an excitation wavelength of 308 nm at 20 °C under constant stirring.

3. Results and discussion

3.1. Diffusion limitation of PCL preparation

The commercial enzyme preparations were mainly in the form of crude enzymes or immobilized enzymes because they possess higher activity and stability than pure enzymes. Crude PCL used in this study was diluted with dextrin and has a protein content of 2 wt% determined by the Bradford assay using bovine serum albumin as the standard. After the removal of dextrin, the enzymatic activity of PCL decreased by 2–3 orders of magnitude. Obviously, the dextrin stabilized and activated PCL. Scanning electron microscopy (SEM) showed that crude PCL was composed of irregular particles with a size of 10–200 μm (Fig. 1(a)). After lyophilization, all spherical particles were

converted into fiber bundles with a diameter of 1 μm and a length of around 500 μm (Fig. 1(b)). The surface area of the PCL preparation increased from 1.77 m^2/g to 62.6 m^2/g . Although there was a 35-fold increase in surface area, the transesterification activity of the biocatalyst only increased from 130 $\mu\text{mol}/(\text{min}\cdot\text{g})$ for crude PCL to 233 $\mu\text{mol}/(\text{min}\cdot\text{g})$ for lyophilized PCL, indicating that less diffusion limitation was not the overriding factor responsible for the increased catalytic efficiency after lyophilization. Then we attempted to further activate crude PCL by the addition of salt to decrease the activity difference between aqueous and nonaqueous media. As shown in Figs. 1(c) and 1(d), after co-lyophilization with phosphate buffer or sodium sulfate, the PCL fibers were destroyed and the surface areas were decreased (17.4 m^2/g for PCL-PB and 15.3 m^2/g for PCL-SS). Although PCL-PB and PCL-SS have similar surface areas, the transesterification activity of PCL-PB (130 $\mu\text{mol}/(\text{min}\cdot\text{g})$) was far less than that of the PCL-SS preparation, which is consistent with past observations and reconfirms the difference between the phosphate buffer and sodium sulfate in salt-induced enzyme activation. These results also indicated that enzyme activation did not originate from decreased diffusion limitation.

3.2. Effect of phosphate buffer (PB) on catalytic activity and the local environment of PCL-PB preparations

Generally, the degree of salt-induced activation strongly depends on the amount of salt and increases with increasing salt content. Although it is one of the most kosmotropic salts, the possible maximum concentration of the phosphate buffer is

Fig. 1. SEM images of crude *Pseudomonas cepacia* lipase (PCL) (a), lyophilized crude PCL without salt (b), crude PCL co-lyophilized with phosphate buffer (PB) (c), and crude PCL co-lyophilized with sodium sulfate (SS) (d).

Fig. 2. Specific activities of PCL-PB preparations with different lyophilization time as a function of PB content. Solid line: in the transesterification resolution reaction of racemic 1-phenylethanol; Dotted line: in the hydrolytic reaction of triacetin; Solid square (■): crude PCL.

much lower than that of a strong electrolyte. This is probably why the activation effect of the kosmotropic buffer is often neglected. Using commercial PCL as a control, we investigated the effect of the phosphate buffer on the catalytic activity of PCL in organic media. Figure 2 shows the catalytic activities of PCL-PB preparations as a function of PB content. Regardless of whether there was the addition of phosphate buffer or not, the PCL-PB preparations showed a similar hydrolytic activity (dotted lines) of around 2600 $\mu\text{mol}/(\text{min}\cdot\text{g})$. This activity is similar to that of crude PCL and lyophilized PB-free PCL, suggesting that the co-lyophilization with the phosphate buffer had hardly affected the structure of PCL. However, the transesterification activity (solid lines) of the PCL-PB preparations was dependent on the PB content and lyophilization time. When the lyophilization time was 72 h, all the PCL-PB preparations showed similar specific activities of around 130 $\mu\text{mol}/(\text{min}\cdot\text{g})$, which is similar to that of crude PCL but lower than that of PB-free PCL. It seemed that the presence of the phosphate buffer exerted a negative effect on the transesterification activity of the PCL preparation. However, when the lyophilization time was shortened to 24 h, the PCL-PB preparations exhibited higher transesterification activities than PB-free PCL. This means that the transesterification activity of PCL-PB preparation is sensitive to the lyophilization time. Moreover, with the increase of PB content, the specific transesterification activity of the PCL-PB preparation increased to the maximum with an optimum PB content of 8 mmol/g PCL.

