PERKIN 1 COMMUNICATION

Enantioselective synthesis of hydroxy ketones through cleavage and formation of acyloin linkage. Enzymatic kinetic resolution *via* C–C bond cleavage

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Both enantiomers of benzoins and (*R*)-2-hydroxy-1-phenylpropanone analogues were obtained in high yield on a preparative scale starting from aromatic aldehydes, *rac*-benzoins and aliphatic aldehydes *via* enzyme-catalysed C–C bond cleavage and C–C bond formation reactions.

About two thirds of all research in the field of biotransformations has been performed using hydrolytic transformations involving amide and ester bonds.[1] On the other hand, enzymatic kinetic resolution *via* C–C bond cleavage has not often been described in the literature so far.[2] Although the enzyme-catalysed hydrolytic cleavage of a carbon–carbon bond adjacent to carbonyl is widespread in biochemistry, it has been applied relatively rarely in biotransformations using isolated enzymes.[3] Exceptions are to some extent transketolase and α-keto acid decarboxylase catalysed reactions.[4]

In this paper a one-step biocatalytic approach for the synthesis of enantiopure 2-hydroxy ketones, an important class of compounds in natural product and drug syntheses,[5] is described using enzymatic kinetic resolution of racemates by C–C bond cleavage and concomitant C–C bond formation.

Benzaldehyde lyase (BAL, EC 4.1.2.38), a thiamin diphosphate (ThDP) dependent enzyme from *Pseudomonas fluorescens* Biovar I, was first reported by Gonzáles and Vicuña.[6] They showed that this strain can grow on benzoin as a sole carbon and energy source, due to the ability of BAL to catalyse the cleavage of the acyloin linkage of benzoin yielding benzaldehyde. The enzyme used in this study was expressed and purified from a recombinant *Escherichia coli* strain. For easier purification a hexahistidine tag was fused to the C-terminus of the enzyme.[7]

When racemic benzoin was reacted with BAL in potassium phosphate buffer at pH 7, only a very small amount of benzaldehyde was formed, probably due to the low solubility of benzoin in aqueous buffer (about 0.15 mM). Addition of 20% DMSO (v/v) as a cosolvent or alternatively 15% polyethylene glycol (PEG 400) (v/v) resulted in enhanced benzaldehyde formation. Analysis of the reaction mixture by chiral phase HPLC showed that the enantiomers of benzoin were unequally converted into benzaldehyde. The highest ee for (*S*)-benzoin was determined as 15% after 18 hours. To verify the assumption that only one enantiomer of benzoin acts as substrate, the reaction was carried out with (*R*)- and (*S*)-benzoin. Only (*R*)-benzoin is converted into benzaldehyde through BAL catalysis, although complete conversion of (*R*)-benzoin was not possible under the conditions tested. Apparently, an equilibrium between cleavage and formation of (*R*)-benzoin exists during this process. (*S*)-Benzoin gave no reaction at all.

The results are in accord with mechanistical investigations of other ThDP-dependent enzymes.[8] Thus, the first step of the catalytic cycle (Scheme 1) is the attack of the ylide form of ThDP **1** on the carbonyl carbon of (*R*)-benzoin to produce an adduct **2**. The enamine **3**, formed after liberation of one molecule of benzaldehyde, is a common intermediate of other ThDP-dependent enzymes catalysing the formation of 2-hydroxy ketones.[8] In the absence of an acceptor aldehyde protonation of **3** results in release of benzaldehyde and regeneration of the ylide **1**. However, in the presence of an acceptor aldehyde the enamine intermediate **3** is able to undergo a C–C bond formation reaction.

From these mechanistic considerations and assuming that cleavage and formation of (*R*)-benzoin are in equilibrium, BAL should also catalyse C–C bond formation. Consequently, BAL-catalysed acyloin condensation with benzaldehyde in aqueous buffer resulted in the formation of (*R*)-benzoin. This reaction demonstrates for the first time the potential of BAL for both C–C bond cleavage and formation.

With other ThDP-dependent enzymes, *e.g.* benzoylformate decarboxylase (BFD) from *Ps. putida*, we observed that addition of DMSO as cosolvent facilitates the formation of acyloins in high yield starting from hydrophobic substrates.[9] Accordingly, addition of DMSO (20%, v/v) to the aqueous medium containing BAL and benzaldehyde resulted in formation of (*R*)-benzoin without excessive deactivation of the enzyme. This conversion worked almost quantitatively and (*R*)-benzoin was obtained in an enantiomerically pure form (ee > 99%). During the reaction most of the benzoin precipitates from the reaction mixture. A small amount of benzaldehyde (1–2%) was present at the end of the reaction (Scheme 2, entry 1).

