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Journal of Molecular Catalysis B: Enzymatic 44 (2007) 32–38
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
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Activity of hydroperoxide lyase under aqueous and micro-aqueous conditions

Dominik L. Jürgen-Lohmann, Raymond L. Legge *

Department of Chemical Engineering, University of Waterloo, Waterloo, Ont., Canada N2L 3G1

Received 29 May 2006; received in revised form 8 August 2006; accepted 9 August 2006
Available online 28 September 2006

Abstract

Hydroperoxide lyases (HPL E.C. 4.1.2.) are part of the lipoxygenase pathway in plants and catalyze the conversion of fatty acid hydroperoxides into oxo acids and short chain aldehydes. These aldehydes have desirable properties for the food and agricultural industry. HPL activity can be modulated by salts and surfactants, but the mechanisms governing the modulation are not fully understood. Recombinant HPL activity was evaluated by use of factorial experimental design investigating the effects of KCl and Triton X-100 on HPL activity with 13-hydroperoxy-octadecadienoic acid (LA-OOH) and 13-hydroperoxy-octadienoyl sulfate (LS-OOH) as substrates. To investigate solubility issues of the two different substrates, an aqueous and a two-phase micro-aqueous reaction medium was used. The highest HPL activity (8.7 μmol min^{-1} mg^{-1}) was achieved under aqueous conditions with high salt (1.5 M) and low surfactant (0%, v/v) concentrations and LA-OOH as a substrate. Maximal activity (2.4 μmol min^{-1} mg^{-1}) under micro-aqueous conditions was achieved with high salt (1.5 M) and high surfactant (0.01%, v/v) concentrations and LS-OOH as a substrate. A significant interaction between salt and surfactant as well as salt and substrate could be identified and a hypothesis for the interaction phenomena is presented.

Keywords: *Escherichia coli*; Hydroperoxide lyase; Fatty acid hydroperoxide; Fatty sulfate hydroperoxide; Micro-aqueous conditions

1. Introduction

Hydroperoxide lyases (HPL) are part of the cytochrome P450 family and catalyze the conversion of fatty acid hydroperoxides to oxo acids and volatile short chain aldehydes. HPL is a constituent of the octadecanoic acid or lipoxygenase pathway. This pathway is one of the most important inducible defense mechanisms in plants. Fatty acids, namely linoleic and linolenic acid, are released as a wound response by the plant and are subsequently converted to their corresponding 9- or 13-hydroperoxides by lipoxygenases of respective regio-selectivity. HPL then catalyzes the reaction to C6 or C9 short chain aldehydes and corresponding oxo acids (Fig. 1) [1].

HPL has received considerable attention since its first description by Vick et al. [2]. Much HPL research was hampered by tedious extraction procedures of HPL from plant sources. Since 2000, recombinantly expressed versions of the enzyme from various sources have been described, resulting in much easier preparation and higher quantities of pure HPL [3,4]. There are several reasons why there is considerable interest in the enzyme. The enzyme represents a target for fundamental research because it is part of a multi-enzyme cascade *in vivo* and has a variety of interesting characteristics in terms of reaction mechanism, catalytic properties and activation phenomena. From a biotechnology perspective, the products of this enzyme, such as hexanal or (3Z)-hexenal, possess desirable organoleptic properties, which make them valuable for use as food flavor additives [1]. Additionally, these aldehydes were shown to increase pest resistance in plants, being potential natural, environmentally friendly agrichemicals [1].

Factors which modulate HPL activity and its spectroscopic properties have been described in the literature. HPL activity has been shown to be enhanced in the presence of non-ionic surfactants, such as Triton X-100, or monovalent salts, such as KCl [5]. Additionally, it was found that Triton X-100 caused a slight conformational change and a spin state transition of the catalytic iron centre of the enzyme [6]. These phenomena

* Corresponding author. Tel.: +1 519 888 4567x6728; fax: +1 519 746 4979.
E-mail address: rllegge@engmail.uwaterloo.ca (R.L. Legge).

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doi:10.1016/j.molcatb.2006.08.003

