Pergamon

Bioorganic & Medicinal Chemistry, Vol. 2, No. 6, pp. 421-428, 1994

0968-0896/94 $7.00 + .00

0968-0896(94)E0032-W

A Kinetic Study and Application of a Novel Carbonyl Reductase Isolated from *Rhodococcus erythropolis*

Thomas Zelinski and Maria-Regina Kula*

Institut für Enzymtechnologie der Heinrich-Heine-Universität Düsseldorf, Forschungszentrum Jülich (KFA), P.O. Box 2050, 52404 Jülich, Germany

Abstract—The newly described carbonyl reductase from *Rhodococcus erythropolis* (*RE*CR) accepts a broad range of substrates. Based on the kinetic constants of a variety of methyl and ethyl ketones a hypothetical model of the substrate-binding site is proposed. Whether a substrate of interest may be reduced by the *RE*CR can be predicted from this model together with the kinetic data. A study of initial velocities and product inhibition is presented, which shows that the kinetics of the *RE*CR follow a Theorell–Chance mechanism. The pro-*R* hydride of NADH is transferred by the enzyme to the *re* face of the carbonyl compounds yielding (*S*)-alcohols. The reduction of methyl 3-oxobutanoate and ethyl 4-chloro-3-oxobutanoate catalyzed by the oxidoreductase lead to the corresponding hydroxy compounds with high enantiomeric purity [enantiomeric excess (*e.e.*) ≥ 99 %]. The synthesis of ethyl (2*R*,3*S*)-3-hydroxy-2-methylbutanoate was accomplished with high diastereoselectivity (diastereomeric excess = 95 %) and enantioselectivity (*e.e.* ≥ 95 %).

Introduction

The stereoselective synthesis of chiral intermediates can be achieved by different approaches. Besides application of non-natural catalysts or the utilization of a readily available natural product as chiral building blocks, enzymes are employed with increasing frequency for the synthesis of chiral compounds at laboratory, pilot and production scale.[1] Such reactions are carried out with whole cells containing the enzyme in their natural environment or with isolated enzymes. The yeast *Saccharomyces cerevisiae* (baker's yeast) has been especially used for reduction of carbonyl compounds.[2–5] Chemical yields and stereospecificity are highly dependent upon the source and physiological state of the microorganism.[6,7] Application of isolated oxidoreductases using pyridine nucleotides as cofactor may overcome these drawbacks. Effective ways for cofactor regeneration either by enzyme or substrate-coupled approaches were developed to minimize the costs for the cosubstrates NADH and NADPH, respectively.[8–10] An important tool for the optimization, control and scale-up of an enzyme-catalyzed reaction is the knowledge of the steady-state kinetic mechanism and the model equations. For example, the productivity of enzymatic conversions could be improved by knowledge of substrate and product inhibition and activation besides the kinetic constants. Detailed kinetic studies have been described for commercially available alcohol dehydrogenases from horse liver (HLADH),[11] *Saccharomyces cerevisiae* (YADH)[12] and *Thermoanaerobium brockii* (*TB*ADH).[13,14] Recently, novel NAD(P)H-dependent oxidoreductases were described reducing various carbonyl compounds. These enzymes exhibit several advantages for application, for example the alcohol dehydrogenases from *Lactobacillus kefir*[15] and *Pseudomonas* sp.[16] yield (*R*)-alcohols, while the carbonyl reductase from *Candida parapsilosis* (*CP*CR) accepts an extremely broad range of substrates and converts ketones to (*S*)-alcohols in the presence of organic solvents.[17] A detailed kinetic study of *CP*CR has been published.[18] Besides *CP*CR we have screened[7] and purified[19] a novel NADH-dependent oxidoreductase from *Rhodococcus erythropolis* (*RE*CR) utilizing a variety of compounds as substrates. We report here the results of a detailed investigation of the kinetics of the enzyme, including the kinetic mechanism, a hypothetical model of the substrate-binding site and the hydride transfer stereochemistry. In addition, examples for the application of the *RE*CR in asymmetric reduction are presented.

To honor Bryan Jones on the occasion of his 60th birthday

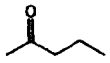
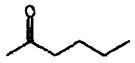
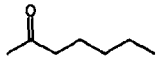
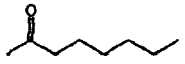
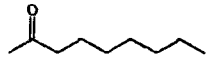
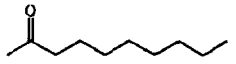
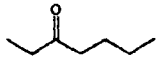
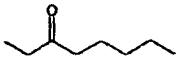
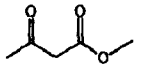
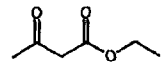
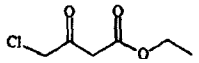
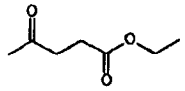
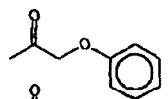
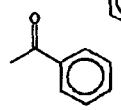
Results

Kinetic constants

Table 1 shows the kinetic constants calculated from the experimental results for the reduction of 16 *RE*CR substrates measured at saturating concentrations of NADH (0.2 mM) in 50 mM triethanolamine–NaOH buffer, pH 7.0. The K_m values in the series of methyl ketones decrease up to the heptyl substituent, whereas the V_{max} values increase up to C-5 side chains. In comparison, ethyl ketones with the same second substituent are reduced with seven to 17-fold slower V_{max} rates and exhibit five to 12-fold increased K_m values than analogous methyl ketones. Keto-esters have comparable K_m values as the ethyl ketones but up to 5-fold higher V_{max} values. The corresponding keto-acids were not reduced by *RE*CR. Aldehydes were reduced with very small reaction rates (≥ 2 %; data not shown).

