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Preparative enantioselective synthesis of benzoins and (*R*)-2-hydroxy-1-phenylpropanone using benzaldehyde lyase

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Abstract

A detailed study of the reaction parameters on the enzymatic activity and stability of benzaldehyde lyase (BAL) catalysed carboligation is presented, like the influence of the cosolvent (DMSO), the role of the cofactor ThDP, the pH of the reaction medium, and the substrate ratio in the case of cross condensation. Surprisingly, an alkaline reaction medium of pH 9.5 accelerates the BAL-catalysed condensation significantly. Under these conditions several (*R*)-benzoins were formed with high productivity of 240 $g\,L^{-1}\,d^{-1}$ and high enantioselectivities (93–99% ee). For the synthesis of (*R*)-2-hydroxy-1-phenyl-propanone (2-HPP) by coupling benzaldehyde and acetaldehyde space-time-yields of 36 $g\,L^{-1}\,d^{-1}$ were obtained with a maximum 2-HPP concentration of 15–20 $g\,L^{-1}$ (97% ee) in 10–15 h.

Keywords: Benzaldehyde lyase; Acyloin condensation; Thiamine-diphosphate; Enzymatic carboligation; Aldehyde

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1. Introduction

Benzaldehyde lyase (BAL; E.C. 4.1.2.38), a thiamine-diphosphate (ThDP)- and Mg^{2+}-dependent enzyme from *Pseudomonas fluorescens* Biovar I, was described more than a decade ago as a catalyst in the cleavage of benzoin and anisoin linkages [1]. In recent years its further synthetic capabilities for producing 2-hydroxy ketones, i.e. benzoins or 2-hydroxy-1-phenylpropanones (2-HPP) in very good yields and high enantioselectivities have been reported [2–10]. Recent studies have mainly focused on the study of the substrate spectrum of BAL-catalysed carboligations. Thus, the coupling of substituted aromatic benzaldehydes to produce benzoins was described, as well as the formation of 2-HPP derivatives by a cross acyloin condensation of an aromatic aldehyde and acetaldehyde [2,3]. In order to evaluate the industrial applicability of BAL-catalysed carboligations, a detailed study of the parameters influencing the activity and the stability of BAL is of pivotal importance. Addressing the challenges of process development issues, in the present paper we report on the influence of cofactors, cosolvents, and especially the pH-influence on the stability and activity of BAL. In addition, we disclose process conditions which allow the synthesis of optically active 2-hydroxy ketones with high reaction rates and high volumetric productivity.

2. Results and discussion

2.1. Benzoin condensation

For initial studies the BAL-catalysed syntheses of (*R*)-benzoins from the respective benzaldehyde derivatives were chosen as test reactions. (Scheme 1). These reactions, which have been described in detail previously [3,4,6] are usually performed in aqueous monophasic media.

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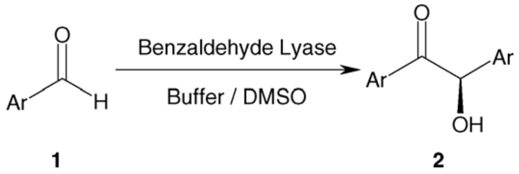

Scheme 1. BAL-catalysed synthesis of benzoins (**2a**) by carboligation of benzaldehyde derivatives.

2.1.1. Influence of cofactors

The influence of the cofactors, ThDP and Mg^{2+}, on the stability of BAL in water and potassium phosphate buffer (KP_i) at pH 8 is shown in Fig. 1. Starting with a lyophilised enzyme preparation with a residual content of 0.015 mmol L^{-1} ThDP and Mg^{2+}, the enzyme looses its activity almost completely within 3 h in water. In the presence of potassium phosphate buffer, with or without Mg^{2+}, a slightly better stability is observed. Only in the presence of both cofactors, a significant enzymatic stabilisation is achieved. Moreover, the addition of DTT, a well-known stabiliser of hydrolases, has also a positive effect on the enzyme stability. A further increase of the cofactor concentration up to 1 mmol L^{-1} had no significant effect (data not shown).

2.1.2. DMSO as a cosolvent

For a preparative large-scale application high substrate concentrations are generally desirable. This demand is difficult to meet with aromatic substrates, such as benzaldehyde, which are only poorly soluble in aqueous media. To overcome this problem, DMSO has been used as cosolvent, although DMSO leads often to some inconveniences concerning the product purification, enzyme inhibition, and deactivation. To minimise those drawbacks, the quantification of the influence of DMSO on BAL-stability was measured as time-dependent enzyme deactivation (Fig. 2). Unexpectedly, the stability of BAL increases with higher DMSO-content, reaching its optimum at about 30 vol.%. At concentrations of >30 vol.%, however, stability decreases.