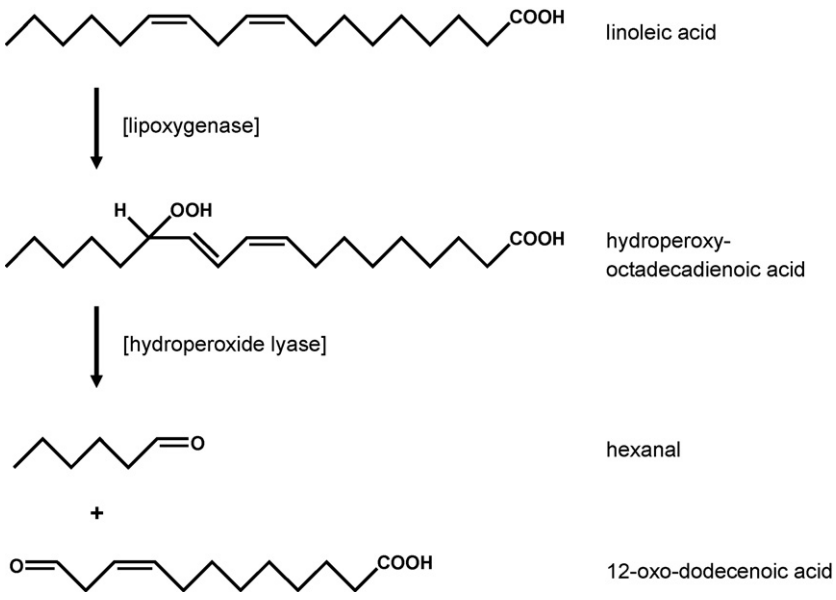

Fig. 1. Schematic representation of the lipoxygenase pathway.

are believed to in part cause enhancement in enzymatic activity although this has not been fully elucidated.

The objective of this study was to garner a better understanding of HPL activation phenomena and to identify optimal conditions for HPL activity. To achieve this objective, a recombinant HPL from bell pepper expressed in *Escherichia coli* was studied under aqueous and micro-aqueous conditions. Substrate effects were also probed by using a fatty acid hydroperoxide and a novel, water soluble fatty sulfate hydroperoxide. Salt and surfactant effects as well as their interactions were evaluated by means of appropriate experimental design.

2. Experimental

2.1. Materials

Lysozyme, IPTG, Triton X-100, Triton X-100 R, δ-aminolevulenic acid, linoleic acid and linoleyl alcohol were purchased from Sigma (Oakville, Ont., Canada). PMSF was obtained from Boehringer Mannheim (Mannheim, Germany) and soybean lipoxygenase was from Fluka (Oakville, Ont., Canada). Ni-NTA agarose was purchased from Qiagen (Mississauga, Ont., Canada). All other chemicals were of analytical grade or better. Transformant *E. coli* M15 carrying the cDNA for bell pepper HPL in pQE-31 (Qiagen, Mississauga, Ont., Canada) were kindly provided by Prof. K. Matsui (Yamaguchi University, Japan).

2.2. HPL expression and purification

HPL expression and purification was carried out according to Delcarte et al. [7]. Briefly, cells were grown in 100 mL TB medium containing 100 mg L^{-1} ampicillin, 50 mg L^{-1} kanamycin and 2.5 mM δ-aminolevulenic acid. A 5% inoculum from a culture that had been grown overnight was used in 100 mL of culture media in a 500 mL shake flask at 37 °C and 250 rpm and grown to an OD of 0.9–1.1; expression was induced by adding IPTG to a final concentration of 0.1 mM. The cultures were incubated at 20 °C and 150 rpm for 48 h following induction.

Cells were harvested from the fermentation broth by centrifugation (10,000 × *g*, 4 °C, 15 min) and the resulting pellet was resuspended in 50 mL phosphate buffer (50 mM, pH 7) and centrifuged (10,000 × *g*, 4 °C, 15 min). Cell lysis was carried out by the addition of 5 mL of lysis buffer (50 mM Tris–HCl, pH 8, 25 mg of lysozyme, 50 μL of 10 mg mL^{-1} PMSF (in ethanol), 25 μL of 1 M $MgCl_2$, 10 μL of DNAseI, 500 mM NaCl and 1% (v/v) Triton X-100) and stirring for 30 min at 4 °C. Cell debris and other solid components were separated from the lysate by centrifugation (25,000 × *g*, 4 °C, 15 min) and the supernatant used for HPL purification.

Ni-NTA affinity chromatography was used for HPL purification. One millilitre of the affinity resin was packed into a column (0.5 cm inner diameter), washed with 5 mL of deionized water and equilibrated with 5 mL buffer 1 (50 mM Tris–HCl, pH 8, 500 mM NaCl, 1% (v/v) Triton X-100). The crude lysate was loaded onto the column, washed with 5 mL buffer 1, 15 mL buffer 2 (50 mM phosphate, pH 6, 500 mM NaCl, 1% (v/v) Triton X-100) and eluted with 10 mL buffer 3 (50 mM acetate, pH 4, 500 mM NaCl, 1% (v/v) Triton X-100 R). The flow rate was kept at about 0.5 mL min^{-1} during the isolation. The HPL containing fraction was identified visually by its brown color and collected in a microcentrifuge tube. The concentration of HPL was determined by the BCA method (Pierce, Rockford, IL) and HPL purity verified by standard SDS-PAGE.