The oxidation of primary alcohols up to 1-octanol could not be detected as judged by the constant NADH adsorption in the assay. Substrates containing secondary hydroxy

Table 1. Kinetic constants of the *R. erythropolis* carbonyl reductase

Substrate	V_{max} [U·mg^{-1}]	K_m [mM]
2-ketones		
	3.5	330
	3.5	260
	4.8	59
	7.7	3.8
	10.4	0.59
	10.3	0.42
	10.8	0.34
	11.1	0.54
3-ketones		
	0.46	18
	1.4	7.3
keto ester		
	2.6	16
	5.5	3.1
	4.2	9.9
	7.6	8.3
aromatic ketones		
	10.6	0.039
	1.7	3.8

functions were converted by the oxidoreductase with 10-fold slower rates compared to the reduction reaction (50 mM triethanolamine–NaOH buffer, pH 7.0) at 2.5 mM NAD^+ concentration.

For the reduced cofactor a 1.5-fold smaller K_m value was found than for the oxidized form (Table 2). Thus, NADH has the greater affinity towards the enzyme. The comparison of the product inhibition constants (K_{ip}) shows that the product inhibition of the oxidation reaction is 2.5-fold higher than the product inhibition of the reduction. Therefore, the oxidation of hydroxy compounds is the unfavoured direction. In contrast, the secondary alcohol dehydrogenase from *Thermoanaerobium brockii* showed high product inhibition by $NADP^+$ and low product inhibition by NADPH.[13] These data suggest that the enzyme from *R. erythropolis* preferentially catalyses the reduction of carbonyl compounds and is therefore called *Rhodococcus erythropolis* carbonyl reductase (*RE*CR), by us.

Structure of the substrate binding site

A hypothetical structure of the active substrate binding site can be deduced from the kinetic constants summarized in Table 1. As suggested for the alcohol dehydrogenase from *T. brockii*,[20] the *RE*CR possesses two hydrophobic substrate binding sites which differ from one another in size and in the affinity towards alkyl and aromatic groups, respectively. One side chain has to be a small group like a methyl residue, whereas the second side chain should be a large, hydrophobic group preferentially with more than five C atoms (Figure 1). The highest affinity of the *RE*CR towards 2-ketones was found for methylheptylketone. Additional stacking or π–π interactions between the hydrophobic pocket and the substrate could be the reason for the 10-fold smaller K_m value of phenoxyacetone in comparison to the aliphatic 2-ketones.

Kinetic reaction mechanism

Studying the patterns of the double reciprocal plots of initial velocity and product inhibition according to Cleland[21] yielded information on the kinetic mechanism. Both plots for the acetophenone reduction at varied NADH and acetophenone concentrations with fixed acetophenone and NADH concentrations, respectively, showed a nearly parallel pattern (Figure 2). The plots of the reverse reaction, the 1-phenylethanol oxidation, both showed an intersecting initial velocity pattern (Figure 3). Two substrate reactions following a Theorell–Chance mechanism give an intersecting pattern for the slow reaction and a nearly parallel pattern for the reverse direction on the condition that the forward and backward initial reaction rates are sufficiently different. The Theorell–Chance mechanism is a sequentially ordered mechanism in which the steady state level of the central ternary complex is insignificant.[22] The product inhibition by NADH with NAD^+ as variable substrate was

Table 2. Kinetic data of the 1-phenylethanol/acetophenone-system

Abbreviation	Value	Dimension	Comment
Reduction of acetophenone			
K_m, NADH	0.091	mM	Michaelis-Menten constant
K_{ip}, NAD^+	0.16	mM	Competitive inhibition constant
K_m, acetophenone	3.8	mM	Michaelis-Menten constant
Oxidation of 1-phenylethanol			
K_m, NAD^+	0.14	mM	Michaelis-Menten constant
K_{ip}, NADH	0.066	mM	Competitive inhibition constant
K_m, (S)-phenylethanol	1.37	mM	Michaelis-Menten constant
K_m, (R)-phenylethanol	no reaction		
K_{ip}, acetophenone	4.1	mM	Competitive inhibition constant

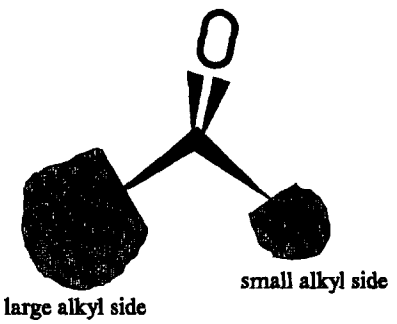


Figure 1. Hypothetical structure of the *RE*CR substrate-binding site. The carbonyl group should be bound to the two hydrophobic pockets, making possible hydride transfer to the *re* face of the keto function to give (*S*)-alcohols.

competitive (Figure 4), also acetophenone inhibiton with 1-phenylethanol as variable substrate (Figure 5). Non-competitive inhibition patterns were found by NADH inhibition with variable acetophenone concentrations (Figure 6) and by acetophenone inhibition with NAD^+ as variable substrate (Figure 7), respectively. Such a product inhibition pattern is compatible with a Theorell–Chance mechanism and a random mechanism with two dead-end complexes, respectively. A Theorell–Chance mechanism is assumed by us based on the initial velocity pattern.

