Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Molecular Catalysis A: Chemical 214 (2004) 107–112
JOURNAL OF MOLECULAR CATALYSIS A: CHEMICAL
www.elsevier.com/locate/molcata

Mandelate racemase activity in ionic liquids: scopes and limitations

Nicole Kaftzik [a], Wolfgang Kroutil [b], Kurt Faber [b], Udo Kragl [a,*]

[a] *Department of Chemistry, Rostock University, Albert-Einstein-Strasse 3a, 18059 Rostock, Germany*
[b] *Department of Chemistry, Organic and Bioorganic Chemistry, Graz University, Heinrichstrasse 28, A-8010 Graz, Austria*

Received 31 July 2003; accepted 17 November 2003

Abstract

Ionic liquids (IL) offer new possibilities for solvent engineering for biocatalytic reactions. The deracemization of (±)-mandelic acid using a lipase-mandelate racemase two-enzyme system was used to investigate the scopes and limitations of ionic liquids as new reaction media for a dynamic resolution approach. Mandelate racemase [EC 5.1.2.2] from *Pseudomonas putida* ATCC 12633 was observed to be active in ionic liquids such as 1,3-dimethylimidazolium methylsulfate ([MMIM][$MeSO_4$]) or 1-butyl-3-methylimidazolium octylsulfate ([BMIM][$OctSO_4$]) at water activities $a_w > 0.74$. Mandelate racemase activity could also be obtained in a biphasic system consisting of water and 1-octyl-3-methylimidazolium hexafluorophosphate ([OMIM][PF_6]) in a ratio of 1:10.

Keywords: Ionic liquids; Dynamic resolution; Mandelate racemase; Water activity

1. Introduction

The controlled racemization of organic compounds constitutes the key to the transformation of racemates into a single stereoisomer in 100% theoretical yield employing dynamic (kinetic) resolution [1–3] or stepwise deracemisation techniques [4], which consists of the coupling of a racemization reaction to an enantioselective transformation.

Since for dynamic resolutions, the racemization and the enantioselective transformation have to be performed in the same vessel, they must be compatible with each other. Unfortunately, the majority of racemization methods described so far require extreme pH and/or temperatures, which makes them incompatible in the presence of biocatalytic enantioselective reactions. This disadvantage may be overcome by enzymatic racemisation which takes place under mild reaction conditions since enzymes are easily compatible with each other, as they generally work under similar reaction conditions [5]. As a consequence, the use of racemases for in situ substrate racemisation combined with biocatalysed kinetic resolution holds great potential for dynamic resolution processes [6].

Recently, mandelate racemase from *Pseudomonas putida* ATCC 12633 [EC 5.1.2.2] was recognised as a valuable catalyst for the racemization of stereochemically stable α-hydroxycarboxylic acids, such as mandelic acid and its derivatives, at neutral pH and ambient temperature [7,8]. These properties allowed it to be combined with a lipase to furnish a two-enzyme deracemization process. Lipases, which in nature catalyse the hydrolysis of triacyl glycerides, readily catalyse reactions such as esterification, transesterification or acylation in anhydrous organic media.

So far, deracemization of (±)-mandelic acid (Fig. 1) was achieved by using a two-enzyme-two-step system consisting of a *Pseudomonas* sp. lipase-catalysed O-acylation of (±)-mandelic acid in diisopropylether followed by mandelate racemase catalysed racemization of the remaining unreacted (*R*)-mandelic acid in aqueous buffer [4]. The whole process, however, could not be turned into a dynamic resolution due to the fact that mandelate racemase was completely inactive in a large variety of organic solvents [9].

For an industrial-scale, a dynamic process which is performed in one reaction vessel combining both biocatalysed reactions is preferred for the ease of handling. During the past decade, ionic liquids (IL) have gained increased

* Corresponding author. Tel.: +49-381-498-6450; fax: +49-381-498-6452.
E-mail address: udo.kragl@chemie.uni-rostock.de (U. Kragl).

