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The hydrolytic activity of *Pseudomonas mendocina* 3121-1 lipase. A kinetic study

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The hydrolytic activity of *Ps. mendocina* 3121-1 (further marked as *Ps. mendocina**) lipase was investigated using p-nitrophenyl butyrate (p-NPB) solutions in selected organic solvents (2-propanol, acetone, 1,4-dioxane and acetonitrile) as well as other soluble p-nitrophenyl fatty acid esters of different chain length in 2-propanol as substrates. In acetone, lower $K_{m\ (app)}$ and higher $V_{max\ (app)}$ were determined than in the other organic solvents used. The calculated $V_{max\ (app)} / K_{m\ app)}$ ratio was found to be higher in the latter case as well. The $k_{cat\ (app)}$ of p-NPB hydrolysis in 1,4-dioxane was shown to be nearly 4-fold higher than in other organic solvents, indicating the hydrophilicity of that solvent to be favourable for the hydrolysis. The kinetic parameters of hydrolysis of other fatty acid esters in the same organic solvent (2-propanol) were determined. This enzyme was shown to display highest activity when hydrolysing substrates of a moderate chain length (p-nitrophenyl laurate, p-NPL) at the same concentration of all esters (0.1 mM). Kinetic parameters were also determined for the hydrolysis of all fatty acid esters used, and the calculated parameters were compared with the determined ones. $K_{m\ (app)}$ was found to be higher for short fatty acid esters, and the lowest characteristics were determined for p-nitrophenyl caprylate (p-NPC), indicating *Ps. mendocina** lipase to be more specific to soluble fatty acid esters of moderate and long chains. No strong dependence of lipase $K_{m\ (app)}$ on fatty acid chain length was determined, while the highest $V_{max\ (app)}$ was determined for p-NPL and p-nitrophenyl myristate (p-NPM), and the lowest one was shown to be for p-nitrophenyl acetate (p-NPA). The $k_{cat\ (app)}$ was found to be higher for p-NPC and p-NPL, showing the lipase specificity to fatty acid esters of a moderate chain. The $V_{max\ (app)} / K_{m\ (app)}$ ratio was different for all substrates used, but for fatty acid esters of moderate and long chains it by far exceeded the ratio for p-NPA and p-NPB. The determined $V_{max\ (app)}$ was compared with the calculated parameter at the substrate concentration close to $K_{m\ (app)}$, and a significant correlation was found. Analysis of kinetic parameters at the substrate concentration close to K_m and 5-fold lower than K_m (at the same "kinetic profile") showed the enzyme to be the most active upon p-NPL at "K_m" profile, and the general dependence of activity on fatty acid chain length was found to be similar to the dependence when fatty acid esters had been used at the same concentration. In contrast, the hydrolytic activity was close for p-NPL and p-NPM and also was not so far from the activity for p-NPP and p-NPS at the substrate concentration in the "below K_m" profile. A slight interfacial activation under formation of micellar structures using p-NPC as the substrate was determined. The kinetic constants of *Ps. mendocina** lipase-catalyzed p-NPB hydrolysis in various alcohols were determined showing the inactivating effect of alcohols at various concentrations. When p-NPB solutions in each of the alcohols mentioned had been used as substrates, the hydrolytic reaction was detected only in most hydrophilic ones. The effect of micellar structure formation on *Ps. mendocina** lipase-catalyzed Tween hydrolysis was investigated. It was found that the efficiency of the hydrolysis changed when the concentration of those detergents exceeded the critical micellar concentration (CMC).

Key words: *Ps. mendocina* lipase, fatty acids p-nitrophenyl esters, hydrolytic activity, organic solvents

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INTRODUCTION

Certain reactions catalyzed by lipases (EC 3.1.1.3) are of great interest for a possible application in biotechnological processes to produce biodegradable and environment-friendly fatty acid esters as specific components of biofuel, biolubricants and bioplasticizers. Control of various parameters of the catalytic process enables to provide a high yield of pure product. The development of industrial biotechnology showed the importance of a detailed analysis of lipolytic reactions. A crucial role of screening for new effective lipolytic enzymes in order to provide favourable processes of biocatalysis to compete with chemical catalysis should also be considered [1–4]. Moreover, substrate specificity of lipases is an attractive feature of the biocatalysts [5–7].

Pseudomonas mendocina 3121-1 (*Ps. mendocina**) lipase was found to possess both esterase and lipase activity [8]. Certain interesting features determined previously [9–11] directed to a detailed analysis of the kinetic parameters of the lipase-catalyzed hydrolysis of soluble fatty acid esters in the presence of organic phase. The results should be crucial for the technological control of enzymatic hydrolysis of oils and fats and of subsequent esterification to produce fatty acid esters with various alcohols.

The purpose of the present report was a kinetic study of *Ps. mendocina** lipase-catalyzed p-nitrophenyl fatty acid esters hydrolysis in organic solvents and comparison of the efficiency of Tween hydrolysis under formation of detergents' micellar structure.

