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Stereochemical control of biocatalytic asymmetric reduction of diethyl 2-oxopropylphosphonate employing yeasts

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Abstract

Nonracemic diethyl 2-oxopropylphosphonate of *R* and *S* configuration and of high enantiomeric purity was obtained by stereocontrolled bioreduction using: *Rhodotorula rubra*, *Rhodotorula glutinis*, *Rhodotorula gracilis* and baker's yeast. The effect of the addition of chemical compounds influencing the stereoselectivity of biotransformation have been investigated.

Keywords: Whole-cell biocatalysis; Enantioselectivity; Phosphonates

1. Introduction

Biotransformations represent an effective and sometimes preferable alternative to the standard synthesis of fine chemicals and optically active compounds. Biocatalysts are excellent catalysts as they can perform regio- and stereospecific reactions under mild conditions and artificial compounds can be accepted as substrates. Asymmetric biocatalysis employs either whole microorganisms or isolated enzymes. Bioconversions with living cells, which have the ability to regenerate their own respective cofactors, are frequently more advantageous. The most popular whole-cell biocatalyst appears yeasts (e.g. *Saccharomyces cerevisiae*), which potential to carry out organic reactions has been explored for nearly a century with the most common application being the asymmetric reduction of ketones by growing or stationary-phase yeast cells [1–4]. We have described preparation of optically pure diethyl α-, β-, γ-, δ-hydroxyphosphonates from the corresponding oxoalkylphosphonates via yeast catalysed bioreduction [5–7]. Resulting hydroxyphosphonates constitute a class of organophosphorus compounds of potential biological activity such as enzymes inhibitors of rennin or human immunodeficiency virus [8–10]. It is well known, that usually physiological activity of chiral compounds differs dependent on the absolute configuration of enantiomers. The objective of work reported here was to established the method of stereochemically controlled bioreduction of diethyl 2-oxopropylphosphonate as a model substrate in water media using living cells of different yeasts as biocatalysts. The elaborations of these procedures resulted in obtaining enantiomerically pure diethyl 2-hydroxypropylphosphonate in both enantiomeric forms.

2. Materials and methods

2.1. Materials

2.1.1. Microorganisms

Fresh baker's yeast were purchased from Wołczyn, Poland (strain PW-A-79002); *Rhodotorula glutinis*

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(DSMZ 10134); *Rhodotorula rubra* (PCM 5023); *Rhodotorula gracilis* (W 1).

2.1.2. Chemicals

All chemicals were purchased from POCH Gliwice, Poland.

2.2. Cultivation medium for Rhodotorula sp.

Medium (DSMZ 186) – was used for *Rhodotorula* sp. cultivation and was composed of (g/dm^3 – distilled water solution): yeast extract (3), maltose extract (3), peptone (5), D-glucose (10). Solution of D-glucose (50%) was autoclaved separately. Medium was autoclaved as described above and then the pH was adjusted to 6.0.

2.3. Methods

2.3.1. Cultivation conditions

The cells of microorganisms were transferred from the slant to the 250 cm^3 flask containing 50 ml of medium and cultivated at 150 rpm at 28 °C for 48 h under aerobic conditions. After incubation, inoculum (1 ml) was transferred to 500 cm^3 flask containing 150 ml of the medium and it was cultivated under the same conditions for six days. The cells pellet, after centrifugation (4500 rpm/5 min), was washed with 0.1 M sodium citrate buffer (pH 6) and prepared for biotransformation.

2.3.2. Biotransformation – general procedure

1 mmol of diethyl 2-oxopropylphosphonate, biocatalyst (30 g of purchased baker's yeast or the cells or *Rhodotorula* sp. after cultivation according to method described above) and depending on down stream, specified additive (ethyl alcohol – 100 μl, *iso*-propyl alcohol – 200 μl, *iso*-propylmethyl ketone – 222 μl, allyl bromide – 106 μl or allyl alcohol – 210 μl, ethyl chloroacetate – 78 μl and methylvinyl ketone – 90 μl) were added to 250 ml flask containing 20 ml of distilled water. After biotransformation, the mixture consisted substrate and desired product – hydroxyphosphonate.

2.3.3. Purification of diethyl 2-hydroxypropylphosphonate

After biotransformation, the biocatalyst was removed by centrifugation and supernatant was extracted twice with ethyl acetate, after that, organic layer was dried and evaporated under reduced pressure resulting in a mixture with about 80% of the mass of the starting compound. Product of the reduction was purified by column chromatography on Merck silica gel 60 (230–400 mesh) using acetonitrile as an eluent. Diethyl 2-hydroxypropylphosphonate: colourless oil; ee 99%; IR (film, cm^{-1}) 3410 (OH), 1245 (PO), 1050 and 1025 (POC); ^{31}P NMR δ 31.71 ppm, ^{1}H NMR δ 3.93–4.12 ppm (m, 4H, P(O)(OCH_2CH$_3$)); δ 4.51–4.54 ppm (m, 1H, CH(OH)); δ 1.18 ppm (t, 6H, J_{HH} = 7.1 Hz, P(O)(OCH$_2$CH_3)); δ 1.28 ppm (dd, 3H, J_{HH} = 6.04 Hz, J_{PH} = 2.43 Hz; CH_3CH(OH)); ^{13}C NMR δ 24.34 ppm (J = 24.26 Hz, CH$_3$); δ 16.43 ppm (J = 4.09 Hz, P(O)(OCH$_2$$CH_3$); δ 62.86 (J = 5.2 Hz, CH(OH)P), δ 63.8 ppm (J = 7.24 Hz, P(O)CH$_2$CH$_3$).