2.3. Substrate synthesis

13-Hydroperoxy-octadecadienoic acid (LA-OOH) and 13-hydroperoxy-octadecadienoyl sulfate (LS-OOH) were

synthesized as HPL substrates. LA-OOH was prepared according to Nunez et al. [8]. A mixture of 40 mg of linoleic acid and 50 mL borate buffer (50 mM, pH 9) were kept at 0 °C in a temperature controlled vessel and subjected to a constant flow of air (5 mL min^{-1}). About 10 mg mL^{-1} of soybean lipoxygenase was added in 100 μL aliquots over 30 min intervals for a total reaction time of 2 h. The pH of the mixture was adjusted to pH 2 with 1 M HCl and the LA-OOH was extracted from the borate buffer with 10 mL aliquots of diethyl ether three times. The organic phase was subsequently dried over anhydrous sodium sulphate and evaporated under nitrogen; the LA-OOH was then reconstituted in a minimal amount of ethanol.

Linoleyl sulfate was prepared from linoleyl alcohol according to Mogul et al. [9]. 0.5 g linoleyl alcohol and 0.35 g sulfamic acid were dissolved in 3 mL dry pyridine. The reaction was carried out at 95 °C for 1.5 h under nitrogen and the reaction stopped by the addition of 15 mL methanol and 1 mL of saturated sodium carbonate. After filtering off waste solids, the methanol was removed *in vacuo* and the residue recrystalized from hot methanol to yield purified linoleyl sulfate. LS-OOH was prepared in the same manner as LA-OOH, using linoleyl sulfate as the substrate for the lipoxygenase reaction.

Substrates were analyzed by TLC (aluminum sheets, silica gel 60 F_{254}, Merck, Darmstadt, Germany) developed in hexane:diethyl ether:acetic acid (60:39:1) mobile phase with iodine detection. Substrate quantification was carried out spectrophotometrically (Cary Bio 1, Varian, Mississauga, Ont., Canada) at a wavelength of 234 nm according to Lambert–Beer with a molar extinction coefficient of 25,000 M^{-1} cm^{-1} [10].

2.4. Enzyme assay

HPL activity was monitored spectrophotometrically (Cary Bio 1, Varian, Mississauga, Ont., Canada) at a wavelength of 234 nm, corresponding to the conjugated double bond of the substrates. Assays were carried out under temperature controlled conditions at 25 °C with mixing. A typical reaction mixture consisted of phosphate buffer (50 mM, pH 7) or hexane, 0–0.01% (w/w) Triton X-100 R, 0–1.5 M KCl (in the case of phosphate buffer), 24 μM LA-OOH or LS-OOH and X μg of purified HPL. The enzyme was added in 20 μL aliquots, containing the appropriate concentration of KCl, resulting in a two-phase system when hexane was used. The total reaction volume was 3 mL. The rate of decrease in absorbance at 234 nm was used to calculate the specific enzymatic activity A in μmol min^{-1} mg^{-1}.

2.5. Experimental design

A full factorial design [11] was used for both aqueous and micro-aqueous conditions. The effects of the numerical factors (surfactant and salt concentration) as well as the categorical factor (substrate type) were assessed. Triton X-100 R concentrations ranged from 0 (−) to 0.01% (w/w) (+) and KCl concentrations from 0 (−) to 1.5 M (+). Design points were assayed in triplicate, center points were replicated twice for each substrate, resulting in 28 runs for each design and 56 runs in total.

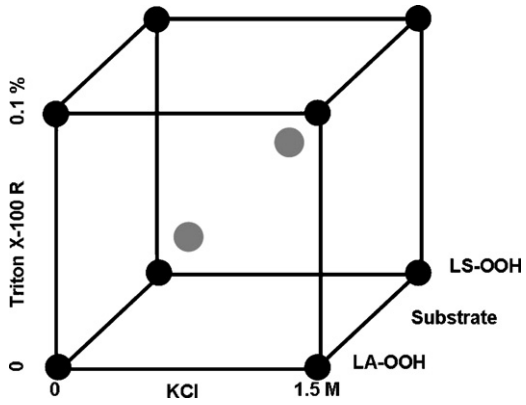

Fig. 2. Schematic representation of the experimental design. Two full factorial designs were used for aqueous and micro-aqueous conditions. Triton X-100 R concentration ranged from 0 to 0.1% (w/w) and KCl concentration from 0 to 1.5 M. LA-OOH and LS-OOH were used as substrates. Design points (black) were assayed in triplicate; center points (grey) in duplicate.

The order of experiments was randomized. A schematic of the experimental design is shown in Fig. 2.

3. Results and discussion

3.1. HPL expression and purification

Expression and purification procedures for HPL from bell pepper, recombinantly expressed in *E. coli*, were used as described by Delcarte et al. [7] without further modification. Results in terms of yield and purity were in accord with the literature. Shake flask cultures (100 mL) yielded 20 mg L^{-1} of purified HPL. HPL purity was assessed by SDS-PAGE (Fig. 3).

