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Preliminary communication

Organometallic substrates of enzymes: lipase catalysed transesterifications in organic solvents via O-stannyl ethers

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Abstract

Lipases from pig pancreas and from *Chromobacterium viscosum* (but not from *Candida cylindracea*) catalyze transesterification reactions between ethyl esters of carboxylic acids and tributylstannyl ethers of primary and secondary alcohols. At concentrations 1 *M* or higher in anhydrous hydrocarbons, use of these nucleophilic derivatives of alcohols gives reaction rates ca. three times higher than those for the corresponding alcohols.

To be of use to organic chemists, enzymes must be able to catalyze reactions with as many different substrates as possible with the highest possible specificity [1]. Thus, lipases have been widely used for regio- and stereo-selective hydrolysis of a variety of esters [1]. Moreover, it is now well established that at least some of these enzymes can work in anhydrous organic solvents [2–4]; this remarkable property enables chemists to use enzymes to catalyse reactions that cannot be carried out in water for thermodynamic reasons. One of these reactions, transesterification (X = H in eq. 1) has been used for stereoselective acylation of alcohols [3,5], esterification of acids [5], and regioselective acylation of diols [6] and sugars [7]. More recently,

$$R^1COOEt + R^2OX \leftrightharpoons R^1COOR^2 + EtOX \quad (1)$$

lipases have been used in organic solvents for the synthesis of peptides [10].

We now report an example of a new class of reactions made possible by the use of enzymes in organic solvents: these reactions involve substrates which cannot be used in water, such as organometallic compounds.

We have undertaken to modify the "classic" transesterification reaction by using, instead of an alcohol (X = H) a nucleophilic derivative such as a trimethylsilyl ether ($X = SiMe_3$) or a tributylstannyl ether ($X = SnBu_3$) as substrate. Three different enzymes were tested for their ability to use these compounds as substrates: lipases (EC. 3.1.1.3) from pig pancreas (PPL), from *Candida cylindracea* (CCL), from

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Table 1

Lipases-catalyzed transesterifications between ethyl carboxylates and alcohols or derivatives of alcohols [a,b]

$R^1COOEt + R^2OX \rightleftharpoons R^1COOR^2 + EtOX$

Ester	Lipase [c] (initial rates, μmol/h/ml)			
	PPL	CVL	CCL	non [d]
R^2 = hexyl, R^1CO = acetyl				
X = H	6.8	0.3	10.0	0.0
$X = SnBu_3$	26.0	11.0	5.0	7.5
R^2 = hexyl, R^1CO = butyryl				
X = H	13.5	2.3	32.0	0.0
$X = SnBu_3$	39.0	13.0	1.0	2.5
$X = SiMe_3$	4.7	2.0	3.2	0.7
R^2 = hexyl, R^1CO = capryloyl				
X = H	6.9			0.0
$X = SnBu_3$	22.5			2.0
R^2 = hexyl, R^1CO = lauryl				
X = H	4.4			0.0
$X = SnBu_3$	17.0			6.2
R^2 = cyclohexyl, R^1CO = acetyl				
X = H	3.0			0.0
$X = SnBu_3$	9.0			0.0
R^2 = cyclohexyl, R^1CO = butyryl				
X = H	5.6	3.5		0.0
$X = SnBu_3$	13.0	11.4		0.2
$X = SiMe_3$	0.3	0.7		0.0

[a] 250 mg/ml of enzyme preparation [c] were added to a solution of ester and alcohol or alcohol derivative (1 *M* each) in benzene or cyclohexane and the suspension was shaken at room temperature. The relative concentrations of esters in the reaction medium were periodically estimated by GC analysis. [b] Silyl ethers: 1 equiv. of trimethylsilyl chloride and 1 equiv. of pyridine were added to a solution of alcohol in cyclohexane. After 15 min at room temperature, the precipitate was filtered off through active charcoal, and the solution was used as it was. The ether was 100% pure when assayed by GC. No trace of the starting alcohol could be detected. Stannyl ethers: 1 equiv. of bis(tributylstannyl) oxide was added to a solution of alcohol in benzene, and the mixture was refluxed during 8 h with azeotropic removal of water [8]. The solution was then used as it was. Virtually no O–H vibration band could be detected when the solution was assayed by IR spectroscopy. [c] Pig pancratic lipase (PPL) and *Candida cylindracea* (CCL) lipase were obtained from Sigma and had specific activities of 16 and 690 units/mg of solid, respectively. *Chromobacterium viscosum* (CVL) lipase was a gift of Finnsugar Biochemicals (Elk grove Village, IL) and had a secific activity of 120 units/mg. CCL was used "straight from the bottle"; PPL was kept under vacuum for two days, which lowered its water content from 5% to 0.5%; CVL was dissolved in water, pH was adjusted to 7 and the solution was freeze-dried: this pH adjustment increases activity of this lipase in organic medium approximately by a factor of 15. [d] No enzyme, or boiled enzyme.

Chromobacterium viscosum (CVL). We found that two out of these three enzymes can use trimethylsilyl and tributylstannyl ethers of alcohols as substrates in the transesterification reaction with various esters (see Table 1). In presence of PPL or CVL, the reaction of a silyl ether with an ester was significantly faster than the uncatalysed reaction. However, these compounds reacted more slowly than the corresponding alcohols and are probably poor substrates for the enzymes.

In contrast, stannyl ethers were found to be much more active than the corresponding alcohols. When these derivatives were used at a concentration of 1 *M* the reaction was three times as fast as that for the free alcohols. The effect was even

larger at higher concentrations. Thus, for these two enzymes, a tributylstannyl ether of an alcohol is an even better substrate than the free alcohol.

The other characteristics of the enzymic reaction in which stannyl ethers were used as substrates are similar to those observed with alcohols [2,6]. Thus, stannyl ethers of primary alcohols are better substrates than those of secondary alcohols; derivatives of tertiary alcohols do not act as substrates at all; the use of activated esters such as trichloroethyl esters as acyl donors leads to higher reaction rates; the concentrations at equilibrium are the same irrespective of whether an alcohol or its stannyl ether is used.

Kinetic parameters for the transesterification reaction catalyzed by porcine pancreatic lipase between ethyl butyrate and hexyl alcohol or its stannyl ether were determined, K_m for ethyl butyrate is very high, namely 5 to 10 molar, and cannot be determined exactly; K_m for hexanol is 15 mM and, surprisingly, the same K_m value was found for the stannyl derivative of this alcohol.

The ability of these enzymes to use tributylstannyl ethers of alcohols as substrates is surprising in view of the size of these ethers compared with their natural substrate water. Alcohols contain the hydroxyl function, and so can be considered as structural analogues of the natural substrate water. The only remaining similarity between water and a tributylstannyl ether of an alcohol is that they both contain a nucleophilic oxygen atom which attacks the acyl enzyme. In this respect stannyl ethers of alcohols can be regarded as functional analogues of water.

The nucleophilicity of an oxygen atom in a molecule is greatly enhanced when bound to tin. This property has found many synthetic applications [8]. Since the enzymic reaction proceeds via a two steps mechanisms [9]: (i) acylation of the enzyme and (ii) hydrolysis (or alcoholysis) of the acyl-enzyme; the overall acceleration of the reaction could be due to the intervention of a powerful nucleophile in the second step. The possibility cannot be excluded, however, that there is a preliminary stannylation of the enzyme, which enhances its nucleophilicity in the first step.

Synthetic applications of this new enzymic reaction are being investigated.

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