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Studies on synthesis of short chain alkyl esters catalyzed by goat pregastric lipase

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Abstract

Goat pregastric lipase, in the form of a suspended enzyme powder, was found to be active in catalyzing the synthesis of alkyl esters in anhydrous organic solvents. The rate of catalyzed synthesis of esters was very dependent on the solvent medium, and maximum activity was found when a hydrocarbon was used as the solvent. The optimal temperature for the catalyzed synthesis ranged from 30 to 40°C and the maximal temperature was 35°C for the synthesis of butyl caproate in isooctane. The selectivity for the carbon-chain length of the fatty acid by the lipase was similar to that seen in hydrolysis reactions in aqueous solution, and the optimal rate of synthesis of alkyl esters was found for synthesis of the esters which had 8 or 10 carbons in the alkyl moieties from the two individual substrates. The rate of synthesis was also dependent on the water content in the system, with maximum activity occurring at 1% w/w water in isooctane.

Keywords: Alkyl esters; Butyl caproate; Enzymatic synthesis; Goat; Pregastric lipase; Reverse micelles

1. Introduction

Fatty acid esters are the essential gradients of natural aroma in various food industries. Traditionally, the flavor esters have been synthesized by means of chemical method, which is still the most economical method, but it suffers from the inevitable by-products generated during high temperature reaction. Direct synthesis of esters from fatty acids and alcohols by enzymatic means has been studied and suggested as a good alternative route to industrial catalysis, since the enzymatic catalysis provides an energy-saving procedure with high selectivity [1–4]. The synthesis of esters by enzymes can be carried out in a native enzyme suspension by a chemically modified form [5,6] or by an enzyme immobilized on a solid support [2,7] in an organic solvent or in a solvent-free system. By these means it has proved possible to synthesize not only flavor esters, but also esters from other families, e.g., monoglycerides, sugar esters and phospholipids which are widely used as emulsifiers in the food and pharmaceutical industries.

Pregastric enzymes of caprine origin have long been used for developing the unique flavor of Italian-style cheeses. Our early work on the function and characteristics of pregastric enzymes has shown that only one major lipolytic

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enzyme existed in the crude enzyme extract from goat and kid [8], suggesting that the crude extract or the partially purified form of the lipase might be used for synthesis of esters. The active lipase component in the enzyme extract showed a strong preference for catalyzing the hydrolysis of the short chain fatty acid moiety during hydrolysis of triacylglycerols or esters and the rate of hydrolysis decreased sharply as the carbon-chain length of the carboxylic moiety increased [9,10]. Since enzymes may be used to catalyze chemical reactions in both the forward and reverse directions, the native characteristics of pregastric lipase suggest that it would be most suitable for synthesizing short chain flavor esters. In this investigation, we have studied the goat pregastric lipase catalyzed synthesis of alkyl esters in organic solvents under various conditions, and the results reveal the versatility of the enzyme, since not only does it hydrolyze lipids or esters in aqueous solution, but it is also active in synthetic reactions under almost anhydrous conditions.

2. Materials and methods

Butanol, hexanol and octanol and butyric, caproic (C6:0), caprylic (C8:0), and capric (C10:0) acids were purchased from The British Drug House (Poole, England). Isooctane (Ajax Chemicals) was distilled and dried over 4 Å molecular sieve. Tris(hydroxymethyl)aminomethane (Tris) was from Sigma and *N*-morpholino-ethanesulfonic acid (Mes) from Serva. Crude goat pregastric lipase (PGL) enzyme extract was provided by NZ Dairy Meats, Eltham, New Zealand.