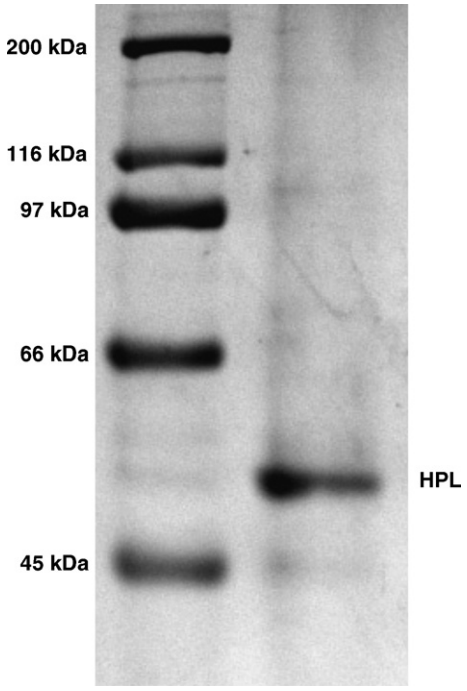

Fig. 3. SDS-PAGE of HPL. Molecular weight standards are shown in the left lane and the HPL preparation (right lane) shows a single band at about 50–55 kDa.

Under denaturing conditions, the gel showed a single band at about 50 kDa, which is expected for a single HPL subunit. Other protein impurities were below detection limits. The integrity of the heme centre of the enzyme was verified by UV–vis spectroscopy, yielding a strong Soret peak with a maximum at 390 nm (data not shown).

3.2. Substrate synthesis

To date HPL activity has been assayed with two different classes of substrates: fatty acid hydroperoxides (e.g. LA-OOH) [1] and hydroperoxy fatty acid(ethanol)amides (e.g. LA-OOH ethanol amide) [12]. Both classes of substrate have little to no water solubility and so the influence of substrate dispersion and effect of surfactants on enzyme activity has not been studied and is not clearly understood. To gain a better understanding of the fundamentals governing HPL activity, and the role that the substrate plays in HPL activity, an alternative, water soluble substrate, as well as enzyme activity under micro-aqueous conditions was investigated.

LA-OOH synthesis was conducted according to the literature producing the anticipated yields, purity and level of conversion. LS-OOH was synthesized enzymatically from linoleyl sulfate. Both substrates were analyzed by UV–vis spectroscopy as reported in Fig. 4 and TLC. The sharp peak centered at 234 nm for both substrates in Fig. 4 verifies the presence of the conjugated double bond introduced through the lipoxygenase reaction. Single spots in the TLC were observed for both substrates at $R_f = 0.73$ for LA-OOH and $R_f = 0.37$ for LS-OOH indicating the difference in substrate properties and their purity.

3.3. HPL activity assay and solvent selection

HPL activity was assayed spectrophotometrically therefore the choice of solvent for the micro-aqueous conditions was limited to solvents with a UV-cutoff of about 220 nm or lower to avoid interference problems with the signal of the conjugated double bond. Another key parameter in the selection of solvents for enzymatic conversions is the octanol/water partition coefficient or log *P*-value. Generally, solvents with a log *P*-value of 4 or larger are considered to be suitable media for enzymatic conversions [13]. The selection of a solvent for any given system, however, depends largely on the properties of the specific enzyme and substrate and is, therefore, often made on an empirical basis. In this study, three different solvents were evaluated: hexane (log *P*, 3.944), methyl tert-butyl ether (1.147) and acetonitrile (−0.447), resulting in a two-phase system for hexane and methyl *tert*-butyl ether and a one-phase system for acetonitrile. HPL activity was observed in hexane and methyl *tert*-butyl ether but not in acetonitrile (Fig. 5). The lack of activity in the acetonitrile may be attributed to the removal of bound water from the enzyme resulting in its inactivation. To our knowledge this is the first report on HPL activity under micro-aqueous conditions in solvents. For practical purposes, the remaining experiments for micro-aqueous conditions were carried out in hexane.

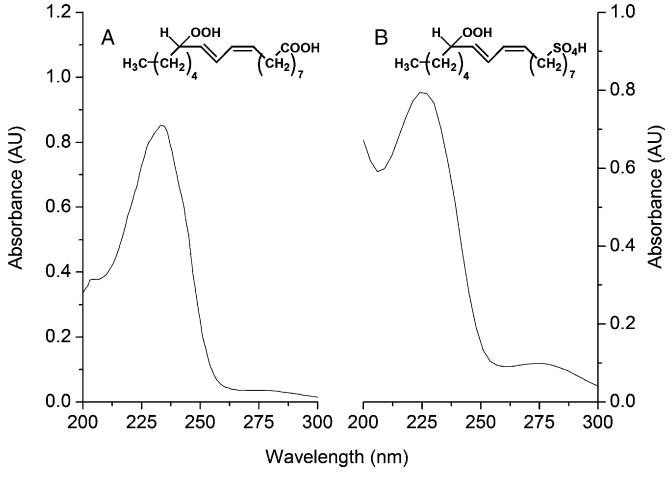

Fig. 4. UV–vis spectra of (A) LA-OOH and (B) LS-OOH.

