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Electrical microenvironment influence on the hydrolytic activity of free and immobilised *Yarrowia lipolytica* lipase

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Summary

The role of electrical properties of interfaces upon the activity of free and immobilised *Yarrowia lipolytica* lipase has been investigated. Sodium taurocholate and Sedipur 400, an anionic polyacrylamide, enhance the negative character of the fatty droplets of substrate and tend to improve the lipolytic activity while the cationic polyacrylamide (Sedipur 900) has opposite effects. Ca^{++} which reduces the fatty droplets charge as Sedipur 900, is however a good activator of the enzyme. The role of electrical properties on the optimum pH of the immobilised enzyme is clearer. Immobilisation of the lipase on a positively charged support shifts its optimum pH to acidic pH by repulsion towards H^{+} ions around the support.

Introduction

Lipases are enzymes that hydrolyse triacylglycerides to fatty acids and glycerol. One characteristic of lipases is that they are more active with insoluble than soluble ester substrates. As a matter of fact, these biocatalysts present a unique property known as interfacial activation (Verger and de Haas, 1976). This phenomenon is expressed by a spectacular enhancement of activity at the oil/water interface (Sarda and Desnuelle, 1958).

Immobilised lipases retain activity and have several advantages over free lipases. These include reuse of the enzyme and possibility for continuous reactions. However, immobilisation presents many physico-chemical problems because of the existence of a new interface, a solid (support)/liquid interface.

For an understanding of lipases, and for optimisation of industrial processes, a thorough knowledge of the interfaces properties is needed. So, in this study, we report on the role of the electrical properties of interfaces upon the activity of both free and immobilised *Yarrowia lipolytica* lipase. Electrical properties have been evaluated by Zeta potential measurement. The effect of various compounds (calcium, sodium taurocholate, polyacrylamides) modifying the fatty droplets and the immobilisation support charges has been investigated. The influence of the electrical properties of the adsorbent on the optimum pH of the immobilised enzyme is also reported as it has been already done for enzymes which act on soluble substrate (Paquot and Hasnaoui, 1986; Thonart and Paquot, 1987).

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Material and methods

Enzyme. Lipase from *Yarrowia lipolytica* was purchased from CWBI, Liège, Belgium.

Activity measurement. The hydrolytic activity was determined on an olive oil emulsion as recommended by Nagaoka *et al.* (1969).

Olive oil emulsion was prepared as follows: 25 ml of olive oil and 75 ml of 2% polyvinyl alcohol solution were emulsified by an ultraturax homogenizer. The reaction mixture composed of 5 ml olive oil emulsion, 4 ml 0.1 M phosphate buffer pH 7 (except for calcium influence study where 0.025 M imidazole buffer pH 7 was prefered because of precipitation of calcium in phosphate buffer) and 1 ml enzyme solution was incubated at 37°C for 10 min. Immediately after incubation, emulsion was destroyed by addition of 20 ml acetone-ethanol mixture (1:1), and liberated fatty acids were titrated with 0.05 M NaOH. One unit of lipase activity was defined as the amount of enzyme that released 1 µmol of fatty acid in 1 min.

Immobilisation. 0.2 g of lipase dissolved in 20 ml of sodium phosphate buffer, pH 7 was mixed at 27°C with 0.1 g of adsorbent, DEAE cellulose or phosphate cellulose. After a determined period of immobilisation, the carrier was then centrifuged (10 min, 3470 G) and the protein content in the supernatant fluid determined by the Bio-rad protein assay based on the Bradford dye binding procedure (Bradford, 1976). Residual activity was also calculated.

Electrokinetic potential measurement. Zeta potential measurement of the support and the fat globules in emulsion was performed on a Lazer Zee Meter (Model 501, Pen Kem Company) following the principle of microelectrophoresis.

Results and discussion

Effect of activators on free lipase activity.

