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Sol–Gel Processes

Xerogel-Encapsulated W110A Secondary Alcohol Dehydrogenase from *Thermoanaerobacter ethanolicus* Performs Asymmetric Reduction of Hydrophobic Ketones in Organic Solvents**

*Musa M. Musa, Karla I. Ziegelmann-Fjeld, Claire Vieille, J. Gregory Zeikus, and Robert S. Phillips**

The use of biocatalysts in organic synthesis has become an effective and sometimes preferable alternative to normal chemical methodologies for the production of optically active compounds.[1,2] The asymmetric reduction of ketones and the kinetic resolution (KR) of racemic alcohols are the most important reactions for producing optically active alcohols that then can be used to synthesize industrially important compounds like natural products.

A practical technique to improve enzyme performance is enzyme immobilization.[3] Most enzyme-immobilization methods involve covalent attachment of the enzyme to an activated group on a solid or gel support, which may result in significant loss of activity. A simple and efficient noncovalent immobilization method is enzyme encapsulation in transparent porous silicate glasses prepared by the sol-gel method.[4] The resulting glasses allow the transport of small molecules, but not enzyme molecules, into and out of the glasses pores.[5] The sol-gel encapsulation of enzymes has a lot of advantages, such as ease of recycling, broad applicability, cost effectiveness, and safety.[3]

Alcohol dehydrogenases (ADHs) are enzymes that catalyze the reversible reduction of aldehydes and ketones to the corresponding alcohols.[6] However, ADHs have not been widely used for synthetic purposes in organic chemistry laboratories in part because they require aqueous media, in which many ketone and alcohol substrates are poorly or not at all soluble; this leads to large reaction volumes and complicated product recovery.[2c,e] An obvious solution for this problem, using organic solvents,[7] was first demonstrated by Klibanov and co-workers.[8]

[*] M. M. Musa, Prof. R. S. Phillips
Department of Chemistry
University of Georgia
1001 Cedar St., Athens, GA 30602 (USA)
and
Department of Biochemistry and Molecular Biology
University of Georgia
B122 Life Sciences Building, Athens, GA 30602 (USA)
Fax: (+1) 706-542-9454
E-mail: rsphillips@chem.uga.edu
K. I. Ziegelmann-Fjeld, Dr. C. Vieille, Prof. J. G. Zeikus
Department of Biochemistry and Molecular Biology
Michigan State University
410 Biochemistry Building, East Lansing, MI 48824 (USA)

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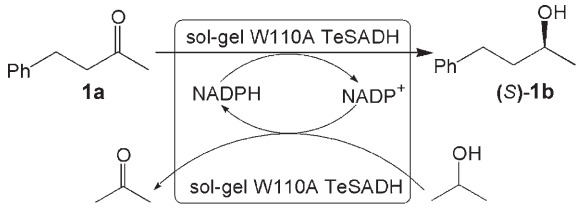
Secondary ADH (EC 1.1.1.2) from *Thermoanaerobacter ethanolicus* (TeSADH), a nicotinamide adenine dinucleotide phosphate ($NADP^+$)-dependent thermostable enzyme,[9,10] is a useful biocatalyst for synthetic applications because it tolerates organic solvents and it accepts ketones and alcohols as substrates with high activities.[11,12] TeSADH obeys Prelog's rule, in which the coenzyme NADPH delivers its *pro-R* hydride from the *Re* face of ketone substrates.[13] Recently, we have reported a new mutant of TeSADH, in which tryptophan-110 was replaced with alanine, W110A TeSADH.[14] Although this mutant is able to reduce phenyl-ring-containing ketones at concentrations of 35 mM to produce their corresponding *S*-configured alcohols in Tris-HCl buffer solution/2-propanol (70:30 v/v; Tris = tris(hydroxymethyl)aminomethane), higher substrate concentrations are required for practical production of optically active alcohols.

Herein, we report the use of encapsulated W110A TeSADH in sol-gel glasses to overcome the aforementioned limitation. In 2003, Gröger et al. reported a practical asymmetric enzymatic reduction of poorly water-soluble ketones by using an ADH-compatible biphasic reaction medium.[15] One problem associated with using mixed aqueous and organic solvents, water-miscible or -immiscible, for enzymatic reactions is the tendency of these solutions to form emulsions in the workup, which causes problems of product separation. If the water, necessary for enzyme activity, is entrapped with the enzyme within the sol gel, the workup procedure can be simplified by using water-immiscible organic solvents, and therefore emulsion formation can be avoided.