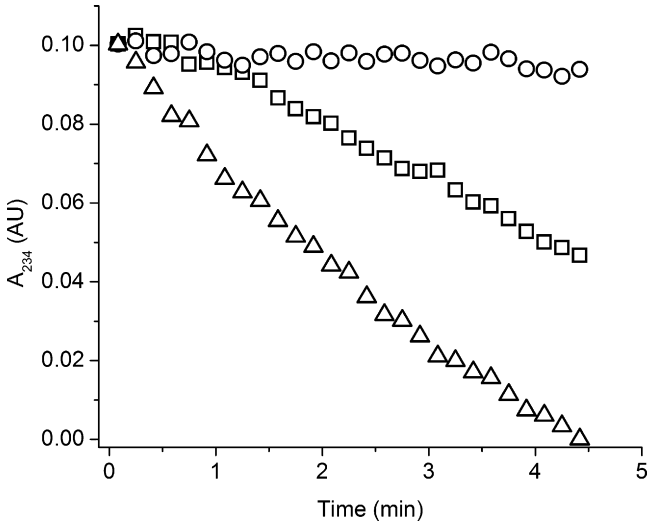

Fig. 5. Activity of HPL in various solvents; the time course of absorbance at 234 nm of LA-OOH in hexane (△), *tert*-methyl butyl ether (□) and acetonitrile (◯). In all cases the LA-OOH concentration was 24 μM and HPL was added in 20 μL of phosphate buffer.

3.4. Experimental design for HPL activity

A factorial experimental design was used to quantify the effects of salt concentration, surfactant concentration, solvent and substrate on HPL activity. In addition to optimizing the number of required experiments, experimental design also reveals possible interactions between the selected factors, which to date with HPL has not been investigated. A Box Cox transformation of the activity data was performed [11]. The data of the aqueous design was transformed using the inverse square root, while the data of the micro-aqueous system was $\log_{10}$ transformed. For both cases, a factorial model was set-up, using a half-normal probability plot to identify significant main effects and interactions. ANOVA showed the factorial model to be significant in both cases. Also, surfactant, salt, surfactant/salt interaction and salt/substrate interaction were identified as significant effects in both cases. The ANOVA results are summarized in Table 1.

Table 1
ANOVA table for HPL activity model under aqueous and micro-aqueous conditions

Source	Aqueous				Micro-aqueous			
	d.f.	MS	$P>F$		d.f.	MS	$P>F$	
Model	5	0.2558	<0.0001	s	5	0.5860	<0.0001	s
Surf.	1	0.0043	0.0319	s	1	1.7522	<0.0001	s
Salt	1	0.3065	<0.0001	s	1	0.0850	0.0002	s
Substr.	1	0.7313	<0.0001	s	1	0.9478	<0.0001	s
Surf. × Salt	1	0.0687	<0.0001	s	1	0.0229	0.0288	s
Salt × Substr.	1	0.1594	<0.0001	s	1	0.1220	<0.0001	s
Curvature	1	0.0089	0.0036	s	1	0.0118	0.1063	ns
Residual	20	0.0008			21	0.0042		
Lack of fit	3	0.0015	0.1250	ns	3	0.0025	0.6505	ns
Pure error	17	0.0007			18	0.0044		
Total	26				27			

s, significant; ns, not significant; surf., surfactant; substr., substrate.

Only the aqueous scenario showed significant curvature but the lack of fit was not significant in either case. To further validate the model, predicted and actual values were plotted for both scenarios (Fig. 6). The predicted values were calculated from the model equations and show good correlation with the experimental values in both cases. The models that were developed can therefore be considered valid in describing the activity modulation of hydroperoxide lyase by salt, surfactant and substrate under aqueous and micro-aqueous conditions.

To visualize and interpret the results, the data was split into four sets for the creation of response surface graphs: LA-OOH as substrate under aqueous conditions (A), LS-OOH under aqueous conditions (B), LA-OOH under micro-aqueous conditions (C) and LS-OOH under micro-aqueous conditions (D) (Fig. 7). The equations describing the four different scenarios are given below:

$$\left(\sqrt{A}\right)^{-1} = 0.52 - 13.75 \cdot \text{Surf.} - 0.12 \cdot \text{Salt} + 14.65 \cdot \text{Salt} \cdot \text{Surf.} \quad \text{(A)}$$

$$\left(\sqrt{A}\right)^{-1} = 1.02 - 13.75 \cdot \text{Surf.} - 0.34 \cdot \text{Salt} + 14.65 \cdot \text{Salt} \cdot \text{Surf.} \quad \text{(B)}$$

$$\log_{10} A = -0.4 + 47.87 \cdot \text{Surf.} - 0.21 \cdot \text{Salt} + 8.23 \cdot \text{Salt} \cdot \text{Surf.} \quad \text{(C)}$$

$$\log_{10} A = -0.18 + 47.87 \cdot \text{Surf.} - 0.03 \cdot \text{Salt} + 8.23 \cdot \text{Salt} \cdot \text{Surf.} \quad \text{(D)}$$