2.1. Purification of goat pregastric lipase

The partial purification of goat PGL followed the method described elsewhere [8], with little modification. The crude goat PGL enzyme-extract (10 g in 400 ml Tris/HCl (50 mM, pH 8.0)), after centrifugation, was loaded onto a Q-Sepharose column (5 cm × 20 cm; Pharmacia XK 50/20 column) with a flow rate of 3.0 ml min^{-1}. After the salt-gradient elution of the ion-exchange chromatograph, the active lipase fraction was collected, then concentrated with 2 M $(NH_4)_2SO_4$. The pellet thus obtained was re-dissolved in Tris/HCl buffer and dialyzed against buffer three times. The enzyme solution was then passed through an Affi-Gel Blue gel column (1.6 × 20 cm) at pH 8 and at a flow rate of 1.0 ml min^{-1}, and the lipase fraction was eluted with 2 M NaCl in 50 mM Tris/HCl. The collected fractions were concentrated by ultrafiltration (Amicon Centriprep® 10 and Centricon® 10) and the concentrated enzyme solution was subjected to gel-filtration as described previously. The lipase fractions from gel-filtration were collected, concentrated, extensively dialyzed against 10 mM Mes buffer (pH 6.5) and freeze-dried. The lipase activity of the final white enzyme-powder was determined as 352.5 U mg^{-1} in tributyrin/lecithin emulsion at pH 6.5, 35°C [8], an activity corresponding to 65% lipase component and 35% other proteins, mainly albumin.

2.2. Enzymatic synthesis of alkyl esters

The lipase-catalyzed synthesis of esters was monitored by gas chromatography. All solvents were dried over 4 Å molecular sieve. Equal volumes of alcohol and fatty acid (1 ml, 200 mM in isooctane) were mixed with the lipase powder (2 mg, 705 U) and powdered molecular sieve (20 mg), and the mixture was placed in a screw-top vial and kept in a horizontal shaker (Model SWB20, Ratek Instruments, Boronia, Australia) at 35°C. At intervals, 100 μl aliquots of the mixture were transferred to 1.5 ml Eppendorf tubes and centrifuged at 10,000 rpm for 1 min to remove the suspended enzyme particles. The supernatant was then diluted 10–50-fold with isooctane and analyzed by gas chromatography on a Hewlett Packard 5890 Series II Gas Chromatograph with HP 3345 autosampler (Hewlett Packard Int., Palo Alto, CA). A

J&W DB-225 column (J&W Scientific (Fisons), Folsom, CA) was used for analysis of the fatty acids and alcohols with short chain lengths. The temperature was programmed for 40°C with a 3-min hold, rising at 8°C min^{-1} to 220°C. For analysis of compounds of which total carbon-number is over 10, an HP-1 column was used and the temperature programme was 40°C for 3 min followed by a rise of 8°C min^{-1} to 200°C. The initial rate of the synthesis of the ester catalyzed by PGL was determined either from the consumption of the substrate or production of the ester. In this investigation, direct analysis of fatty acids and aliphatic alcohols was used to prevent possible complications which may have occurred upon derivatisation. All the kinetic data were obtained by triplicate experiments. Providing the concentration of the sample was controlled at < 5 mM, then the peak area of the FID response was proportional to the concentration of the individual fatty acid or alcohol. At concentrations > 5 mM, the peak shape started to show asymmetry and the linear relationship broke down. The DB-225 column was mainly used for analysis of short chain fatty acids (C4–C8). However, for the longer chain fatty acids, the higher oven temperature which was required would have shortened the lifetime of the column. Thus, an HP-1 column was chosen for analysis of the longer chain fatty acids (C10–C14).

2.3. Determination of water content

The water content in the medium was determined by Karl Fisher titration. The titration was performed with a 736 GP Titrino (Metrohm, Herisau, Switzerland) at room temperature. Hydranal®-Solvent (10 ml) was placed in a microtitration cup and pre-conditioned by the Hydranal®-Composite until the system was fully dried, and the system was then standardized by injecting a known weight of water (20–50 mg). The water content in the substrate solutions was determined by injecting 0.2–0.3 ml of the sample and titrating it with the composite reagent. The water content of all the solutions used in these experiments was 0.01% (w/w), which is close to the detection limit of the analysis.

3. Results and discussion

Even though many lipases have been found to have catalytic ability in an anhydrous medium, there is no report in the literature on catalytic activity of pregastric enzymes in organic solvents. There was some difficulty in measuring the activity of crude, commercial PGL in reactions involving synthesis of esters, since the total percentage of lipase was very low (0.7–1.0% w/w) [8] compared with the other proteins present in the commercial source. Increasing the quantity of crude enzyme used in the experiment caused different errors, mainly due to the adsorption of substrates and/or products onto the other proteins. Thus, it was necessary to use a more highly purified form of PGL in order to eliminate errors in analysis. In this investigation, therefore, the partially purified freeze-dried goat PGL enzyme powder with specific activity was 352.5 U mg^{-1} was used. The composition of this enzyme powder was 65% lipase component and 35% other proteins (mainly albumin). Further purification [8] did not give higher specific activity in organic media, since the more purified form was more prone to inactivation in an organic phase.