MATERIALS AND METHODS

Materials. Lipase from *Ps. mendocina** was provided by the Institute of Biochemistry, Vilnius, Lithuania. p-Nitrophenyl fatty acid esters, Tweens 20, 40, 60, 80, 85, stabilized lipase substrate, organic solvents, alcohols, and Tris were from Sigma. Gum arabic was from Merck. Acetic, boric and orthophosphoric acids, NaOH were from Lachema.

METHODS

The standard spectrophotometric assay. The hydrolytic activity of the lipase upon p-NPA, p-NPB solutions in 2-propanol, acetone, 1,4-dioxane, acetonitrile and upon p-NPC, p-NPL, p-NPM, p-nitrophenyl palmitate (p-NPP) and p-nitrophenyl stearate (p-NPS) solutions in 2-propanol was investigated. The change of optical density at λ = 400 nm for 3–6 min at 30 °C and pH 7.6 or 9.0 was determined. 100 mM of a universal (ub) or Britton–Robinson buffer composed of acetic, ortho-boric and orthophosphoric acids 1 : 1 : 1 providing the buffering capacity at a wide pH range was used. The final concentration of the enzyme was 0.04–1 μg/ml, of the substrate 0.1–0.5 mM, of organic solvents 1–77% (v/v) [12]. A spontaneous hydrolysis of p-nitrophenyl fatty acid esters in blank samples was considered as control. The molar extinction coefficient 1.03 was determined for 0.15 mM p-NPB. One unit of the hydrolytic activity corresponded to the amount of enzyme releasing 1 μmol of p-nitrophenol per minute under standard conditions.

Kinetic parameters of the lipase-catalyzed hydrolysis of various p-nitrophenyl fatty acid esters. 1. The hydrolytic activity was determined using p-NPB solutions in 2-propanol, acetone, 1,4-dioxane and acetonitrile. The final concentration of p-NPB was 0.03–1.02 mM and of organic solvent 1% (v/v). The hydrolysis was monitored for 3 min at 30 °C and pH 9.0. 2. Solutions of substrates with a various chain length of fatty acids were used at the following concentrations (μM): p-NPA 100–800; p-NPB 30–1020; p-NPC 1–10; p-NPL 5–50; p-NPM, p-NPP and p-NPS 10–50.

The extinction coefficients were determined measuring the optical density at λ = 340 nm using the following concentrations of p-nitrophenyl fatty acid esters (μM): p-NPB 30–1020; p-NPC 2–10; p-NPL, p-NPM, p-NPP and p-NPS 0.5–5. The measurement was carried out at 30 °C and pH 9.0.

The hydrolysis of various p-nitrophenyl fatty acid ester solutions in 2-propanol at the substrate concentration in the profiles "close to K_m" and "below K_m" was investigated. The reaction was performed for 3 min at 30 °C, 100 mM ub, pH 9.0. The final concentration of the enzyme was 0.04 μM and of 2-propanol 1% (v/v). Km – "close to K_m" profile; <Km – "below K_m" profile. Substrate concentration (μM): Km: p-NPA 500; p-NPB 300; p-NPL, p-NPM and p-NPS 100; p-NPP 50; <Km: p-NPA 100; p-NPB 60; p-NPL, p-NPM and p-NPS 20; p-NPP 10.

Apparent catalytic constants ($k_{cat\ (app)}$, min^{-1}) of the hydrolysis at alcohol concentrations of 10, 20 and 45% (v/v) were determined. Enzymatic activity was investigated using methanol, ethanol, 1-butanol, 1-pentanol, 1-octanol, 2-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol and phenylmethanol. The catalysis was monitored using p-NPB solution in 2-propanol in the presence of another alcohol or using the substrate solution in a respective alcohol. The activity was detected at 30 °C, pH 7.6 in the presence of alcohol directly in the reaction mixture at a concentration of 0.5–2.25% (v/v) or after pre-incubation of the enzyme at the alcohol concentration of 10–45% (v/v) at 30 °C for 30 min. $K_{m\ (app)}$ for p-NPB was determined as 280 ± 30 μM.

The standard titrimetric assay. The analysis of Tween hydrolysis was as described [13]. Tween 20, 40, 60, 80 and 85 as the lipase substrates were used in aqueous media at the following final concentrations: twice below CMC, close to CMC, five-fold exceeding CMC and by far exceeding CMC (11–44 mM). The mixture containing the substrate and 50 mM Tris-HCl buffer, pH 7.9 (30 °C) (final volume

8 ml) was adjusted to pH 8.0 by 1N NaOH, and such pH was maintained for 3 min by titration with 43 mM NaOH solution (blank). Then 1 ml of the lipase solution (4 μg/ml) in the buffer was added, and the lipolytic reaction was tested for 1–30 min by titration as mentioned above (sample). One unit of the lipolytic activity corresponded to the amount of the enzyme releasing 1 mmol of fatty acid per minute under standard conditions.

Statistics. Four measurements were provided in each experiment, and the results are presented as means ± S. E. M.

