Activity and selectivity of W110A secondary alcohol dehydrogenase from *Thermoanaerobacter ethanolicus* in organic solvents and ionic liquids: mono- and biphasic media

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The asymmetric reduction of hydrophobic phenyl-ring-containing ketones and the enantiospecific kinetic resolution of the corresponding racemic alcohols catalyzed by *Thermoanaerobacter ethanolicus* W110A secondary alcohol dehydrogenase were performed in mono- and biphasic systems containing either organic solvents or ionic liquids. Both yield and enantioselectivity for these transformations can be controlled by changing the reaction medium. The enzyme showed high tolerance to both water-miscible and -immiscible solvents, which allows biotransformations to be conducted at high substrate concentrations.

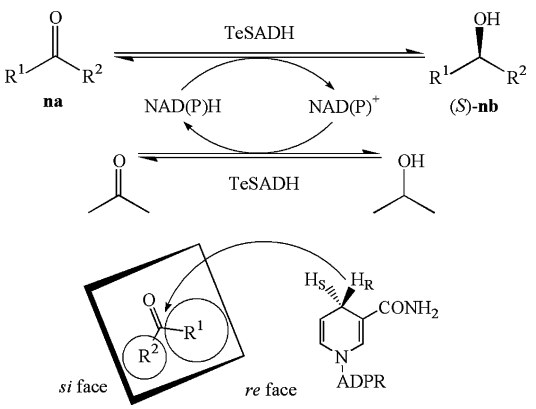
Introduction

Using biocatalysts in organic synthesis has become an effective methodology for the production of optically active compounds due to the high chemo-, regio-, and enantioselectivities of enzymes.[1] The natural environment for biocatalysts is an aqueous medium, which, in most cases, does not satisfy organic chemists, because most interesting substrates and products are either insoluble or only sparingly soluble in aqueous media. One solution for this limitation is the use of organic solvents.[2] A similar, recently developed solution is the use of room temperature ionic liquids (ILs) as solvents, which are known as environmentally friendly because they are nonvolatile and nonflammable.[3] Both organic solvents and ILs can be used as cosolvents with aqueous media as either monophasic or biphasic systems to enhance the solubility of hydrophobic substrates in biocatalytic transformations.

Alcohol dehydrogenases (ADHs, EC 1.1.1.X, X = 1 or 2) are enzymes that catalyze the reversible reduction of ketones and aldehydes to the corresponding alcohols.[4] There has been great interest in the use of ADHs in asymmetric synthesis to produce enantiomerically pure alcohols, which are important building blocks in pharmaceutical and agricultural compounds.[5] Gröger *et al.* reported a practical method for asymmetric reductions of poorly water-soluble ketones using *Rhodococcus erythropolis* ADH in water/*n*-heptane (4 : 1, v/v) biphasic systems with satisfactory conversions.[6] Recently, Gonzalo *et al.* reported a method for enzymatic reduction of ketones catalyzed by *Rhodococcus ruber* ADH-A in micro-aqueous media.[7] This method allowed substrate concentrations as high as 2.0 M.

Most enzymatic reactions that have been used in organic synthesis involve lipases because of their availability, thermal stability and high tolerance to organic solvents.[1,2] Several reports have shown that the activity and enantioselectivity of lipases can be controlled by changing the reaction medium.[2c,8] It is of great interest to check the performance of ADHs in nonaqueous media.

We have been studying *Thermoanaerobacter ethanolicus* secondary ADH (TeSADH, EC 1.1.1.2), a nicotinamide-adenine dinucleotide phosphate ($NADP^+$)-dependent enzyme.[9] This enzyme is thermally stable, it accepts ketones and alcohols as substrates with high activities, and it resists denaturation in organic solvents.[10] For the above-mentioned reasons, TeSADH is a useful biocatalyst for synthetic applications.[11] 2-Propanol and acetone can be used as cosubstrates in the reduction and oxidation pathways, respectively, to regenerate the coenzyme. This therefore makes the process catalytic as shown in Scheme 1. Recently, we designed a new TeSADH mutant, where tryptophan-110 was replaced with alanine (W110A TeSADH).[12] This mutant accepts phenyl-ring-containing ketones and their corresponding alcohols as substrates with high enantioselectivities and enantiospecificities.[13] The enzyme TeSADH and its mutant W110A TeSADH obey Prelog's rule, in which NADPH delivers its *pro-R* hydride from the *re* face of the ketone (Scheme 1).[14] We have also shown that



Scheme 1 Prelog's rule for predicting the stereochemical outcome for ADH-catalyzed asymmetric reduction.