Sol-gel-encapsulated W110A TeSADH was prepared as previously reported,[5,16] although with some modifications. The sol gel was kept in Tris-HCl buffer solution medium until it was used as a wet sol gel (hydrogel). The asymmetric reduction of 4-phenyl-2-butanone (**1a**) to (*S*)-4-phenyl-2-butanol ((*S*)-**1b**), a precursor for the synthesis of bufeniode and labetalol (antihypertensive agents),[17] was used as a model in the screening reactions in this study. The hydrogel-encapsulated W110A TeSADH was used to reduce **1a** to (*S*)-**1b** in several different solvent systems (Table 1). The reduction carried out in aqueous buffer solution gave almost the same yield as with the free enzyme.[14a] However, the same sol gel was reused three more times to give 56%, 30%, and 10% conversion, respectively. It was necessary to add 2.0 mg of $NADP^+$ for every new reaction because $NADP^+$ molecules either escape from the pores of the sol-gel glasses or become inactivated during turnover.[18a] The asymmetric reduction of **1a** was also carried out in Tris-HCl

Table 1: Asymmetric reduction of **1a** by using sol-gel-encapsulated W110A TeSADH in different media.[a]



Solvent	Hydrogel		Xerogel	
	Conv. [%][b]	*ee* [%][c]	Conv. [%][b]	*ee* [%][c]
50 mM Tris-HCl pH 8.0[d]	93 (1st)	98	92	98
	56 (2nd)	98		
	30 (3rd)	98		
	10 (4th)	98		
50 mM Tris-HCl /CH_3CN (1:1)[d]	81 (1st)	97	–	–
	43 (2nd)	97	–	–
hexane	80	96	74	97
diisopropyl ether	40	97	–	–

[a] Unless otherwise stated, all reactions were performed at 50 °C by using sol-gel samples containing W110A TeSADH (0.43 mg) and $NADP^+$ (3.0 mg) encapsulated in sol gel, **1a** (0.34 mmol), 2-propanol (600 μL), and 2.0 mL solvent. [b] % conversion was determined by GC. [c] *ee* values were determined by chiral stationary-phase GC for the corresponding acetate derivative.[24] [d] 50 mM Tris-HCl buffer solution, pH 8.0 (3.5 mL) and 2-propanol (1.5 mL). [e] 50 mM Tris-HCl buffer solution, pH 8.0 (1.5 mL), CH_3CN (1.5 mL), and 2-propanol (600 μL).

buffer solution/acetonitrile/2-propanol (41:41:18 v/v) to produce (*S*)-**1b** in good yield (81 %). When the same sol gel was reused, the yield was lower (43 %). This indicates that W110A TeSADH is not inactivated by polar solvents. In all cases (*S*)-**1b** was produced with high enantioselectivity (> 96 % *ee*).

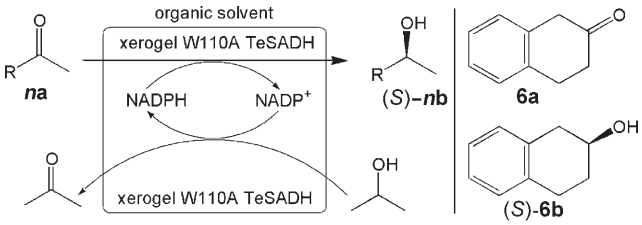
The asymmetric reduction of **1a** to (*S*)-**1b** was also performed in hexane and diisopropyl ether to give good to moderate conversions (80 % and 40 %, respectively) by using hydrogel-encapsulated W110A TeSADH (Table 1). Although **1a** was reduced with a higher yield in the aqueous medium by using sol-gel-encapsulated W110A TeSADH, the use of organic solvents makes the process more efficient by allowing high concentrations of substrates (≈ 140 mM) to be used. It also makes this asymmetric reduction accessible to hydrophobic substrates.

The W110A TeSADH hydrogel was dried in air for 24 h to form a xerogel ($SiO_2 \cdot n\,H_2O$). When this xerogel was used for asymmetric reduction of **1a** in Tris-HCl buffer solution/2-propanol (70:30 v/v), it gave the same conversion as that achieved by the hydrogel (Table 1). Asymmetric reduction of **1a** by using the xerogel-encapsulated W110A TeSADH in hexane gave 74 % conversion as compared with 80 % with the hydrogel form. These results indicate that the xerogel retains the essential water molecules required for enzyme activity.[18b] Use of the xerogel instead of the hydrogel is preferable as it simplifies the workup procedure.[18c]

The lower yield for the asymmetric reduction with a sol-gel-encapsulated enzyme compared with the reduction using free enzyme[14a] could be due to the slow diffusion of substrate, product, and co-substrate into and out of the sol-gel glasses. Regardless, the use of sol-gel-encapsulated ADHs is of great advantage for several reasons beside the ease of the workup procedure. First, it makes these enzymes more stable than the free form, which makes them more attractive to organic chemists. Second, it allows the reuse of the enzyme. Third, it might allow these redox reactions to be mixed in situ with other organic reactions.