It is known that the hydrophobicity of organic solvents greatly influences the activity of biocatalysts acting in either biphasic systems or in anhydrous media. Polar solvents will undoubtedly replace the water on the protein surface and alter the tertiary structure, thereby possibly causing inactivation of the enzyme [11]. Most lipases which have been investigated, in their native form or in an immobilized state, have been reported to be rigid and active in a variety of organic solvent systems [7]. Naraya and Klibanov [12] reported that solvent polarity (water immiscibility) was irrelevant to efficiency for three lipases and one protease during

transesterification reactions between esters and alcohols. Laane et al. [13], however, reported that the partition ratio of substrates between the solvent and enzyme surface also depends on the properties of the solvent. For substrates such as short chain alcohols or acids, the 'effective' concentration of substrates on the enzyme surface will be less in the presence of a polar solvent, and higher activity can usually be obtained in the presence of non-polar solvents (log $P > 2$) (P is the partition coefficient between 1-octanol/water) [13] rather than polar ones. Thus, direct synthesis of esters from alcohols and acids is directly dependent on solvent polarity.

A series of different solvents, namely hexane, isooctane, MTBE (methyl *t*-butyl ester), diethyl ether, acetone, pyridine, THF, and toluene were tested for PGL catalyzed synthesis of esters. Hydrocarbons (hexane and isooctane) were found to be the best media, while the reaction rate in ethers (diethyl ether, MTBE) was decreased to 25–30% of the rate in the hydrocarbon solvents. Since the pregastric enzyme has probably only two internal disulfide bonds [14], it is capable of adopting a reasonably flexible conformation when subjected to a change in environment. Thus, in summary, the activity of enzymes in organic solvents may be affected by two factors: (1) the disruption of the tertiary structure or active site of the enzyme by solvent molecules, and (2) thermodynamic solvation and desolvation of the substrate and/or product molecules into/from the enzyme-active site. It is still arguable as to what mainly affects enzyme activity.

Pregastric lipase was found to be active only for more volatile short chain substrates, and thus the addition of molecular sieve was used to remove water from the system and decrease interference from the hydrolysis reaction. Fig. 1 shows a typical profile for ester production catalyzed by goat PGL and the reaction reached ~90% completion after incubation for 48 h. The data points for decrease in percentage of fatty acid substrate showed more scattering than those for the increasing ester product, due mainly to the asymmetric peak shape at higher concentrations of injected sample. Thus, quantification of produced ester was the method primarily used for determination of the rate of the enzyme-catalyzed reaction. The enzyme activity was represented by the initial rate of synthesis of the ester up to 10% production, during which period the reverse reaction would have been insignificant. The initial rate did not change significantly in the absence of a drying agent under the experimental conditions, although the reaction usually only reached 60% completion.

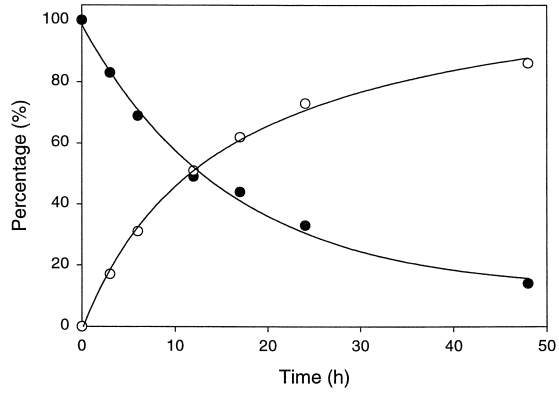


Fig. 1. Kinetics of production of butyl butyrate catalyzed by goat PGL in isooctane, 35°C: butyl butyrate (○); butyric acid (●).