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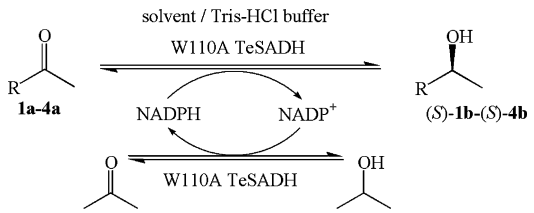
Table 1 Asymmetric reduction of phenyl-ring-containing ketones by W110A TeSADH in water-miscible solvents (monophasic).[a]



Substrate	R	Product[b]	Solvent	Conv. (%)[c]	ee (%)[d]
1a	$Ph(CH_2)_2$	(*S*)-**1b**	[bmim][BF_4]	86	98
			DMF	95	95
			Acetonitrile	94	97
			tert-Butanol	85	97
2a	$PhOCH_2$	(*S*)-**2b**	[bmim][BF_4]	96	97
3a	4-$MeOC_6H_4(CH_2)_2$	(*S*)-**3b**	[bmim][BF_4]	40	87
			DMF	35	86
			Acetonitrile	28	94
4a	$PhCH_2$	(*S*)-**4b**	[bmim][BF_4]	88	38
			DMF	97	56
			Acetonitrile	90	62

[a] Conditions: substrate (0.244 mmol), $NADP^+$ (1.33 mg), solvent (1.0 mL), W110A TeSADH (0.48 mg), Tris-HCl buffer (1.0 mL, 50 mM, pH 8.0), and 2-propanol (600 μL). [b] The absolute configuration was confirmed by coinjection of their acetate derivatives with both (*S*) and (*R*)-acetates made before either by enantioselective reduction or enantiospecific oxidation using W110A TeSADH.[13] [c] % Conversion was determined by GC. [d] % ee values were determined on the corresponding acetate by a GC equipped with a chiral column as described.[13]

xerogel-encapsulated W110A TeSADH can be used in organic solvents as a solution to the solubility problem for hydrophobic phenyl-ring-containing ketones and their corresponding alcohols.[15] In the same report, we noticed that the reaction's enantioselectivity was higher when substrates such as phenylacetone were reduced in organic solvents than in aqueous media.[15]

In this paper, we report the results of asymmetric reductions and oxidations using W110A TeSADH in ILs as environmentally friendly solvents, representing a solution for the problem of poor solubility of the substrates and products. 1-Butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF_4]) was used as a cosolvent with Tris-HCl buffer in monophasic systems. 1-Butyl-3-methylimidazolium bis((trifluoromethyl)sulfonyl)imide ([bmim][NTf_2]), which is water-immiscible, was used as the nonaqueous phase in biphasic systems. Those transformations were also conducted in water-miscible and water-immiscible organic solvents for comparison. Yields and stereoselectivities for each W110A TeSADH-catalyzed biotransformation were compared in the different solvent systems to show that both could be affected by changing the reaction medium.

Results and discussion

Asymmetric reduction using W110A TeSADH in monophasic systems

The asymmetric reductions of phenyl-ring-containing ketones catalyzed by W110A TeSADH were conducted in media containing Tris-HCl buffer and water-miscible nonaqueous solvents to enhance the solubility of hydrophobic substrates. 4-Phenyl-2-butanone (**1a**) was reduced in high yield and high enantioselectivity to produce (*S*)-4-phenyl-2-butanol ((*S*)-**1b**) in a monophasic medium containing [bmim][BF_4], 50 mM Tris-HCl buffer (pH 8.0), and 2-propanol (38 : 38 : 24 [v/v/v]). 2-Propanol was used as a cosubstrate to regenerate NADPH from the oxidized $NADP^+$ and therefore make the process catalytic. Although an IL cosolvent was used, 2-propanol had to be used in excess to shift the equilibrium to the reduction direction. The same results were obtained when water-miscible organic solvents such as dimethyl formamide (DMF), acetonitrile, or *tert*-butanol were used as cosolvents (Table 1). This means that W110A TeSADH remains active in water-miscible organic solvents or ILs in high concentrations (>60% by volume), which is remarkable for an ADH. With only a few exceptions in the literature,[16] ADHs are observed to be unstable under these conditions.[4c,d] Under every condition, (*S*)-**1b** was produced in high yield and enantioselectivity.