When studying the enzymatic initial rate a linear increase of activity was observed up to this concentration (data not shown), indicating that the maximal BAL activity is not still achieved under these conditions. Exceeding the solubility limit of benzaldehyde (50 mmol L^{-1}), we found that BAL keeps its activity constant up to about 120–130 mmol L^{-1} in aqueous buffer and DMSO (30 vol.%) which can be regarded as the highest not deactivating concentration of benzaldehyde. Higher concentrations of benzaldehyde led to a progressive decrease of activity, so that at 200 mmol L^{-1} benzaldehyde no activity was detected any more.

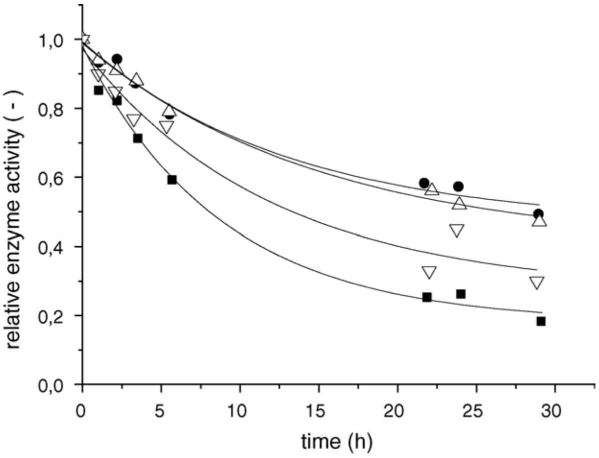

Fig. 2. Influence of the DMSO-concentration (vol.%) on the stability of BAL in 50 mM KP_i buffer (pH 8.0) with various concentrations of DMSO. (■) 0% DMSO, (●) 20% DMSO, (△) 30 vol.% DMSO, (▽) 40% DMSO and 0.5 mmol L^{-1} ThDP, 0.5 mmol L^{-1} Mg^{2+}, 20 °C.

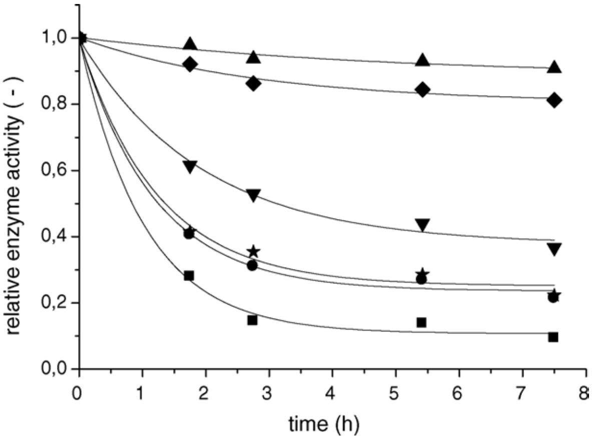

Fig. 1. Influence of cofactors on the stability of BAL (buffer pH 8.0, 0.5 mmol L^{-1} ThDP, 0.5 mmol L^{-1} Mg^{2+}, 1 mmol L^{-1} DTT, 0 °C), (■) water, (●) KPi, (⋆) $KPi_i + Mg^{2+}$, (▼) KPi_i + ThDP, (♦) $KPi_i + Mg^{2+}$ + ThDP, and (▲) $KPi_i + Mg^{2+}$ + ThDP + DTT.

2.1.3. pH-dependency

BAL-catalysed carboligations are routinely performed at a neutral to slightly alkaline pH [3–6]. However, to our knowledge a detailed pH-study has not been published yet. Such pH-profile is depicted in Fig. 3. Remarkably, the highest carboligation activity was achieved at pH 9.5. At pH >10 a sharp decrease in activity is observed.

However, pH 9.5 might cause a lower stability of the enzyme. Thus, despite the high activity an unfavourable enzymatic overall performance would occur. Therefore the applicability of these conditions was investigated on a preparative scale.

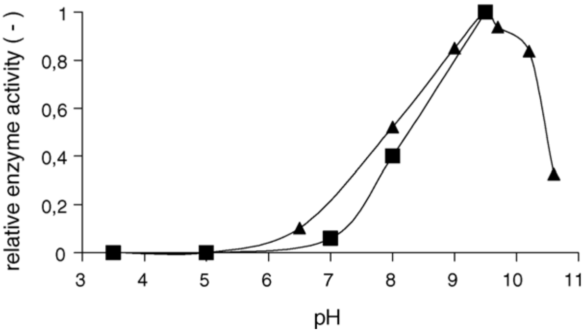

Fig. 3. Influence of the pH in the BAL-catalysed synthesis of benzoin (**2a**) (▲) and 2-HPP (**3**) (■). Relative initial activity corresponds to an activity of 10 μmol min^{-1} mL^{-1} in the case of benzoin (**2a**) and 5 μmol min^{-1} mL^{-1} in case of 2-HPP.