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‘Watching’ lipase-catalyzed acylations using ^{1}H NMR: competing hydrolysis of vinyl acetate in dry organic solvents

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Abstract

Lipase-catalyzed acetylations of 1-phenylethanol with vinyl acetate were monitored in situ by ^{1}H NMR spectroscopy. Surprisingly, even under dry conditions (no added water) the major reaction was hydrolysis of the vinyl acetate, not acetylation of the substrate. Because this competing hydrolysis consumes water and releases acetic acid, the reaction conditions in lipase-catalyzed acylations are not constant, but vary with the reaction time. Addition of a chiral shift reagent reveals the enantiomeric purity of the starting alcohol and allows calculation of the enantiomeric ratio, *E*, for the reaction. © 1999 Elsevier Science Ltd. All rights reserved.

Many new applications of lipases to organic synthesis involve reactions in organic solvents.[1] The most common are enantioselective acetylations of racemic or prochiral alcohols using vinyl acetate. These reactions are fast, the lipase is easy to recover by filtration, and the enantioselectivity is sometimes higher than for hydrolysis of the corresponding acetate in water. To maximize the rate and enantioselectivity of these reactions researchers vary the solvent type, the acyl donor type, and water activity.[2] However, optimization remains a largely trial and error process.

To speed up reaction optimization in organic solvents, we used ^{1}H NMR spectroscopy to monitor reactions in situ. This monitoring allowed us to ‘watch’ all the components of the reaction, especially vinyl acetate and acetaldehyde, which are difficult to monitor by HPLC or GC. This experiment revealed a surprisingly large amount of hydrolysis even under dry conditions. Furthermore, addition of a chiral shift reagent revealed the enantiomeric purity of the remaining starting material thereby allowing calculation of the enantiomeric ratio, *E*, for the reaction.

As a test reaction, we used the lipase-catalyzed acetylation of 1-phenylethanol (1-PE) with vinyl acetate (Fig. 1).[3] As expected, the resonances for the starting materials (1-PE and vinyl acetate) decreased, while the resonances for the products (1-PE acetate and acetaldehyde) increased as a function of time. However, the amount of acetaldehyde (aldehyde proton resonance at 9.2 ppm) increased three times faster than the amount of ester (methine resonance at 5.9 ppm) indicating that hydrolysis competes

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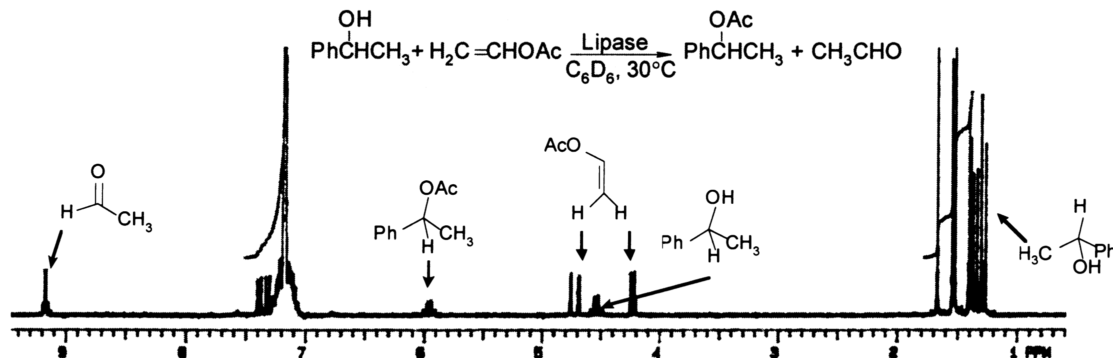

Figure 1. ^{1}H NMR spectrum of a *Burkholderia cepacia* lipase-catalyzed acetylation of 1-phenylethanol (1-PE) in C_6D_6 at ~15% conversion. The relative area of the signals at 9.2 ppm (acetaldehyde) and at 4.2 and 4.7 ppm (vinyl acetate) reveals consumption of acyl donor, while the relative areas of the signals at 5.9 ppm (methine proton of 1-PE acetate) and 4.6 ppm (methine proton of 1-PE) reveals the conversion of alcohol to acetate. This reaction consumed three times more acyl donor than alcohol indicating competing hydrolysis of the acyl donor

with acetylation of 1-PE even under these dry conditions.[4] A similar reaction under dry conditions using CAL-B[5] (Novo SP525) showed that hydrolysis occurred twice as fast as acetylation (Fig. 2a). In another reaction, the solution was equilibrated with a salt/salt hydrate pair $Na_2SO_4/Na_2SO_4 \cdot 10H_2O$ for one hour to adjust the thermodynamic water activity[6] to a relatively high value (a_w=0.76).[7] Hydrolysis now occurred six times faster than acetylation (Fig. 2b). The rate of conversion was similar in both cases.

