HYDROLYSIS OF OLIVE OIL BY PANCREATIC LIPASE IN BIPHASIC ORGANIC–AQUEOUS SYSTEM

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Six ethylene oxide-based surfactants were tested for their suitability to mediate lipase action on olive oil in a water–organic biphasic system. In Slovasol EL only it was possible by lipase to catalyze hydrolysis of the substrate over a wide range of isooctane and water content in the system. The Michaelis–Menten parameters of lipase measured for this system are comparable with those measured in a reversed emulsion of water in an oil–isooctane solution. The hydrolysis rate was not increased, however, in the presence of isooctane.

Many attempts have been made to convert water-insoluble substrates to highly valuable materials by using enzymes or microbes in the presence of organic solvents[1–6]. One of the most attractive subjects of technical interest is lipase (triacylglycerol hydrolase EC 3.1.1.3) catalyzed hydrolysis[7] or transesterification[8,9] of fats and oils designed to improve their properties[10]. The lipase catalyzed reactions, however, involve several problems.

First, lipase converts its water insoluble substrate in two-phase systems which vary in the procedure of their preparation. Since lipase is almost completely adsorbed from an aqueous solution by emulsified liquid long chain triacylglycerol particles[11], its activity is greatly dependent on the physical state of the substrate[12]. Lipase action in a heterogenous system is controlled by the concentration of the substrate molecules available. This concentration under constant experimental conditions is proportional to the size of the interface. Any factor affecting the affinity of the enzyme for the interface may also be important.

Fats and oils are natural substrates for lipase catalyzed reactions. However, they form in water inferior emulsions with great particles. In the presence of surfactants and organic solvents an increase in stability[13] and reaction rate[14] is observed. The best results were obtained when the catalysis was effected by the enzyme entrapped in reversed micelles of water-surfactant in organic solvents[15]. The reversed micelles, however, are formed under strictly defined conditions only and a few surfactants and organic solvents are suitable for micelle formation.

This paper describes the investigation of the water-isooctane-surfactant system suitable for action of pancreatic lipase over wide range of concentrations of non-aqueous phase.

EXPERIMENTAL

Materials

Lipase (triacylglycerol hydrolase, EC 3.1.1.3) type II crude from porcine pancreas was purchased from Sigma Chemical Co. Refined oil, saponification value 200 and acid value 0·1, was used as a substrate. The molecular weight of ricine oil determined from the saponification value was 840. All surfactants were a gift of the producer CHZMP Nováky, Czechoslovakia: Slovasol EL (ricine oil + 20 molecules of ethylene oxide), Slovasol SF 10 (fatty alcohol + 10 molecules of ethylene oxide), Slovapon N (blend of oxyethylated fatty alcohols), Slovanic (copolymers of ethylene oxide and propylene oxide), Slovasol S (fatty alcohol + 4—9 molecules of ethylene oxide). All the other chemicals were of analytical purity and were purchased from Lachema Brno, Czechoslovakia.

Lipase assay. Lipase activity was assayed in four reaction systems of different hydrophobicity:

A) 0·5 g olive oil, 8·7 ml isooctane, 0·005 g Slovasol EL, 0·8 ml of lipase solution 2 mg/ml in sodium cholate (1·6% w/v in water, pH 9). Sodium cholate was used for the stabilization of the enzyme[12].

B) 0·5 g olive oil, 2·7 ml isooctane, 0·005 g Slovasol, 6·0 ml sodium cholate solution (1·6% w/v in water, pH 9) and 0·8 ml lipase solution.

C) 0·5 g olive oil, 0·005 g Slovasol EL, 8·7 ml sodium cholate (1·6% w/v in water, pH 9) and 0·8 ml lipase solution. The reaction mixture was homogenized in rotating homogenizer 15 min at 2 000 rpm. After 20 h incubation at 28°C free fatty acids were determined as follows: Into 10 ml of a mixture of ethanol and diethyl ether (1 : 1) the reaction blend (1 ml) was added and titrated with 10 mM ethanolic potassium hydroxide using phenolphtalein as an indicator using an ABU 12 (Radiometer, Copenhagen, Denmark) microburet. Under these conditions the amount of free fatty acids released was a linear function of time; after 20 h about 6% of all hydrolysable ester bonds of olive oil were cleaved. The initial rates of lipase catalyzed reactions were therefore determined as a tangent of the straight line.

D) Arabic gum solution, 10% (32 ml), 8 g of crushed ice and 10 ml of olive oil were mixed for 15 min in a rotating homogenizer at 2 000 rpm and 25°C. Into 5 ml of this reaction blend 5 ml of sodium cholate solution and 0·8 ml of lipase solution (2 mg ml^{-1} in sodium cholate) was added. After 30 min incubation 1 ml of this mixture at 28°C was diluted with 10 ml of boiled water and titrated with 10 mM aqueous solution of potassium hydroxide using an autotitrator TTT 60 (Radiometer, Copenhagen, Denmark). The initial rates of the reaction were determined as above.

Methods

Determination of Michaelis–Menten parameters. Parameters of the Michaelis–Menten equation were evaluated from Lineweaver–Burk reciprocal plot. Varying amounts of olive oil were added into reaction mixtures *A*—*D*. The reaction rates were measured at 28°C and the free fatty acids were determined by acid-base titration.

Test of surfactants. When the surfactants were tested for their ability to enhance lipolysis in the presence of isooctane the following reaction mixture was used: olive oil (1 ml), isooctane (8·5 ml) lipase (0·5 ml) 2 mg ml^{-1} in sodium cholate (1·6% w/v in water, pH 9) and 0·1 g of a surfactant. This mixture was incubated at 28°C for 20 h in a homogenizer. The reaction products were detected by TLC chromatography on Silufol silicagel plates (Lachema, Brno, Czechoslovakia) developed with petroleum ether, diethyl ether and acetic acid (90 : 10 : 1 v/v/v) as mobile phase. The spots were visualized by iodine vapors.