(A)–(D) show that salt concentration is the predominant effect in the aqueous system ((A) and (B)), whereas surfactant concentration is the predominant effect in the micro-aqueous system ((C) and (D)). Fig. 7A depicts the scenario that has been previously described in the literature [5,6]. With increasing salt concentration from 0 to 1.5 M, HPL activity increases from 3.7 to 8.7 μmol min^{-1} mg^{-1}. Upon increasing the surfactant concentration (0–0.01%) HPL activity increases from 3.7 to 6.8 μmol min^{-1} mg^{-1}. Both effects are, therefore, somewhat comparable in magnitude within the observed range of concentrations. However, due to the interaction of both phenomena, HPL activity is only 5.6 μmol min^{-1} mg^{-1} at high salt and surfactant concentrations. This interaction has not been previously described in the literature and is contradictory to the assumption that the salt and surfactant effects would be additive.

Fig. 7B shows the HPL activity with the novel substrate LS-OOH under aqueous conditions. In the absence of Triton X-100

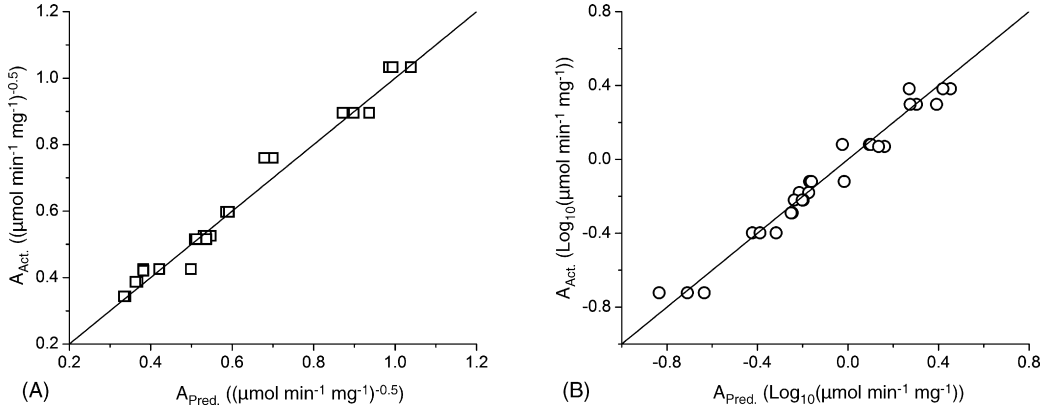

Fig. 6. Model predictions for aqueous and micro-aqueous conditions; predicted and actual values are plotted for aqueous (A, □) and micro-aqueous (B, ◯) conditions. The solid line (–) represents the ideal prediction.

