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Journal of Molecular Catalysis B: Enzymatic 22 (2003) 7–12

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

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(*R*)-Oxynitrilase-catalyzed synthesis of (*R*)-2-trimethylsilyl-2-hydroxyl-ethylcyanide

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Received 10 August 2002; received in revised form 18 November 2002; accepted 20 November 2002

Abstract

The enantioselective synthesis of (*R*)-2-trimethylsilyl-2-hydroxyl-ethylcyanide with (*R*)-oxynitrilase from defatted almond meal in a biphasic system was successfully performed. The influences of some factors on the reaction were investigated systematically. Diisopropyl ether was found to be the best for this reaction among all the organic solvents explored. The optimal aqueous phase content, concentrations of crude enzyme, acetyltrimethylsilane, acetone cyanohydrin, shake speed, buffer pH, reaction temperature were 13% (v/v), 5% (w/v), 0.02 M, 0.04 M, 150 rpm, 5.0, 40 °C, respectively, under which the initial reaction rate, substrate conversion and product e.e. were 5.87 mmol/l h, 99.5 and 99.0%.

Keywords: (*R*)-2-Trimethylsilyl-2-hydroxyl-ethylcyanide; (*R*)-Oxynitrilase; Asymmetric synthesis; Organosilicon; Cyanohydrin

1. Introduction

The synthesis of optically active aldehyde cyanohydrins by oxynitrilase-catalyzed addition of hydrogen cyanide to prochiral aldehydes has been the goal of extensive and successful studies during recent years [1–6]. The preparation of optically active ketone cyanohydrins, on the other hand, has been less studied [7–9]. Enantiopure ketone cyanohydrins could be converted to tertiary α-hydroxy acids, which are useful starting materials or synthetic intermediates for many chiral natural products [10–12].

Organosilicon compounds show unique chemical and physical properties compared to their carbon analogues due to the specific characteristics of silicon atom, and therefore they not only play an important role in asymmetric synthesis and functional materials, but also are bioactive and could be used for the synthesis of drugs with better pharmacological effect, higher selectivity and lower toxicity than their carbon counterparts. Recently, many investigations have been carried out in order to exploit biotransformation of organosilicon compounds because of their great interest both in fundamental study of enzymology and in the production of useful organosilicon compounds [13–15]. We have already reported the bioconversion of organosilicon compounds with alcohol dehydrogenase and lipase [16–18]. In these cases, the silicon atom served as a more effective atom than the carbon atom to enhance the activity and enantioselectivity of the enzymes.

In this paper, we describe the first study on the synthesis of optically active silicon containing aliphatic ketone cyanohydrin (2-trimethylsilyl-2-hydroxyl-ethylcyanide) using acetone cyanohydrin as

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1381-1177/02/$ – see front matter
PII: S1381-1177(02)00283-7

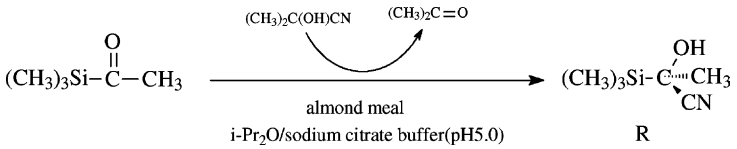

Fig. 1. The synthesis of 2-trimethylsilyl-2-hydroxyl-ethylcyanide.

transcyanation agent and powdered, defatted almond meal as biocatalyst (Fig. 1).

2. Results and discussion

2.1. Organic solvent effect

As shown in Table 1, the more hydrophobic the organic solvent, the higher the initial reaction rate. This could be explained by the higher concentration of HCN owing to the higher concentration of acetone cyanohydrin in aqueous phase when more hydrophobic solvent is used as the organic phase. Two competitive reactions (enantioselective enzymatic reaction and non-enantioselective chemical reaction) take place simultaneously. Too much HCN in the aqueous phase might inhibit the enzymatic but enhance the chemical reaction, thus leading to a low product e.e. This could account for the low product e.e. in the cases with *n*-hexane and cyclohexane as the organic phase. Why the reaction becomes less enantioselective when ethylacetate is used as the organic phase is to be studied. Among the four organic solvents, diisopropyl ether is most suitable for the reaction.