1381-1169/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcata.2003.11.034

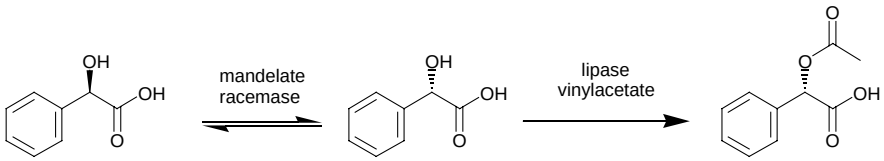

Fig. 1. Deracemization of (±)-mandelic acid via a lipase-mandelate racemase two-enzyme system: *Pseudomonas* sp. lipase catalysed O-acylation of (±)-mandelic acid and by mandelate racemase catalysed racemisation of the remaining unreacted (*R*)-mandelic acid.

attention as new solvents and reaction media for performing all types of reactions with sometimes remarkable results [10–12]. Due to their special properties and possible advantages, ionic liquids have become of interest as new solvents for biocatalytical reaction as well. In the last 2 years a number of papers have been published on first results of the use of ionic liquids as reaction media for enzymatic reactions. In these examples, ionic liquids such as [BMIM][PF_6] or [BMIM][BF_4] have been used to replace organic solvents in thermolysin- and lipase-catalysed reactions [13,14]. Besides, other enzymes as well as whole cell processes have been described [15–21]. First reviews on biocatalysis in ionic liquids are also available [22–24]. In some cases remarkable results with respect to yield, (enantio) selectivity or enzyme stability were observed. Therefore, ionic liquids seemed to be a promising solvent system to perform the dynamic resolution described above. Herein we report the first example of the use of a mandelate racemase in ionic liquids.

2. Experimental

2.1. Materials

(±)-, D- and L-mandelic acid were purchased from Fluka (Vienna) and were of analytical grade. Mandelate racemase was prepared by fermentation of Pseudomonas putida ATCC 12633 on glucose and (±)-mandelic acid, a partially purified enzyme preparation showing a specific activity of 107 U mg^{-1} (towards D-mandelic acid at 20 °C and pH 7.5) was obtained [25]. Lyophilised *Pseudomonas* sp. Lipase PS was purchased from Amano (Japan). The organic solvents and vinylacetate as acyl-donor were obtained from Merck (Vienna). The ionic liquids were a gift from P. Wasserscheid (Aachen University). They are also available from Solvent Innovation GmbH (Cologne).

2.2. Racemization experiments

All racemisation experiments were performed in 1.5 ml eppendorf tubes. (*R*)-Mandelic acid (2 mg ml^{-1}, 0.013 mol l^{-1}) was dissolved in each ionic liquid and lyophilised mandelate racemase (2 mg ml^{-1}, 214 U mg^{-1}) was added. The mixture was shaken in a thermomixer at 30 °C and 130 rpm for either 24 or 48 h. The samples were analysed by HPLC.

2.3. Equilibration and measurement of water activity

For the reactions in the presence of different % v/v of water, ionic liquids which are completely miscible with water were used and the amount of ionic liquid was reduced and replaced by water. In the case of [BMIM][$(CF_3SO_2)_2N$] the substrate solutions were equilibrated over saturated salt solutions in closed vessels based on [26]. The salts used were LiBr ($a_w = 0.06$), LiCl ($a_w = 0.11$), $MgCl_2$ ($a_w = 0.33$), $Mg(NO_3)_2$ ($a_w = 0.53$), NaCl ($a_w = 0.74$) and K_2SO_4 ($a_w = 0.97$). To control the final equilibration state in both case, the water activity (a_w) was measured with an a_w measuring instrument from Novasina (Novasina AW SPRINT, Axair Ltd. Switzerland).