3.1. Temperature profile

If water molecules were the only factor which affected the thermostability, we might expect significant enhancement of stability in anhydrous media. However, Fig. 2 shows that the optimal temperature for synthesis of butyl caproate by goat PGL was 35°C, and the rate decreased when the temperature was increased. Even though there are some reports stating that pancreatic lipase [15] and subtilisin [16] are extremely thermostable in anhydrous media, there are also some other conflicting results reported for *Candida rugosa* [7] and *Mucor miehei* [17] in either their native enzyme powder or immobilized form. It was found through this investigation that the temperature profile for

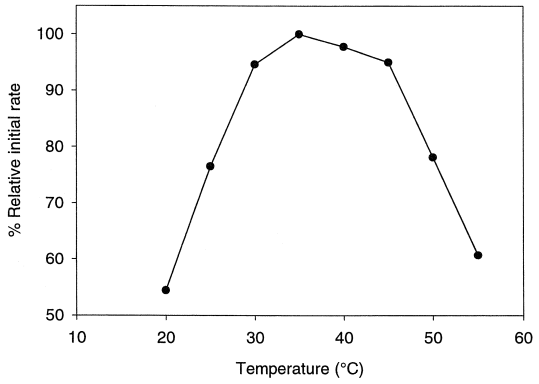


Fig. 2. The effect of temperature on the initial rate of synthesis of butyl butyrate catalyzed by goat PGL. The data were normalized to the rate data at 35°C.

the enzyme-catalyzed ester synthesis reactions was similar to that for the hydrolysis reaction [8], and the optimal temperature was found within the range 35–45°C. Thus, water is unlikely to be the only factor affecting thermo-inactivation; the presence of polar substrates, such as fatty acids and alcohols, may also act as inhibitors which will denature the lipase upon an increase in temperature.

3.2. Effect of alcohol class

Fig. 3 shows the relative rate of synthesis of alkyl caproates catalyzed by goat PGL at 35°C. It was found that PGL showed the highest activity for butanol among the alcohols tested. Based on the hypothesis for the acyl-enzyme intermediate [18], the alcohol is involved in the deacylation step as a nucleophile and attacks the bound acylated moiety. The rate of synthesis for a particular fatty acid will be dependent on its accessibility to the active site, while the selection of alcohols for the enzyme-catalyzed synthesis will be less dependent on the active site, and more dependent on the region which surrounds the active site. Since alcohols are not involved in the acylation process for forming the acyl-enzyme intermediate, the net rate of synthesis of an ester is then decided by the diffusion of alcohol molecules into the active site of the enzyme. Smaller aliphatic alcohol molecules have the advantage of being able to diffuse into the active site of the enzyme more readily than do bulky ones, and this will lead to a decrease in reactivity as the chain length of the alcohol increases. This trend has previously been found for other lipases, e.g., *M. miehei*, *Aspergillus*, *C. rugosa*, and *Rhizopus arrhizus* [19] with maximum activity being seen for C4:0–C6:0 alcohols. However, when ethanol was used as a nucleophile, the rate of formation of ethyl butyrate was lower than that for butyl butyrate, a result probably due to denaturation of the enzyme by ethanol.

Our results show that diffusion is not the only reason for PGL catalyzed synthesis of alkyl esters, which also depends on the size of the pocket of the active site of the enzyme (see below). Steric effects also play an important role in the synthesis of esters. Table 1 demonstrates the relative rate for a variety of alcohols. Steric hindrance becomes a major factor, while a substrate with a methyl group in the α-position reacts more slowly than one which is β-substituted, and the rate of reaction for the secondary alcohol decreases to 50% of that for the straight-chain alcohol. Similar conclusions have also been made for an immobilized enzyme, *M. miehei* [20]. In that case, the reactivity of a variety of alcohols was dominated by their thermodynamic properties rather than by the nature of the enzyme active-site.

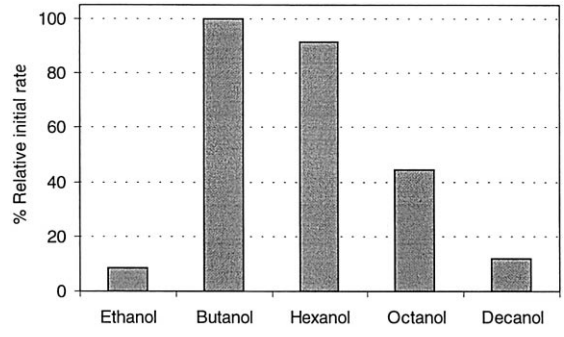
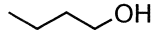
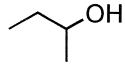
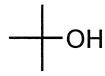
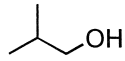
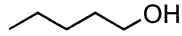
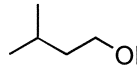
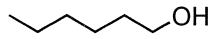


Fig. 3. Effect of carbon chain length of alcohols on the rate of synthesis of alkyl caproates catalyzed by goat pregastric lipase in isooctane at 35°C.