Impressed with the activity of W110A TeSADH in media containing such a high percentage of nonaqueous water-miscible solvents, we investigated the asymmetric reductions of other phenyl-ring-containing ketones using [bmim][BF_4], DMF, or acetonitrile as the cosolvent and 2-propanol as the cosubstrate. Using [bmim][BF_4] as the cosolvent, phenoxy-2-propanone (**2a**) was reduced to (*S*)-phenoxy-2-propanol ((*S*)-**2b**) with high yield and enantioselectivity. The asymmetric reduction of 4-(4′-methoxyphenyl)-2-butanone (**3a**) using [bmim][BF_4] as the cosolvent afforded 4-(4′-methoxyphenyl)-2-butanol ((*S*)-**3b**) in 40% yield and 87% ee. Similar results were obtained when DMF or acetonitrile were used as the cosolvent; however the % ee was higher when acetonitrile was used as cosolvent. 1-Phenyl-2-propanol ((*S*)-**4b**) was obtained from the asymmetric reduction of 1-phenyl-2-propanone (**4a**) in high yield and 38% ee when [bmim][BF_4] was used as the cosolvent, in agreement with the results obtained previously for the asymmetric reduction of **4a** using W110A TeSADH in monophasic medium using 2-propanol as both the cosolvent and cosubstrate.[13] However, when the same asymmetric reduction was conducted in DMF or acetonitrile,

an improved ee was noticed (Table 1). Similar enantioselectivity enhancements for secondary alcohol dehydrogenase from *Thermoanaerobacterium sp.* KET4B1 were repoted by Simpson and Cowan.[17] The improved enantioselectivities noticed when DMF or acetonitrile were used as cosolvents in the asymmetric reduction of **4a** can be explained by differences in solvation of the enzyme active site proposed previously.[15,18] For **1a**, **3a** and **4a**, it was noticed that their asymmetric reduction was achieved with higher enantioselectivity using acetonitrile as the cosolvent than using DMF. The results in Table 1 show that the enantioselectivities of the asymmetric reduction reactions catalyzed by W110A TeSADH in media containing Tris-HCl buffer, 2-propanol as the cosubstrate, and [bmim][BF_4] as the cosolvent are similar to those in Tris-HCl buffer and 2-propanol as both the cosolvent and cosubstrate.[13]

Asymmetric reduction using W110A TeSADH in biphasic systems

The asymmetric reductions of hydrophobic ketones catalyzed by W110A TeSADH were conducted in biphasic media with either ILs or organic solvents as the nonaqueous phase. The W110A TeSADH-catalyzed asymmetric reduction of **1a** to (*S*)-**1b** was investigated in a biphasic system containing [bmim][NTf_2], a water-immiscible IL, 50 mM Tris-HCl buffer (pH 8.0), and 2-propanol as the cosubstrate (Table 2). This reaction was also investigated in a series of water-immiscible organic solvents (Table 2). (*S*)-**1b** was produced with high enantioselectivities in all cases; however the percentage conversions were different from one solvent to the other. The asymmetric reduction in the biphasic system containing [bmim][NTf_2] as the nonaqueous phase had a lower yield than in the biphasic systems containing cyclohexane, hexane, heptane, or di*iso*propyl ether (DIPE), but it had a higher yield than in the biphasic systems containing toluene and *tert*-butyl methyl ether (TBME). Eckstein *et al.* reported the first example of asymmetric reduction using ADH in a biphasic system containing [bmim][NTf_2].[3d] They also reported that taking advantage of the partition coefficients of 2-propanol and acetone, 2-propanol preferably remains in the aqueous phase and improved ADH-catalyzed reduction yields are obtained. We believe that the partition coefficients of 2-propanol and acetone between aqueous medium and [bmim][NTf_2] are not the only factor that controls the percentage conversion in these biphasic systems because the partition coefficients of ketone substrates and their corresponding alcohols also play an important role. It is always good to consider environmentally friendly IL solvents as substitutes to organic solvents but they are not always the best in terms of percentage yield. Reducing the 2-propanol concentration when hexane was used as the organic solvent resulted in decreases in both percentage conversion and ee, which can be explained due to time-dependent racemization as the possibility of reversibility increases. The asymmetric reduction of **1a** catalyzed by xerogel-encapsulated W110A TeSADH, prepared as described,[15] was also conducted in [bmim][NTf_2] as the solvent to produce only about 10% of (*S*)-**1b** after 48 hours.