2.2. Aqueous phase content effect

It has been reported that the influence of the aqueous phase content varies widely [19,20]. In this reaction, acetone cyanohydrin was used as transcyanation agent. Relatively high water content is necessary for the decomposition of acetone cyanohydrin at a reasonable rate [21]. Fig. 2 shows the variation of the product e.e. and the initial reaction rate with aqueous phase content. At low water content, acetone cyanohydrin decomposed slowly, and therefore, HCN concentration in aqueous phase is rather low, resulting in the low initial reaction rate. On the other hand, an excess of water accelerates the non-enantioselective chemical reaction, resulting in a decrease in the product e.e.

2.3. Crude enzyme concentration effect

As can be seen in Fig. 3, both initial reaction rate and product e.e. increase with the increase of the crude enzyme concentration when it is below 5% (w/v). This is due to the higher enzymatic reaction rate at higher enzyme concentration. Further increase in the crude enzyme concentration, however, results in a decrease in both initial reaction rate and product e.e., which is in good accordance with the previous report [3]. The reason for this is that obvious aggregation of the crude enzyme at high concentration (>5% (w/v)) led to a low enzymatic activity due to mass transfer limitation. Clearly, there exists a mass transfer rate limiting enzyme concentration ($C_{E,MTL}$), and under the conditions used, the $C_{E,MTL}$ is 5% (w/v) for acetyltrimethylsilane.

Table 1
Effect of organic solvent on the reaction

Organic solvent	Log *P*	V_0 (mmol/l h)	*t* (h)	Conversion (%)	e.e. (%)
Ethyl acetate	0.68	0.39	23	74.1	86.4
Diisopropyl ether	1.9	0.94	23	99.7	97.7
Cyclohexane	3.0	1.84	20	93.1	73.8
n-Hexane	3.5	1.90	20	94.2	72.6

The reaction was initiated by addition of 3% (w/v) almond meal in the biphasic system of 13% (v/v) of citrate buffer (0.1 M, pH 5.0) and organic solvent at 30 °C, 150 rpm. The reaction mixture contained 0.02 M acetyltrimethylsilane and 0.04 M acetone cyanohydrin.

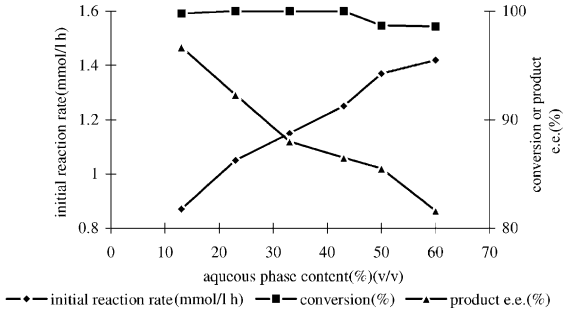

Fig. 2. Effect of aqueous phase content on the reaction. This was investigated in the biphasic system of citrate buffer (0.1 M, pH 5.0) and diisopropyl ether at 30 °C and 150 rpm. The reaction mixture contained 0.02 M acetyltrimethylsilane, 0.04 M acetone cyanohydrin, and 3% (w/v) almond meal.

2.4. Substrate concentration effect

It is well known that, thermodynamically, high substrate concentration pushes the reaction towards the synthesis of the product, and that substrate inhibition, however, may occur at excessively high substrate concentration. Therefore, it is important to investigate the influence of substrate concentration on the reaction. It has been found that the optimal acetyltrimethylsilane concentration was 0.02 M and the product e.e. dropped sharply at higher substrate concentration (Table 2). Substrate inhibition might be the main reason for this.

2.5. Buffer pH effect

The buffer pH has a significant influence on enzymatic enantioselectivity and activity [7,21]. At the same time, the effect of pH on the formation of CN^- is apparent [21]. At low pH ($\leq$6.0), the formation of HCN proceeds slowly, which contributes to a high product e.e. As can be seen in Fig. 4, the maximal initial reaction rate was achieved at pH 5.4, which is identical with Griengl's report [22]. High product e.e. and low initial reaction rate at low pH ($\leq$5.0) demonstrate the depression of non-enzymatic reaction at low pH.