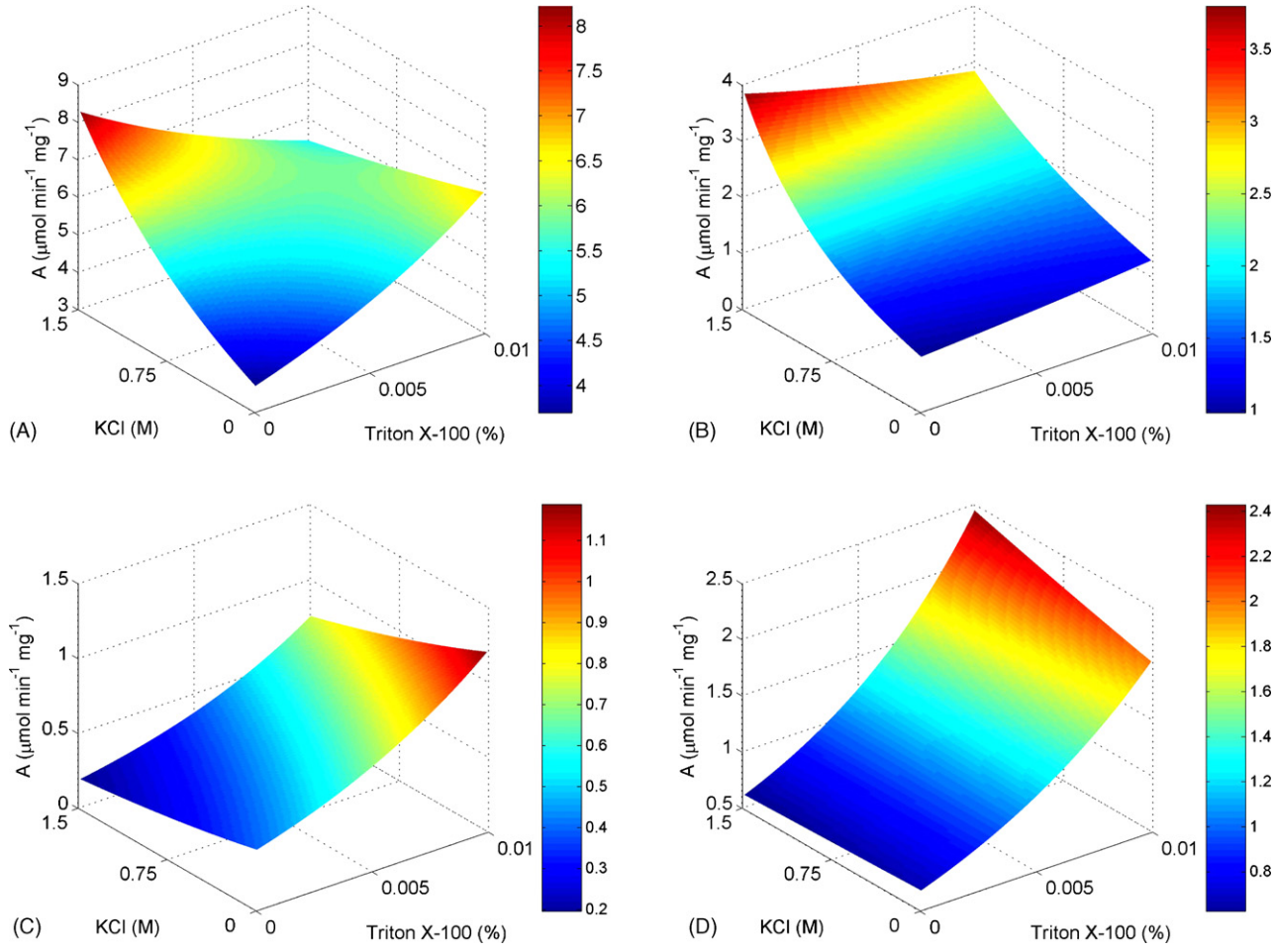

Fig. 7. Response surface plots for HPL activity under various conditions. The plots show HPL activity as calculated by the experimental design model under aqueous conditions with LA-OOH (A) and LS-OOH (B) as a substrate and under micro-aqueous conditions with LA-OOH (C) and LS-OOH (D) as a substrate.

R, HPL activity increases from 0.96 to 3.8 μmol min^{-1} mg^{-1} over the range of KCl concentrations studied. The surfactant effects on the other hand, show only a slight effect on activity and only at higher salt concentrations.

Fig. 7C and D show the activity response for HPL under micro-aqueous conditions. It can be clearly seen, that for both substrates, Triton X-100 R has the largest influence on enzyme activity. For LA-OOH activity increases from 0.39 to 1.2, for LS-OOH from 0.66 to 1.99 μmol min^{-1} mg^{-1}. A contrary salt-effect is visible for LA-OOH under micro-aqueous conditions. HPL activity decreases with increasing salt concentration (e.g. from 0.4 to 0.19 μmol min^{-1} mg^{-1} at low Triton X-100 R concentrations) throughout the whole range of surfactant concentrations tested. For LS-OOH, the salt effect is only visible at higher surfactant concentrations and is consistent with the observations for LS-OOH under aqueous conditions, but much smaller: HPL activity increases from 1.99 to 2.38 μmol min^{-1} mg^{-1} at high Triton X-100 R concentrations. Maximal activity (8.7 μmol min^{-1} mg^{-1}) is achieved under aqueous conditions with high salt and low surfactant concentrations and LA-OOH as a substrate. Maximal activity (2.4 μmol min^{-1} mg^{-1}) under micro-aqueous conditions is achieved with high salt and surfactant concentrations and LS-OOH as a substrate.

Fig. 7 reveals the complexity of effects in this system. The influence of several factors has to be considered for interpretation. For example, the surfactant Triton X-100 R affects HPL directly, but also has an effect on the solubility of sparsely water soluble components such as LA-OOH. Under aqueous conditions (scenarios (A) and (B)) the predominant effect of salt can be attributed to a possible increase in HPL stability. HPL is believed to be a multimeric enzyme in its native state [1] and high salt concentrations are known to affect inter-subunit interaction [14]. HPL could therefore be kept in a stable native conformation resulting in a higher activity.

Under micro-aqueous conditions (scenarios (B) and (C)), the presence of salt has less of an effect on HPL activity. Under micro-aqueous conditions the salt would remain exclusively in the aqueous phase; the enzyme will also likely be located in this phase or at the interface of the phases. The limited volume of available water could therefore be responsible for a more rigid conformation of HPL, so the presence of the salt would have a reduced effect on the enzyme. Another possible explanation is that the enzyme was located at the interface and that this interfacial positioning caused a more native and stable/active conformation. This is known to be the case for some membrane associated enzymes and enzymes involved in lipid transformations [15].