The asymmetric reductions of **2a** and **3a** catalyzed by W110A TeSADH were conducted in a biphasic system containing [bmim][NTf_2] to produce (*S*)-**2b** and (*S*)-**3b** with yields and enantioselectivities comparable to those achieved previously in aqueous media with 2-propanol as the cosolvent.[13] Under the same conditions, (*S*)-**4b** was produced from the asymmetric reduction of **4a** with high yield and higher ee than obtained in monophasic systems with 2-propanol or [bmim][BF_4] as the cosolvent (Table 1 and Table 2).[13] The same enantioselectivity enhancement was noticed when the asymmetric reduction of **4a** was conducted in water-immiscible organic solvents such as hexane, toluene, or DIPE using xerogel-encapsulated W110A TeSADH.[15] This enantioselectivity enhancement can be explained by differences in solvation of the enzyme active site as proposed previously.[15,18] It can also be explained as the result of the substrate concentration in the aqueous phase in biphasic systems being lower than in the monophasic systems containing 2-propanol, water-miscible IL, or organic solvent as the cosolvent with 2-propanol as the cosubstrate.

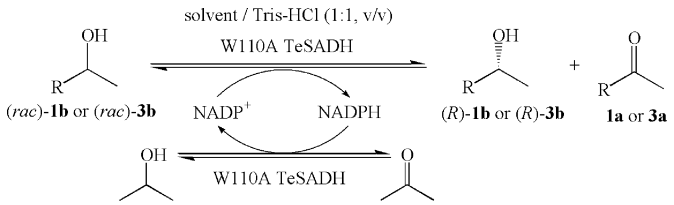
The use of biphasic systems in enzymatic transformations is of great interest not only because of the ease of work-up, but also because the enzyme and its cofactor are dissolved in the aqueous phase, where the reaction takes place, while the reactant, product, cosubstrate, and coproduct are all distributed in the two phases, in most cases preferentially in the nonaqueous phase. This distribution reduces the possibility of enzyme inhibition by the

Table 2 Asymmetric reduction of phenyl-ring-containing ketones by W110A TeSADH in biphasic systems[a]

Substrate	R	Product[b]	Solvent	2-Propanol (eq.)	Conv. (%)[c]	ee (%)[d]
1a	$Ph(CH_2)_2$	(*S*)-**1b**	[bmim][NTf_2]	16	65	97
			DIPE	16	94	99
			TBME	16	59	96
			Toluene	16	36	97
			Cyclohexane	16	83	97
			Hexane	16	95	96
			Hexane	8	87	94
			Hexane	4	69	81
			Heptane	16	92	96
2a	$PhOCH_2$	(*S*)-**2b**	[bmim][NTf_2]	16	96	>99
3a	4-$MeOC_6H_4(CH_2)_2$	(*S*)-**3b**	[bmim][NTf_2]	16	52	88
4a	$PhCH_2$	(*S*)-**4b**	[bmim][NTf_2]	16	> 99	60

[a] Conditions: substrate (0.244 mmol), $NADP^+$ (1.33 mg), water-immiscible solvent (1.0 mL), W110A TeSADH (0.48 mg), Tris-HCl buffer (1.0 mL, 50 mM, pH 8.0), and 2-propanol. [b] The absolute configuration was confirmed by coinjection with both (*S*)- and (*R*)-alcohols made beforehand.[13] [c] % Conversion was determined by GC. [d] % ee values were determined on the corresponding acetate by a GC equipped with a chiral column as described.[13]

Table 3 Enantiospecific oxidation of phenyl-ring-containing alcohols by W110A TeSADH in biphasic media.[a]



Substrate	R	Product[b]	Solvent	Acetone (eq.)	*c* (%)[c]	ee (%)[d]	*E*-value[e]
rac-**1b**	$Ph(CH_2)_2$	(*R*)-**1b**	[bmim][NTf_2]	4	44	80	> 100
			TBME	4	31	38	17
			DIPE	4	44	75	97
			Toluene	4	18	22	> 100
			Cyclohexane	4	48	83	49
			Hexane	4	48	78	28
rac-**3b**	4-$MeOC_6H_4(CH_2)_2$	(*R*)-**3b**	[bmim][NTf_2]	4	52	61	7
			TBME	4	19	22	39
			DIPE	4	41	52	12
			Toluene	4	20	25	> 100
			Hexane	4	58	9	1.3
			Hexane	8	66	51	2.7
			Hexane	16	63	80	6.4