RESULTS AND DISCUSSION

Kinetic parameters of the hydrolysis of p-nitrophenyl fatty acid esters. Organic solvents have been shown more or less to affect the hydrolytic activity of lipases including the substrate specificity. Kinetic parameters of the reaction usually show the effect [14, 15]. In this report, hydrolytic activity of *Ps. mendocina** lipase was investigated using p-NPB solutions in four selected organic solvents or soluble p-nitrophenyl fatty acid esters of different chain length in 2-propanol as substrates. The first experimental series show the effect of organic solvent on the solvatation of the substrate and of the enzyme that also should affect protein structure, whereas the second series illustrates the influence of the solvent on the enzyme–substrate interaction [14]. The kinetic parameters of p-NPB hydrolysis were determined in 2-propanol, acetone, 1,4-dioxane and acetonitrile. Results are presented in Table 1.

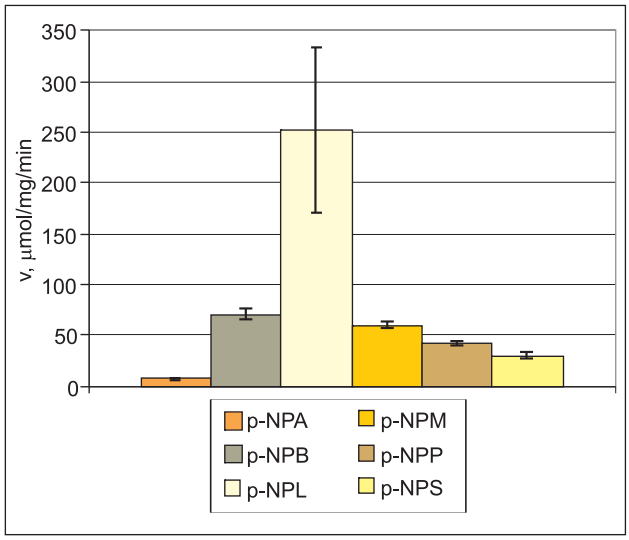

Fig. 1. *Ps. mendocina** lipase-catalyzed hydrolysis of various p-nitrophenyl fatty acid esters at the same concentration of the substrate. Solutions of substrates in 2-propanol were used. Final concentration of the substrate was 0.1 mM, of the enzyme 0.04 μg/ml and of the solvent 1% (v/v). The reaction was performed for 3 min at 30 °C and pH 9.0

The catalytic process of lipolytic enzymes cannot be characterized by the classical Michaelis–Menten model, consequently, the kinetic parameters are considered as apparent. Those parameters are influenced by the substrate hydrophobicity, partition of the reaction products and other factors [16]. It is evident (Table 1) that acetone caused a lower $K_{m\,(app)}$ and a higher $V_{max\,(app)}$, than the other organic solvents used. The calculated $V_{max\,(app)}$ / $K_{m\,(app)}$ ratio was found to be higher in this case as well. Hydrophobic solvents affect substrate solvatation and usually cause a lower K_m than hydrophilic ones. V_{max} can illustrate the role of organic solvent in enzyme conformation, structure and catalytic action. Thus, acetone most probably influenced both the solvatation of p-NPB and the structure of *Ps. mendocina** lipase. Evaluation of V_{max} as a real maximal rate of the enzymatic reaction could indicate that lipases act similarly in the majority of organic solvents tested. Our experimental results confirmed this fact. A more pronounced variation of $K_{m\,(app)}$ than of $V_{max\,(app)}$ in different organic solvents was found for other lipases [17]. We showed that $k_{cat\,(app)}$ of p-NPB hydrolysis in 1,4-dioxane was nearly 4-fold higher than in other organic solvents, indicating the hydrophilicity of this solvent to be favourable for the hydrolysis.

It was already mentioned that the second experimental series was provided to show the substrate specificity of the lipase. Kinetic parameters of the hydrolysis of various p-nitrophenyl fatty acid esters in the same organic solvent (2-propanol) were determined. Results are illustrated in Fig. 1. We showed the enzyme to display highest activity upon p-NPL, i. e. a substrate of a moderate chain length

Table 1. **The kinetic parameters of *Ps. mendocina** lipase-catalyzed hydrolysis of p-NPB solutions in various organic solvents. Final concentrations of the substrate were 0.03–1.02 mM, and the concentration of organic solvent was 1% (v/v). The parameters were determined at 30 °C and pH 9.0**

Organic solvent	logP[a]	$K_{m\,(app)}$, μM	$V_{max\,(app)}$, μmol/mg/min	$V_{max\,(app)}$ / $K_{m\,(app)}$[b], min^{-1}	$k_{cat\,(app)}$, min^{-1}
2-Propanol	0.05	280 ± 30	454 ± 25	1.6	0.05
Acetone	–0.23	165 ± 40	654 ± 43	4.0	0.05
1,4-Dioxane	–1.1	320 ± 80	400 ± 33	1.3	0.19
Acetonitrile	–0.33	230 ± 30	399 ± 33	1.7	0.04

[a] Measure of hydrophobicity of organic solvent, where P is the partition coefficient of the organic solvent between 1-octanol and water.
[b] The ratio of calculated parameters.