The order of addition of substrates cannot be deduced from the initial velocity and the product inhibition patterns. Additional experiments like isotopic exchange or dead-end inhibition studies are needed to clarify this point.

Hydride transfer stereospecificity

The methylene carbon (C-4) of the dihydronicotinamide ring of NADH is a prochiral center, with the pro-*R* hydrogen (H_R) and the pro-*S* hydrogen (H_S), respectively. If one of the two prochiral hydrogens at the C-4 is replaced with deuterium (^2H), a new chiral center is introduced to the ring. Oxidoreductases catalyzing the reduction of NAD^+ to NADH were classified into A and B stereospecificity depending on the position of the hydrogen transferred H_R or H_S, respectively.[23]

The identification of the transmitted hydride was accomplished by NMR analysis of the diastereotopic methylene atoms of the C-4 ^2H NADH produced by *RE*CR catalyzed $^2H_8$-2-propanol oxidation (Figure 8). The chemical shifts of the C-4 hydrogens are different and were found at 2.77 ppm for the H_R hydrogen of (4*S*) ^2H NADH and 2.67 ppm for the H_S hydrogen of (4*R*) ^2H NADH, respectively.[24] The isolated C-4 ^2H NADH showed one doublet at 2.67 ppm indicating a deuterium transfer from $^2H_8$-2-propanol to the *re* face of NAD^+ yielding (4*R*) ^2H NADH. Thus, *RE*CR belongs to the oxidoreductases of Group A stereospecificity.

The hydride attack at the carbonyl group takes place from the *re* side resulting in (*S*)-hydroxy compounds.[25] The stereochemistry of the hydride transfer is shown in Figure 9. Thus, the *RE*CR follows the 'Prelog-rule' that means the hydride transfer follows a favourable stereochemical course with the least non-bonded repulsion between cofactor and substrate.[26] The *RE*CR showed the same stereochemistry as yeast, horse liver and *Thermoanaerobium brockii* alcohol dehydrogenase and *Candida parapsilosis* carbonyl reductase,[18] but was found to differ from the alcohol dehydrogenases from *Pseudomonas* sp. PED, PADH[16] and *Lactobacillus kefir*.[27]

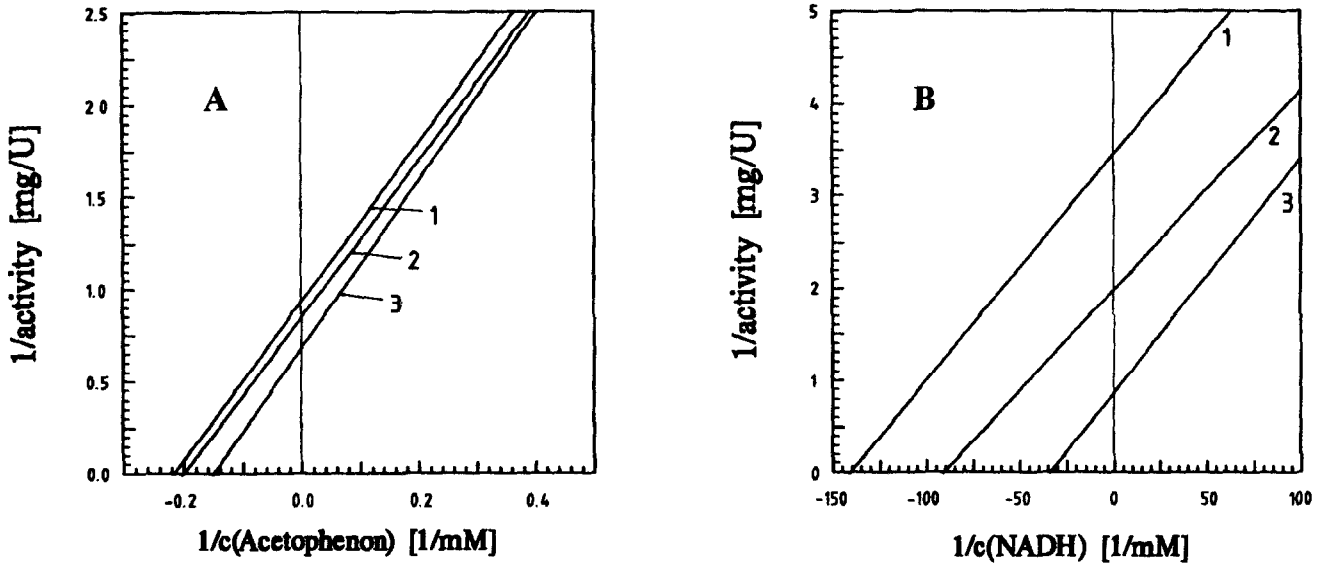


Figure 2. Determination of the initial velocity patterns of *RE*CR for the reduction reaction by double reciprocal plots with (A) acetophenone as variable substrate. NADH 0.05 (1), 0.1 (2), 0.2 (3) mM and (B) NADH as variable substrate. Acetophenone 1 (1), 2 (2), 10 (3) mM.