[a] Conditions: substrate (0.17 mmol), $NADP^+$ (1.0 mg), solvent (750 μL), Tris-HCl buffer (750 μL, 50 mM, pH 8.0) containing W110A TeSADH (0.24 mg), and acetone. [b] The absolute configuration was confirmed by comparing the retention time with that for the *S* enantiomer. [c] % Conversion was determined by GC. [d] % ee values were determined on the corresponding acetate by a GC equipped with a chiral column as described.[13] [e] *E*-value was calculated as described in the experimental section.

substrate, product or solvent. Another advantage of using biphasic systems for enzymatic reactions is the ability to recycle the enzyme easily.

Enantiospecific kinetic resolution using W110A TeSADH in biphasic systems

It is of great interest to generate both enantiomers of chiral alcohols with high optical purities. Because most ADHs follow Prelog's rule,[4d] producing (*S*)-enantiomers in most cases, it is important to develop methods that produce the *anti*-Prelog enantiomers using ADHs. One way to do this is to use an (*S*)-selective ADH in the oxidation direction (*i.e.* kinetic resolution [KR]). If, instead of 2-propanol, acetone is used as the cosubstrate in ADH-catalyzed biotransformations, KR will be achieved, thus stereospecifically converting the (*S*)-enantiomer to the corresponding ketone and leaving the (*R*)-enantiomer with a maximum theoretical yield of 50% with high ee. We have shown that W110A TeSADH can be used to catalyze the stereospecific KR of a series of phenyl-ring-containing alcohols to produce their (*R*)-enantiomers with moderate to high ee in aqueous media containing acetone as both the cosolvent and cosubstrate.[13] Because acetone is known to inhibit ADHs and it cannot be used as a cosolvent at high concentrations, substrate and product solubilities remain major issues. For this reason, using an alternative cosolvent that can minimize the amount of acetone needed for enantiospecific KR might be advantageous.

The enantiospecific KR of *rac*-**1b** catalyzed by W110A TeSADH was investigated in a biphasic system containing [bmim][NTf_2] as the nonaqueous phase with acetone as the cosubstrate. (*S*)-**1b** was converted enantiospecifically to **1a**, leaving (*R*)-**1b** as the unreacted enantiomer with a high *E*-value (Table 3). Acetone was used as a cosubstrate in relatively low concentrations (4 eq.) instead of being used as a cosolvent as previously.[13] The same asymmetric transformation was conducted in several water-immiscible organic solvents (Table 3) to produce (*R*)-**1b** with high *E*-value. In agreement with the results obtained in Table 2 for asymmetric reduction of **1a**, enantiospecific KR of *rac*-**1b** gave higher yields when either [bmim][NTf_2], hexane, or DIPE were used as the nonaqueous phase than when either TBME or toluene were used. This result is a clear indication that the partition coefficients of 2-propanol and acetone are not the only factors that control the equilibrium in asymmetric redox reactions catalyzed by ADHs. In all cases the *E*-value was higher than 17, which indicates that this reaction is a very specific KR.[19]

We decided to carry out the enantiospecific KR of *rac*-**3b** under the previous conditions to clarify the observed change of the enantioselectivity upon reaction medium used for these biotransformations. Since **3a** was reduced to (*S*)-**3b** with lower enantioselectivity than **1a** using W110A TeSADH, it is expected that the *E*-value for enantiospecific KR of *rac*-**3b** will be less than that for *rac*-**1b**. In agreement with the results obtained for enantiospecific KR of *rac*-**1b** in biphasic systems, enantiospecific KR of *rac*-**3b** in a biphasic system gave a higher percentage conversion with either [bmim][NTf_2], DIPE, or hexane as the non-aqueous phase than with toluene or TBME. The lowest *E*-value was obtained with hexane but this *E*-value increased dramatically when the acetone concentration was increased, which can be explained as the result of reducing the possibility of reversibility by increasing the amount of acetone used. Although toluene showed low percentage conversion in both the reduction and oxidation

directions, it exhibited the highest stereospecificity (*i.e.*, the highest *E*-value for substrates *rac*-**1b** and *rac*-**3b**).