When (*R*)-benzoin was reacted with BAL in the presence of acetaldehyde (Scheme 2, entry 2) quantitative formation of (*R*)-2-hydroxy-1-phenylpropanone [(*R*)-2-HPP] in an optically pure form occurred. The same reaction starting from (*S*)-benzoin failed. Repeating this reaction with *rac*-benzoin afforded (*R*)-2-HPP (ee > 99%) and (*S*)-benzoin (ee > 99%) after separation of the products by column chromatography (Scheme 2, entry 3).

As expected from these results, BAL-catalysed reaction of benzaldehyde and acetaldehyde also gave (*R*)-2-HPP in very high yield (95%, ee > 99%) (Scheme 2, entry 4). Monitoring of the reaction by HPLC showed that the formation of (*R*)-benzoin occurred first prior to a decrease of (*R*)-benzoin accompanied by an increase of (*R*)-2-HPP formation.

Since structural information about the enzyme is still missing, a structure-based discussion of the observed stereocontrol is not yet possible. Nevertheless, the C–C bond formation is most probably a consequence of a selective attack by the enamine intermediate **3** on an acceptor aldehyde yielding (*R*)-benzoin and (*R*)-2-HPP. The detailed mechanism of this reaction and the influence of the added cosolvent is under investigation.

Besides benzaldehyde, a wide range of aromatic aldehydes

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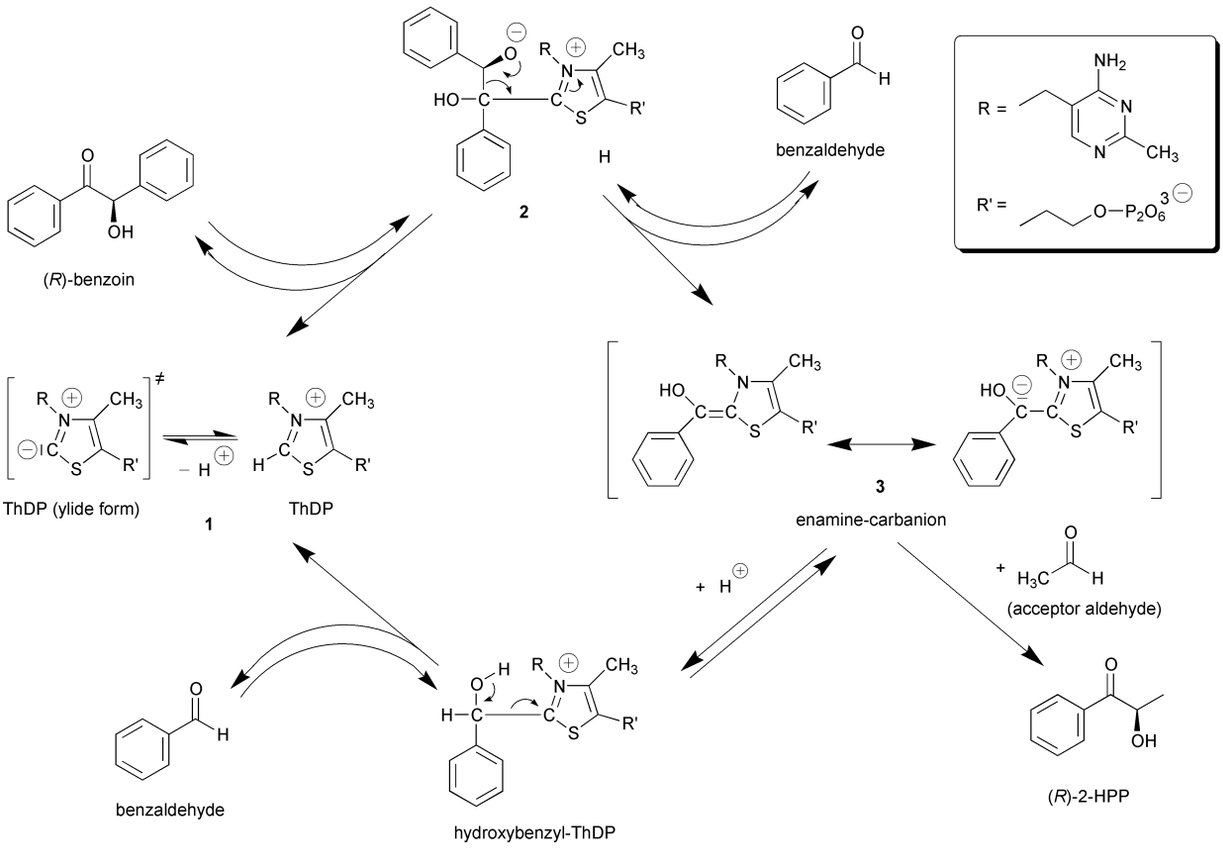
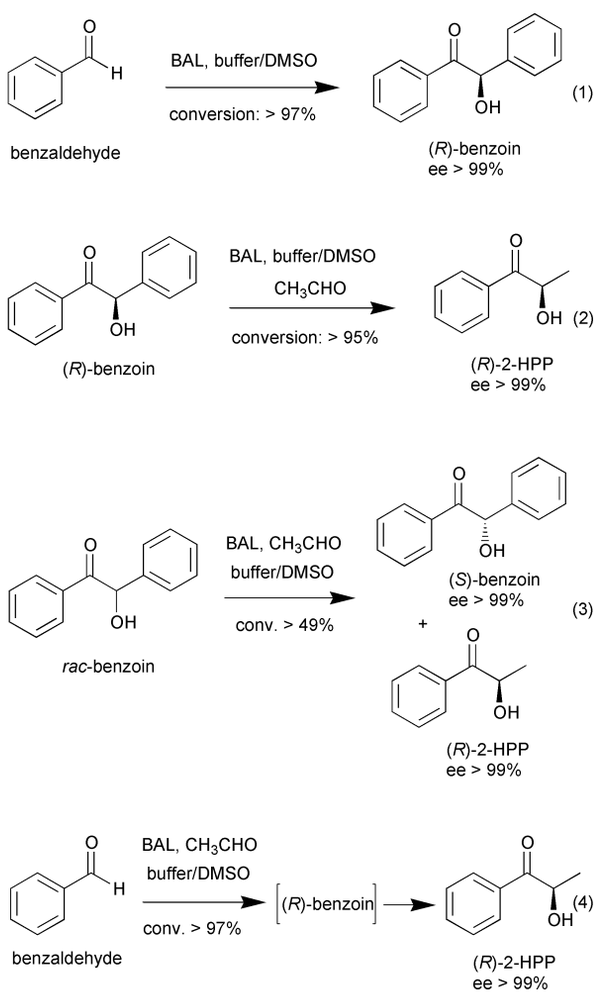


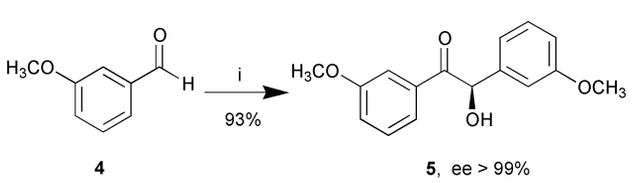
Scheme 1 Proposed mechanism for the BAL-catalysed acyloin formation and cleavage based on observations with other ThDP-dependent enzymes.[8]



Scheme 2 Different types for reactions catalysed by BAL.

substituted at the *ortho*-, *meta*-, and *para*-position with electron-releasing as well as electron-withdrawing properties are accepted as substrates. Substituents tolerated in the *ortho*-, *meta*-, and *para*-position are, for example, methoxy, chloro and fluoro. Additionally, different aromatic and heteroaromatic benzoin-like acyloins are accepted as substrate for the kinetic racemic resolution *via* C–C bond cleavage. Also, in contrast to BFD-catalysed reactions, the aliphatic acceptor aldehyde substrate is not restricted to acetaldehyde; longer chain aliphatic aldehydes (*e.g.* propionaldehyde, butyraldehyde) are accepted by BAL as well as olefinic aldehydes (*e.g.* cinnamaldehyde).