A previous study on lipase from *Yarrowia lipolytica*, formerly called *Candida paralypolytica* showed that this enzyme could be activated by some compounds, sometimes unidentified, from the culture medium (Ota and Yamada, 1966). Activators seem to differ with the shape of the substrate (Ota and Yamada, 1967). Figure 1 clearly shows that calcium and taurocholate activate the lipase from *Yarrowia lipolytica* of a free state. The effects of these compounds are well known and referred in the literature (Constantin *et al.*, 1960; Homsen and Brockman, 1976) but their optimum concentrations vary with the operating conditions. Calcium insolubilizes the hydrolysis product while the bile salt (taurocholate) participate in the emulsification of fat and, therefore, is essential for the lipase action. The effects of calcium and taurocholate on the electrical properties of the olive oil emulsion are opposite (figure 1).

Calcium reduces the electrical charge of the fatty droplets near zero whilst taurocholate strengthens the negative charge even in a buffer medium. Therefore, it is reasonable to think that taurocholate does not favour electrostatic interactions between enzyme and substrate. In the contrary, at high concentration (> 1.2 %), it could induce a negative potential barrier around the fatty droplets, desorbing the lipase from the interface and consequently reducing the lipolysis.

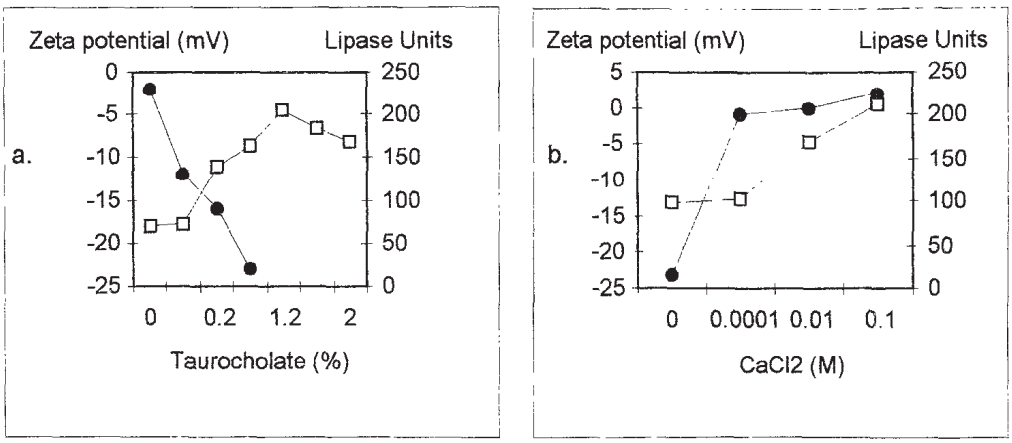

Figure 1: Taurocholate (a.) and calcium (b.) influence on the free enzyme activity (□) and Zeta potential of the olive oil emulsion (●).

The influence of two polyacrylamides with opposite charges upon the enzymatic activity has also been investigated. As illustrated in table 1, the anionic compound (Sedipur 400) enhances the negative character of the fatty globule and tends to improve the lipolytic activity while the cationic compound (Sedipur 900), has unfavourable effects. This is not in good agreement with the recent observation of Skagerlind *et al.* (1995) about *Rhizomucor miehei* lipase acting on emulsions stabilised by different surfactants. Indeed, it was demonstrated that the lipase bound to droplets stabilised by a cationic surfactant but not to those stabilised by a negatively charged surfactant. So in the anionic system, almost no enzymatic activity was observed. Apparently, this phenomenon could be reversed by the presence of counterions (Skagerlind *et al.*,1992).

Table 1: Effect of anionic and cationic polyacrylamides on *Yarrowia lipolytica* lipase activity and on Zeta potential of emulsion.

	Sedipur 400		Sedipur 900	
Concentration (%)	Activity (LU)	Zeta potential (mV)	Activity (LU)	Zeta potential (mV)
0	102	-6	102	-6
0.001	108	-12	63	-1
0.005	121	-12	50	+2
0.01	105	-12	48	+8
0.02	97	-14	44	+14
0.05	105	-13	39	+21

Lipase immobilisation on chromatographic supports.