Table 2. **The kinetic parameters of *Ps. mendocina** lipase-catalyzed hydrolysis of various p-nitrophenyl fatty acid ester solutions in 2-propanol. Substrates were used at concentrations of (μM): p-NPA 100–800; p-NPB 30–1020; p-NPC 1–10; p-NPL 5–50; p-NPM, p-NPP and p-NPS 10–50. Final concentration of 2-propanol was 1% (v/v). The parameters were determined at 30 °C and pH 9.0**

Substrates	Parameters		C near $K_{m(app)}$	$V_{max(app)}$*	$V_{max\,(app)} / K_{m(app)}$**, min^{-1}	$k_{cat(app)}$, min^{-1}	$k_{cat(app)} / K_{m(app)}$, $\mu M^{-1}min^{-1} \times 10^{-4}$
	$K_{m(app)}$, μM	$V_{max(app)}$, μmol/mg/min					
p-NPA	430 ± 40	32 ± 2	500	46 ± 2	0.07	0.03	0.7
p-NPB	280 ± 30	454 ± 25	300	472 ± 10	1.6	0.05	1.8
p-NPC	20 ± 10	384 ± 65	30	400 ± 148	19	2.11	1055.0
p-NPL	70 ± 20	1066 ± 108	100	1096 ± 100	15	1.11	158.6
p-NPM	80 ± 20	847 ± 92	100	700 ± 80	11	0.52	65.0
p-NPP	40 ± 10	276 ± 54	50	268 ± 50	7	0.21	52.5
p-NPS	80 ± 20	264 ± 37	100	256 ± 30	3	0.16	20.0

* Calculated parameter.

** The ratio of calculated parameters.

when the same 0.1 mM concentration of all esters was used. It should be noted that all the soluble fatty acid esters varied in hydrophobicity, consequently, each substrate was in a different physico-chemical state, providing also a different profile of the reaction kinetics. In this approach, kinetic parameters were also determined for the hydrolysis of all the p-nitrophenyl fatty acid esters used, and the calculated parameters were compared to the determined ones. Data are summarized in Table 2.

It is evident that $K_{m\,(app)}$ was higher for short p-nitrophenyl fatty acid esters, and the lowest parameter was determined for p-NPC. The parameters for *Ps. cepacia* lipase-catalyzed p-NPA hydrolysis were shown to be K_m 640 ± 60 μM and V_{max} 337 ± 9 μmol/mg/min [18]. It could be concluded that *Ps. mendocina** lipase is more specific to soluble p-NPR substrates of moderate and long chains, particularly to p-NPC. On the other hand, we showed that there was no strong dependence of the lipase $K_{m\,(app)}$ on fatty acid chain length in the substrates, while the tendency of a lower parameter with the elongation of the chain in triacylglycerols (TAGs) was noted [19]. Moreover, the highest $V_{max\,(app)}$ was determined for p-NPL and p-NPM, and the lowest parameter was shown to be for p-NPA. The $k_{cat\,(app)}$ constants were found to be higher for p-NPC and p-NPL. So, it was evident that *Ps. mendocina** lipase was specific of p-nitrophenyl fatty acid esters of a moderate chain. The $V_{max\,(app)} / K_{m\,(app)}$ ratio was different for all the substrates tested, but for p-nitrophenyl fatty acid esters of moderate and long chain it exceeded the ratio determined for p-NPA and p-NPB [20].

The determined $V_{max\,(app)}$ was compared with the calculated parameter at the substrate concentration close to the $K_{m\,(app)}$ determined, and a correlation was found (Table 2). Thus, it finally could be summarized that in hydrolysis *Ps. mendocina** lipase preferred soluble substrates of a moderate chain length. A similar specificity was shown for certain other lipases [21–23].

Analysis of the kinetic parameters confirmed the suggestion that hydrolysis of different p-nitrophenyl fatty acid esters proceeded at different "kinetic profile" when substrates were used at the same concentration of 0.1 mM. Next, analysis was carried out at the concentrations of each substrate close to K_m and 5-fold lower than K_m. Results are presented in Fig. 2.

Obviously, the lipase was most active when hydrolysing p-NPL at the "K_m" profile, and the general dependence of the activity on the chain length of fatty acid in p-nitrophenyl esters was found to be similar to that found using p-nitrophenyl fatty acid esters at the same concentration (Fig. 1). In contrast, the hydrolytic activity was close for p-NPL and p-NPM and also was not significantly higher than the activity for p-NPP and p-NPS at the substrate concentration in the "below K_m" profile (Fig. 2). So, it should be assumed that the lipase specificity for p-nitrophenyl fatty acid esters is necessary to be determined at the same kinetic profile rather than using the same concentration of all substrates.