2.4. Kinetic resolution of (R,S)-mandelic acid

All kinetic resolution experiments were performed in 1.5 ml eppendorf tubes. Rac-mandelic acid (8.3 mg ml^{-1}, 0.055 mol l^{-1}) was dissolved in [BMIM][$OctSO_4$] or [OMIM][PF_6]. Lyophilized lipase PS (8.3 mg ml^{-1}) and vinylacetate (250 μl ml^{-1}, 2.71 mol l^{-1}) were added. The mixtures were shaken using a thermomixer at 30 °C and 130 rpm for 24 h. To study the influence of water on the conversion of (*S*)-mandelic acid in [BMIM][$OctSO_4$] the amount of the ionic liquid was reduced and replaced by water. The influence of water on the conversion of (*S*)-mandelic acid in [OMIM][PF_6] was studied using a two-phase system consisting of [OMIM][PF_6] and water in different ratios. The samples were analysed by thin layer chromatography. Chloroform/methanol/acetic acid (60/10/1) was used as mobile phase (R_f of (*S*)-acetyl mandelic acid = 0.4). Detection was done by colouring the acetyl mandelic acid as a molybdate complex.

2.5. Dynamic resolution experiments

Dynamic resolution experiments were performed in 1.5 ml eppendorf tubes as a biphasic reaction. (*R*)-Mandelic acid (5 mg ml^{-1}, 0.033 mol l^{-1}) was dissolved in 1 ml [OMIM][PF_6], lyophilized lipase PS (8.3 mg ml^{-1}) was suspended and vinylacetate (250 μl ml^{-1}, 2.71 mol ml^{-1}) was added. The reaction was started by adding 100 μl of an aqueous mandelate racemase solution (70 mg ml^{-1}) as the second phase. The reaction mixture was shaken in a thermomixer at 30 °C and 130 rpm for 24 h.

The formation of (*S*)-acetyl mandelic acid was analysed by thin layer chromatography. Therefore, 3 M HCl was added until the reaction mixture reached a pH of 2. The sample was then extracted three times with either ethyl acetate or diisopropyl ether and the organic phase was separated and used for analysis.

2.6. Sample preparation for HPLC-analysis

3 M HCl was added to the sample until the reaction mixture reached pH 2. The sample was then extracted three times with either ethyl acetate or diisopropyl ether. The organic phase was separated, dried ($NaSO_4$) and the solvent was evaporated under reduced pressure. The residue was resuspended in *n*-heptane/*i*-propanol (80:20) and analysed by HPLC.

2.7. HPLC-analysis

The extracts were analysed by HPLC as previously described [7], using a Chiracel OD-H column (Daicel 0.46 cm × 25 cm) at 18 °C, *n*-heptane/*i*-propanol/trifluoroacetic acid (80:20:0.1 v/v) was used as mobile phase at 0.5 ml min^{-1}; 11.5 min (*S*); 13.5 min (*R*). The enantiomeric excess, conversion and reaction rate were calculated from the HPLC data.

3. Results and discussion

3.1. Racemization experiments: mandelate racemase activity in ionic liquids and influence of water activity

Eleven different ionic liquids were used as solvents to investigate the influence on the mandelate racemase activity (Table 1) to racemise (*R*)-mandelic acid under anhydrous conditions. The activity was determined by the enantiomeric excess of the (*R*)-enantiomer of the mandelic acid after 24 h. While the mandelic acid was soluble in all neat ionic liquids (sometimes the mixture has to be warmed up to 50 °C before used, and then cooled down for use), the lyophilised mandelate racemase preparation was not soluble in any of the ionic liquids. In neat ionic liquids no activity was observed at all. In the ionic liquids a hexafluorophosphate anion, the enzyme preparation formed aggregates, which might be the reason that no activity was observed.

Reactions, which are catalysed by enzymes in non-conventional media, are often affected by the nature of the solvent as well as by its water content [27]. The water content of the reaction media plays an important role in enzyme catalysis, because it influences the flexibility of the protein, which is responsible for its activity and selectivity [28].

To investigate the influence of water the ionic liquids [EMIM][CH_3–C_6H_4–SO_3], [BMIM][BF_4], [PMIM][BF_4], [MMIM][$MeSO_4$] and [BMIM][$OctSO_4$] were chosen, since these compounds are miscible with water in all proportions. As shown in Table 1, the enzyme activity varies significantly with the nature of the ionic liquid and the water content leading to a total loss of activity for the ionic liquids [EMIM][CH_3–C_6H_4–SO_3], [BMIM][BF_4], [PMIM][BF_4], even at high water contents up to 70% (v/v). A racemisation of (*R*)-mandelic acid was only obtained in the presence of 90% (v/v) water. However, a racemization of (*R*)-mandelic acid was still observed in [MMIM][$MeSO_4$] in the presence of 50% (v/v) water and in [BMIM][$OctSO_4$] the mandelate racemase was even active in the presence of 20% (v/v) water.