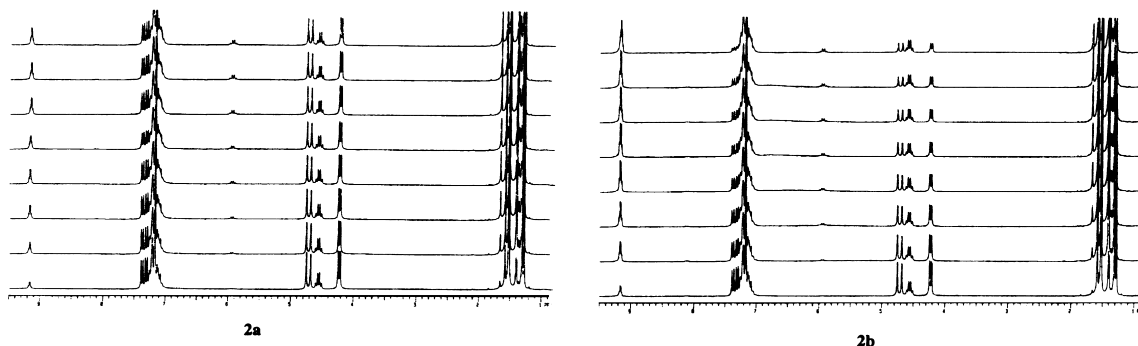

Figure 2. ^{1}H NMR spectra of *Candida antarctica* lipase B-catalyzed acetylation of 1-PE as a function of time. The stacked plots show spectra from 0.6–4.6 h under dry conditions (a), and at a water activity of 0.76 in (b). Both reactions show hydrolysis of vinyl acetate as well as acetylation of 1-PE. Hydrolysis is detected because the signal at 9.2 ppm (acetaldehyde) increases faster than the signal at 5.9 ppm (methine of 1-PE-acetate). Hydrolysis is three times faster than acetylation under dry conditions and six times faster in the wetter reaction mixture (a_w=0.76)

Although hydrolysis of the product ester is also conceivable, we observed no ester hydrolysis even after 32 h (~50% conversion, CAL-B, a_w=0.76). Under some conditions researchers observed spontaneous acetylation of alcohols with vinyl acetate,[8] but we observed no spontaneous reaction under our conditions (Fig. 3a). Omitting the substrate, 1-PE, increased the rate of hydrolysis of vinyl acetate by a factor of 1.2 at a_w=0.76 (Fig. 3b). Both the lack of hydrolysis without lipase and the apparent competition between the hydrolysis and acylation reactions strongly suggests that the hydrolysis of vinyl acetate is a lipase-catalyzed reaction. Thus, the major reaction in lipase-catalyzed acetylations with vinyl acetate is not the desired acetylation, but the competing hydrolysis of vinyl acetate. This hydrolysis is 2–3 times faster than acetylation even under dry conditions.[9] This hydrolysis changes the reaction conditions because it consumes water and releases acetic acid. This phenomenon may account for changes in

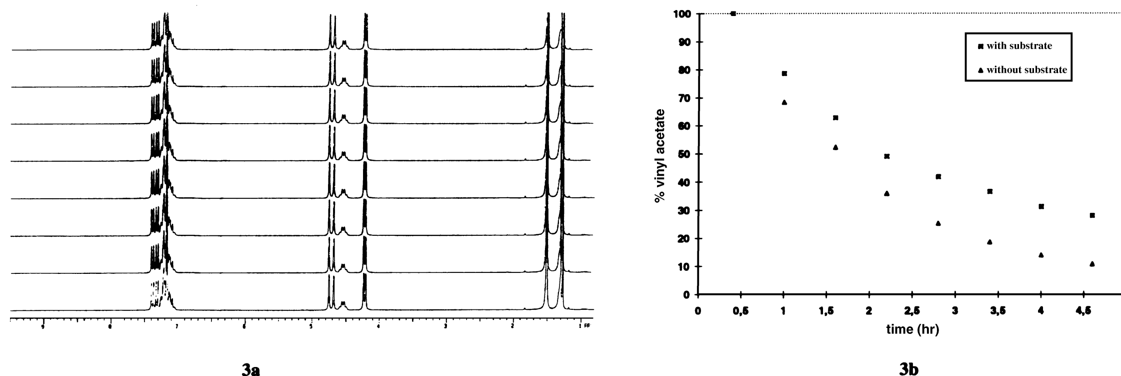

Figure 3. Control experiments with the CAL-B-catalyzed acetylation of 1-phenylethanol with vinyl acetate at a_w=0.76. (a) No reaction, neither hydrolysis nor ester formation, occurs in the absence of the enzyme. (b) In the absence of substrate alcohol, the hydrolysis of vinyl acetate is 1.2 times faster

