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Quantitative transformation of racemic 2-hydroxy acids into (*R*)-2-hydroxy acids by enantioselective oxidation with glycolate oxidase and subsequent reduction of 2-keto acids with D-lactate dehydrogenase

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Abstract

The enzymatic resolution of chiral 2-hydroxy acids **1** by enantioselective oxidation with molecular oxygen in the presence of glycolate oxidase from spinach (*Spinacia oleracea*) and subsequent asymmetric reduction of 2-oxo acids **2** with D-lactate dehydrogenase from *Lactobacillus leichmannii* leads to enantiomerically pure (*R*)-2-hydroxy acids in up to 89% yield based on the racemate. © 1998 Elsevier Science Ltd. All rights reserved.

Optically active 2-hydroxy acids are important building blocks for the asymmetric synthesis of glycols,[1a] halo esters[1b] and epoxides.[1c] Several chemical[2] and enzymatic[3] methods have been described previously on the synthesis of optically active α-hydroxy acids. Recently, we have reported[4] the enzymatic resolution of chiral 2-hydroxy carboxylic acids by enantioselective oxidation with molecular oxygen, catalyzed by the glycolate oxidase (GOX) from spinach (*Spinacia oleracea*). This novel enzymatic transformation affords optically active 2-hydroxy acids in excellent ee values. Nevertheless, the enantioselective oxidation of the (*S*)-2-hydroxy acid to corresponding 2-oxo acid **2** (Scheme 1, Method **A**) provides only a maximum yield of 50% for the (*R*)-2-hydroxy acid, based on the racemate. For synthetic applications and economic reasons it would be desirable to prepare the (*R*)-2-hydroxy acids quantitatively from their racemic precursors by a biocatalytic process.

The microbial D-lactate dehydrogenase (D-LDH, E.C. 1.1.1.28), which catalyzes *in vivo* the NADH-dependent interconversion of pyruvate and D-lactate in anabolic and catabolic pathways,[5] is a useful enzyme in organic synthesis; it reduces a range of 2-oxo acids to the corresponding (*R*)-2-hydroxy acids.[6] Therefore, we have coupled the glycolate–oxidase-catalyzed oxidation of racemic 2-hydroxy acids with the asymmetric reduction of 2-oxo acids by D-lactate dehydrogenase from *Lactobacillus leichmannii*

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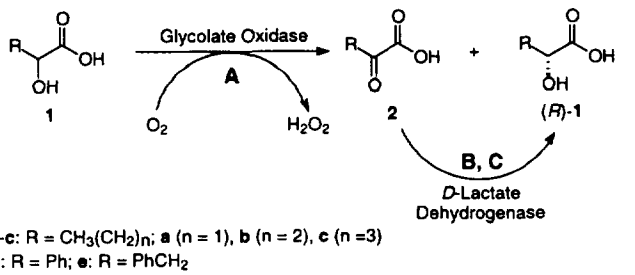

Scheme 1. Oxidation of 2-hydroxy acids with molecular oxygen catalyzed by the glycolate oxidase from spinach (*Spinacia oleracea*) (Method **A**) and reduction of 2-oxo acids by the D-lactate dehydrogenase (Methods **B** and **C**)

for the efficient oxidoreductative transformation to (*R*)-2-hydroxy acids (Scheme 1, Method **B** and **C**). Herein, we report for the first time such a tandem biocatalytic transformation of racemic 2-hydroxy acids.

The results of the biocatalytic transformation of the 2-hydroxy acids **1a–e** are given in Table 1, wherein conversion and enantiomeric excess of the known[6–9] 2-hydroxy carboxylic acids **1** were assessed by gas chromatography. The enzymatic oxidation of the racemic 2-hydroxy acids **1** with glycolate oxidase by molecular oxygen were carried out as reported earlier.[4] Subsequently, the D-lactate dehydrogenase was added together with catalytic amounts of NADH for enantioselective reduction of the 2-oxo acid **2** to corresponding (*R*)-2-hydroxy acid **1**. D-Lactate dehydrogenase contains an air-sensitive thiol group, but it is stable when used in an inert atmosphere in the presence of reducing agents such as dithiothreitol to prevent autooxidation. The cosubstrate NADH is recycled by the system formate/formate dehydrogenase, so that the product, CO_2, of the regeneration system does not complicate the workup.