Table 1
Effect of different classes of alcohols on the initial rate of synthesis of alkyl caproates catalyzed by goat PGL in isooctane at 35°C

Alcohol	Structure	% initial rate
n-butanol		100
sec-butanol		51
tert-butanol		~0
iso-butanol		15
pentanol		96
iso-amyl alcohol		97
hexanol		92

Geranyl esters, essential fragrance compounds used in the food, cosmetic and pharmaceutical industries, have also been synthesized by some microbial enzymes [21,22]. Goat pregastric lipase revealed little activity to catalyze the esterification of the bulky alcohol, geraniol, using butyric acid. The activity was less than 1% of that for butanol. The micro-environment around the active site of goat PGL seems to be too small and it only allows small nucleophiles, e.g., water or short chain alcohols, to reach and deacylate the acyl-enzyme intermediate. In contrast to most microbial lipases, possibly the intrinsic nature of PGL therefore restricts the ability of the enzyme to perform a wide range of applications in the synthesis of esters.

The effect of an electron-donating or electron-withdrawing substituent on the alcohol also needs to be considered. It has been reported [7] that the presence of electron-donating substituents, e.g., hydroxy groups, results in a higher reactivity than do electron-withdrawing substituents, e.g., nitro groups, as would be expected, if the mechanism involves nucleophilic attack.

In summary, the effect of the alcohol class on the rate of enzyme-catalyzed synthesis of esters can be predicted by their thermodynamic properties, e.g., carbon-chain length (size), steric effects and electronegative effects, and depends little on the native catalytic triad of the enzyme. The finding that the relative rates of synthesis of esters from different classes of alcohols were very similar and independent of the type of lipase [20] confirms our conclusion that the extent of lipase-catalyzed ester synthesis is independent of the class of the alcohol.

3.3. Effect of carbon-chain length of fatty acids

Chain-length selectivity of fatty acids is usually dependent on the native properties of the enzymes, and is usually equivalent to that seen in hydrolysis reactions. Since the lipase-catalyzed synthesis reaction involve an acyl-enzyme intermediate, the dependency on the chain length of the fatty acids will mostly be accounted for by the affinity between the fatty acid and enzyme itself.

Pregastric lipases prefer shorter chain triacylglycerols for hydrolysis in aqueous solution, and the activity decreases as the chain length increases [10]. In an anhydrous system, the synthesis of an ester catalyzed by PGL follows a similar trend, with the rate decreasing as the chain length increases. Fig. 4 shows the relative

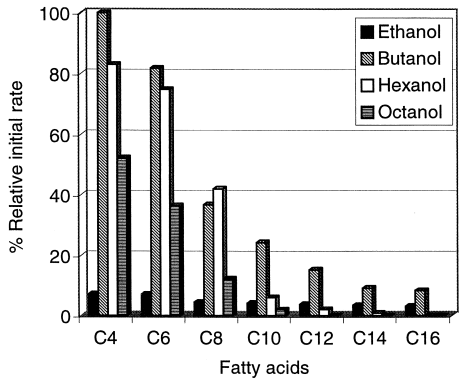


Fig. 4. Effect of carbon-chain length of fatty acids on the rate of esterification between 100 mM fatty acids and aliphatic alcohols catalyzed by goat PGL in isooctane at 35°C.

rate of the synthesis of butyl esters against the carbon chain length of the fatty acids. For a given alcohol, e.g., butanol, the initial rate of synthesis of the butyl esters decreased as the carbon-chain length of the fatty acids increased. However, the chain length of the fatty acid is not the only factor which affects the enzyme-catalyzed reaction and the chain length of the alcohol also plays an important role in overall selectivity. In the case of ethanol as substrate, the rate of the synthesis of the esters is relatively independent of the chain length of the fatty acids, however when the chain length of the alcohol increases, the longer chain fatty acids become disfavored. This result suggests that the acylation step in goat PGL-catalyzed synthesis of ester is not rate-determining although it is in most other lipase-catalyzed reactions, and it is the extent of the deacylation step which affects the overall rate of reaction.