A series of phenyl-ring-containing ketones were reduced by using xerogel-encapsulated W110A TeSADH in hexane as a solvent and 2-propanol as a co-substrate to produce their corresponding *S* alcohols with good yields and high enantioselectivities (Table 2). All reactions were performed at

Table 2: Asymmetric reduction of phenyl-ring-containing ketones by using xerogel W110A TeSADH in organic solvents.[a,b]



n	R	Solvent	Conv. [%][c,d]	*ee* [%][c,e]
1	$Ph(CH_2)_2$		74 (99)	97 (> 99)
2	$PhOCH_2$		> 99 (> 99)	> 99 (> 99)
3	*p*-$MeOC_6H_4(CH_2)_2$		61 (87)	94 (91)
4	$PhCH_2$	hexane	80 (95)	69 (37)
	$PhCH_2$	toluene	24	55
	$PhCH_2$	diisopropyl ether	37	73
	$PhCH_2$	*tert*-butyl alcohol	38	63
5	*p*-$MeOC_6H_4CH_2$		67 (97)	> 99 (> 99)
6	(see structure under table heading)		94 (> 99)	76 (71)

[a] All reactions were performed at 50 °C by using W110A TeSADH (0.43 mg) and $NADP^+$ (3.0 mg) encapsulated in xerogel, substrate (0.34 mmol), 2-propanol (600 μL), and 2.0 mL hexane. [b] The absolute configuration was determined as described previously.[14a] [c] Results of reduction with free W110A TeSADH in 50 mM Tris-HCl buffer solution (pH 8.0)/2-propanol (70:30 v/v) are given in parentheses.[14a] [d] % conversion was determined by GC. [e] *ee* values were determined by chiral stationary-phase GC for the corresponding acetate derivative.[24]

140 mM substrate concentrations. 1-Phenoxy-2-propanone (**2a**) was reduced with a very high yield and enantioselectivity to produce (*S*)-1-phenoxy-2-propanol ((*S*)-**2b**). (*S*)-4-(4′-Methoxyphenyl)-2-butanol ((*S*)-**3b**) was obtained from the enantioselective reduction of 4-(4′-methoxyphenyl)-2-butanone (**3a**) with a moderate yield and a higher enantioselectivity when compared with the same alcohol produced by asymmetric reduction with free W110A TeSADH in Tris-HCl buffer solution (Table 2).[14a] Although 1-phenyl-2-propanone (**4a**) was reduced to (*S*)-1-phenyl-2-propanol ((*S*)-**4b**) with high yield but a rather low *ee* value (37 %) in aqueous

media,[14a] we were pleased to obtain a good yield and significantly improved enantioselectivity (69% *ee*) by using xerogel W110A TeSADH in hexane. The asymmetric reduction of **4a** was also performed by using xerogel W110A TeSADH in toluene, *tert*-butyl alcohol, and diisopropyl ether to produce (*S*)-**4b** with 55, 63, and 73% *ee*, respectively. This indicates that the solvent can affect the enzyme enantioselectivity.[19] The lower yield in toluene (Table 2) may be due to competitive inhibition of aromatic ketone binding by toluene. The enantioselectivity of the reduction of **4a** by W110A TeSADH correlates neither with the hydrophobicity nor with the dipole moment of the solvent. This is consistent with the recent study of ADH-catalyzed reactions in biphasic systems by Filho et al.,[20] who reported that a single physicochemical parameter does not predict the biocompatibility of organic solvents but rather the solvent functionality would be of great significance. 1-(4′-Methoxyphenyl)-2-propanone (**5a**) was reduced by using the xerogel W110A TeSADH with a lower yield but the same *ee* value when compared with that using the free enzyme, which produces (*S*)-1-(4-methoxyphenyl)-2-propanol ((*S*)-**5b**). The cyclic ketone, 2-tetralone (**6a**), was reduced to the corresponding (*S*)-2-tetralol ((*S*)-**6b**) by the xerogel with yields comparable with that produced by using free W110A TeSADH in aqueous medium. However, the *ee* value of (*S*)-**6b** was improved in hexane by using the xerogel (Table 2).