As reported in the literature, *Ps. mendocina* lipase has been characterized as lacking interfacial activation and the "lid" in contrast to most of other lipases. Our investigation of the effect of substrate concentration on the hydrolysis of p-nitrophenyl fatty acid esters showed a slight interfacial activation when p-NPC was used as a substrate (Fig. 3). Thus, it is worth noting that *Ps. mendocina** lipase could also be activated under a changed physical state of certain substrates. On the other hand, it could be argued that the lipase *Ps. mendocina** examined in this report showed different features compared to the enzyme of the strain already described.

The fact that *Ps. mendocina** lipase was activated during the hydrolysis of p-NPC raises two questions: 1) why the interfacial activation was not observed for other p-nitrophenyl fatty acid esters? 2) could the point of the formation of the substrate micelles was reached in the range of p-NPC concentrations used? Consequently, we determined the

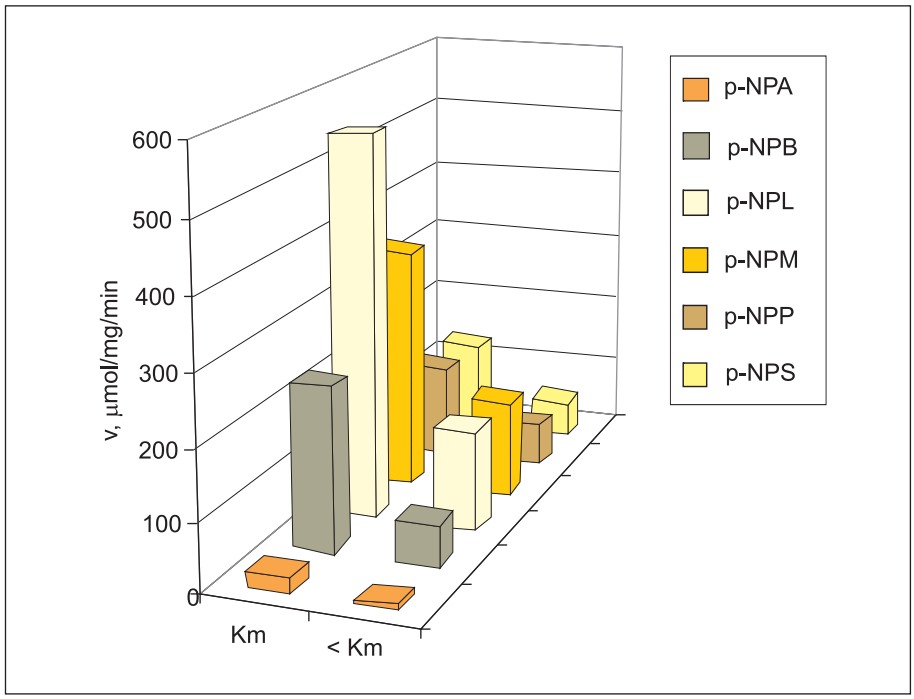

Fig. 2. *Ps. mendocina** lipase-catalyzed hydrolysis of various p-nitrophenyl fatty acid esters at profiles "K_m" and "below K_m". Solutions of substrates in 2-propanol were used. Final concentration of 2-propanol was 1% (v/v). Km – "K_m" profile; <Km – "below K_m" profile. Substrate concentration (µM): "K_m" profile: p-NPA – 500; p-NPB – 300; p-NPL, p-NPM and p-NPS – 100; p-NPP – 50; "below K_m" profile: p-NPA – 100; p-NPB – 60; p-NPL, p-NPM and p-NPS – 20; p-NPP – 10. The reaction was performed for 3 min at 30 °C and pH 9.0

dependence of the molar extinction coefficients ε_{340} on the substrate concentration for all the p-nitrophenyl fatty acid esters used, except high-soluble p-NPA. Data showed the formation of micelles to occur at the following ester concentrations: p-NPB 150–200 µM; p-NPC 3–5 µM; p-NPL, p-NPM and p-NPP ~1 µM; p-NPS <0.5 µM. Summarizing the data of these experimental series, it could be concluded that: i) no interfacial activation of *Ps. mendocina** lipase was noted under formation of micellar structure of a short-chain substrate (p-NPB), while the activation was observed at that point using the substrate of a moderate chain (p-NPC); ii) the kinetic curves of the other substrates did not illustrate a possible activation as their concentration was at the range exceeding critical micellar concentration (CMC); iii) the lack of interfacial activation of lipases could be caused not only by the structural features of these enzymes, but also by a very low CMC of the substrate below which the activity should be minimal.

The effect of alcohols on the kinetics of p-NPB hydrolysis. Spectrophotometric assay can be used not only for continuous measurements of the hydrolysis reaction, but also for determination of the final product by the ended reaction. In this case, organic solvents or their mixtures (usually alcohols) are used. We investigated the role of linear and branched aliphatic and aromatic alcohols of different chain length in *Ps. mendocina** lipase hydrolytic activity upon p-NPB providing the efficiency of inactivation. The kinetic constants of the reaction in various alcohols are summarized in Table 3. Apparently, in the presence of hydrophilic alcohols, the slowest hydrolytic reaction was at their concentration of 1% (v/v), while the reaction rate dropped with increasing the amount of other alcohols (especially of aromatic phenylmethanol) except 1-octanol. The reaction rate constants were lower in most cases when the lipase had been pre-incubated with an alcohol for 30 min prior to hydrolysis.