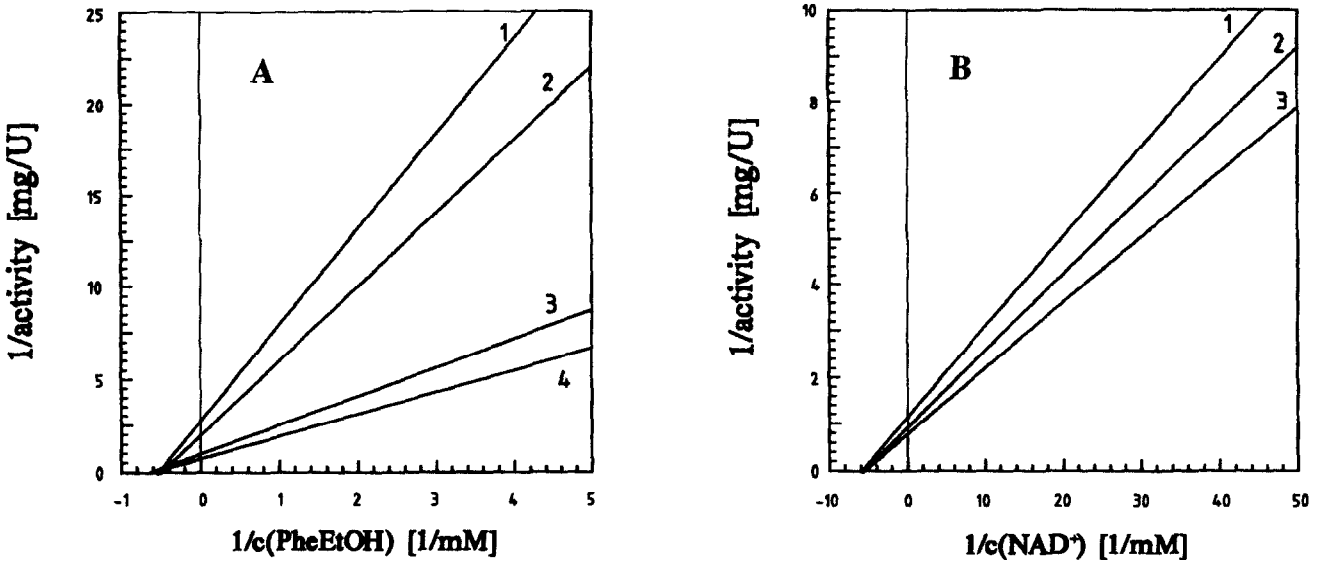


Figure 3. Determination of the initial velocity patterns of *RE*CR for the oxidation reaction by double reciprocal plots with (A) 1-phenylethanol (PheEtOH) as variable substrate. NAD^+ 0.05 (1), 0.1 (2), 0.5 (3), 2.5 (4) mM and (B) NAD^+ as variable substrate. 1-Phenylethanol 1.25 (1), 2.5 (2), 12.5 (3) mM.

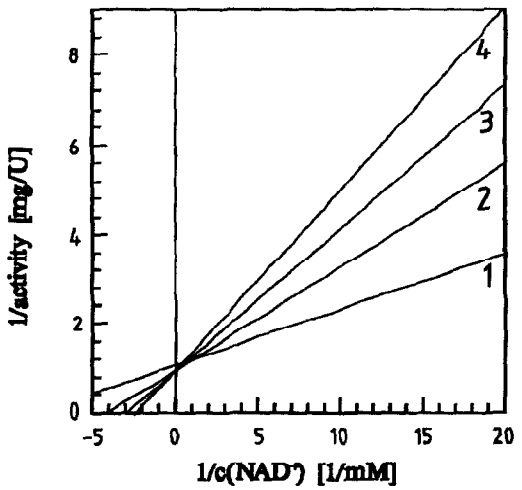


Figure 4. Product inhibition of *RE*CR by NADH with NAD^+ as variable substrate. 1-Phenylethanol 12.5 mM, NAD^+ 0–2.5 mM, NADH 0 (1), 0.05 (2), 0.1 (3), 0.15 (4) mM.

