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Microemulsion-Based Gels for Lipase-Catalyzed Ester Synthesis in Organic Solvents

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Microemulsions are clear, stable, isotropic mixtures of oil, water, and surfactant, frequently in combination with a cosurfactant. These systems are currently of interest to the pharmaceutical scientist because of their considerable potential to act as drug delivery vehicles by incorporating a wide range of drug molecules. The purpose of this work is to solubilize in AOT [sodium bis(2-ethylhexyl)sulphosuccinate] water-in-oil microemulsions at two different R-values the Chromobacterium viscosum (CV) lipase and lipoprotein lipase ex Pseudomonas and to use them to catalyze the lactonization of 16-hydroxyhexadecanoic acid at 40°C. CV lipase is also immobilized in gelatin-containing microemulsion-based gels (MBGs) with retention of catalytic activity. These lipase-containing MBGs proved to be novel solid-phase catalysts for use in apolar organic solvents. CV lipase-containing MBGs are used to synthesize, on a preparative scale, a variety of different esters under mild conditions.

1. Introduction

There is much current interest in the properties of enzymes solubilized in water-in-oil microemulsions.[1–4] It is possible to solubilize enzymes in microemulsion media containing very small amounts of water so that the enzymes retain activity. Such media can alter the equilibrium positions of reactions through reagent partitioning and, in the case of hydrolysis/condensation reactions, by lowered water activity. Many condensation-type reactions require forcing conditions or are extremely slow; the use of microemulsion media containing enzymes offers the possibility of specific synthesis under mild conditions. Hence, the use of enzymes in microemulsions can permit the catalysis of reactions in a direction opposite to that normally observed in aqueous solution, a process often referred to as "reverse enzyme synthesis."[4] Microemulsions offer considerable advantages over other multiphase systems. For example, (i) it is possible to mix polars and nonpolar reactants in the same reaction mixture (in our first example, oleic acid and glycerol); (ii) the equilibrium position in the reaction can be affected both by partitioning of the reactants/products and by changes in the water activity in the dispersed phase (in one of our reactions the water activity is varied by mixing with glycerol as cosolvent); (iii) the products of the reaction may be physically separated, for example, using the semipermeable hollow fiber reactor as described by Liithi and Luisi.[5] The most commonly used w/o microemulsion systems consist of dispersions of nanometer-sized water droplets stabilized by a surfactant skin in an apolar solvent such as heptane or isooctane (Figure lA). The more recent development of gelatin containing microemulsion-based organo-gels[6–10] has led to their use as a matrix for the immobilization of lipases.[11–15] The physical characterization of MBGs has been carried out by a number of groups using a variety of techniques including tracer diffusion, electrical conductivity, NMR, X-ray, and small angle neutron scattering. For high R-value systems (R = $[H_2O]$/[surfactant]) or the type used in our studies, the proposed model is of a continuous gelatin/water three-dimensional network, coexisting with excess w/o microemulsion droplets (**Figure 1**B). We have immobilized lipase from Chromobacterium Viscosum and from Pseudomonas sp. in sodium bis(2-ethylhexyl)sulphosuccinate (AOT)* w/o microemulsions and MBGs. We report here the use of lipase for lactone synthesis as well as preparative-scale regio- and stereo-selective syntheses using CV lipase containing MBGs.

2. Results and Discussion

2.1. Lactone Synthesis

CV lipase and lipoprotein lipase ex Pseudomonas were solubilized in microemulsions of 20 mol dm^{-1} AOT in *n*-heptane and found to catalyze the lactonization of 16-hydroxyhexadecanoic acid in reasonable yield, typically around 50% after 8 hr. As indicated by the CV lipase data in **Figure 2**, lactonization was observed in both low and high R value microemulsions (10 and 40, respectively). The rates of reaction were always noticeably faster in the R = 10 microemulsion systems as compared to the R = 40 systems for both lipases, but the final yields of lactone in all four cases were very similar. It is notable that the substrate