To compare the enzyme's activity in dependence of the water present in solvents of different polarities, however, it is more precise to use the water activity a_w [29]. Fig. 2 shows the water amount in the reaction mixture as a function of water activity for the ionic liquids [MMIM][$MeSO_4$] and [BMIM][$OctSO_4$]. Even when both reaction mixtures contain the same amount of water, the water activity is always lower in the presence of [MMIM][$MeSO_4$]. This might be due to the stronger interactions between the methyl sulfate ions and the water molecules.

Fig. 3 shows the activity of the mandelate racemase in [MMIM][$MeSO_4$] and [BMIM][$OctSO_4$] as a function

Table 1
Mandelate racemase activity in the presence of ionic liquid and in dependence of different water amounts

Ionic liquid	(*R*)-Enantiomer ee (%)						
Water amount (% (v/v))	0	10	20	30	50	70	90
[MMIM][$MeSO_4$]	100	100	100	100	89	63[a]	14[a]
[EMIM][CH_3–C_6H_4–SO_3]	100	100	100	100	100	100	<90
[EMIM][$(CF_3SO_2)_2N$]	100						
[BMIM][BF_4]	100	100	100	100	100	100	<90
[BMIM][$OctSO_4$]	100	100	61	55[a]	31[a]	33[a]	78[a]
[BMIM][$(CF_3SO_2)_2N$]	100						
[PMIM][BF_4]	100	100	100	100	100	100	<90
[HMIM][PF_6]	100						
[OMIM][PF_6]	100						
[NMIM][PF_6]	100						

Conditions: 0.013 mol l^{-1} (*R*)-mandelic acid; 214 U ml^{-1}; 30 °C; 130 rpm; 24 h.
[a] 0.5 h.

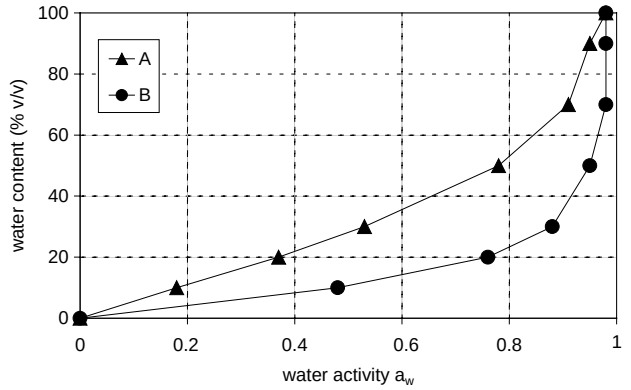

Fig. 2. Water activity (a_w) as function of water content (% (v/v)) in different water–ionic liquid mixtures at 25 °C; (A) [MMIM][$MeSO_4$], (B) [BMIM][$OctSO_4$].

of water activity. In both cases the enzyme activity decreased by reducing the water activity. While the graph for [MMIM][$MeSO_4$] describes an almost linear correlation between enzyme activity and water activity, the graph of [BMIM][$OctSO_4$] shows a significant deviation from this linear behaviour. With higher amounts of the ionic liquid the activity of the enzyme drops and reaches less then 20% although the water activity is nearly constant. This behaviour is reproducible. Finally the water activity could be reduced to a_w: 0.75 while the enzyme was still active. At higher enzyme concentration (up to 8 mg ml^{-1} in the reaction mixture) the conversion reaches values of nearly 47% but the reaction velocity is still lower in the presence of the ionic liquid than in water.