The results in Table 1 show that for the one-pot reaction (Method **B**) of the 2-hydroxy acids **1a–c** with the glycolate oxidase and the D-lactate dehydrogenase the (*R*)-2-hydroxy acids **1a–c** are obtained in moderate to high enantiomeric excesses (ee values 67–91%) and 84–93% yields, based on the racemate (Table 1, entries 1–4, 8, 9, 12, 13). However, the (*R*)-2-hydroxy acids **1a–c** could not be obtained enantiomerically pure by this method; presumably the gylcolate oxidase catalyzes the reduction of the 2-oxo acids **2a–c** to the (*S*)-2-hydroxy acids **1a–c** under these anaerobic conditions. Thus, we have carried out the reaction of **2a** according to Method **B**, but without the addition of D-LDH (Table 1, entry 5). Indeed, the GOX-catalyzed reduction of the 2-oxo acid **2a** to the enantiomerically pure (*S*)-2-hydroxy acid **1a** at 100% conversion reveals that the glycolate oxidase and the D-lactate dehydrogenase compete under anaerobic conditions for the 2-oxo acid **2** as substrate. To circumvent this loss in enantioselectivity, we have modified our biocatalytic transformation of the racemic 2-hydroxy acids **1**. After the enzymatic resolution of the 2-hydroxy acids **1a,b** with glycolate oxidase, the acids **1a,b** and **2a,b** were separated from the aqueous enzyme solution and added to the D-lactate dehydrogenase medium (Method **C**). In this way, the (*R*)-2-hydroxy acids **1a,b** may be obtained essentially enantiomerically pure at 85–89% yield, based on the racemate (Table 1, entries 6, 7, 10, 11).

Furthermore, we have investigated the enzymatic resolution of the mandelic acid **1d** and the phenyllactic acid (**1e**) with glycolate oxidase. While the mandelic acid **1d** was not accepted by the glycolate oxidase (Table 1, entry 14), the phenyllactic acid **1e** was oxidized by molecular oxygen in the presence of glycolate oxidase (Table 1, entries 15, 16). Nonetheless, the enzyme activity towards this substrate is remarkably diminished. Thus, the enzymatic resolution of the phenyllactic acid **1e** may not be achieved with affordable amounts of the enzyme and within a reasonable reaction time (Table 1, entry 16).

In summary, we have shown that the tandem enzymatic transformation of racemic 2-hydroxy acids **1** with glycolate oxidase and D-lactate dehydrogenase from *Lactobacillus leichmannii* leads to enantiomerically pure (*R*)-2-hydroxy acids **1** in up to 89% yield based on the racemate. Thus, this novel

Table 1
Conversion of racemic 2-hydroxy acids into (*R*)-2-hydroxy acids catalyzed by the glycolate oxidase (Method **A**) and the D-lactate dehydrogenase (Methods **B** and **C**)

entry	substrate (mmol)	method[a]	GOX[b] (IU)	D-LDH[c] (IU)	time (h)	conversion[d] (%)	ee (%)[e] (*R*)-**1**
1	**1a** (0.8)	**B**	3		25	49	95
2				700	39	98	91
3	**1a** (0.4)	**B**	2		47	50	>99
4				470	24	100	86
5	**2a** (0.1)[f]	**B**	2		18	100	>99[g]
6	**1a** (0.4)	**C**	2		42	50	>99
7				450	24	100	>99
8	**1b** (0.4)	**B**	2		47	48	92
9				700	168	100	72
10	**1b** (0.4)	**C**	2		42	50	>99
11				900	168	100	94
12	**1c** (0.4)	**B**	3		113	46	96
13				700	93	100	67
14	**1d** (0.07)	**A**	2		22	0	–
15	**1e** (0.01)	**A**	5		48	45	81
16	**1e** (0.3)	**A**	5		10 d	36	55

[a] Method **A**: Enzymatic resolution of the 2-hydroxy acids **1** with glycolate oxidase; Method **B**: The *D*-LDH was added to the glycolate oxidase medium; Method **C**: The *D*-LDH was added to the mixture of acids **1** and **2**, which have been separated from the glycolate oxidase medium. [b] Pure glycolate oxidase from Sigma. [c] *D*-Lactate dehydrogenase was obtained as gift sample from Boehringer Mannheim. [d] Conversion was determined by GC analysis (DB-Wax column; error limit ± 2%). [e] Enantiomeric excess (ee values) was determined by multidimensional GC analysis [column 1: DB-Wax; column 2: 30% heptakis(2,3-diethyl-6-*tert*-butyldimethylsilyl)-β-cyclodextrin in PS-086]; error limit ± 2%.[f] Carried out according to Method **B**, but without the addition of *D*-LDH. [g] The (*S*)-2-hydroxy acid **1a** was obtained.

biocatalytic method enables the clean preparation of enantiomerically pure (*R*)-2-hydroxy acids **1** almost quantitatively from the racemic precursors.