2.3.4. Determination of optical purity of diethyl 2-hydroxypropylphosphonate

Optical purity of the product was assayed using quinine as chiral discriminator by achieving a shift difference ($\Delta\delta$) of ^{31}P NMR signals (experimental error is about 1%) [11] (e.g. Fig. 1).

2.3.5. Determination of absolute configuration of diethyl 2-hydroxypropylphosphonate

Absolute configuration was determined according to described results of work of Hammerschmidt and coworkers [12]. Optical rotation was measured as $[\alpha]^0_{578} = +7.5^0$ (for S isomer) and -6.0^0 (for R), $c = 2$ in methanol.

2.3.6. Assay of the microbial activity

Reductive activity of living microbial cells was assayed under biotransformation conditions, using acetophenone (1.75 ml/l) as a standard substrate, presence of the phenylethyl alcohol after biotransformation was monitored by HPLC (Beckman Gold Nouveau System, ODS column – Cosmosil 5C18 (filled with 5 μ particles of silica as support), 4.6 mm × 150 mm; eluent: 0.0017

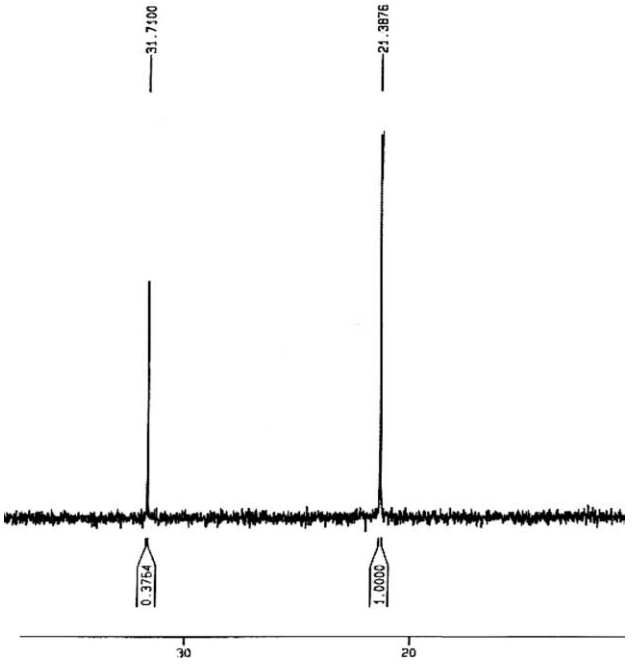

Fig. 1. ^{31}P NMR spectra (with quinine) (δ 31.71 ppm for the product and 21.38 ppm for the substrate) for biotransformation catalysed by baker's yeast without any additives (Table 2).

M MES–Na buffer (pH 8.7) and acetonitrile – v/v – 1:1; flow – 1 ml/min; $\lambda = 220$ nm) [13].

2.3.7. Determination of optical purity and absolute configuration of phenylethanol

Absolute configuration and optical purity of 1-phenylethanol were assayed using gas chromatography (chromatograph Ai Cambridge GC94M, chiral column: Chirasil – Val 25 m × 0.25 mm D × 0.16 μm, conditions: initial temperature = 40 °C, temperature changed gradually at rate = 0.6 °C per minute to 90 °C) [14]. Under these conditions, the elution times for the enantiomers of 1-phenylethanol were as follows: *R*, 64.10 min; *S*, 65.16 min.