2.6. Shake speed effect

Shake speed influences the diffusion and partition of the substrate and the product in the reaction system and thus the initial reaction rate, substrate conversion and product e.e. As could be seen in Fig. 5, when shake speed was lower than 150 rpm, the initial reaction rate increased rapidly with the increase of shake speed, indicating that mass transfer was the rate limiting step. The optimal shake speed was thought to be 150 rpm, above which little increase in the initial

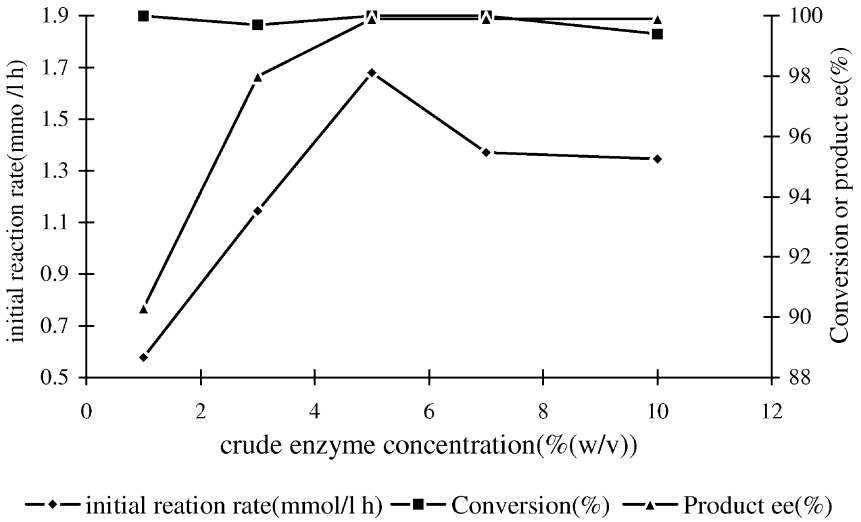

Fig. 3. Effect of crude enzyme concentration on the reaction. The reaction was performed in the biphasic system of 13% (v/v) of citrate buffer (0.1 M, pH 5.0) and diisopropyl ether at 30 °C and 150 rpm. The reaction mixture contained 0.02 M acetyltrimethylsilane and 0.04 M acetone cyanohydrin.

Table 2
Effect of substrate concentration on the reaction

Acetyltrimethylsilane concentration (M)	Acetone cyanohydrin concentration (M)	t (h)	Conversion (%)	e.e. (%)
0.01	0.02	30	100	>99
0.02	0.04	30	98.9	>99
0.04	0.08	48	99.7	95.8
0.08	0.16	48	96.8	90.6
0.16	0.32	48	82.7	82.0
0.20	0.40	48	71.9	78.6

The reaction was initiated by addition of 5% (w/v) almond meal in the biphasic system of 13% (v/v) of citrate buffer (0.1 M, pH 5.0) and diisopropyl ether at 30 °C and 150 rpm.

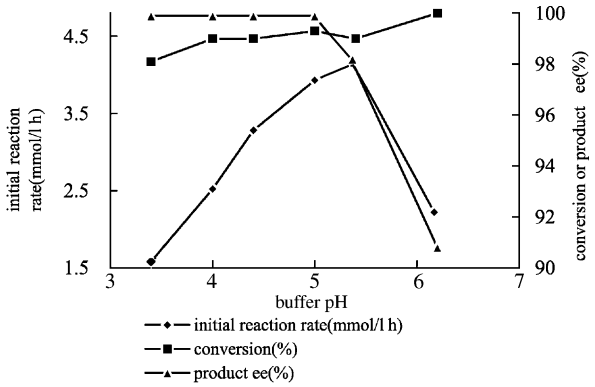

Fig. 4. Effect of the buffer pH on the reaction. The reaction was initiated by addition of 5% (w/v) almond meal in the biphasic system of 13% (v/v) of citrate buffer (0.1 M) and diisopropyl ether at 30 °C and 150 rpm. The reaction mixture contained 0.02 M acetyltrimethylsilane and 0.04 M acetone cyanohydrin.

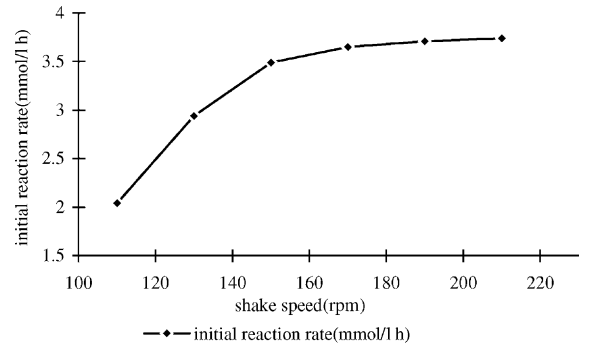

Fig. 5. Effect of shake speed on the initial reaction rate. The reaction was initiated by addition of 5% (w/v) almond meal in a flask (50 ml) containing the biphasic system of 13% (v/v) of citrate buffer (1.5 ml, 0.1 M, pH 5.0) and diisopropyl ether (10 ml) at 30 °C. The reaction mixture contained 0.02 M acetyltrimethylsilane and 0.04 M acetone cyanohydrin.

