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Review

Synthetic potential of thiamin diphosphate-dependent enzymes [1]

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Abstract

Thiamin diphosphate-dependent enzymes (mainly pyruvate decarboxylase, transketolase, benzoylformate decarboxylase) are increasingly being used to perform regio- and enantioselective reactions in chemoenzymatic syntheses. To utilize enzymes for unphysiological reactions and to yield novel products, a broad substrate spectrum is desirable. We give an overview of the use of these enzymes in biotransformations and in chemoenzymatic syntheses including multi-enzyme approaches which involve thiamin diphosphate-dependent enzymes as biocatalysts to obtain pharmaceutical compounds as ephedrine and glycosidase inhibitors, sex pheromones as *exo*-brevicomin, ^{13}C-labelled metabolites, and other intermediates as 1-deoxyxylulose 5-phosphate, a precursor of vitamins and isoprenoids.

Keywords: Thiamin diphosphate; Transketolase; Pyruvate decarboxylase; Benzoylformate decarboxylase: 1-deoxyxylulose 5-phosphate; Acyloin condensations; Phenylacetyl carbinol; Site-directed mutagenesis; α-hydroxyketones

1. Introduction

Due to their intrinsic chiral function, enzymes are finding increasing acceptance as catalysts in pure and applied chemistry. As the demand for enantiomerically pure compounds, e.g., pharmaceutical products, is increasing, enzymes will also be looked at for further applications. Especially in the field of carbohydrate chemistry, the inherent multifunctionality of sugars is an enormous challenge for organic chemists who have to approach carbohydrate syntheses with protective groups in order to prevent undesired reactions of the various hydroxyl-, keto-, or phosphate groups. So far, mainly lyases and aldolases have been used to synthesize complex sugars, sugar analogues and other biologically important natural compounds [1–8]. Thiamin diphosphate (ThDP)-dependent enzymes include the potential of both breaking and formation of C–C bonds [9–11] and have been used for quite a while as catalysts in chemoenzymatic syntheses [3,7,8,12,13] (for recent reviews, see Refs. [14,15]). The spectrum

Abbreviations: 3D, three-dimensional; BFD*Ps.p.*, Benzoylformate decarboxylase from *Pseudomonas putida*; DHAS, dihydroxyacetone synthase; DXS, 1-deoxyxylulose 5-phosphate synthase; 2-HPP, 2-hydroxypropiophenon; (*R*)-PAC, (*R*)-1-hydroxy-1-phenylpropan-2-one; PDC*S.c.*, pyruvate decarboxylase from *Saccharomyces cerevisiae*; PDC*S.u.*, pyruvate decarboxylase from *Saccharomyces uvarum*; PDC*Z.m.*, pyruvate decarboxylase from *Zymomonas mobilis*; RAMA, rabbit muscle aldolase; *S.* sp., *Saccharomyces* species; ThDP, thiamin diphosphate; wt, wild-type; TKT, transketolase

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[1] Dedicated to Professor Hideaki Yamada in honor of his 70th birthday.

