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Behaviour of lipase immobilized on Amberlite IRA-410

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Two different lipases were immobilized on the strongly basic ion-exchange resin Amberlite IRA-410 in its Cl form and used to hydrolyse n-butylacetate. Operational stability and dependenc.! of the rate on substrate concentration were examined at room temperature in order to establish the behaviour of the immobilized lipase.

Immobilized enzymes are enzymes which are physically confined or localized in a ce1tain defined region of space with retention of their catalytic activites and which can be used repeatedly and continuously.1

Several techniques can be used to prepare immobilized enzyme:2-4 phys:cal adsorbtion to a solid phase, entrapment within a gel matrix, containment behind a semipermeable membrane, covalent attachment to an inert support, incorporation directly into a polymer and intermolecular crosslinking of enzyme molecules.

Lipases (triacylglycerol acylhidrolases, E.C. 3.1.1.3.), are fully active on aggregated substrates -emulsions, micelles or monomolecular films -but rather inactive on monodisperse substrates.s

Two main hypotheses have been proposed to explain this property of lipolytic enzymes. One involves a change in enzyme conformation when the enzyme adsorbs at the oil-water interface while the other implies an increase in substrate concentration at the interface.6 A study of the properties of lipases immobilized on hydrophobic supports should give more information about how lipases act. Several studies were done6-9 in this field. Conventional immobilization methods lead to a very low activity (1% or less) of lipases. It was demonstrated that lipases strongly adsorb on hydrophobic surfaces' 1.12 and the resulting activity could be greatly enhanced by the adsorption step.13

This paper reports the behaviour of lipases from hog pancreas and *Candida cylindracea* immobilized on the strongly basic ion-exchange resin Amberlite IRA-410 in its Cl form. Lipase from *Candida cylindracea* immobilized on this support was used to study the behaviour ot lipases in the hydrolysis of n-butylacetate in water.

EXPERIMENTAL

Two different lipases, one from hog pancreas ("Fluka") and the other from *Candida cylindracea* ("Meito Sangyo") were immobilized on the resin Amberlite IRA-410 ("Fluka", d< lmm) using the previously employed procedure, as follows:14

Resin (d<l mm) was kept in distilled water for 24 h and then rinsed several times with deionized water. After introducing 10 cm3 resin to 25 cm3 of 1% glutaraldehide solution ("Fluka") for 30 min, with constant mixing at room temperature, and rinsing again with deionized water, 10 cm3 of lipase solution were then added to the resin and mixed (250 rpm) on the ice bath for 4 h for lipase from the pancreas and for three different periods of time for lipase from *Candida cyli11dracea*. After immobilization, resin was rinsed with deionized water until UV absorbtion at 255 nm become insignificant (less then 5% of the initial enzyme solution). Such an immobilized enzyme was used to examine the hydrolysis of 11-butylacetate.

The immobilization of lipase from *Candida cylindracea* was followed UV spectrophotometrically to reach the optimum time of immobilization and to examine the dependence of the hydrolysis of n-butylacetate on the substra te zero concentration. The stability of immobilized enzymes was also investigated.

Hydrolysis was followed by volumetric titration of the whole system after one hour with potassium hydroxide (0.05 M, with phenolphtalein as indicator). Deionized water was obtained by Millipore system.

A blank was done under the same conditions for each probe. The activity of the free enzyme from *Candida cylindracea* was also examined. 10 mg of lipase were added to a mixture of 3 cm3 of n-BuAc and 7 cm3 of deionized water, agitated on a shaker for 1 hour and then titrated with 0.05 M KOH. In the same manner activity of free lipase from hog pancreas was examined (10 mg of lipase in a mixture of 5 cm3 of 11-BuAc and 5 cm3 of deionized water).

RESULTS

In the tirst part of the experiment, dealing with the lipase from pancreas immobilization was performed on the strongly basic ion-exchange resin Amberlite IRA-410 without previous activation into OH form. The behaviour of the immobilized lipase from pancreas (0.1% and 0.2% solutions) with reuse is shown in Figs. l and 2.