Table 3
Effect of reaction temperature on the reaction

T (°C)	V_0 (mmol/l h)	t (h)	Conversion (%)	e.e. (%)
40	5.87	19	99.5	>99
35	3.56	26	99.5	>99
30	3.39	31	98.5	>99
25	2.84	47	98.7	>99
15	0.84	55	89.3	>99

The reaction was initiated by addition of 5% (w/v) almond meal in the biphasic system of 13% (v/v) of citrate buffer (0.1 M, pH 5.0) and diisopropyl ether at 150 rpm. The reaction mixture contained 0.02 M acetyltrimethylsilane and 0.04 M acetone cyanohydrin.

reaction rate was observed with further increase in shake speed.

2.7. Reaction temperature effect

Temperature influences the activity, selectivity and stability of a biocatalyst and the equilibrium of a reaction as well significantly. The initial reaction rate, the maximum conversion and the product e.e. were explored at different temperatures (Table 3). Within the range from 15 to 40 °C, higher temperature enhanced the initial reaction rate, and substrate conversion. Temperature had no effect on the product e.e., which was against the previous study [23]. Good thermo stability of crude (*R*)-oxynitrilase from almond meal was observed.

3. Materials and methods

3.1. Materials

Almond meal was ground, then defatted with ethyl acetate and stored at 4 °C for use. The specific activity

of (*R*)-oxynitrilase is 2826 U/g as measured by following the cleavage of rac-mandelonitrile into benzaldehyde spectrophotometrically at 280 nm. *n*-Nonane, acetyltrimethylsilane were purchased from Sigma and Aldrich (USA). Acetone cyanohydrin was from TCI (Japan). All other chemicals were from commercial sources and of analytical grade.

3.2. Enzymatic reaction

The reaction was carried out in a biphasic system. Diisopropyl ether (10 ml) containing 0.02 M acetyltrimethylsilane, 0.04 M acetone cyanohydrin and 0.01 M *n*-nonane (used as internal standard) was mixed with citrate buffer (1.5 ml, 0.1 M, pH 5.0) containing powdered, defatted almond meal (unless specified). The mixture was incubated within a flask (50 ml) capped with a septum and shaken in a water bath shaker.

3.3. Assay of reaction mixture

At predetermined time intervals, 0.4 ml sample was taken from the organic phase with a syringe (more than 99% of substrate was dissolved in the organic phase and no side product was detectable by GC equipped with different columns) and assayed by HP 4890 gas chromatography with a flame ionization detector and a chiral column (20% permethylated β-cyclodextrin 30 m × 0.32 mm, HP, USA). The column temperature was programmed from 80 to 139 °C at the rate of 7 °C/min and further increased to 142 °C at the rate of 1 °C/min. The retention-times of acetyltrimethylsilane, *n*-nonane, acetone cyanohydrin, (*S*)-product, and (*R*)-product were 2.851, 3.651, 6.323, 9.715 and 9.780 min, respectively. GC peaks of the (*R*) and (*S*) enantiomers of the product were assigned on the basis of the known (*R*) enantioselectivity of the enzyme.

3.4. NMR spectral analysis

Kugelrohr distillation gave 2-trimethylsilyl-2-hydroxyl-ethylcyanide (65–70 °C, 8 mm). NMR spectra were obtained using $CDCl_3$ solutions ($CHCl_3$ taken as δ: 7.24) and a Brucker AVANCE Digital 400 MHz Spectrometer. ^{1}H NMR δ: 0.18 (s, 9H), 1.54 (s, 3H), 2.85 (br s, 1H). ^{13}C NMR δ: −5.0, 23.3, 59.9, 123.1.

4. Conclusion

Optically active silicon containing aliphatic (*R*)-ketone-cyanohydrin (*R*)-2-trimethylsilyl-2-hydroxyl-ethylcyanide could be efficiently synthesized with (*R*)-oxynitrilase from defatted almond meal in a water/organic solvent biphasic system.

Acknowledgements

This work was supported by grants from the Chinese National Science Foundation (20076019) and the Natural Science Foundation of Guangdong Province (000444).

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