When p-NPB solutions in each alcohol were used as substrates, the hydrolytic reaction was observed only in most hydrophilic ones. This result should indicate the significance of factors such as substrate solubility, different manner of partition of reaction mixture components in the presence of water-miscible and immiscible alcohols and other factors that should be considered for optimization of lipase-catalyzed hydrolysis reactions [24, 25]. On the other hand, the stability of lipase catalytic function might be essential under a longer interaction of the enzyme with alcohols.

The effect of Tweens on *Ps. mendocina lipase hydrolytic activity.** The effect of the formation of micellar structure on *Ps. mendocina** lipase-catalyzed hydrolysis of Tweens was investigated. We have previously shown the enzyme to be active for those non-ionic detergents [10].

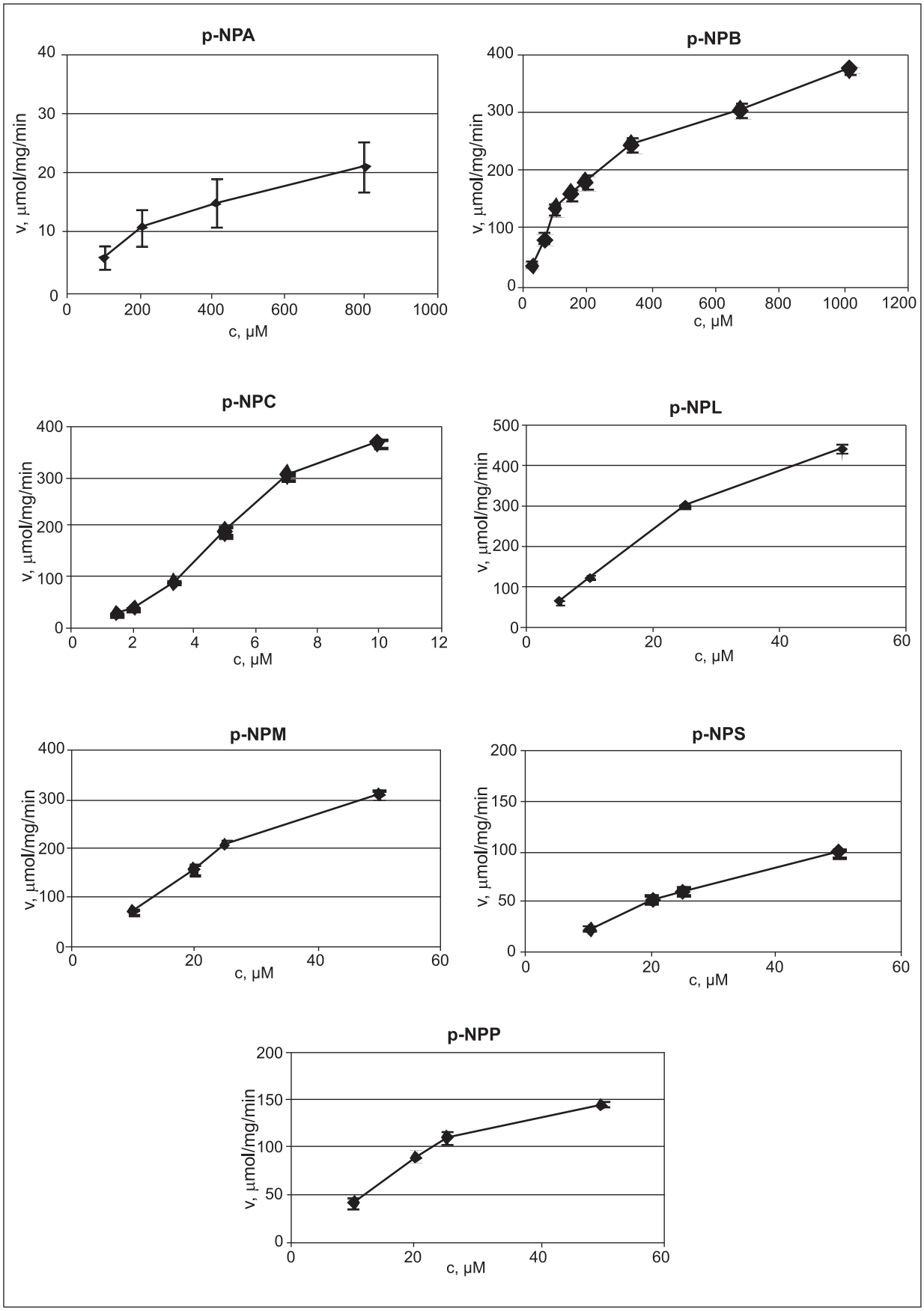

Fig. 3. The effect of substrate concentration on the hydrolysis efficiency of *Ps. mendocina** lipase-catalyzed p-nitrophenyl fatty acid esters. The reaction was performed for 3 min at 30 °C and pH 9.0 at a final concentration 0.04 µM of the ezyme and 1% (v/v) of 2-propanol