If one supposes that the acylation step is dependent on the affinity between the fatty acids and enzyme and that deacylation of the acyl-enzyme intermediate by alcohols is a purely thermodynamically-controlled nucleophilic reaction, then it is expected that for an enzymatic reaction for which the acylation step is rate-determining, the relative rate of synthesis of esters using fatty acids of different carbon chain length will be more dependent on the carbon chain length of the fatty acid and less dependent on different types of alcohols. However, interpretation of our finding, with respect to the relationship between the chain lengths of fatty acids and alcohols, seems to be consistent with the fact that the active site of PGL is composed of a cavity large enough to accommodate substrates whose total carbon-chain length occupies a space equivalent to 10 $-CH_2-$ moieties. A similar spatial arrangement of the active site has been also suggested for bile-salt stimulated human milk lipase [23]. Thus, even though the longer chain fatty acids may intrude into this active site, they will also block the pathway for approach by the alcohols to form the products from the acyl enzyme intermediate, thereby causing a decrease in the net rate of production of esters.

3.4. Effect of substrate concentration

In an aqueous emulsion system, the rate-determining step for enzyme-catalyzed hydrolysis is acylation of the enzyme, since, in the presence of excess water, the rate of deacylation will be faster than the rate of acylation. In other words, the relative activity of the enzyme against different substrates will be dependent on the interaction between the substrate and the active site of the native enzyme. However, in the enzyme-catalyzed synthesis of esters in organic solvents, both substrates are soluble in a single phase and have the same opportunity to reach the enzyme active site, and thus the kinetics of the reaction will be more complicated than those of the hydrolysis reaction.

Fig. 5 shows the rate of synthesis of butyl caproate for different concentrations of the acid and alcohol substrates (which were always kept equal). The rate of synthesis increased with an increase in substrate concentration, and reached a maximum rate at 250 mM. When the substrate concentrations were greater than 250 mM, the initial rate started to decrease. The loss of activity at high alcohol concentrations might be influenced by its dehydrating effect on the surface of the enzyme in organic media, which would

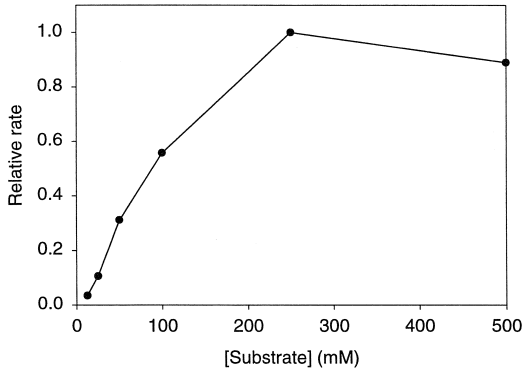


Fig. 5. Effect of the concentration of the substrates, butanol and caproic acid at equal molar concentrations, on the initial rate of synthesis of butyl caproate catalyzed by goat PGL in isooctane at 35°C. Data are normalized to the value for 250 mM substrates.

cause inhibition of lipase activity. Similarly, an increase in acid concentration may also change the catalytic environment. In the unbuffered organic solvent, an increase in fatty acid content will introduce a pH effect into the system. PGL was found to have little activity at pH < 5 during the hydrolysis reactions, and thus we can expect that the rate will be slower when the caproic acid content ($pK_a = 4.8$) is high. In a solvent-free medium with a 1:1 molar ratio of acid to alcohol, the rate of catalysis is approximately the same as that in 25 mM alcohols and acids. Thus, the main drawbacks in using pregastric enzymes in organic media are the requirement for an apolar medium and the inefficiency of the catalysts in a water-free system [24]. The dominant factor that emerges is the partition of the substrate between the bulk solvent phase and the enzyme active site.

When the solvent was isooctane, increasing the concentrations of substrate led to formation of a biphasic micellar system. Some difficulties were experienced in performing enzyme-catalyzed synthesis of esters or transesterification reactions in reversed micellar systems. In aqueous solution, the activity of goat PGL is inhibited by the presence of the ionic surfactants, AOT and CTAB. Therefore it is not surprising that, even when these surfactants are incorporated into reversed micelles, the activity of goat PGL remains inhibited. This behavior contrasts with that seen for the other lipases which have been studied, e.g., *C. rugosa* [25] and *Chromobacterium viscosum* lipase [26], whose catalysis is enhanced in the presence of most of the common surfactants used for the formation of reversed micelles.