Then, PCL-PB preparations with the PB content of 8 mmol/g PCL were prepared under different lyophilization conditions.

Fig. 3. Thermogravimetric/differential thermogravimetric data of PCL-PB preparations with different lyophilization conditions. Heating rate 5 $^{\circ}\text{C}/\text{min}$, in a nitrogen atmosphere. (1) PCL-PB-a; (2) PCL-PB-b; (3) PCL-PB-c.

As shown in Table 1, PCL-PB-a with extended lyophilization (72 h) showed the lowest transesterification activity (187 $\mu\text{mol}/(\text{min}\cdot\text{g})$) and PCL-PB-b with a shortened lyophilization (24 h) showed a moderate transesterification activity (566 $\mu\text{mol}/(\text{min}\cdot\text{g})$). By diminishing the rate of water sublimation, the transesterification activity of PCL-PB-c can be further enhanced (1375 $\mu\text{mol}/(\text{min}\cdot\text{g})$). To rule out enzyme deactivation, the enzymatic activities of these three PCL-PB preparations in aqueous media were also assayed. All the PCL-PB preparations showed a similar hydrolytic activity to crude PCL, meaning that PCL did not get denatured upon extended lyophilization. To probe the effect of lyophilization on the local environment of PCL, we performed the thermogravimetric analysis of these three PCL-PB preparations (Fig. 3). This method can differentiate between adsorbed and bonded water by the temperature range of the weight loss. Generally, a weight loss below 120 $^{\circ}\text{C}$ is attributed to the removal of adsorbed water [27], and a weight loss in the temperature range 150–230 $^{\circ}\text{C}$ is attributed to the elimination of bound water and the dissociation of the quaternary structure of the protein [28]. Loosely adsorbed water affects enzyme flexibility, and tightly bound water takes part in constructing the quaternary structure of the protein, which are essential for the enzyme structure and catalytically active conformation [22].

As shown in Fig. 3, PCL-PB-b has the largest weight loss below 120 $^{\circ}\text{C}$. Moreover, the water molecules in PCL-PB-b were removed in two steps, namely, at 45 and 70 $^{\circ}\text{C}$, which is similar to the removal of crystallization water in $\text{Na}_2\text{HPO}_4\cdot 12\text{H}_2\text{O}$. In contrast to the removal of the adsorbed water of PCL-PB-b, that

Table 1

Weight losses and transesterification activities of PCL-PB preparations with different lyophilization conditions.

Enzyme preparation ^a	Weight loss (%)		PCL content (10 ⁻³)	Weight loss per enzyme		Activity ($\mu\text{mol}/(\text{min}\cdot\text{g})$)
	Below 120 $^{\circ}\text{C}$	150–230 $^{\circ}\text{C}$		Below 120 $^{\circ}\text{C}$	150–230 $^{\circ}\text{C}$	
PCL-PB-a	4.4	9.2	9.4	4.7	9.8	187
PCL-PB-b	9.6	6.6	8.2	11.7	8.1	566
PCL-PB-c	4.1	5.5	7.6	5.4	7.2	1375

^a PCL-PB-a: lyophilized for 72 h; PCL-PB-b: lyophilized for 24 h; PCL-PB-c: lyophilized for 24 h with diminished rate of water sublimation.

of PCL-PB-a and PCL-PB-c occurred by only one step. These results indicate that part of the water molecules in PCL-PB-b were probably present as crystallization water of the phosphate buffer and do not take part in enzymatic catalysis. Compared to PCL-PB-b and PCL-PB-c, PCL-PB-a has larger weight losses in the temperature range 150–230 °C and 230–450 °C, which may explain the diminished catalytic activity of PCL-PB-a in organic media.