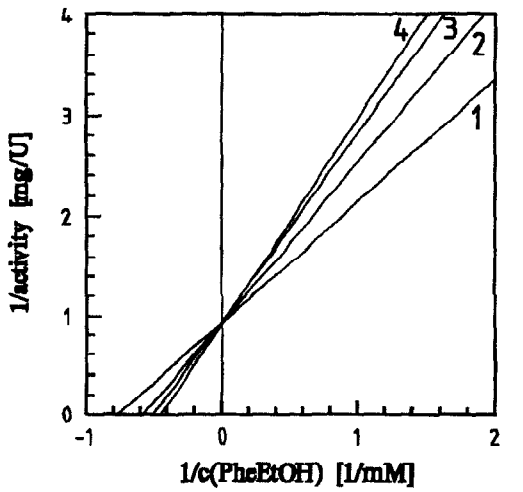


Figure 5. Product inhibition of *RE*CR by acetophenone with 1-phenylethanol as variable substrate. NAD^+ 2.5 mM, 1-phenylethanol 0–12.5 mM, acetophenone 0 (1), 0.625 (2), 1.25 (3), 2.5 (4) mM.

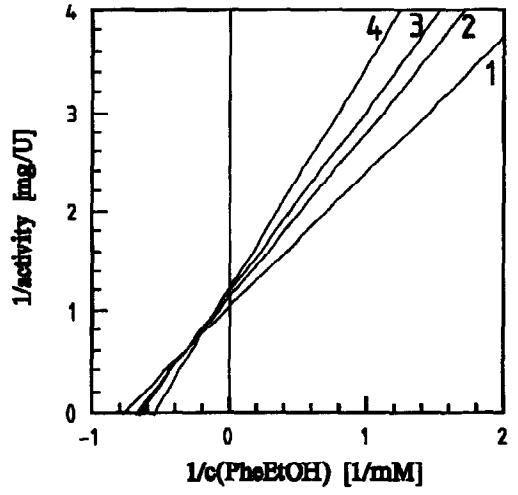


Figure 6. Product inhibition of *RE*CR by NADH with 1-phenylethanol as variable substrate. NAD^+ 2.5 mM, 1-phenylethanol 0–12.5 mM, NADH 0 (1), 0.05 (2), 0.1 (3), 0.15 (4) mM.

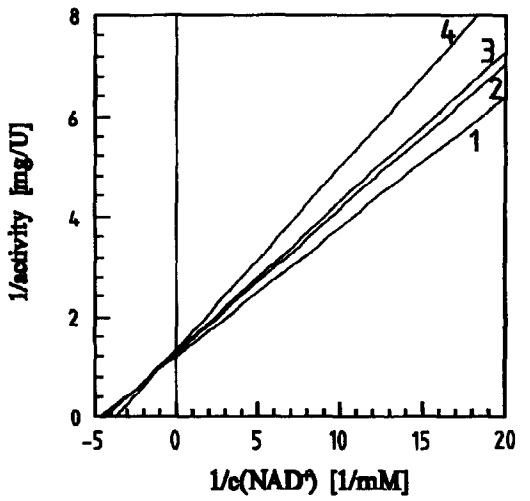


Figure 7. Product inhibition of *RE*CR by acetophenone with NAD^+ as variable substrate. 1-Phenylethanol 12.5 mM, NAD^+ 0–2.5 mM, acetophenone 0 (1), 0.625 (2), 1.25 (3), 2.5 (4) mM.

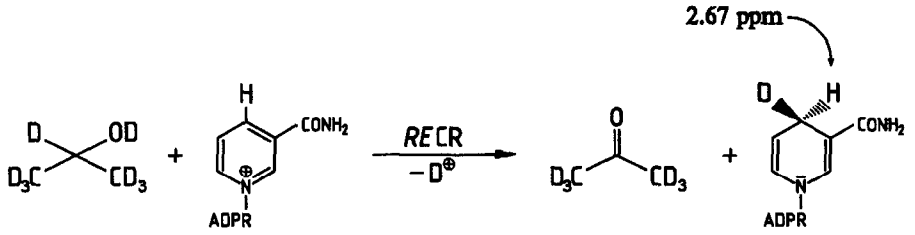


Figure 8. Scheme of the *RE*CR catalyzed NAD^+ -reduction by $^2H_8$-2-propanol. The pro-*S* hydrogen of the methylene group of the reduced nicotinamide ring shows a ^1H NMR signal at 2.67 ppm.

Application of R. erythropolis *carbonyl reductase*

The results of chiral reductions catalyzed by *RE*CR in the preparative scale are shown in Table 3. Cofactor regeneration was accomplished with an enzyme coupled system using formate dehydrogenase (FDH) and sodium formate as second substrate (Figure 10). The oxidoreductase was recovered by ultrafiltration in 80–95 % yield after the reaction was terminated. All substrates were reduced with high stereoselectivity yielding (*S*)-configurated hydroxy compounds with enantiomeric excesses of more than 99 % as determined by chiral gas chromatography.

It was found that ethyl 4-chloro-3-oxobutanoate slowly but irreversibly deactivated the FDH presumably by alkylation of an essential thiol group. Thus, the FDH had to be added in intervals during the preparative reaction.

NMR analysis of ethyl (2*R*,3*S*)-3-hydroxy-2-methyl-butanoate synthesized by *RE*CR reduction from the racemic keto ester showed a *syn*:*anti* ratio[28] of 25:1 corresponding to a diastereomeric excess of 95 %. Further differentiation between the two *syn* enantiomers (2*S*,3*R*) and (2*R*,3*S*) was accomplished by measurement of the optical rotation ($[\alpha]_D^{22}$ = +7.0 ° (*c* = 1.0; $CHCl_3$)) in comparison with known literature data.[29] Ethyl 2-methyl-3-oxobutanoate reduction resulted in the (2*R*,3*S*) configurated product with a *d.e.* of 95 % and an *e.e.* of >95 %.