the enantioselectivity at different conversions.[10] We recommend that careful optimizations of lipase-catalyzed acetylations with vinyl acetate include both a buffer for the water content (e.g. a salt/salt hydrate pair) to minimize the effect of water consumption and an organic phase pH buffer to minimize the effect of acetic acid release. Another possibility is the addition of a non-reactive organic base, e.g. Et_3N, for neutralizing any acid released as described by Rakels et al.[11] and more recently by Parker et al.[12]

A simple extension of this ^1H NMR monitoring technique also permits measurement of the enantiomeric ratio. We added a chiral shift reagent, praseodymium–tris(heptafluorocamphorato) complex [Pr(hfc)$_3$] to an aliquot of the reaction mixture. This reagent shifted the resonances for the methyl group of 1-PE (1.3 ppm), which is already furthest upfield, even further upfield while separating the signals for both enantiomers. The more commonly used europium shift reagents were not suitable because they shifted resonances downfield, leading to overlapping signals. The conversion of 1-PE into the corresponding acetate was calculated from the resonances at 4.6 ppm and 5.9 ppm. The enantiomeric ratio was calculated from the conversion and the enantiomeric purity of the remaining starting material. The measured enantiomeric ratios agreed with those in the literature although they were measured for slightly different reaction conditions (Table 1).[13]

Table 1
Enantioselectivity of several lipases measured by an extension of the ^1H NMR monitoring technique

Lipase	Reaction Time	c [%]	ee_S [%]	E-value (Lit.)
PCL (Amano PS)	152 min	23.8	31.2	>100 (>100)[13a)]
CRL (Sigma, L 1754)	5 days	5.4	4.4	8.07 (11-13)[2a)]
CAL-A (Chirazyme L-5)	152 min	7	1.2	1.03 (no ref.)
CAL-B (Novo Nordisk SP525)	152 min	20	25	>100 (>500)[13c)]
CLL (Amano L)	7 days	0	nr	nr (no ref.)
RML (Amano MAP)	152 min	9.6	10.6	>100 (>100)[2a)]
PPL (Sigma)	152 min	16.6	19.9	>100 (>100)[2a),13b)]

Notes: nr = not possible to measure since no reaction occurred. PCL = *Burkholderia cepacia* lipase, CRL = *Candida rugosa* lipase CAL = *Candida antarctica* lipase (type A and B), CLL = *Candida lipolytica* lipase, RML = *Rhizomucor miehei* lipase, PPL = porcine pancreatic lipase. (no ref.)= no reference available.

The main strength of this method is the ability to monitor all reactants and products at the same time without derivatization and without repeated sample taking. It is very close to actually ‘watching’ the reaction. This method could also be applied to study the influence of different solvents or acyl donors.

Currently it is used in our laboratory in the development of a method to synthesize enantiomerically pure drug precursors. This method for determination of the enantiomeric ratio for reactions in organic media is complementary to existing methods.

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2. For recent examples: (a) Ottolina, G.; Bovara, R.; Riva, S.; Carrea, G. *Biotechnol. Lett.* **1994**, *16*, 923–928. (b) Orrenius, C.; Norin, T.; Hult, K.; Carrea, G. *Tetrahedron: Asymmetry* **1995**, *6*, 3023–3030. (c) Ke, T.; Wescott, C. R.; Klibanov, A. M. *J. Am. Chem. Soc*. **1996**, *118*, 3366–3374. (d) Wehtje, E.; Costes, D.; Adlercreutz, P. *J. Mol. Catal.* **1997**, *3*, 221–230. (e) Straathof, A. J. J.; Jongejan, J. A. *Enzyme Microb. Technol*. **1997**, *21*, 559–571. (f) Secundo, F.; Ottolina, G.; Riva, S.; Carrea G. *Tetrahedron: Asymmetry* **1997**, *8*, 2167–2173.
3. Reactions were carried out in 5 mm NMR tubes and monitored on a 200 MHz spectrometer. For reactions under dry conditions, benzene-d_6 and the reactants were dried by filtering through a short silica gel column, enzymes were dried over $CaCl_2$ overnight in vacuo. For reactions with added salts/salt hydrates, the reaction mixture was equilibrated for 1 h before adding enzyme.
4. Hydrolysis also released acetic acid. The acidic proton resonance was not seen, but the methyl group resonance was seen at 1.5 ppm. However, this resonance could not be accurately integrated due to overlap from other resonances.
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