RESULTS AND DISCUSSION

Six surfactants (Slovasol EL; Slovasol, SF 10 Slovapon N; Slovanic; Slovasol SF and Slovasol S) produced by CHZWP Nováky, Czechoslovakia, were tested for their suitability to mediate lipase action on olive oil in the water–isooctane system. The reaction can be catalyzed by lipase in Slovasol EL only (Fig. 1). The rate of the oil hydrolysis was strongly dependent on the concentration of Slovasol EL in the reaction system composed of oil (5% w/v), isooctane (87% v/v) and water (8% v/v) (Fig. 2). A sharp maximum of initial velocity of hydrolysis at 0·05% concentration of Slovasol EL was observed.

However, at the optimal concentration of Slovasol EL, the rates of hydrolysis at higher concentrations of water were only about twice higher than the rates at lower concentrations of water in the system (Fig. 3). This observation and the fact that droplets about 3 μm in diameter were formed during the homogenization suggest that Slovasol EL is a good emulsifier for the oil substrate under these conditions.

Kobayashi et al.[16] and Mukataka et al.[17] have found that the hydrolysis by *Candida cylindricaea* lipase was accelerated in a water substrate–surfactant system if hydrophobic organic solvents, such as isooctane, heptane and hexane were added in suitable amounts. However, only a small acceleration was observed when porcine pancreatic lipase was used for the hydrolysis of olive oil. We observed that hydrolysis by

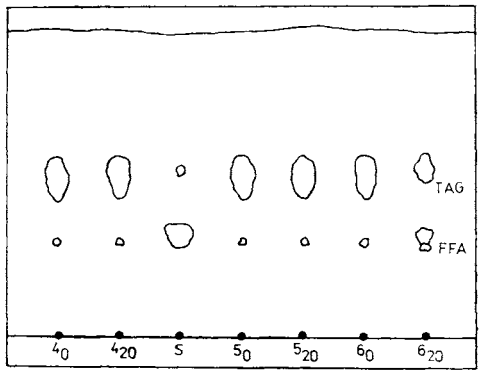

FIG. 1

TLC chromatography of reaction mixtures. The mixtures contained 10% of olive oil, 82% of isooctane, 8% of water, 1·6 mg of lipase and 0·01% w/v of surfactant: **4** Slovasol SF, **5** Slovasol S, **6** Slovasol EL. **S** is a standard solution of free fatty acids of olive oil. Time of the incubation at 28°C is indicated by the TAG triacylglycerols, FFA free fatty acids

lipase is more efficient in systems with high or zero concentrations of isooctane and the K_m – values are smaller than in systems with medium concentration of isooctane. Table I indicates that Slovasol EL forms suitable substrate emulsions for the lipase catalyzed reactions in systems with both high and low concentration of isooctane. This is important from the practical point of view, since Slovasol EL should also be

TABLE I

Michaelis–Menten kinetic parameters for lipase catalyzed hydrolysis of olive oil in various water–isooctane surfactant systems (see Materials and Methods, r is correlation coefficient)

System	K_m μmol ml^{-1}	k_{cat} μmol h^{-1} mg^{-1}	k_{cat}/K_m ml h^{-1} mg^{-1}	r
A	53	13·8	0·264	0·982
B	203	45·9	0·226	0·970
C	18	48·5	0·276	0·998
D	6	11·9	2·01	0·960

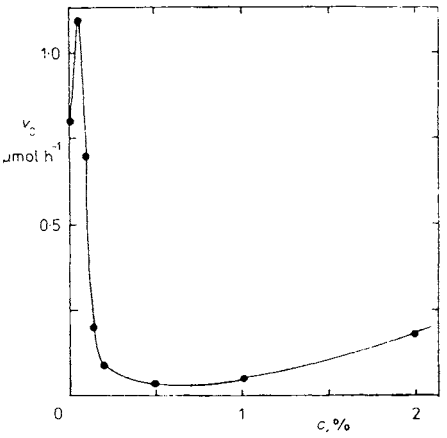

FIG. 2

Effect of Slovasol EL concentration c on initial rate of olive oil hydrolysis (v_0). The reaction mixture consisted of 5% of olive oil, 87% v/v of isooctane and 8% v/v water and lipase (0·16 mg ml^{-1}). Incubation was carried out at 28°C

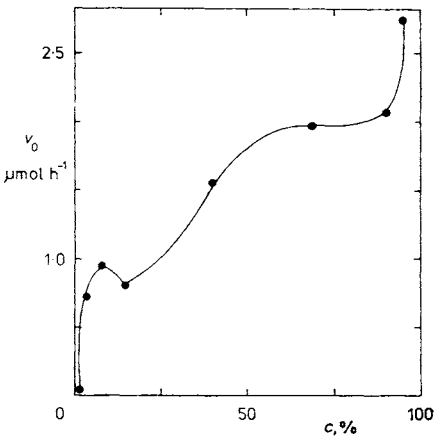

FIG. 3

Effect of water concentration in isooctane on initial rate of olive oil hydrolysis. The concentrations were: Slovasol EL 0·05% (w/v), olive oil 5% (w/v) and lipase (0·08 mg . ml^{-1}). Incubation was at 28°C

suitable for transesterifications catalyzed by lipase in systems with a low water content.

Arabic gum yields more suitable substrate emulsions[18] in water than Slovasol EL (the ratio k_{cat}/K_m is much higher – Table I, system *D*). However, arabic gum cannot be used in isooctane containing emulsions since it is precipitated by isooctane.

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