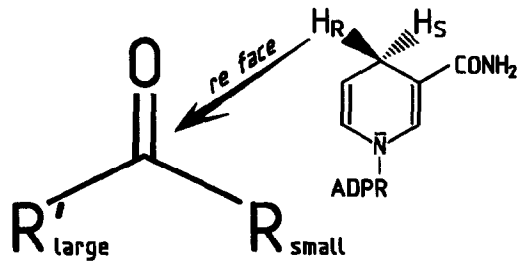
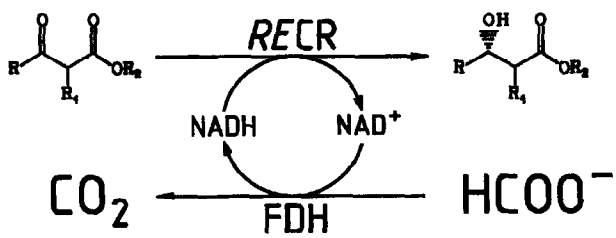


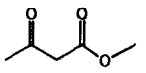
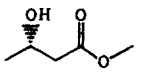
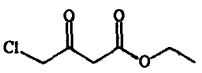
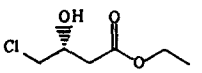
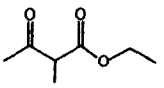
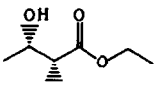
Figure 9. Stereochemistry of the hydride transfer from NADH to carbonyl functions.



R = -CH_3, -CH_2Cl; R_1 = -H, - CH_3; R_2 = -CH_3, -CH_2CH_3

Figure 10. Enzymatic synthesis of 3-hydroxyacid esters by *RE*CR-catalyzed reduction with an enzyme-coupled cofactor regeneration system. FDH: formate dehydrogenase.

Table 3. Products prepared from *RE*CR catalyzed reductions with enzyme-coupled coenzyme regeneration (10 mmole scale)

Substrate	Product	ee [%]	de [%]	Conversion [%]	Recovered activity [%]
		> 99[a]	-	90[b]	95
		> 99[a]	-	100[c]	80
		> 95[d]	95[e]	49[b]	90

[a]Enantiomeric excess and absolute configuration was determined by gas chromatography on a chiral cyclodextrin phase (Lipodex®E) by comparison to authentic enantiomers or racemic compounds. The D-enantiomers elute before the L-enantiomers.
[b]Yield was determined by GC analysis.
[c]Yield was determined by optical rotation. eDiastereomeric excess was determined by ^{1}H-NMR analysis.

Discussion

The new enzyme isolated from *Rhodococcus erythropolis* catalyzes the reduction of a broad substrate range including a variety of compounds useful for synthetic chemistry. Reduction of all tested carbonyl compounds yielded (*S*)-configurated hydroxy functions with high enantiomeric excess. A decreased stereoselectivity for short chain 2-ketones as found for the secondary alcohol dehydrogenase from *Thermoanaerobium brockii*[20] was not detected. Also, only the (*S*)-enantiomers of various optically pure alcohols were oxidized to the corresponding ketones.[19]

The kinetic study presented offers detailed information about the substrate-binding site of the novel carbonyl reductase. The prediction, will a new interesting substrate be reduced by *RE*CR catalysis, should be possible from these data. A correlation between enzyme activity and substrate structure attributes like hydrophobicity and the Taft's steric parameters according to Hansch and Bjorkroth[30] was not found. Additional steric data about the enzyme, especially from the substrate-binding side must be known to build a quantitative model for the *RE*CR-catalyzed reductions.

A valuable goal in enzyme-catalyzed reductions is the simultaneous building of two chiral centers. Rapid racemization of the 2-alkylated 3-ketoacid ester in water at the C_2-atom is a prerequisite to obtain only one of the four possible diastereomers by reduction of the carbonyl group.[31] The *RE*CR catalyze the diastereoselective reduction of ethyl 2-methyl-3-oxobutanoate with high *d.e.* of 95 % and great enantioselectivity (*e.e.* > 95 %). To our knowledge only two enzymes catalyze the NADH-dependent diastereoselective reduction of 2-alkylated 3-ketoacid esters, the *RE*CR and the *CP*CR, as we reported previously.[32]

Further work is in progress to describe the time course of the *RE*CR-catalyzed product formation by a mathematical model using the information on kinetic constants and the kinetic mechanism, presented here.

Experimental

NMR spectra were obtained on a Varian VXR 300 spectrometer (Varian GmbH, Darmstadt, Germany). Shifts are reported as ppm relative to the internal references tetramethylsilane (TMS) or sodium 3-trimethylsilyl-2,2,3,3-^{2}H-propionate (TSP) dependent on the solvent used ($CDCl_3$ or D_2O, respectively). $[\alpha]_D$ Was measured on a polarimeter model 241 MC (Perkin Elmer GmbH, Überlingen, Germany) using a 10 cm path-length cell.