The low enantioselectivity observed in the reduction of **4a** and **6a** is a result of binding of these substrates in alternative ways within the large pocket of the active site,[14a] allowing NADPH to deliver its *pro-R* hydride to either the *Re* face or the *Si* face of the substrate. The improvement in enantioselectivity observed when these substrates are reduced by the xerogel W110A TeSADH in organic solvents is likely due to differences in solvation of the enzyme active site.[21] In an aqueous environment, the binding of a large substrate must displace solvent water from the active site. The binding of the substrate in the "wrong" orientation may actually displace more water, making it favorable entropically.[22] In a non-aqueous medium, this entropic advantage would be diminished. We have previously proposed that active-site solvation plays a significant role in the stereospecificity of aliphatic secondary alcohols by TeSADH.[23]

To our knowledge, this is the first report of a preparative-scale asymmetric reduction by using xerogel-encapsulated ADH in pure organic solvent media. This study clearly demonstrates that the misconception that practical non-aqueous enzymology is limited to hydrolases is false.

In summary, the tolerance of TeSADH to high concentrations of organic solvents allows asymmetric reduction of phenyl-ring-containing hydrophobic ketones by using xerogel-encapsulated W110A TeSADH. Sol-gel immobilization is a convenient method not only for reusing the enzyme but also for making the enzyme accessible to a wide variety of water-insoluble substrates by switching the traditional aqueous medium to organic media. This new method allows for the use of high concentrations of substrates that are crucial for large-scale synthetic applications. Reusable catalysts for chemo-, regio-, and enantioselective asymmetric reduction may be of industrial interest.

Experimental Section

Commercial grade solvents were used without further purification. $NADP^+$, tetramethyl orthosilicate (TMOS), **1a**–**3a**, and **6a** were used as purchased from commercial suppliers. Compounds **4a** and **5a** were prepared as described previously.[25]

Gene expression and purification of W110A TeSADH: W110A TeSADH was expressed in recombinant *Escherichia coli* HB101-(DE3) cells and purified as described.[14b]

Preparation of sol-gel-encapsulated W110A TeSADH: The silica sol was prepared by mixing TMOS (2.10 g), distilled water (0.47 g), and HCl (0.04 M, 3 drops). The mixture was then sonicated until one layer was formed. The gels were prepared by mixing the above sol (1.0 mL) with enzyme stock (1.0 mL) in a 10 mL round-bottomed flask. The enzyme stock was prepared in 50 mM Tris-HCl buffer solution (pH 8.0) such that the concentration of the enzyme was 0.43 mg mL^{-1} and that of $NADP^+$ was 3.0 mg mL^{-1}. The sol gel was then left in the same flask and closed with Parafilm at room temperature for 48 h to allow gel aging. In the case of the hydrogel, the gel was then used without further treatment. The hydrogel was dried at room temperature in air for 24 h to give hydrated silica $SiO_2 \cdot n H_2O$, the so-called xerogel.

Asymmetric reduction using xerogel-encapsulated W110A TeSADH in organic solvents: Unless otherwise mentioned, all reactions were performed with W110A TeSADH (0.43 mg) and $NADP^+$ (3.0 mg) encapsulated in sol gel, substrate (0.34 mmol), 2-propanol (600 μL), and organic solvent (2.0 mL) in a 10 mL round-bottomed flask equipped with a magnetic stirrer. The reaction mixture was stirred at 50 °C for 12 h. The sol gel was then removed by filtration and washed with ethyl acetate (2 × 2 mL). The combined organic solvent was then concentrated under vacuum, and the remaining residue was analyzed by chiral-column GC to determine the yield. The residue was then converted into the corresponding acetate ester derivative to determine the *ee* value of the product alcohol by GC.[23]

Capillary GC measurements were performed on a Varian 3300 GC equipped with a flame-ionization detector and a Supelco β-Dex 120 chiral column (30 m, 0.25 mm (internal diameter), 0.25 μm film thickness) by using He as the carrier gas. All products were isolated and characterized as described previously.[14a] Their absolute configurations were determined by coinjection on a chiral-column GC with their *S*- or *R*-configured alcohols, which were prepared by asymmetric reduction of the corresponding ketones or kinetic resolution of the corresponding racemates by using free W110A TeSADH.[14a]

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