It is not easy to conclude which solvent is the best for a specific ADH-catalyzed biotransformation where a cosubstrate (here, either 2-propanol or acetone) is needed to regenerate the coenzyme. At this time, we can conclude that the selectivity of ADH-catalyzed transformations can be controlled by changing the reaction medium; however there is no correlation between either the hydrophobicity or dielectric constant of the cosolvent and the enantioselectivity of TeSADH. This is consistent with previous studies for ADH-catalyzed reactions in both water-miscible and -immiscible organic solvents.[15,17,20]

Experimental

General

Capillary gas chromatographic measurements were performed on a GC equipped with a flame ionization detector and a Supelco β-Dex 120 chiral column (30 m, 0.25 mm [i.d.], 0.25 μm film thickness) using helium as the carrier gas. ^{1}H NMR and ^{13}C NMR spectra were recorded on 400 MHz spectrometer at room temperature in $CDCl_3$ using either solvent peak or tetramethylsilane as internal standard. All reactions were performed in a 10 mL round-bottomed flask equipped with a magnetic stirrer and condenser. Commercial grade solvents were used without further purification. $NADP^+$, *Candida antarctica* Lipase B (Novozyme 435), phenylacetic acid, acetic anhydride, and $NaBH_4$ were used as purchased from commercial sources. Ketones **1a–3a** and *rac*-**1b** were used as purchased from commercial suppliers. The ketone **4a** was prepared as described.[21] *rac*-**3b** was prepared by reducing **3a** with $NaBH_4$ as described.[22]

Gene expression and purification of W110A TeSADH

W110A TeSADH was expressed in recombinant *Escherichia coli* HB101(DE3) cells and purified as described.[12]

Determination of absolute configuration

The absolute configurations of both alcohol enantiomers were determined by co-injection on a chiral GC column with samples prepared previously either by asymmetric reduction or oxidation using TeSADH or by KR using Novozyme 435 as described.[13] All % ee values were determined on the acetate form by a GC equipped with a chiral column as described.[13]

Preparation of [bmim][BF_4] and [bmim][NTf_2]

[bmim][BF_4] was prepared as described.[23] To prepare [bmim][NTf_2], [bmim][BF_4] (3.94 g, 17.4 mmol) and bis(trifluoromethane)sulfonimide lithium salt (5.0 g, 17.4 mmol) were mixed in distilled water (7.0 mL) in a 50-mL round bottomed flask. The mixture was stirred at room temperature for 2 h to form two layers. After removing the water under vacuum, methylene chloride (20 mL) was added to the residue, which contains [bmim][NTf_2] and $LiBF_4$. The solution was then filtered and dried with Na_2SO_4. The solvent was then removed under vacuum to produce the ionic liquid [bmim][NTf_2] as a colorless oil. Spectral data were consistent with those reported.[24]

General procedure for asymmetric reduction using W110A TeSADH in monophasic systems

A mixture of substrate **na** (0.244 mmol), $NADP^+$ (1.33 mg), water-miscible organic solvent or IL (1.0 mL), Tris-HCl buffer (1.0 mL, 50 mM, pH adjusted to 8.0 at 25 °C) containing W110A TeSADH (0.48 mg), and 2-propanol (quantities described in Table 1) was stirred at 50 °C for 24 h. The reaction mixture was extracted with diethyl ether (3 × 2 mL). The combined organic layers were dried with Na_2SO_4. A sample was injected in a GC to determine the percentage conversion. The solvent then was removed under vacuum and the remaining residue was treated with pyridine and acetic anhydride to convert the product alcohol to the corresponding acetate as reported,[22] which was analyzed by a chiral column GC to determine the percentage ee.

General procedure for asymmetric reduction using W110A TeSADH in biphasic systems

A mixture of substrate **na** (0.244 mmol), $NADP^+$ (1.33 mg), solvent (1.0 mL), Tris-HCl buffer (1.0 mL, 50 mM, pH adjusted to 8.0 at 25 °C) containing W110A TeSADH (0.48 mg), and 2-propanol (quantities described in Table 2) was stirred as two layers at maximum speed to keep a suspension at 50 °C for 24 h. The two layers were then separated and the aqueous layer was extracted with diethyl ether (3 × 2 mL). The combined organic layers were combined with the original organic layer and dried with Na_2SO_4 (In the case of [bmim][NTf_2], the IL layer was extracted with hexane (6 × 2 mL), then the combined hexane layers were combined with the organic layers from the aqueous layer extraction). The product was then analyzed as described above.