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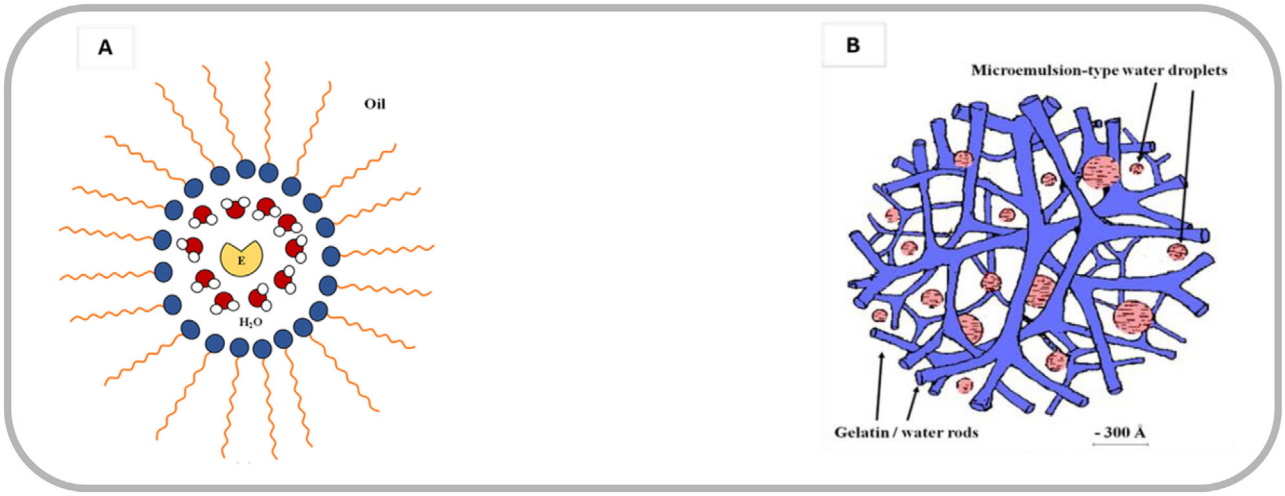


Figure 1. A, Schematic representation of hydrophilic enzyme molecule incorporated into the water core of a water in oil microemulsion droplet and B, a proposed model for a gelatin-containing microemulsion-based gel.

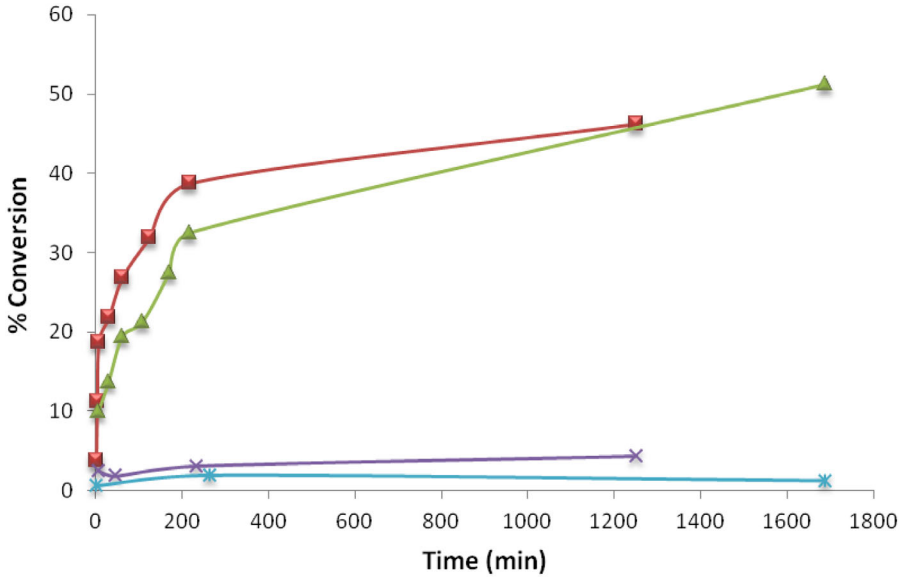


Figure 2. – Lactone synthesis catalysed by CV lipase in AOT microemulsions at R = 10 (■) and R = 40 (▲) at 40°C. Microemulsion composition [AOT] = 0.20 mol dm^{-3}, [CV lipase] overall = 200 μg mL^{-1} an [16-hydroxylhexadecanoic acid] = 2 × 10^{-3} mol dm^{-3}. No enzyme was present in the control experiments at R = 10 (X) and R = 40 (※).

concentration used in these experiments is higher than that achievable in the parent oil itself by a factor of ca. 40, this improved solubility being a general feature of microemulsion systems. The concentration of substrate was deliberately chosen to take into account the droplet concentration in the microemulsion itself. By choosing a substrate/droplet ratio of approximately one, the lactonization reaction should be promoted in comparison to the competing linear esterification/polymerization reactions.

2.2. Ester synthesis using lipase-containing MBGs

CV lipase immobilized in MBGs is capable of accepting a variety of structurally different molecules as substrates. This is illustrated by the examples in **Table 1** where linear, unsaturated, branched, aromatic, and cyclic molecules were all successfully esterified with either octanol or decanoic acid. The CV lipase-containing MBGs were also used to catalyze regio-selective acylation of diols as shown in the examples of **Table 2**.

These syntheses are particularly interesting because 1,3-butanediol and 1,5-hexanediol are water-soluble diols. For this reason, at the beginning of the reaction, a three-phase system forms which, after 30 min or so, becomes two-phase (MBG and external oil) as the pelleted MBG absorbs the diol. The resulting swollen MBG pellets subsequently reduce in volume to their original size as the esterification reaction progresses. Finally, the observed high regio-selectivity towards the formation of the terminal monoester (isomer A of Table 2) is presumably caused by steric hindrance reasons, being the A:B isomer ratio slightly

Table 1. Examples of batch-type syntheses carried out at 25°C using 10 mL of pelleted lipase-containing MBG in 30 mL of n-heptane containing 0.33 mol L^{-1} substrates. The second substrate was either octanoic or decanoic acid, [CV lipase]ov = 250 μg mL^{-1} of MBG.