In the case of goat PGL, in the presence of phospholipids and Tween, which did not inhibit the activity of the enzyme in aqueous solution, a complete absence of activity was observed in the presence of these surfactants in reversed micelles. We have shown that pregastric lipase is active in a pure organic phase, but it seems to be quickly inactivated at an oil–water interface [27], a behavior which is very unusual within the lipase family.

3.5. Dependency on water content of the catalyzed rate of ester-synthesis

The role of water in enzyme function in organic solvents is one of the major questions in non-aqueous enzymology. It has been proven that water molecules provide an essential ingredient for the enzyme to retain the activity, but on the other hand, the enzyme also catalyzes the hydrolysis reaction which mitigates against successful synthesis of esters. Fig. 6 shows that the relative rates of synthesis of butyl caproate in

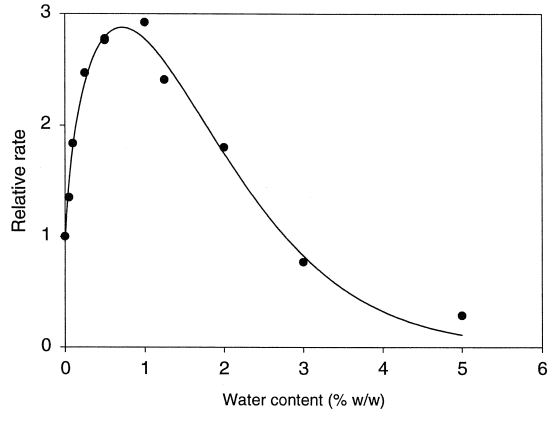


Fig. 6. Effect of water content on the initial rate of synthesis of butyl caproate catalyzed by goat PGL in isooctane at 35°C.

solvents of different water content. Surprisingly, the rate of synthesis was enhanced about three-fold in the presence of 1% w/w water compared with anhydrous conditions, and the rate decreased as the concentration of water was increased. Only 30% activity was found in 5% w/w water compared with that in an anhydrous medium.

The results suggest that a limited amount of water is essential for goat PGL-catalyzed synthesis of esters, and that enhancement of activity is probably due to the hydration effect which turns the inactive form of the powdered enzyme into its active conformation by interaction with water molecules. High concentrations of water, however, initiated the reverse hydrolysis reaction and decreased the net production of esters. Stimulation of enzyme activity in the presence of a limited concentration of water has also been reported for oxidase and dehydrogenase [28] and more than 1000-fold enhancement of activity was achieved in 0.6% w/w water content. Thus, we may conclude that not all enzymes are retained in their active conformation in freeze-dried powders and that access to their active site may be hindered by neighbouring proteins. The addition of water will then help to re-orient the enzyme conformation or the geometry between the enzyme and proteins, thereby creating a more 'effective' enzyme concentration.

The size of suspended enzyme powders of porcine pancreatic lipase and *Pseudomonas cepacia* lipase in an organic phase has been measured by electronic microscopy and it was suggested that there were no pores or channels which allowed substrates/products to diffuse in/out, and thus only the enzyme on the surface of the aggregate was involved in catalysis [29]. The increase in water content also stimulated aggregation of the enzyme particles and decreased the enzyme activity. However, this trend did not apply to this present investigation, a result which implies that the extent of activation of the enzyme by water molecules is much greater than the inhibition caused by aggregation. Moreover, in high concentrations of water, ca. $>5\%$ w/w, a sufficient amount of water would form a biphasic system and the rate of the catalyzed reaction would then be dependent upon the diffusion of substrates/products between the two phases.

In this study, several fundamental studies have been made in an attempt to identify the parameters governing the catalytic behaviour of PGL in an organic medium. The results have shown that goat PGL, and probably the whole family of preduodenal lipases, have the ability to catalyze the synthesis of simple esters in a water-free system. Regardless of the dependency on the carbon-chain length of fatty acids, which, in turn, is quite dependent on the nature of the enzyme, the turnover rate for goat PGL-catalyzed synthesis of alkyl esters is comparable with that of other microbial lipases with a high yield for conversion (80–90%) in 20–40 h [4]. However, it is difficult to compare this value with the results from other researchers because of the different assay methods and different reaction conditions used, e.g., temperature, water content, solvent type and substrates. Thus, in studies of enzyme activity in anhydrous media, the variation due to the different reaction conditions will be much greater than expected from only a change in the enzyme.

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