Further insights into the effect of lyophilization on the transesterification activity of PCL-PB preparations were obtained by correlating the accurate weight losses and specific activities (Table 1). Weight losses of PCL-PB preparations in the temperature range 150–230 °C decreased with increasing transesterification activity, that is, weight loss in the temperature range 150–230 °C was more important to catalytically active conformation of the PCL. Obviously the weight loss in the temperature range 150–230 °C included not only the removal of protein-bonded water but also damaged the protein-salt interaction. Due to the low concentration of the phosphate buffer surrounding the PCL, the amount of water was probably not enough to activate enzyme fully. If the freeze-drying time was long (e.g., more than 2 d), the presence of PB in contact with the enzyme surface can remove protein-bonded water and affect the catalytically active conformation by specific protein-anion interactions. A shortened freeze-drying time or diminished rate of water sublimation can artificially increase the amount of water in the PCL-PB preparations. On the one hand, additional water molecules increase enzyme flexibility, but on the other hand, they stabilize the PCL structure and increase the transition state polarity with the aid of protein-water and protein-anion interactions, thus decreasing the activation energy and increasing the catalytic activity of PCL preparation in organic media.

3.3. Effect of sodium sulfate (SS) on catalytic activity and the local environment of PCL-SS preparations

To compare the effect of strong electrolytes and weak electrolytes, crude PCL co-lyophilized with various amounts of sodium sulfate were prepared. Sulfate is a typical kosmotropic anion (B coefficient = 0.208), which exhibits a profound acti-

Fig. 4. Specific transesterification activity of PCL-SS preparations as a function of SS content. Lyophilization time of all the PCL-SS preparations were 24 h.

vating effect on the catalytic activity of PCL [16]. Figure 4 shows the catalytic efficiency of PCL-SS preparations in the transesterification reaction as a function of SS content. With the increase of SS content, the catalytic activities of the PCL-SS preparations increased and reached an optimum at the SS content of 20 mmol/g PCL, about 6-fold higher than crude PCL. Further increase of SS content would result in decreased catalytic activity of the PCL-SS preparation. To probe the relation between SS content and the water content of the PCL-SS preparations, the thermogravimetric analysis of the PCL-SS preparations was performed. After the addition of SS, weight losses below 120 °C and in the temperature range 150–230 °C dramatically decreased (Fig. 5(a)), while the weight loss per enzyme increased slightly (Fig. 5(b)). Viewing the weight losses below 120 °C and in the temperature range 150–230 °C as the measure of the amount of adsorbed water and bound water, the trend that the amount of water per enzyme increased with increased SS content is consistent with the report of salt-activated subtilisin [17]. However, too much SS would affect the catalytic activity of the PCL preparation, probably due to restricted internal diffusion.

In addition, PCL-SS preparations (with 20 mmol SS per gram crude PCL) under different lyophilization conditions were prepared. Table 2 shows the weight losses and transesterification activities of the three PCL-SS preparations. On increasing the lyophilization time to 72 h for PCL-SS-a, or decreasing the lyophilization time to 24 h for PCL-SS-b, or even diminishing the rate of water sublimation in the lyophilization time of 24 h for PCL-SS-c, the catalytic activity of the PCL preparations changed slightly. That is, the lyophilization condition has little influence

Fig. 5. Weight loss (a) and weight loss per enzyme (b) of PCL-SS preparations as a function of SS content.

Table 2

Weight losses and transesterification activities of PCL-SS preparations under different lyophilization conditions.

Enzyme preparation ^a	Weight loss (%)		PCL content (10 ⁻³)	Weight loss per enzyme		Activity ($\mu\text{mol}/(\text{min}\cdot\text{g})$)
	Below 120 °C	150–230 °C		Below 120 °C	150–230 °C	
PCL-SS-a	2.1	5.2	4.8	4.4	10.8	626
PCL-SS-b	2.4	5.3	4.7	5.0	11.1	884
PCL-SS-c	6.3	4.6	4.2	15	10.9	1086

^a PCL-SS-a: lyophilized for 72 h; PCL-SS-b: lyophilized for 24 h; PCL-SS-c: lyophilized for 24 h with diminished rate of water sublimation.

on the catalytic activity of the PCL-SS preparations. Altering the lyophilization condition of PCL-SS preparation mainly affects the amount of adsorbed water, while the weight losses of the PCL-SS preparations in the temperature range 150–230 °C changed little. With a shortened lyophilization or diminished rate of water sublimation, the amount of adsorbed water of the PCL-SS preparation increased, and it gave a slight increase in catalytic activity. These results indicated that the use of kosmotropic sulfate in enzyme activation is due to a strong affinity to water. The presence of 20 mmol SS per gram PCL can afford enough water to maintain the catalytic activity of PCL. Extended lyophilization cannot remove water molecules from the protein and shortened lyophilization cannot affect the protein-salt interaction. This also explains why the transesterification activity of the PCL-SS preparations was mainly dependent on the SS content rather than the lyophilization condition.