Table 3. **The apparent catalytic constants of lipase-catalyzed p-NPB hydrolysis in the presence of alcohols. The activity was detected at 30 °C and pH 7.6 in the presence of alcohol directly in the reaction mixture at a concentration of 0.5–2.25% (v/v) or when the enzyme had been pre-incubated with alcohol at a concentration of 10–45% (v/v) at 30 °C for 30 min. $K_{m\,(app)}$ for p-NPB 280 ± 30 μM**

Time course, min	Alcohol concentration, % (v/v)					
	10		20		45	
	0	30	0	30	0	30
	$k_{cat\,(app)}$, min^{-1}					
Without alcohol	0.22	0.22				
Methanol	0.18	0.05	0.13	0.04	0.18	0.06
Ethanol	0.24	0.06	0.14	0.07	0.18	0.07
1-Butanol	0.16	0.03	0.15	0.02	0.11	0.09
1-Pentanol	0.16	0.04	0.14	0.03	0.08	0.06
1-Octanol	0.10	0.06	0.12	0.10	0	0
2-Propanol	0.19	0.04	0.15	0.07	0.22	0.02
2-Methyl-1-Propanol	0.20	0.04	0.14	0.04	0.12	0.09
3-Methyl-1-Butanol	0.17	0.04	0.13	0.05	0.07	0.05
Phenylmethanol	0.16	0.05	0.06	0.03	0.05	0.04

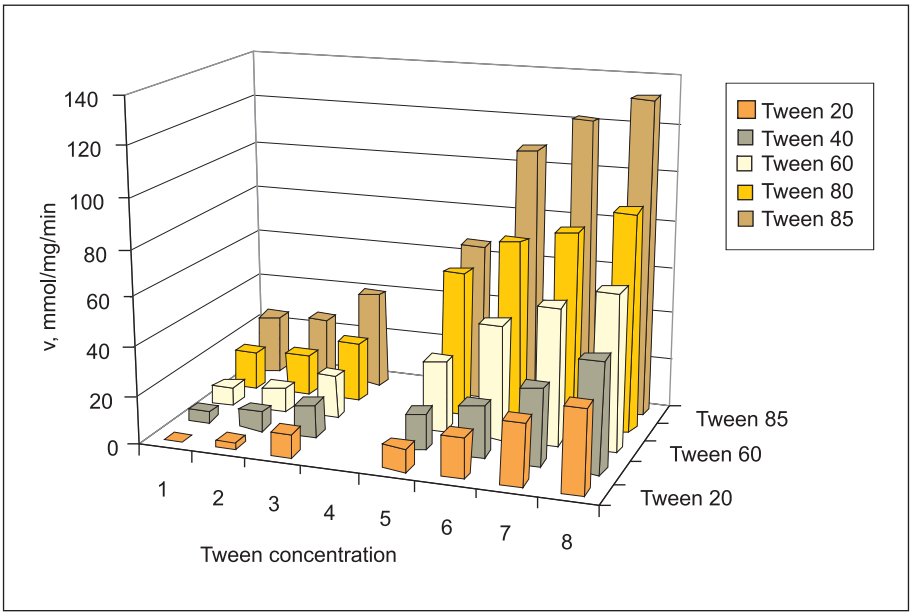

Fig. 4. The hydrolytic activity of *Ps. mendocina** lipase when non-ionic detergents Tweens were used as substrates. The activity was determined by the standard titrimetric (continuous) method in the presence of Tweens at the following final concentrations: twice below CMC (1), close to CMC (2), five-fold exceeding CMC (3) and by far exceeding CMC (11–44 mM, 5–8, respectively). CMC (mmol/L) are: 0.049 for Tween 20; 0.023 for Tween 40; 0.021 for Tween 60; 0.010 for Tween 80; CMC for Tween 85 was not determined

Results are presented in Fig. 4. Here we showed that the hydrolytic effect of *Ps. mendocina** lipase on Tweens depended on the chain length of fatty acid in the detergent as well as on Tween concentration and physical state. It is evident that the efficiency of the hydrolysis changes at the point where the Tween concentration exceeds CMC. The fact should be considered when Tweens are used for stabilization of the lipase substrate. It could be assumed that Tweens might be used at a concentration below CMC, which is low for these detergents.

The variation of the lipase effect on different Tweens could also be explained by the difference of their hydrophilic / lipophilic balance (HLB) – the measure of hydrophobicity [14]. It should be noted that the higher the hydrophobicity of the detergents, the higher the hydrolytic activity of *Ps. mendocina** lipase.