As an example of the usefulness of BAL-catalysed reactions on a preparative scale the synthesis of (*R*)-3,3′-dimethoxybenzoin (**5**) was performed (Scheme 3).[10] Starting from



Scheme 3 *Reagents and conditions*: i, 3.2 g (24 mmol) 3-methoxybenzaldehyde, 40 ml DMSO, 160 ml potassium phosphate buffer (50 mM, pH 7.0), ThDP (0.15 mM), 6.6 mg BAL (200 U), 6 h, rt.

3-methoxybenzaldehyde (**4**) and 6.6 mg of BAL, after 6 hours at room temperature the optically pure product **5** was isolated in 93% yield on a 3 gram scale.

In summary, the method described here demonstrates the high potential of the enzyme-catalysed kinetic resolution of racemates *via* C–C bond cleavage and C–C bond formation reactions. The reactions work well in organic–aqueous medium, overcoming the solubility problem of lipophilic substrates and opening the way for large-scale preparations. The enantiopure products are obtained in high yield starting from simple, readily available aromatic aldehydes, *rac*-benzoins and aliphatic aldehydes. In this way, BAL represents a link between the related BFD and transketolase, two well known ThDP-dependent

enzymes.[4] It will be exciting to see whether catalytic activities of BAL and BFD can be interconverted by mutagenesis studies.

Experimental

Preparation of BAL

Hexahistidine-tagged BAL was obtained from recombinant *E. coli* SG13009 cells following a procedure described previously.[8a] One unit of activity is defined as the amount of enzyme which catalyses the cleavage of 1 μmol benzoin (1.5 mM) into benzaldehyde in potassium phosphate buffer (50 mM, pH 7.0, containing $MgSO_4$ (2.5 mM), ThDP (0.15 mM) and 15% PEG 400 (v/v)) in 1 min at 30 °C.

Representative example of the synthesis of benzoin derivatives: (*R*)-benzoin

Benzaldehyde (318 mg, 3 mmol) was dissolved in a mixture of DMSO (20 mL) and potassium phosphate buffer (80 mL, 50 mM, pH 7.0, containing $MgSO_4$ (2.5 mM) and ThDP (0.15 mM)). After addition of BAL (20 U) the reaction was allowed to stand at 25 °C for 48 h and a further 20 U of BAL was added. After 62 h the conversion was determined as 97% (GC-MS). The reaction mixture was extracted with dichloromethane (250 mL) and the organic layer washed with water (25 mL) and brine (25 mL) and dried over Na_2SO_4. Evaporation of the solvent and purification of the crude product by crystallisation afforded 305 mg (96%) (*R*)-benzoin (mp 134 °C); $[\alpha]_D^{22}$ −115 (*c* 1.5, CH_3COCH_3), ee > 99%; lit.[11] $[\alpha]_D^{25}$ −118.3 (*c* 2.4, CH_3COCH_3) for ee 99% (from commercially available compound); HPLC: (Chiralpak AD, isohexane–propan-2-ol; 90 : 10, 0.75 ml min^{-1}, 20 °C, 21 bar) t_R: 26.95 min.

Representative example of the synthesis of (*R*)-2-hydroxy-1-phenylpropanone derivatives: (*R*)-2-hydroxy-1-phenylpropanone [(*R*)-2-HPP]

Benzaldehyde (212 mg, 2 mmol) was dissolved in a mixture of DMSO (20 mL) and potassium phosphate buffer (80 mL, 50 mM, pH 7.0, containing $MgSO_4$ (2.5 mM) and ThDP (0.15 mM)). To this solution 88 mg (2 mmol) acetaldehyde was added. After addition of BAL (20 U) the reaction was allowed to stand at 25 °C. After 24 h 20 U of BAL and 176 mg (4 mmol) of acetaldehyde were added. This was repeated every 24 h. After 96 h the conversion was determined as 97% (GC-MS). Work-up according to the former procedure afforded 285 mg (95%) (*R*)-2-HPP, $[\alpha]_D^{22}$ +76 (*c* 2, $CHCl_3$), ee > 99%; lit.[12a] $[\alpha]_D^{22}$ +70 (*c* 1.7, $CHCl_3$) for ee 89%; lit.[12b] $[\alpha]_D^{20}$ +58 (*c* 2, $CHCl_3$) for ee 62%; lit.[5g] $[\alpha]_D^{20}$ +82 (*c* 2, $CHCl_3$) for ee 96%; HPLC: (Chiralpak AD, isohexane–propan-2-ol; 90 : 10, 0.75 ml min^{-1}, 20 °C, 21 bar) t_R: 15.78 min.

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