3. Results and discussion

Different yeasts (baker's yeast, *R. rubra*, *R. glutinis*, *R. gracilis*) which are known from their reductive activity [7] were used for the evaluation of possibility to obtain optically pure enantiomers of diethyl 2-hydroxypropylphosphonate in stereochemically controlled processes. As it is well established in literature there are at least two sort of chemical additives affecting the activity of particular enzymes: designed as exogenous sources of hydrogen for cofactor regeneration or as an enzyme inhibitors [15–19]. We have tested six of them: ethyl, *iso*-propyl and allyl alcohols, allyl bromide, methylvinyl ketone and *iso*-propylmethyl ketone, which was found in our previous work, as a compound increasing chemical yield of diethyl 1-oxophosphonates microbial reduction [7]. First of all, we have checked the reductive activity of the chosen microorganisms against the standard substrate, acetophenone, in biotransformation conditions. As it is shown in Table 1 there are no differences between microorganisms if considering absolute configuration of the product – phenylethyl alcohol, which was the same for every strain of yeasts and for particular additive. Bioreduction of acetophenone in water media afforded either the *S* isomer of phenylethyl alcohol (without any additives or for allyl alcohol – Table 1) or *R* isomer when allyl bromide was used or lack of the reaction was observed for ethyl chloroacetate and methylvinyl ketone, which probably inhibit the reductive activity of dehydrogenases which are involved in acetophenone biotransformation. The rest of additives used, have no significant influence on the bioprocess. Subsequently we have investigated the biotransformation of diethyl 2-oxopropylphosphonate. Experiments done showed, that only baker's yeast and *R. rubra* were active against model oxophosphonate (Table 2). It is necessary to stress that results described in Table 2 are in contradiction with those described for acetophenone bioreduction if considering the additives as the enzymes inhibitors. In the case of diethyl 2-oxopropylphosphonate, the lack of the reaction was observed when allyl bromide and allyl alcohol were used. As it is shown in Table 2, the possibility of stereocontrol of bioreduction of diethyl 2-oxopropylphosphonate was achieved when baker's yeast were employed. It is noteworthy that the best chemical yield (50% for *S(+)* isomer and baker's yeast) was obtained when biotransformation was carried out without any additives. However, the presence of ethyl chloroacetate and methylvinyl ketone allowed to reverse the stereospecificity of the process, product was of *R(−)* configuration, but the chemical yield of reaction decreased (Table 2). It has to be stressed, that for *R. rubra* ethyl chloroacetate turned out to be a factor which definitely increased the chemical yield of bioreduction of model phosphonate, but unfortunately the only enantiomer obtained for these yeasts was of *R*-configuration, independent on biotransformation conditions (Table 2).

The attention has to be turned on one more result, which implied from the comparison of the activity of baker's yeast and *R. rubra* toward acetophenone and oxophosphonate in the process carrying out without any additives (Fig. 2).

As it is shown in Fig. 2, biotransformation catalysed by baker's yeast resulted only in enantiomer *S*, independent on the substrate used, whereas when employed *Rhodotorula rubra*, the products of the bioconversion were of opposite absolute configuration: *S* – in the case of phenylethyl alcohol and *R* – for hydroxyphosphonate. It seems, that enzymes present in both

Table 1
The influence of additives on enantioselectivity of bioreduction of acetophenone to 1-phenylethyl alcohol[a]

Additive	*S. cerevisiae*	*R. rubra*	*R. glutinis*	*R. gracilis*
No additive	*S*	*S*	*S*	*S*
Methylvinyl ketone	–	–	–	–
Allyl bromide	*R*	*R*	*R*	*R*
Allyl alcohol	*S*	*S*	*S*	*S*
Ethyl chloroacetate	–	–	–	–

[a] 1-Optical purity range from 95% to 99%; "–" lack of the reduction.

Table 2
The influence of additives on enantioselectivity of bioreduction of diethyl 2-oxopropylphosphonate[a]

Additive	Absolute configuration/chemical yield (%)	
	S. cerevisiae	*R. rubra*
No additive	*S*(+)/50	*R*(−)/10
Ethyl chloroacetate	*R*(−)/30	*R*(−)/40
Methylvinyl ketone	*R*(−)/20	

[a] 1-Optical purity of diethyl 2-hydroxypropylphosphonate was 99% (e.g. Fig. 1).

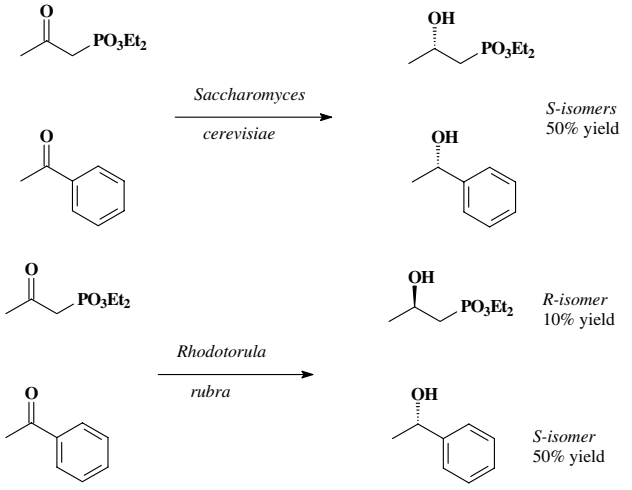

Fig. 2. Comparison of the enantiospecificity of baker's yeast and *R. rubra* toward acetophenone and model oxophosphonate in bioreduction carrying out without any additives.

kinds of yeast and involved in these particular bioreductions, represent different families of dehydrogenases, that is why they have a wide range of possible applications, including stereocontrolled reduction of phosphonates.

Summing up, efforts undertaken allowed to obtain both enantiomers of diethyl 2-hydroxyproylphosphonate with quite good chemical yield and of high enantiomeric purity (over 90%) by alteration the activity of particular enzymes in baker's yeasts, which turned out to be the most versatile biocatalysts from the rest of microorganisms employed.

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