Enzyme assays

The assay mixture contained in a total volume of 1.00 mL 8 mM carbonyl compound, 0.2 mM NADH in glycylglycine–piperazine buffer (100 mM, pH 5.5) and limiting amounts of enzyme. The consumption of the reduced coenzyme was followed in a Shimadzu UV-160 spectrophotometer (Düsseldorf, Germany) at 334 nm and 30 °C using a molar absorption coefficient of 6180 M^{-1} cm^{-1} for calculation.

Kinetic analysis was performed in a 96-well microtiter plate using a microplate reader with thermocontrol (Thermomax, Molecular Devices, Menlo Park, U.S.A.) and flat bottom plates (Neolab, Düsseldorf, Germany). The plates were pretreated with 0.2 % (v/v) Triton X-100 to eliminate adsorption of hydrophobic substrates or enzyme. Measurements of activity were performed in quadruplicate using a total volume of 200 µL of the following incubation mixture for each well: 50 µL coenzyme solution of different concentration, 50 µL substrate of different concentration, 50 µL 200 mM glycylglycine–piperazine buffer pH 7.0 and 50 µL of dilute enzyme solution (11 µg protein). Enzyme assays were initiated within 5 s by addition of enzyme solution employing an 8-

channel microdispenser (model 868, Dynatech, Germany). All reactions were performed at 25 °C. The reaction was linear for about 5 min. The consumption of the reduced coenzyme was followed for 2 min at 340 nm using a molar absorption coefficient of 6220 M^{-1} cm^{-1} and 0.67 cm path length, for calculation.

One Unit (U) of enzyme activity is defined as the amount of enzyme which catalyzes the reduction of 1 µmol of NADH per min under the conditions specified.

Protein concentration

Protein concentrations were measured by the method of Bradford[33] using bovine serum albumin as standard. Specific activity is expressed as units per mg of protein.

Hydride transfer stereochemistry

Partially purified *RE*CR (3.5 U) was added to a mixture of 1 mL $^2H_8$-2-propanol, 150 mg NAD^+ and 50 mL 25 mM ammonium hydrogen carbonate buffer pH 8.0 and stirred at room temperature. When the A_{260}/A_{340} ratio was less than 3.0, the reaction was stopped by removing the enzyme in an ultrafiltration cell equipped with a YM-10 membrane (Amicon, Witten, Germany). The filtrate was applied to a DEAE-Sephacel column (Pharmacia, Freiburg, Germany; 2.6 cm x 8.5 cm) previously equilibrated with 25 mM ammonium hydrogen carbonate buffer pH 8.0. NAD^+ was eluted with 50 mM ammonium hydrogen carbonate, and NADH was eluted with 250 mM ammonium hydrogen carbonate buffer, both pH 8.0. Those NADH fractions having A_{260}/A_{340} ratio of less than 2.4 were pooled and lyophilized. The residue was lyophilized twice from ^2H_2O to remove excess H_2O.

Data handling

Kinetic parameters were estimated from kinetic measurements by means of non-linear regression methods according to Rosenbrock[34] applied to suitable kinetic models (initial reaction rate for noncompetitive substrate inhibition, noncompetitive, uncompetitive and competitive product inhibition and Michaelis–Menten kinetics). Alternatively, kinetic constants were estimated by the statistical method developed by Wilkinson[35] or a Marquardt algorithm; both produced identical results. The kinetic data were replotted double-reciprocally to obtain the initial velocity and the inhibition patterns. For all calculations an IBM AT with a math coprocessor was employed.

Enzymatic conversions

The enzymatic transformations were carried out at room temperature with stirring (120 rpm) in a total volume of 100 mL. The reduction systems with enzyme-coupled cofactor regeneration consisted of the following compounds: 1 M sodium formate, 0.05 mM NAD^+, 100 mM carbonyl compound (40 mM in the case of ethyl 4-chloro-3-oxobutanoate) 1 U *RE*CR, 4 U FDH and 100 mM triethanolamine buffer pH 7.5. At various times samples were removed, products were isolated by extraction with chloroform and analyzed by chiral gas chromatography.

Chiral gas chromatography

Product samples were derivatized with trifluoroacetic anhydride according to König *et al.*[36] and an aliquot (1 µL) was applied on a chiral Lipodex®E γ-cyclodextrin column (25 m x 0.25 mm ID, Macherey and Nagel, Düren, Germany). The temperature of the injector of the chromatograph (GC 9A, Shimadzu, Düsseldorf, Germany) was set at 250 °C. The detection was carried out with a flame ionization detector. Separation of the hydroxy compounds was performed isothermal at 80 °C. The retention times of the trifluoroacetyl derivates of the authentic enantiomers were as follows: methyl (*R*)-3-hydroxybutanoate, 10.5 min; methyl (*S*)-3-hydroxybutanoate, 14.4 min; ethyl (*S*)-4-chloro-3-hydroxybutanoate, 48.9 min; ethyl (*R*)-4-chloro-3-hydroxybutanoate, 50.6 min.

References and Notes

1. Wong, C. H. *Science* **1989**, *244*, 1145.

2. Roberts, S. M. In *The Enzyme Catalysis Process*, pp. 443–463, Cooper, A.; Houben, J. L.; Chien, L. C., Eds; Plenum Press; New York, 1988.