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PSEUDOMONAS MENDOCINA 3121-1 LIPAZĖS HIDROLIZINIS AKTYVUMAS. KINETINIŲ PARAMETRŲ TYRIMAS

Santrauka

Ps. mendocina 3121-1 (toliau žymima *Ps. mendocina**) lipazės hidrolizinis aktyvumas tirtas kaip substratus naudojant p-nitrofenilbutirato (p-NPB) tirpalus įviriuose organiniuose tirpikliuose (2-propanolyje, acetone, 1,4-dioksane ir acetonitrile) bei kitų skirtingo grandinės ilgio riebalų rūgščių p-nitrofenolio esterių tirpalus 2-propanolyje. Palyginus su kitais tirpikliais, acetone nustatyta mažesnė $K_{m\ (app)}$ ir didesnis $V_{max\ (app)}$, taip pat apskaičiuotas didesnis $V_{max\ (app)}$ / $K_{m\ (app)}$ santykis. Katalizinė konstanta $k_{cat\ (app)}$, hidrolizuojant p-NPB tirpalą 1,4-dioksane, net keturis kartus viršijo šį rodiklį, kai buvo naudojami kiti tirpikliai. Tai rodo, kad tirpiklio hidrofiliškumas palankus hidrolizės reakcijai. Kinetiniai parametrai nustatyti hidrolizuojant kitus tirpius riebalų rūgščių esterius ir naudojant vieną tirpiklį – 2-propanolį. Nustatyta, kad, esant vienodai (0,1 mM) substratų koncentracijai, fermentas aktyviausias hidrolizuojant p-nitrofenilauratą (p-NPL), t. y. iš vidutinio grandinės ilgio riebalų rūgšties sudarytą substratą. Buvo ištirti šių substratų hidrolizės kinetiniai parametrai, o apskaičiuoti parametrai palyginti su eksperimentiškai nustatytais. Didesnė $K_{m\ (app)}$ nustatyta, kai hidrolizuojami trumpagrandžiai riebalų rūgščių p-nitrofenolio esteriai, p-nitrofenilkaprilato (p-NPK) ši konstanta buvo mažiausia. Vadinasi, *Ps. mendocina** lipazė savitesnė tirpiems vidutinio ir didesnio grandinės ilgio riebalų rūgščių esteriams. Nepastebėta aiškios $K_{m\ (app)}$ dydžio priklausomybės nuo riebalų rūgšties grandinės ilgio substrate, bet didžiausias $V_{max\ (app)}$ nustatytas p-NPL ir p-nitrofenilmiristato (p-NPM), o mažiausias – p-nitrofenilacetato (p-NPA) hidrolizės

atveju. Nustatyta didesnė p-NPK ir p-NPL hidrolizė $k_{cat\ (app)}$ rodo tiriamos lipazės savitumą vidutinio grandinės ilgio riebalų rūgščių esteriams. $V_{max\ (app)}$ / $K_{m\ (app)}$ santykis skirtingas hidrolizuojant visus tirtus substratus, tačiau vidutinio ir didesnio grandinės ilgio riebalų rūgščių esterių atveju jis daug didesnis, negu esant p-NPA ir p-NPB. Nustatytas $V_{max\ (app)}$ palygintas su apskaičiuotuoju, kai substratų koncentracija artima $K_{m\ (app)}$, ir rasta reikšminga koreliacija. Kinetinius parametrus ištyrus vadinamuosiuose skirtinguose „kinetiniuose režimuose“, t. y. kai substrato koncentracija artima K_m arba yra 5 kartus mažesnė už šią konstantą, nustatyta, kad „K_m režime“ lipazė aktyviausia naudojant p-NPL. Šiuo atveju fermento aktyvumo priklausomybė nuo riebalų rūgšties grandinės ilgio buvo panaši, kaip ir naudojant tą pačią visų tirtų substratų koncentraciją. Kita vertus, pakeitus kinetinį režimą į „mažiau už K_m“, hidrolizės efektyvumas, naudojant p-NPL ir p-NPM, buvo labai panašus ir palyginti nedaug tesiskyrė nuo aktyvumo, nustatyto naudojant ilgagrandžius substratus. Kaip substratą naudojant p-NPK ir formuojantis jo micelinėms struktūroms, pastebėtas fermento aktyvumo padidėjimas sąlyčio su substratu paviršiuje. Nustatytos ir palygintos *Ps. mendocina** lipazės katalizuojamos p-NPB hidrolizės įvairiuose alkoholiuose kinetinės greičio konstantos rodo alkoholių slopinamąjį poveikį fermento aktyvumui. Ištirtas Tween detergentų micelinių struktūrų formavimosi poveikis fermento aktyvumui. Nustatyta, kad šių junginių hidrolizės efektyvumas padidėja, kai jų koncentracija viršija kritinę micelių susidarymo koncentraciją (KMK).

Raktažodžiai: *Ps. mendocina* lipazė, riebalų rūgščių p-nitrofenolio esteriai, hidrolizinis aktyvumas, organiniai tirpikliai