It has recently been shown that reactions catalysed by isolated lipases have resulted in improved enantioselectivity, improved conversion or higher stability when such reactions have been performed in ionic liquids such as [BMIM][PF_6]; [BMIM][BF_4] or [BMIM][$(CF_3SO_2)_2N$] [30–33]. The equilibration of initial water activity in such non-water miscible ionic liquids has been described recently [26]. The method of controlling water activity by equilibration over saturated salt solutions was transferred from organic media to ionic liquids and it could be shown that the kinetic resolution of (*R,S*)-1-phenylethanol catalysed by a lyophilised lipase from *Pseudomonas* sp. was markedly influenced by the water activity and had higher selectivity in [BMIM][$(CF_3SO_2)_2N$] at low water activities ($a_w < 0.53$) than in methyl *tert*-butylether [26]. Following this report, the racemisation of (*R*)-mandelic acid was also studied in dependence of solvent's initial water activity using [BMIM][$(CF_3SO_2)_2N$] as pure solvent. To our disappointment even at high water activities (a_w: 0.97) this kind of ionic liquid caused a denaturation of the enzyme and no activity could be found for the mandelate racemase at all.

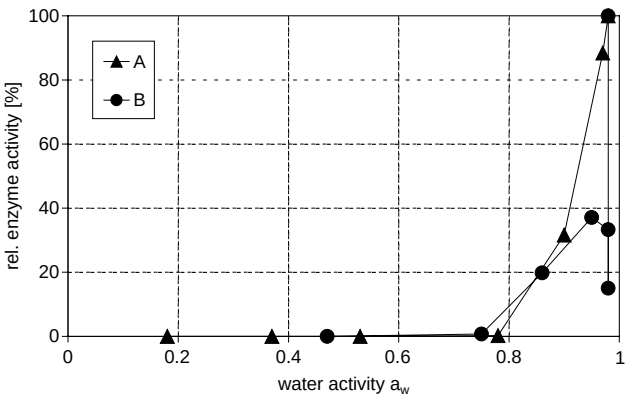

Fig. 3. Mandelate racemase activity as function of water activity in different ionic liquids; starting concentrations: 0.013 mol l^{-1} (*R*)-mandelic acid; 214 U ml^{-1} lyophilized mandelate racemase; 30 °C; 130 rpm; 24 h. (A) [MMIM][$MeSO_4$]; (B) [BMIM][$OctSO_4$].

The non-water miscible ionic liquids such as [BMIM][$(CF_3SO_2)_2N$] or ionic liquids possessing a hexafluorophosphate anion also offer the possibility for a biphasic reaction. Therefore, a biphasic system containing an aqueous phase for the mandelate racemase catalysed racemisation and an ionic liquid for the lipase catalysed kinetic resolution was investigated. It is worth to note, that the contact with the aqueous phase leads to an equilibration of initial water activity in the ionic liquid. In the case of lipase catalysed reactions, at high water activities the hydrolysis is favoured against the acetylation. Therefore, the water activity in the ionic liquid has to be kept low. In the biphasic system the water activity in the ionic liquid can be controlled over the phase ratio. To investigate the influence of phase ratio on the mandelate racemase activity, the ionic liquid [OMIM][PF_6] was chosen. The phase ratio of water:[OMIM][PF_6] was varied from 1:1 to 1:20. Activity for the mandelate racemase could be obtained up to a ratio of 1:10. At this point the conversion from (*R*)- to the (*S*)-enantiomer was already low, but the enzyme is still active. At higher mandelate racemase concentrations (up to 24 mg ml^{-1} based on the volume of the whole reaction mixture) a conversion of 42% was reached after 24 h but the reaction velocity is still much lower than in a pure aqueous system.

In summary, activity for the mandelate racemase could be obtained in [BMIM][$OctSO_4$] at a water activity a_w: 0.75 and in a biphasic system consisting of water and [OMIM][PF_6] in a ratio of 1:10.