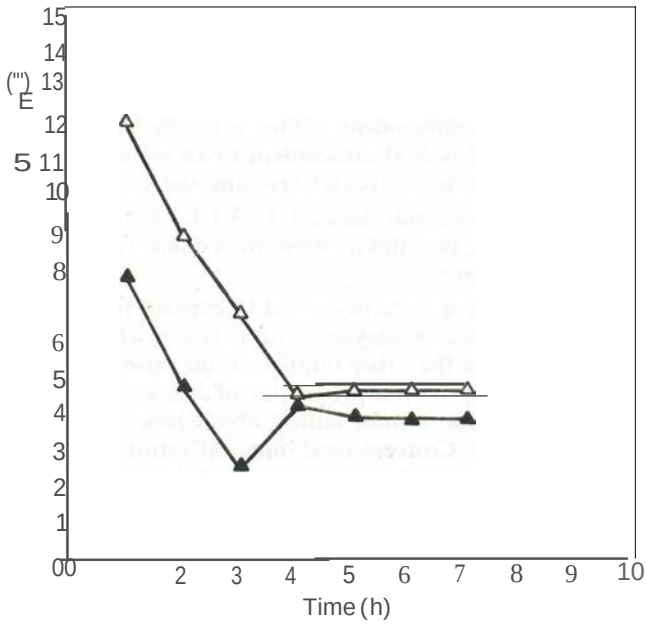

Fig. 1. Behaviour of lipase from hog pancreas (4 h immobilization, 0.1% lipase solution, *Vs* = 10 cm3, 50% of n-BuAc, 200 rpm, room temperature, time = 1 h)

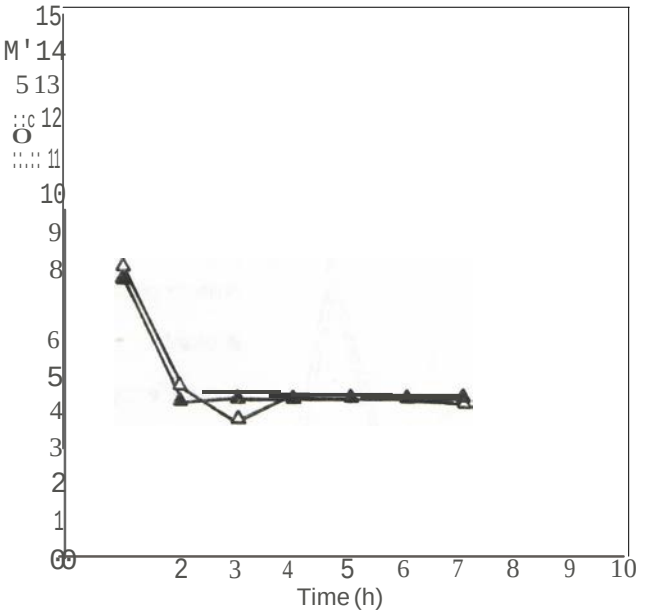

Fig. 2. Behaviour of lipase from hog pancreas (4 h immobilization, 0.2% lipase solution, *Vs* — 10 cm3, 50% of 11-BuAc, 200 rpm, room temperature, time = 1 h)

Table I gives the data concerning the amount of immobilized enzyme (lipase from *Candida cylindracea)* for three different periods of time of immobilization and activity of immobilized enzyme prepared as described above.

TABLE I. Immobilization of lipase from *Candida cyli11dracea* on Amberlite IRA-410 (UV = 255 nm, 200 rpm, room temperature, time = 1 h)

Immobilization time/h	2	4	6
Amount of immobilized enzyme/mg	0.7	2.8	1.4
Activity, cm3 KOH	0.5	5.7	1.4

Table II gives the stability of lipase from *Candida cylindracea* immobilized on Amberlite IRA-410 dependi ng on the percentage cf n-BuAc in the reaction mixture.

TABLE II. Stability of lipase from *Candida cyli11dracea* immobilized Amberlite IRA-410 (4 h immobilization, 0.1% lipase solution, 200 rpm, room temperature, time = 1 h)

Initial mass % of 11-BuAc in deionized water	10	20	30	40	50	60	80	100
Number of probes with retained activity	10	8	4	7	7	3	3	2

Data from experiments in which the influence was followed of the initial substrate concentration on the hydrolysis of n-BuAc by the lipase from *Candida cylindracea* immobilized on the ion-exchange resin are presented in Fig. 3.

The activity of the free (soluble) lipase from *Candida cylindracea* was 2.0 cm3 0.05 M KOH and of the free lipase from hog pancreas was 1.0 cm3 0.05 M KOH.