3.4. Polarity change of crude PCL co-lyophilized with PB and SS

PCL-SS-a: lyophilized for 72 h; PCL-SS-b: lyophilized for 24 h; PCL-SS-c: lyophilized for 24 h with diminished rate of water sublimation. Fluorescence spectroscopy is commonly used to probe the environmental polarity and local structure in sol gel-derived nanocomposite materials and silica-based hybrid materials [29,30]. Here, we attempted to use fluorescence spectroscopy to study in situ the salt-incorporated PCL preparations in organic media. 2-(4'-Amino-2'-hydroxy phenyl)benzoxazole (4-AHBO) that emits fluorescence by the excited state intramolecular proton transfer mechanism (ESIPT) [31] was used as the fluorescent probe to study the polarity

change of salt-incorporated PCL in organic media. The fluorescence spectrum of 4-AHBO is sensitive to the environmental polarity (e.g. various organic media, Figure not shown), but it is unclear whether the addition of the salt-incorporated PCL preparation can further alter the local polarity surrounding 4-AHBO. Figure 6 presents the fluorescence emission spectra of 4-AHBO in *n*-hexane suspended with lyophilized PCL and PB. There are two distinct peaks, one (I_1) at short wavelengths ascribed to a typical conformer in polar solvent and the other one (I_2) due to the excited keto tautomer common in non-polar solvents. Although there is no evidence that the fluorescent probe was at the enzyme/solvent interface, the obvious shift in the dual fluorescence emission of 4-AHBO suggested that the environment surrounding 4-AHBO was changed by the addition of lyophilized PCL and PB. The presence of PCL and PB enhanced the polarity of the environment surrounding 4-AHBO, resulting in enhanced emission from the I_1 peak at 378 nm. The intensity ratio I_1/I_2 is related to the local polarity surrounding 4-AHBO [30], so the increase in the I_1/I_2 ratio indicated the increased polarity of the enzyme preparation surrounding 4-AHBO.

Figure 7 shows the effect of PCL-PB preparations on the I_1/I_2 ratio of 4-AHBO in *n*-hexane. When a set of PCL-PB preparations with different PB contents and a lyophilization time of 24 h were dispersed into the 4-AHBO-hexane system, the I_1/I_2 ratio is typically around 0.6–0.8, and there was no obvious difference among the PCL-PB preparations. In these systems, the enzyme preparation cannot be fully dispersed, and the polarity is mainly determined by the solvent composition rather than the enzyme preparation. The addition of the PCL-PB preparation resulted in a minimal change in the I_1/I_2 ratio of 4-AHBO in *n*-hexane. On the contrary, when a set of PCL-PB preparations with a lyophilization time of 72 h were dispersed into the

Fig. 6. Steady state fluorescence spectra of 4-AHBO in *n*-hexane suspended with lyophilized PCL and PB. (1) 4-AHBO without PCL and PB; (2) 4-AHBO in *n*-hexane suspended with lyophilized PB; (3) 4-AHBO in *n*-hexane suspended with lyophilized PCL.

Fig. 7. I_1/I_2 ratio of 4-AHBO versus PB content of PCL-PB preparation with different lyophilization time.

Fig. 8. Fluorescence spectra of 4-AHBO in *n*-hexane suspended with PCL-SS preparations with different SS contents. Lyophilization time of all the PCL-SS preparations was 24 h.

4-AHBO-hexane system, the I_1/I_2 ratio increased with the increasing PB content, indicating that 4-AHBO was sensitive to the more polar environment of the enzyme preparations. There are similar trends in the I_1/I_2 value of 4-AHBO and transesterification activity of the PCL-PB preparation. It turns out that the I_1/I_2 value is associated with the polarity of the enzyme preparation and PB incorporation increased the polarity of the PCL-PB preparations.