The effect of Triton X-100 R on HPL is different for scenarios A and B. For LA-OOH, a beneficial effect can be seen, whereas the effect of surfactant on the activity for the water soluble LS-OOH substrate is minimal at higher salt concentrations. It is reasonable that the predominant mode of action of surfactant in aqueous systems is an increase in substrate solubility. The direct activity modulation of HPL, in which the surfactant is believed to interact with HPL, may still be in place, but does not show the same magnitude as the salt effect or the influence of Triton X-100 R on LA-OOH solubility.

The importance of Triton X-100 R in the two-phase system can be seen in Fig. 7C and D. Several modes of action could operate simultaneously. Triton X-100 R could increase the activity by increasing the interface surface area and/or it could be involved in facilitating mass transfer of substrate and products between the two phases. The salt/surfactant interaction is most prominent in scenario A (Fig. 7A). Triton X-100 R is a non-ionic surfactant so a direct interaction of salt and surfactant molecules seems unlikely. It may be that both factors enhance HPL activity by non-compatible exclusive mechanisms. The salt/substrate interaction as identified by ANOVA (Table 1) can not be seen from Fig. 7, because the data was blocked by substrates. However, a direct interaction of anionic substrate molecules (carboxylates and sulfates) with K^+ ions at high salt concentrations seems likely. Polarity of the substrate head group was found to be a crucial feature for good HPL activity [12]. High salt concentrations could mask the head group charge, hence diminishing HPL activity to some extent.

4. Conclusions

The objective of the work presented here was to develop a better understanding of the factors which modulate HPL activity by using novel reaction media and substrates. HPL activities under micro-aqueous conditions and with the novel substrate LS-OOH are described for the first time. The effect of salt, surfactant, solvent and substrate on HPL activity was assessed by means of experimental design. It was found that HPL activity is maximal at high salt and low surfactant concentrations under aqueous conditions with LA-OOH as a substrate. Furthermore, an influence of the surfactant Triton X-100 on solubility properties of the system and resulting HPL activity were identified. The interaction of different effects was found to be significant, which is in contrast to previously reported findings [5]. Specifically, a salt/surfactant interaction under aqueous conditions with LA-OOH as a substrate revealed the complexity of the investigated effects, which do not combine additively.

This work represents a step towards the use of HPL for the production of value added compounds. The results obtained can aid in the choice of reaction conditions and in the design of a suitable immobilization supports, which will ultimately be needed for feasible biotransformation reactions.

Acknowledgements

Funding in the form of an NSERC Discovery Grant to RLL is gratefully acknowledged. The authors would like to thank Dr. Kenji Matsui, Yamaguchi University, Japan and Dr. Jérôme Delcarte, CRA-W Gembloux, Belgium for providing the transformant *E. coli* strains and giving valuable insight into HPL expression and purification.

References

[1] M.A. Noordermeer, G.A. Veldink, J.F.G. Vliegenthart, ChemBioChem 2 (2001) 494.
[2] B. Vick, D. Zimmerman, Plant Physiol. 56 (1975) 84.
[3] K. Matsui, C. Ujita, S. Fujimoto, J. Wilkinson, B. Hiatt, V. Knauf, T. Kajiwara, I. Feussner, FEBS Lett. 481 (2000) 183.
[4] K. Matsui, C. Miyahara, J. Wilkinson, B. Hiatt, V. Knauf, T. Kajiwara, Biosci. Biotechnol. Biochem. 64 (2000) 1189.
[5] T. Koeduka, M. Stumpe, K. Matsui, T. Kajiwara, I. Feussner, Lipids 38 (2003) 1167.
[6] M.A. Noordermeer, G.A. Veldink, J.F.G. Vliegenthart, FEBS Lett. 489 (2001) 229.
[7] J. Delcarte, M.L. Fauconnier, P. Jacques, K. Matsui, P. Thonart, M. Marlier, J. Chromatogr. B 786 (2003) 229.
[8] A. Nunez, G. StArmand, T.A. Foglia, G.J. Piazza, Biotechnol. Appl. Biochem. 25 (1997) 75.
[9] R. Mogul, E. Johansen, T.R. Holman, Biochemistry 39 (2000) 4801.
[10] B.A. Vick, Lipids 26 (1991) 315.
[11] D.C. Montgomery, Design and Analysis of Experiments, John Wiley & Sons Inc., New York, 2001.
[12] M. van der Stelt, M.A. Noordermeer, T. Kiss, G. van Zadelhoff, B. Merghart, G.A. Veldink, J.F.G. Vliegenthart, Eur. J. Biochem. 267 (2000) 2000.
[13] C. Laane, S. Boeren, K. Vos, C. Veeger, Biotechnol. Bioeng. 30 (1987) 81.
[14] E.Y. Chi, S. Krishnan, T.W. Randolph, J.F. Carpenter, Pharm. Res. 20 (2003) 1325.
[15] M.H. Gelb, J.H. Min, M.K. Jain, BBA-Mol. Cell Biol. L. 1488 (2000) 20.