1. Experimental

1.1. Materials

The glycolate oxidase (GOX) and the catalase were purchased from Sigma. D-Lactate dehydrogenase (D-LDH) and formate dehydrogenase (FDH) were obtained as gift samples from Boehringer Mannheim and used without further purification. The racemic 2-hydroxy acids **1a–e** were purchased from Fluka and Sigma.

1.2. General procedure for the enzymatic resolution of the 2-hydroxy acids ***1d–e*** *with glycolate oxidase (Method A)*

These reactions were performed in an Amicon Ultrafiltration Cell (Model 8050), in which the filtration membrane was substituted by a Teflon sheet. The glycolate oxidase was added to 50 mL of a solution (pH 7.8) of the 2-hydroxy acid **1** (cf Table 1), Tris(hydroxymethyl)-aminoethane (tris-HCl, 0.1 M), flavin mononucleotide (FMN, 1 mM) and catalase (1600 IU). The resulting mixture was stirred at 15°C under O_2 (0.4 MPa). Aliquots (1 mL) were removed and worked up to monitor the progress of substrate conversion. For this purpose, the solution was acidified with 6 N hydrochloric acid (pH 3) and extracted with ethyl ether (3×5 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure (20°C, 17 Torr). The acids **1** and **2** were converted to their methyl esters by treatment with diazomethane and submitted to GC analysis.

1.3. General procedure for the quantitative transformation of the racemic 2-hydroxy acids ***1*** *with glycolate oxidase and* D*-lactate dehydrogenase*

Method **B**: The enzymatic resolution of the racemic 2-hydroxy acids **1a–c** was performed as described above. At 50% conversion, the buffer was adjusted to pH 7.5. After bubbling nitrogen gas through the solution for 15 min, the D-lactate dehydrogenase (cf Table 1) was added, together with ammonium formate (1.5 equiv. based on the 2-oxo acid **2**), tris–HCl (0.1 M), dithiothreitol (1 mM), FDH (13–25 IU) and catalytic amounts of NAD (0.002 mmol). The resulting mixture was stirred at room temperature under N_2 (0.4 MPa). Aliquots (1 mL) were removed and worked up as described above to monitor the progress of substrate conversion. After the complete conversion of the 2-oxo acid **2**, the mixture was extracted with ethyl ether (3×25 mL) and the combined organic layers were dried over Na_2SO_4. The solvent was removed (20°C, 17 Torr) and the 2-hydroxy acid **1a–c** was obtained in 84–93% yields.

Method **C**: The enzymatic resolution of the racemic 2-hydroxy acids **1a** and **1b** was performed as described under Method **A**. At 50% conversion, the solution was acidified with 6 N hydrochloric acid (pH 3) and extracted with ethyl ether (3×25 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure (20°C, 17 Torr) to yield the 2-hydroxy acids **1a,b** and the 2-oxo acids **2a,b** as a crude mixture in 92–96% yield. The acids were added to a solution (pH 7.5) of ammonium formate (1.5 equiv. based on the 2-oxo acid **2**), tris–HCl (0.1 M), dithiothreitol (1 mM), D-LDH (cf Table 1), FDH (13 U) and a catalytic amount of NAD (0.002 mmol). The resulting mixture was stirred at room temperature (ca. 20°C) under N_2 (0.4 MPa). Aliquots (1 mL) were removed and worked up as described above to monitor the progress of substrate conversion. After complete conversion of the 2-oxo acid **2**, the mixture was extracted with ethyl ether (3×25 mL) and the combined organic layers were dried over Na_2SO_4. The solvent was removed (20°C, 17 Torr) and the 2-hydroxy acid **1a,b** was obtained in 85–89% yields.

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