3.2. Kinetic resolution of (R,S)-mandelic acid

The kinetic resolution of (*R,S*)-mandelic acid catalysed by a lipase from *Pseudomonas* sp. was performed both in [BMIM][$OctSO_4$] in the presence of 20% (v/v) water (a_w: 0.75) and in the biphasic system using [OMIM][PF_6] and water. The conversion of the lipase catalysed reaction was markedly influenced by the amount of water as well in [BMIM][$OctSO_4$] as in the biphasic approach. While the mandelate racemase needs at least a water activity a_w: 0.75 to be active in [BMIM][$OctSO_4$], however, measurable conversion for the lipase catalysed reaction could only be obtained when the water activity of the media was reduced to

a_w: 0.47. Therefore, a combination of the mandelate racemase catalysed reaction and the lipase catalysed reaction in one phase using an ionic liquid such as [BMIM][$OctSO_4$] was impossible.

Therefore, the lipase catalysed kinetic resolution of (±)-mandelic acid was performed in the biphasic system using [OMIM][PF_6] and the influence of the phase ratio on the conversion was investigated. Again the phase ratio water:[OMIM][PF_6] was varied from 1:1 to 1:20. A detectable formation of the (*S*)-acetyl mandelic acid could be obtained in a reaction system consisting of water and [OMIM][PF_6] in a ratio of 1:9. As mentioned above the mandelate racemase was still active in the biphasic system at a phase ratio of 1:10. This encouraging result led us to combine both enzymatic reactions to perform a dynamic resolution of (±)-mandelic acid.

3.3. Dynamic resolution experiments

After performing both enzyme catalysed reactions separately, for the first time both reactions were performed together in the same reaction system. While the ionic liquid contained the mandelic acid, the lipase and the acyl-donor, the mandelate racemase was dissolved in the aqueous phase. During the reaction a precipitation of protein material was observed and it could be figured out to be denaturated mandelate racemase. Analysis of the reaction mixture relating to the formation of (*S*)-acetyl mandelic acid showed, that the mandelate racemase was totally inactive under this reaction conditions. It turned out, that the mandelate racemase was affected by the vinylacetate used as acyl-donor for the lipase catalysed acylation. So far a promising reaction system was found using an ionic liquid and water to perform a biphasic reaction, but nevertheless a dynamic resolution of (±)-mandelic acid could not be realised because the mandelate racemase was not compatible with the acyl-donor used.

4. Conclusions

We have shown, for the first time, that *P. putida* mandelate racemase catalysed the racemisation of (*R*)-mandelic acid in ionic liquids. Reaction rates and therefore enzyme activity is markedly influenced by the water activity of the reaction media. In general, water activity less than 0.8 caused a loss of enzyme activity. A combination of the mandelate racemase catalysed racemisation of (*R*)-mandelic acid and the lipase catalysed kinetic resolution of (*S*)-mandelic acid to (*S*)-acetyl mandelic acid in [BMIM][$OctSO_4$] at a water activity a_w: 0.75 was not successful, because at such a high water activity no conversion for the lipase catalysed reaction could be obtained. However, in a biphasic system consisting of [OMIM][PF_6] and water in a ratio of 1:10 both, a measurable racemase activity and the formation of a measurable amount of (*S*)-acetyl mandelic acid was observed. At least the realisation of both reactions in the same vessel at the same time was not possible, because the mandelate racemase was incompatible with the vinylacetate used as acyl-donor for the lipase catalysed kinetic resolution of (*S*)-mandelic acid.

It has been shown that a number of other acyl-donors can be used for lipase catalysed acylation with nearly similar reaction velocities and conversions [34]. If an acyl-donor could be found which is compatible with the mandelate racemase the biphasic approach using an ionic liquid and water in the right ratio offers a great potential to realise the combination of two enzymatic reactions for a dynamic resolution process.

Acknowledgements

Financial support by the Deutscher Akademischer Austauschdienst (DAAD, Germany) and the *Fonds der Chemischen Industrie* is gratefully acknowledged.