General procedure for asymmetric kinetic resolution using W110A TeSADH in monophasic and biphasic systems

A mixture of substrate (*rac*)-**nb** (0.17 mmol), $NADP^+$ (1.0 mg), solvent (750 μL), Tris-HCl buffer (750 μL, 50 mM, pH adjusted to 8.0 at 25 °C) containing W110A TeSADH (0.24 mg), and acetone (quantities described in Table 3) was stirred at 50 °C for 24 h. The reaction mixture was then worked up and its contents were analyzed as described above for the asymmetric reduction. *E*-values were calculated from the formula $E = \ln[(1 - c)(1 - ee_s)]/\ln[(1 - c)(1 + ee_s)]$, where c is percentage conversion of alcohol to ketone, and ee_s is enantiomeric excess of the unreacted (*R*)-alcohol.[25]

Conclusions

The high tolerance of W110A TeSADH to elevated concentrations of both organic solvents and ILs allows asymmetric redox reactions to be conducted in both directions by choosing the appropriate cosubstrate (*i.e.*, 2-propanol or acetone) and by using high concentrations of hydrophobic substrates. The enantioselectivity and yield of the reactions catalyzed by W110A TeSADH can be controlled by changing the reaction medium. The partition coefficients of 2-propanol and acetone in a biphasic system containing an organic solvent or IL are not the only factors that control the equilibrium in the asymmetric transformations catalyzed by ADHs. This study also shows that ADH selectivity

can be tuned by changing the reaction medium. The efficient production of both enantiomers of optically active alcohols is of great interest as they are building blocks for the synthesis of pharmaceutically important molecules.

