SUPPORTING INFORMATION

Practical Asymmetric Enzymatic Reduction through Discovery of a Dehydrogenase-Compatible Biphasic Reaction Media

by

Harald Gröger, Werner Hummel, Stefan Buchholz, Karlheinz Drauz, Tien Van Nguyen,
Claudia Rollmann, Hendrik Hüsken, Kofi Abokitse

Supporting Information to Figure 1:

Investigation of the enzymatic activity of the formate dehydrogenase (FDH) from *Candida boidinii* (mutant: C23S, C262A; expression in *E. coli*; see also reference [8]) after storage in aqueous organic solvent mixtures

General protocol for the measurement of the enzyme activity:

At first, 100 μL of a formate dehydrogenase solution (formate dehydrogenase (FDH) from *Candida boidinii* (mutant: C23S, C262A; expression in *E. coli*; see also reference [8]) are dissolved in 10 mL of a solvent mixture containing 10-20%(v/v) of an organic solvent (an overview about the investigated solvents is given in Table S.I.1 below). The samples are stored for a duration of up to 3 days, and regularly samples of 100 μL are taken from the solution.

These samples are analyzed with respect to its enzymatic activity by spectrophotometrically measuring the reduction of NAD^+ to NADH at 340 nm ($\epsilon_{340} = 6.22 \text{ mM}^{-1} \text{ cm}^{-1}$) with sodium formate (spectrophotometer: JASCO V-530 UV/VIS). The activity was measured at 30°C in a cuvette (1 mL) containing 500 μL of a sodium formate solution (0.8M; pH 8.2; 0.1M phosphate buffer), and 500 μL of a NAD^+ -solution (0.4mM). The reaction was started by the addition of the enzyme solution (10 μL of the 100 μL solution). One unit of FDH activity was defined as the amount of enzyme that converted 1 μmol of NAD^+ per minute.

The resulting course of the enzymatic activities of the FDH in dependency of the storage time are described in Table S.I.1 below.

Table S.I.1: Remaining activity of the formate dehydrogenase (FDH) from *Candida boidinii* (mutant: C23S, C262A; expression in *E. coli*; see also reference [8]) after storage in aqueous organic solvent mixtures

Added Organic Solvent	Amount of solvent [% (v/v)]	Activity of FDH ^e [%]
Ethanol ^{a)}	10	41
Ethanol ^{b)}	20	4
<i>n</i> -Butanol ^{a)}	10	1
Tetrahydrofuran ^{a)}	10	5
Methy- <i>tert</i> -butylether (MTBE) ^{b)}	10	23
Methy- <i>tert</i> -butylether (MTBE) ^{b)}	20	28
Ethylacetate ^{a),d)}	10	<1
Ethylacetate ^{a),d)}	20	<1
Toluene ^{b)}	10	39
<i>n</i> -Hexane ^{c)}	10	92
<i>n</i> -Hexane ^{c)}	20	90
<i>n</i> -Heptane ^{c)}	10	79

a) after 1d or less; b) after ca. 2d (45-48h); c) after ca. 3d (66-69h); d) not shown in Figure 1 ; e) As a formate dehydrogenase, the formate dehydrogenase (FDH) from *Candida boidinii* (mutant: C23S, C262A; expression in *E. coli*; see also reference [8]) was used.

Supporting information to Table 1:

Enzymatic reduction with different type of substrates (according to Table 1):

A general procedure is as follows: At a reaction temperature of 30°C, 10U of the (*S*)-alcohol dehydrogenase from *Rhodococcus erythropolis* (expression in *E. coli*; see also reference [3i]), and 10U of the formate dehydrogenase from *Candida boidinii* (mutant: C23S, C262A; expression in *E. coli*; see also reference [8]) are added to a solution of 0.5 mmol of the ketone component, 2.5 mmol sodium formate (171.6 mg), and 0.1 mmol of NADH (70.2 mg) in a solvent mixture, consisting of 10 mL of *n*-heptane, and 40 mL of a phosphate buffer (50 mM; pH 7.0). After stirring the reaction mixture for 21h, the organic phase was separated and the aqueous phase is extracted with 3 x 50 mL of methyl *tert*-butyl ether. The collected organic phases are dried over magnesium sulfate, and after filtration and evaporation of the volatile components in vacuo the resulting oily crude product is analyzed with respect to the conversion rate (*via* NMR, HPLC). The enantiomeric excess (ee) was determined by chiral GC chromatography.

Supporting information to Figure 2:

Enzymatic reduction at higher substrate concentrations (according to Figure 2) exemplified for the reaction with a substrate concentration of 100 mM:

At a reaction temperature of 30°C, 10U of the (*S*)-alcohol dehydrogenase from *Rhodococcus erythropolis* (expression in *E. coli*; see also reference [3i]), and 10U of the formate dehydrogenase from *Candida boidinii* (mutant: C23S, C262A; expression in *E. coli*; see also reference [8]) are added to a solution of 0.5 mmol of *p*-chloroacetophenone (83.9 mg), 2.5 mmol sodium formate (171.6 mg), and 0.1 mmol of NADH (70.2 mg) in a solvent mixture, consisting of 1 mL of *n*-heptane, and 4 mL of a phosphate buffer (50 mM; pH 7.0). After stirring the reaction mixture for 21h, the organic phase was separated and the aqueous phase is extracted with 3 x 5 mL of methyl *tert*-butyl ether. The collected organic phases are dried over magnesium sulfate, and after filtration and evaporation of the volatile components in vacuo the resulting oily crude product is analyzed with respect to the conversion rate (*via* NMR, HPLC).

In addition, for the asymmetric reduction at a substrate concentration of 100 mM the product (*S*)-**4a** has been purified from the crude product and isolated *via* TLC-chromatography (eluent: ethylacetate / *n*-hexane (25/75); thin-layer-chromatography plate: Merck TLC plate 20x20 cm, silica gel 60 F254; R_f -value: 0.21).