After that, 4-AHBO was used to probe the PCL-SS series. As shown in Fig. 8, the intensity of the peak at shorter wavelength (I_1) dramatically increased, suggesting that the addition of the PCL-SS preparation significantly increased the local polarity surrounding 4-AHBO. Varying of the amount of SS showed a similar correlation between the polarity surrounding 4-AHBO and enzyme activity leading up to an optimum. That the I_1/I_2 ratio of 4-AHBO, as well as the local polarity of enzyme preparation, correlates with enzymatic activity suggested that the incorporation of SS affected the polarity of the enzyme preparation and increased the enzymatic activity in organic media.

These results demonstrated the special advantage of 4-AHBO in probing the local polarity of the salt-incorporated enzyme preparation in organic solvent. Therefore, this 4-AHBO-hexane system can be used for studying the influence of the salt species and salt content on the local polarity of the enzyme preparation with thorough freeze-drying.

4. Conclusions

The correlation of the catalytic activity and local environment in crude PCL co-lyophilized with a phosphate buffer (PB) or sodium sulfate (SS) was investigated. The transesterification activity of the salt-incorporated PCL increased with increasing amount of phosphate buffer or sodium sulfate up to an optimum. Optimizing the lyophilization condition significantly improved the transesterification activity of the PCL-PB preparation; the effect of SS content on the catalytic activity of PCL-SS preparations was more obvious. The high transesterification activity of the PCL preparation measured in the presence of kosmotropic SS was also obtained in the presence of PB by controlling the lyophilization process. There are similar trends between the local polarity and transesterification activity of salt-incorporated crude PCL as a function of salt content. A highly active enzyme preparation exhibits a strong polar nature, indicating that the salt increases the polarity of the enzyme preparation and activates the enzyme to some extent.

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Graphical Abstract

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JIN Qianru, JIA Guoqing, WANG Xiuli, LI Can *

Dalian Institute of Chemical Physics, Chinese Academy of Sciences

The catalytic activity of *Pseudomonas cepacia* lipase (PCL) in organic media was drastically enhanced by the addition of a kosmotropic phosphate buffer or sodium sulfate. This activation is probably due to the polar environment formed by the salt-bound water surrounding the PCL.

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磷酸缓冲盐和硫酸钠对洋葱假单胞菌脂肪酶的非水相激活

金倩茹, 贾国卿, 王秀丽, 李 灿*

中国科学院大连化学物理研究所催化国家基础重点实验室, 辽宁大连116023

摘要: 研究了kosmotropic型磷酸缓冲盐和硫酸钠对洋葱假单胞菌脂肪酶(*Pseudomonas cepacia* lipase, PCL)非水相催化性能的影响。以往磷酸缓冲盐被用来调控体系的pH值, 其掺杂量对酶的催化活性无明显影响, 而适量硫酸钠的掺杂则可有效提高酶在非水相的催化活性。本文研究发现, 通过精确调控冻干过程, 磷酸缓冲盐掺杂能够将PCL在有机相中的转酯化活性提高近10倍, 达到其水相本征活性的50%, 这一激活效果甚至高于硫酸钠掺杂。利用热重方法分析了盐掺杂PCL的含水量和蛋白结构, 并将失重结果同其在有机相中的催化活性相关联, 发现PCL在磷酸缓冲盐和硫酸钠掺杂下的催化构型与蛋白含水量及其周围盐环境具有不同的依赖关系。利用2-(4'-氨基-2'-羟基苯基)苯并恶唑作为荧光探针, 研究了磷酸缓冲盐和硫酸钠掺杂的PCL悬浮于有机相对荧光探针发射光谱的影响, 发现盐掺杂酶制剂的存在能够大大增加荧光探针稳定于极性溶剂的构型含量, 这可能与蛋白周围掺杂盐键和的水分子有关。如果用探针分子稳定于极性溶剂和非极性溶剂的构型比值间接表示悬浮酶制剂的极性结构, 在正己烷体系中硫酸钠掺杂的PCL具有比磷酸缓冲盐掺杂的PCL大得多的极性, 且酶制剂的极性大小与其非水相转酯化活性之间具有相似的变化趋势。上述研究结果表明, 掺杂盐对粗PCL酶制剂的激活可能部分归因于掺杂盐键和的水分子在蛋白周围构筑的极性环境。

关键词: 洋葱假单胞菌脂肪酶; 酶激活; 磷酸缓冲盐; 硫酸钠; 热重分析; 局部极性; 荧光探针

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*通讯联系人. 电话: (0411)84379070; 传真: (0411)84694447; 电子信箱: canli@dicp.ac.cn

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