Immobilisation was performed as described in Material and Methods on an anionic exchange resin, DEAE cellulose and on a cationic resin, phosphate cellulose. Fixation yield reached 78% after 30 minutes and 100% after 24 hours on DEAE cellulose. Residual activity of the adsorbed enzyme was about 30% after 30 minutes but decreased to 15% after 24 hours. Reuse of the immobilised lipase was enabled without desorption or loss of activity. Fixation yield on the cationic exchange resin were inferior: only 20% after one hour and 57% after 24 hours. For this time of immobilisation, residual activity was only 8%. The poor adsorption yield obtained with phosphate cellulose are imputed to the electrical properties of *Yarrowia* lipase which has its isoelectric point near to 5.2. So, for the rest of our research, we only used DEAE cellulose as immobilisation support.

Electrical properties of the immobilised enzyme and optimum pH.

Through fixing on the DEAE cellulose by ionic interactions, the lipase counterbalances the positive charge of the support. This explains that Zeta potential of DEAE Cellulose decreases with the enzyme/support ratio (table 2).

Table 2: Influence of *Yarrowia lipolytica* lipase on the Zeta potential of DEAE Cellulose.

Lipase/ support ratio	Zeta potential (mV)
0	114
0.085	105
0.1	97
0.2	68
0.5	60
1	48

The electrical properties of the support have a significant effect on the optimum pH of the lipase. As shown in figure 2, optimum pH of the free enzyme is around 7 whereas optimum pH of the immobilised enzyme is about 6. Moreover, both curves have the same profile. They are only shifted from one pH unit.

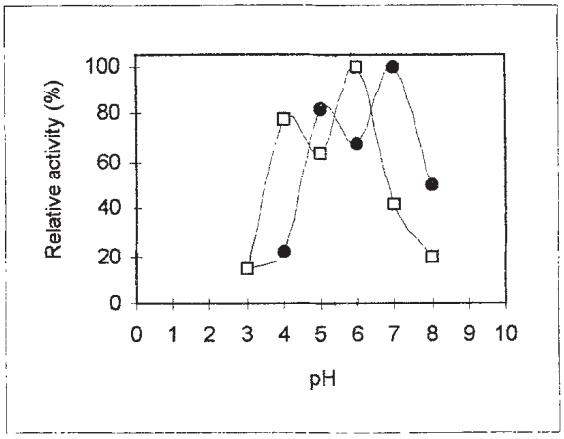

Figure 2: pH influence on free (●) and immobilised (□) *Yarrowia lipolytica* lipase.

The positive charges of DEAE Cellulose exert a repulsion towards H^+ ions from the medium. The pH around the support is therefore higher than the pH measured in the solution. An apparent shifting of the optimum pH to acid pH is then observed. Such a behaviour has already been noted for β-galactosidase, an enzyme for which the substrate (lactose) is soluble (Paquot and Hasnaoui, 1986).

Effects of activator on the immobilised enzyme activity

Despite the fact that it considerably modifies the fatty globule charge, calcium affects the support Zeta potential to a lesser extent. However, it remains a strong activator of the immobilised lipase. An improvement factor of 3 is obtained with a 0.01 M $CaCl_2$ concentration. An opposite effect is observed with taurocholate.

The anionic polyacrylamide added during the immobilisation process, counterbalances the electrical charges in the immobilised enzyme microenvironment while improving its residual activity (table 3).

Table 3: Anionic polyacrylamide effect on residual activity and Zeta potential of the lipase adsorbed on DEAE Cellulose.

Sedipur 400 content (ppm)	Residual activity (%)	Zeta potential (mV)
0	34	19
10	42	1
100	58	0

Conclusions

In this study, it has been underlined that anionic compounds such sodium taurocholate and a polyacrylamide which strengthen the negative charges of fatty droplets, have a favourable effect on the lipase lipolytic activity. A cationic polyacrylamide has an opposite influence. However, calcium which reduces the electrical charges has favourable effect on the activity. The action mechanisms are very different and it will be relevant to study synergy or antagonisms between the various activators.

The role of the electrical properties upon the optimum pH of the immobilised enzyme is clearer. Modifying its microenvironment by immobilisation on a solid support, it is possible to shift the optimum pH from at least one unit.

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