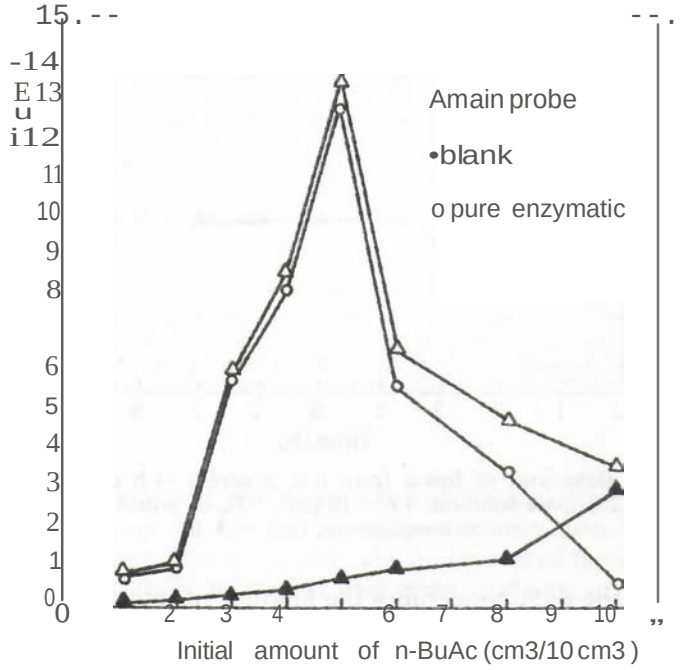

Fig. 3. Influence of the initial substrate concentration on the hydrolysis of 11-BuAc using lipase from *Candida cylindracea* immobilized on Amberlite IR A-410 (4 h immobilization, 0.1% lipase solution, 200 rpm, time = 1 h, room temperature)

DISCUSSION

Though immobilization of lipase from hog pancreas was not moniton:d spectrophotometrically but by the activity of so prepared immobilized enzyme, we can say that the amount of immobilized enzyme is the same. The immobilized enzyme prepared in this way decreases its activity rapidly in the first tew experiments and afte1that it is more or less stable. Comparing the activity of free and immobilized lipase Lrom hog pancreas one can say that the activity of the immobilized enzyme is more than 5 times greater than that of the fiee lipase (4.5 cm3 to 1.0 cm3).

Preparation of immobilized enzymes on an ice bath showed that the optimum time is 4 h, though at room temperature it can be done faster. The amount of immobilized enzymes and the activity of enzymes immobilized in this way are the bigest and we used this 01 preparation procedure ror subsequent experiments.

Also, the stability ot immobilized enzyme ptepared in this way is satistactory· With very low concentrations of n-butylacetate it is possi ble to use it almost 10 times. An increase in the percentage of n-butylacetate to 50% does not ettect the sta-

bility much but when over 50% stability drops significantly and in pure n-butylacetate it is possible to use enzymes prepared in such a way only twice with the retention ot activity.

The dependence of the hydrolysis of n-butylacetate on the initial substrate concentration in the range of 10% to 100% of n-butylacetate in a sample showed that lipase from *Candida cylindracea* is inhibited by the substrate so that the possible mechanism is as follows.1s

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \qquad K_1 = \frac{k_1}{k_2}$$

$$ES + S \underset{k_4}{\overset{k_3}{\rightleftarrows}} ES \qquad K_2 = \frac{k_3}{k_4}$$

$$ES \xrightarrow{k_5} E + P$$

Experiments with the free lipase from *Candida cylindracea* showed that the amount of liberated acetic acid was less than in an equal probe with the immobilized lipase, even if there was more free anzyme present. This could lead to a conclusion that the immobilized enzyme had enhanced activity compared to the free one. The activity of IO mg 01 the free enzyme expressed as cm3 of 0.05 M KOH was 2.0 and of immobilized one for the same conditions 5,7 but with 3 times less enzyme; this led to the conclusion that the immobilized enzyme was more than 8 times a better catalyst. This enhanced activity was the result of lipase binding to a hydrophobic support as already reported by several authors.6.11-13

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HJBO.D;

IIOHAIIIAELE JIHIIA3E HMOBHJIHCAHE HA AMBEPJIHTY IRA-410

.D;YIIIAH MHJHH, rOP.n;AHA r. HHKOJIH'.ll., MHJIHQA MHIDH'.ll.-BYKOBH'.ll. H .D;PArOJLYli BYKOBH'JI.

Texuo11owKo-t.1efiiwrypu11m fljaKy11iUeiii, Ym1sep3Uiiiem y Seoipagy, Kapuelujesa 4, il. *lip. 494, 11001 Geolpag*

.D;se pll3Jlll'ilfre mma3e Ue,D;Ha HJ muncpeaca, a .D;pyra HJ *Candida cylindracea)* HMo6rumcaHe cy Ha jaKo 6a3Hoj joHOHJM eILHBa'IKoj cMoJJH Amberlite IRA-410 6eJ npeTXo,D;Hor rrpesoljeH>a cMone y OH 06.llll'.K n: Kopm:nTeHe Ja XK.D;poJIHJy n-6YTKJiau;eTaTa Ha co6Hoj TeMIIepazypK. HcITH-THBaHa je orrepau;KoHa CTa6HJIHoCT TaKO HMo6KJJHcaHHX mma3a, a Ja mma3y HJ *Candida cy/in-dracea* KCIIHTaH je H YTHD;aj IIOJia3He KOHD;eHTpau;Hje cyrrcrpaTa Ha KOHBePJHjy. TaKolje je KJBpmeHo rropeljeJLe ca CJI06oromM mma3aMa.

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