3. Christen, M.; Crout, D. H. G. *J. Chem. Soc. Chem. Commun.* **1988**, *4*, 264.

4. Christen, M.; Crout, D. H. G.; Holt, R. A.; Morris, J. G.; Simon, H. *J. Chem. Soc. Perkin Trans. I* **1992**, 491.

5. Sato, T.; Fujisawa, T. *Biocatalysis* **1990**, *3*, 1.

6. Sih, C. J.; Chen, C. S. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 570.

7. Peters, J.; Zelinski, T.; Kula, M. R. *Appl. Microbiol. Biotechnol.* **1992**, *38*, 334.

8. Chenault, H. K.; Whitesides, G. M. *Appl. Biochem. Biotechnol.* **1987**, *14*, 147.

9. Hummel, W.; Kula, M. R. *Eur. J. Biochem.* **1989**, *184*, 1.

10. Wandrey, C. In *Proc. 4th European Congress of Biotechnology*, Vol. 4, pp. 171–188, Neijssel, O. M.; van der Meer, R. R.; Luyben, K. C., Eds; 1987.

11. Scrimgeour, K. G. In *Chemistry and Control of Enzyme Reactions*, pp. 137-143, Academic Press; London, 1977.

12. Wratten, C. C.; Cleland, W. W. *Biochemistry* **1963**, *2*, 935.

13. Peters, J.; Kula, M. R. *Biotechnol. Appl. Biochem.* **1991**, *13*, 363.

14. Ford, J. B.; Askins, K. J.; Taylor, K. B. *Biotechnol. Bioeng.* **1993**, *42*, 367.

15. Hummel, W. *Biotechnol. Lett.* **1990**, *12*, 403.

16. Bradshaw, C. W.; Fu, H.; Shen, G. J.; Wong, C. H. *J. Org. Chem.* **1992**, *57*, 1526.

17. Peters, J.; Minuth, T.; Kula, M. R. *Enzyme Microb. Technol.* **1993**, *15*, 950.

18. Peters, J.; Minuth, T.; Kula, M. R. *Biocatalysis* **1993**, *8*, 31.

19. Zelinski, T.; Peters, J.; Kula, M. R. *J. Biotechnol.* **1994**, *33*, 283.

20. Keinan, E.; Hafeli, E. K.; Seth, K. K.; Lamed, R. J. *J. Am. Chem. Soc.* **1986**, *108*, 162.

21. Cleland, W. W. In *Investigations of Rates and Mechanisms of Reactions*, Vol. 6, pp. 792–821, Bernasconi, C. F., Ed.; Wiley; New York, 1986.

22. Theorell, H.; Chance, B. *Acta Chem. Scand.* **1951**, *5*, 1127.

23. You, K. S. *Crit. Rev. Biotechnol.* **1984**, *17*, 313.

24. Arnold, L. J.; You, K.; Allison, W. S.; Kaplan, N. O. *Biochemistry* **1976**, *15*, 4844.

25. According to the nomenclature of Cahn, Ingold and Prelog the great alkyl chain has a higher priority than the small alkyl chain. In the case of ethyl 4-chloro-3-oxobutanoate the priorities are reversed, however. Thus, the reduction product has (*R*)-configuration, for formal reasons.

26. Prelog, V. *Pure Appl. Chem.* **1964**, *9*, 119.

27. Bradshaw, C. W.; Hummel, W.; Wong, C. H. *J. Org. Chem.* **1992**, *57*, 1532.

28. For simplicity, we use the definition of Nakamura[37] that the (2*R*,3*R*)- and (2*S*,3*S*)-isomer are the *anti*-isomers and the (2*R*,3*S*)- and (2*S*,3*R*)-isomers are the *syn*-isomers, respectively. We prefer the *syn/anti* expression rather than the *threo/erythro* notation because the latter is subject to the substituent dependent priority rules.

29. Frater, G.; Müller, U.; Günther, W. *Tetrahedron* **1984**, *40*, 1269.

30. Hansch, C.; Bjorkroth, J. P. *J. Org. Chem.* **1986**, *51*, 5461.

31. Poppe, L.; Novak, L. *Selective Biocatalysis. A Synthetic Approach*, Weinheim, 1992.

32. Peters, J.; Zelinski, T.; Minuth, T.; Kula, M. R. *Tetrahedron: Asymmetry* **1993**, *4*, 1683.

33. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.

34. Hoffmann, U.; Hofmann, H. *Einführung in die Optimierung mit Anwendungsbeispielen aus dem Chemie-Ingenieur-Wesen*, Weinheim, Germany, 1971.

35. Wilkinson, G. N. *Biochem. J.* **1961**, *80*, 324.

36. König, W. A.; Krebber, R.; Mischnick-Lübbecke, P. *J. High Resolut. Chromatogr. Chromatogr. Commun.* **1989**, *12*, 732.

37. Nakamura, K.; Miyai, T.; Nagar, A.; Babu, B. R.; Ando, T.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 298.

(*Received* 18 *October* 1993; *accepted* 20 *January* 1994)