References

[1] R.S. Ward, Tetrahedron: Asymmetry 6 (1995) 1475.
[2] R. Noyori, M. Tokunaga, M. Kitamura, Bull. Chem. Soc. Jpn. 68 (1995) 36.
[3] F.F. Huerta, A.B.E. Minidis, J.-E. Bäckvall, Chem. Soc. Rev. 30 (2001) 321.
[4] U.T. Strauss, K. Faber, Tetrahedron: Asymmetry 10 (1999) 4079.
[5] E. Adams, Adv. Enzymol. Relat. Areas Mol. Biol. 44 (1976) 69.
[6] B. Schnell, K. Faber, W. Kroutil, Adv. Synth. Catal. 345 (2003) 653.
[7] M. Goriup, U.T. Strauss, U. Felfer, W. Kroutil, K. Faber, J. Mol. Catal. B: Enzymatic 15 (2001) 207.
[8] U. Felfer, U.T. Strauss, W. Kroutil, W.M.F. Fabian, K. Faber, J. Mol. Catal. B: Enzymatic 15 (2001) 213.
[9] M. Pogorevc, H. Stecher, K. Faber, Biotechnol. Lett. 24 (2002) 857.
[10] P. Wasserscheid, T. Welton (Eds.), Ionic Liquids in Synthesis, Wiley-VCH, Weinheim, 2002.
[11] T. Welton, Chem. Rev. 99 (1999) 2071.
[12] K.R. Seddon, J. Chem. Tech. Biotechnol. 68 (1997) 351.
[13] M. Erbeldinger, A.J. Mesiano, A.J. Russel, Biotechnol. Progr. 16 (2000) 1129.
[14] R.M. Lau, F. van Rantwijk, K.R. Seddon, R.A. Sheldon, Org. Lett. 2 (2000) 4189.
[15] J.A. Laszlo, D.L. Compton, Biotechnol. Bioeng. 75 (2001) 181.
[16] P. Lozano, T. de Diego, J.P. Guegan, M. Vaultier, J.L. Iborra, Biotechnol. Bioeng. 75 (2001) 563.
[17] T.L. Husum, C.T. Jorgensen, M.W. Christensen, O. Kirk, Biocatal. Biotrans. 19 (2001) 331.
[18] M. Eckstein, M. Sesing, U. Kragl, P. Adlerkcreutz, Biotechnol. Lett. 24 (2002) 867.
[19] N. Kaftzik, P. Wasserscheid, U. Kragl, Org. Process. Res. Dev. 6 (2002) 553.
[20] S.G. Cull, J.D. Holbrey, V. Vargas-Mora, K.R. Seddon, G.J. Lye, Biotechnol. Bioeng. 69 (2000) 227.
[21] J. Howarth, P. James, J.F. Dai, Tetrahedron Lett. 42 (2001) 7517.
[22] R.A. Sheldon, R.M. Lau, M.J. Sorgedrager, F. van Rantwijk, K.R. Seddon, Green Chem. 4 (2002) 147.
[23] F. van Rantwijk, R.M. Lau, R.A. Sheldon, Trends Biotechnol. 21 (2003) 131.
[24] U. Kragl, M. Eckstein, N. Kaftzik, Curr. Opin. Biotechnol. 13 (2002) 565.
[25] H. Stecher, U. Felfer, K. Faber, J. Biotechnol. 56 (1997) 33.

[26] M. Eckstein, P. Wasserscheid, U. Kragl, Biotechnol. Lett. 24 (2002) 763.
[27] G. Carrea, R. Riva, Angew. Chem. 39 (2000) 2226.
[28] A.M. Klibanov, Nature 409 (2001) 241.
[29] R.H. Valivety, P.J. Halling, Biochem. Biophys. Acta 1118 (1992) 218.
[30] S. Schöfer, N. Kaftzik, P. Wasserscheid, U. Kragl, Chem Commun. (2001) 425.
[31] K.-W. Kim, B. Song, M.-Y. Choi, M.-J. Kim, Org. Lett. 3 (2001) 1507.
[32] R.M. Lau, F. van Rantwijk, K.R. Seddon, R.A. Sheldon, Org. Lett. 2 (2000) 4189.
[33] P. Lozano, T. de Diego, D. Carrie, M. Vaultier, J.L. Iborra, Biotechnol. Lett. 23 (2001) 1529.
[34] T. Miyazawa, S. Kurita, S. Ueji, T. Yamada, S. Kuwata, J. Chem. Soc. Perkin Trans 1 (1992) 2253.