Acknowledgements

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References

1 (*a*) K. Faber, *Biotransformation in organic chemistry*, 5th ed., Springer-Verlag, New York, 2004; (*b*) R. N. Patel, *Biocatalysis in the pharmaceutical and biotechnology industries*, CRC Press LLC, Boca Raton, Florida, 2007.
2 (*a*) A. M. Klibanov, *Curr. Opin. Biotechnol.*, 2003, **14**, 427–431; (*b*) A. M. Klibanov, *Nature*, 2001, **409**, 241–246; (*c*) G. Carrea and S. Riva, *Angew. Chem., Int. Ed.*, 2000, **39**, 2226–2254.
3 (*a*) K. Schroer, E. Tacha and S. Lutz, *Org. Process Res. Dev.*, 2007, **11**, 836–841; (*b*) T. Matsuda, Y. Yamagishi, S. Koguchi, N. Iwai and T. Kitazume, *Tetrahedron Lett.*, 2006, **47**, 4619–4622; (*c*) Z. Yang and W. Pan, *Enzyme Microb. Technol.*, 2005, **37**, 19–28; (*d*) M. Eckstein, M. Villela, A. Liese and U. Kragl, *Chem. Commun.*, 2004, 1084–1085; (*e*) S. Park and R. J. Kazlauskas, *Curr. Opin. Biotechnol.*, 2003, **14**, 432–437.
4 (*a*) N. Blanchard and P. Weghe, *Org. Biomol. Chem.*, 2006, **4**, 2348–2353; (*b*) T. Daußmann, T. C. Rosen and P. Dünkelmann, *Eng. Life Sci.*, 2006, **6**, 125–129; (*c*) E. Garcia-Urdiales, I. Alfonso and V. Gotor, *Chem. Rev.*, 2005, **105**, 313–354; (*d*) W. Kroutil, H. Mang, K. Edeger and K. Faber, *Curr. Opin. Chem. Biol.*, 2004, **8**, 120–126.
5 (*a*) J. Zhang, B. Witholt and Z. Li, *Adv. Synth. Catal.*, 2006, **348**, 429–433; (*b*) K. Edegger, W. Stampfer, B. Seisser, K. Faber, S. F. Mayer, R. Oehrlein, A. Hafner and W. Kroutil, *Eur. J. Org. Chem.*, 2006, 1904–1909; (*c*) D. Zhu, Y. Yang and L. Hua, *J. Org. Chem.*, 2006, **71**, 4202–4205; (*d*) H. Gröger, F. Chamouleau, N. Orologas, C. Rollmann, K. Drauz, W. Hummel, A. Weckbecker and O. May, *Angew. Chem., Int. Ed.*, 2006, **45**, 5677–5681; (*e*) R. L. Hanson, S. Goldberg, A. Goswami, T. P. Tully and R. N. Patel, *Adv. Synth. Catal.*, 2005, **347**, 1073–1080.
6 H. Gröger, W. Hummel, S. Buchholz, K. Drauz, T. V. Nguyen, C. Rollmann, H. Hüsken and K. Abokitse, *Org. Lett.*, 2003, **5**, 173–176.
7 G. D. Gonzalo, I. Lavandera, K. Faber and W. Kroutil, *Org. Lett.*, 2007, **9**, 2163–2166.
8 (*a*) T. Itoh, Y. Matsushita, Y. Abe, S. Han, S. Wada, S. Hayase, M. Kawatsura, S. Takai, M. Morimoto and Y. Hirose, *Chem.–Eur. J.*, 2006, **12**, 9228–9237; (*b*) K. W. Kim, B. Song, M. Y. Choi and M. J. Kim, *Org. Lett.*, 2001, **3**, 1507–1509.
9 (*a*) D. C. Burdette and J. G. Zeikus, *Biochem.J.*, 1994, **302**, 163–170; (*b*) D. C. Burdette, C. Vieille and J. G. Zeikus, *Biochem. J.*, 1996, **316**, 115–122.
10 (*a*) V. T. Pham and R. S. Phillips, *J. Am. Chem. Soc.*, 1990, **112**, 3629–3632; (*b*) V. T. Pham, R. S. Phillips and L. G. Ljungdahl, *J. Am. Chem. Soc.*, 1989, **111**, 1935–1936.
11 (*a*) C. Heiss, M. Laivenieks, J. G. Zeikus and R. S. Phillips, *Bioorg. Med. Chem.*, 2001, **9**, 1659–1666; (*b*) C. Heiss and R. S. Phillips, *J. Chem. Soc., Perkin Trans. 1*, 2000, 2821–2825.
12 K. I. Ziegelmann-Fjeld, M. M. Musa, R. S. Phillips, J. G. Zeikus and C. Vieille, *Protein Eng. Des. Sel.*, 2007, **20**, 47–55.
13 M. M. Musa, K. I. Ziegelmann-Fjeld, C. Vieille, J. G. Zeikus and R. S. Phillips, *J. Org. Chem.*, 2007, **72**, 30–34.
14 V. Prelog, *Pure Appl. Chem.*, 1964, **9**, 119–130.
15 M. M. Musa, K. I. Ziegelmann-Fjeld, C. Vieille, J. G. Zeikus and R. S. Phillips, *Angew. Chem., Int. Ed.*, 2007, **46**, 3091–3094.
16 (*a*) B. Kosjek, W. Stampfer, M. Pogorevc, W. Goessler, K. Faber and W. Kroutil, *Biotechnol. Bioeng.*, 2004, **86**, 55–62; (*b*) B. Kosjek, W. Stampfer, S. M. Glueck, M. Pogorevc, U. Ellmer, S. R. Wallner, M. F. Koegl, T. M. Poessl, S. F. Mayer, B. Ueberbacher, K. Faber and W. Kroutil, *J. Mol. Catal. B*, 2003, **22**, 1–6.
17 H. D. Simpson and D. A. Cowan, *Protein Peptide Lett.*, 1997, **4**, 25–32.
18 (*a*) R. S. Phillips, *J. Mol. Catal. B*, 2002, **19–20**, 103–107; (*b*) C. Heiss, M. Laivenieks, J. G. Zeikus and R. S. Phillips, *J. Am. Chem. Soc.*, 2001, **123**, 345–346.
19 B. Martín-Matute and J. E. Bäckvall, *Curr. Opin. Chem. Biol.*, 2007, **11**, 226–232.
20 M. Villela Filho, T. Stillger, M. Muller, A. Liese and C. Wandrey, *Angew. Chem., Int. Ed.*, 2003, **42**, 2993–2996.
21 J. A. King and F. H. McMillan, *J. Am. Chem. Soc.*, 1951, **73**, 4911–4915.
22 A. Ghanem and V. Schuring, *Tetrahedron: Asymmetry*, 2003, **14**, 57–62.
23 D. M. Wolfe and P. R. Schreiner, *Eur. J. Org. Chem.*, 2007, 2825–2838.
24 C. Thomazeau, H. Olivier-Bourbigou, L. Magna, S. Luts and B. Gilbert, *J. Am. Chem. Soc.*, 2003, **125**, 5264–5265.
25 C. S. Chen, Y. Fujimoto, G. Girdaukas and C. J. Sih, *J. Am. Chem. Soc.*, 1982, **104**, 7294–7299.