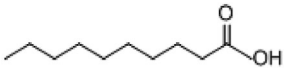
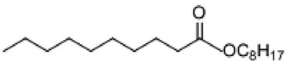
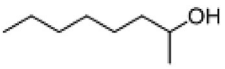
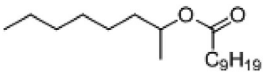
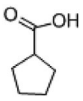
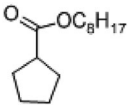
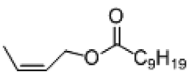
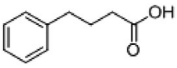
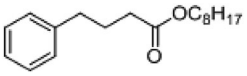
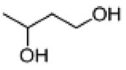
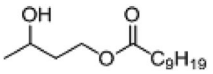
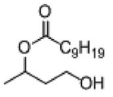
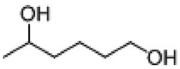
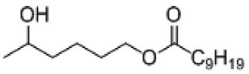
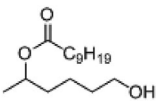
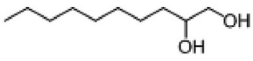
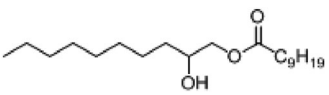
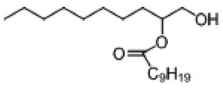
Principle Substrate	Product	Yield (%)	Time
		93	4 hours
		45	5 days
		83	7 days
		91	1 days
		92	5 days

Table 2. Examples of regio-selective monoester synthesis by CV lipase in a batch-type synthesis performed at 25°C using 10 mL of pelleted MBG in 30 mL of *n*-heptane containing 0.33 mol L^{-1} substrates. [CV lipase]ov = 250 μg mL^{-1} of MBG.

Diol	Monoesters synthesided		Ratio of A:B
	A	B	
			75:25
			98:2
			97:3

lower when the carbon chain length of the substrate (diol) is shorter.

3. Conclusions

Microemulsion system proved to be an effective method to trap polar enzymes in order to solubilize them in apolar organic solvent. In this paper, we demonstrated that AOT microemulsion of CV and lipoprotein Lipases were able to catalyze the 16-hydroxylhexadecanoic acid lactonization in *n*-heptane with a high substrate % conversion. In particular, the reduction of R-value resulted in a slightly faster enzyme activity. We also evaluated the use of the microemulsions-based gel (MGB) to immobilize lipases as an effective alternative to the conventional w/o microemulsion system. The enzyme containing MBGs not only possess many advantages with respect to conventional w/o microemulsion systems, but they also have the added benefits of allowing a straightforward product isolation and an easy catalyst re-use. These properties are due to the insolubility of MBGs in their parent oils (and many others beside), and to the absence of any appreciable loss of surfactant, water, or enzyme into the substrate-containing organic solvent. Moreover, same lipase containing MBGs are able to catalyze tandem reactions involving different substrates, because any product and/or substrate left in the gel after a synthesis may be easily partitioned out using fresh aliquots of organic solvent.

4. Experimental Section

CV lipase and lipoprotein lipase ex Pseudomonas were purchased from Genzyme Biochemicals Ltd, UKs, with specific activities of 3300 and 1800 units mg^{-1} of powder, respectively, sodium bis(2-ethylhexyl)sulphosuccinate (AOT) and gelatin (Bloom 300) were purchased from Sigma and used without further purification. The identity of

the lactone product was confirmed using an HP 5890 Series II GC with a DB17 capillary column coupled to an HP 5971 Series Mass Selective Detector. Lactone synthesis was subsequently monitored on a 6.8 m DB17 capillary column using FID detection. Octyl decanoate synthesis was monitored by HPLC using a C18 NovapakTM column eluted with acetonitrile. Lipase-containing microemulsions were prepared by mixing the appropriate volume of an aq. lipase solution to a reverse micellar solution of AOT in n-heptane. Dissolution of 16-hydroxyhe-xadecanoic acid was achieved by co-sonication with a low R-value ($R = [H_2O]/[surfactant]$) microemulsion rather than with a reverse micelle solution. Lipase-containing MBGs were prepared by mixing an enzyme-containing microemulsion with an aq. gelatin solution above the gel transition temperature which was subsequently allowed to cool down to room temperature. The final MBG composition (w/v) was as follows: 14% gelatin, 24% water, and 8.9% AOT with n-heptane as the oil phase. MBGs were stored at -20°C and pelleted prior to use. A batchwise method of synthesis was generally employed whereby the lipase-containing MBG was immersed in an external oil phase containing 0.33 mol dm^{-3} each of the acid and alcohol.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

ester synthesis, reverse enzyme synthesis, water-in-oil microemulsion

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