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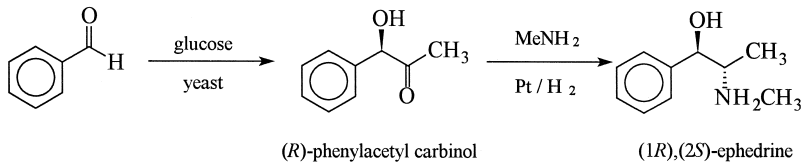
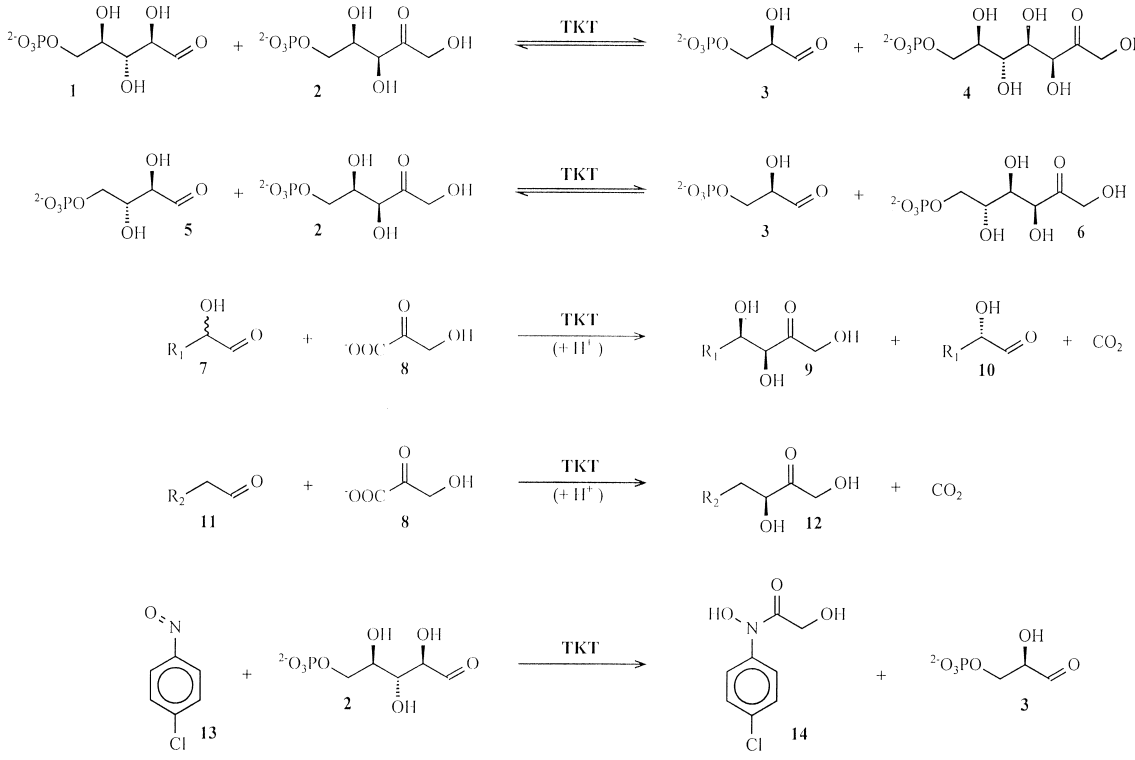


Scheme 1. Synthesis of (1*R*,2*S*)-ephedrine from benzaldehyde and glucose via phenylacetylcarbinol, using fermenting yeast.

of reactions catalyzed by ThDP-enzymes encompasses nonoxidative decarboxylations of α-keto acids (α-keto acid decarboxylases), oxidative decarboxylation of α-keto acids (pyruvate oxidase), carboligation [transketolase (TKT), α-keto acid decarboxylases, acetolactate synthase] as well as the cleavage of C–C bonds (TKT, benzaldehyde lyase). All enzymes have in common a ThDP-bound 'active aldehyde' intermediate formed either by decarboxylation (e.g., of pyruvate through action of pyruvate decarboxylase, PDC) or by transfer from a suitable donor compound (e.g., from xylulose-5-phosphate by TKT). Some of the enzymes, in side-reactions, are also capable of performing an acyloin-type condensation reaction leading to chiral α-hydroxyketones. This makes them interesting as catalysts for biotransformations.

One of the first biotransformation processes to be commercialized at all, is the production with whole yeast cells of phenylacetylcarbinol, a precursor of L-ephedrine [16] (Knoll-procedure), a process which is in use since the 1930s (Scheme 1). It has been established meanwhile



Scheme 2. Physiological and nonphysiological reactions catalyzed by TKT. R_1 and R_2 denote variable residues. Adapted from Ref. [14].

[17,18] that the underlying principle of reaction (benzaldehyde plus pyruvate yielding phenylacetylcarbinol) is being catalyzed by PDC. From the various ThDP-dependent enzymes, we will concentrate mainly on TKT and related transferases (dihydroxyacetone synthase, DHAS, 1-deoxyxylulose 5-phosphate synthase, DXS), on PDC and benzoylformate decarboxylase (BFD) as only these have been applied in chemoenzymatic syntheses. For an in-depth treatise on other ThDP-enzymes and their substrate spectra, see Refs. [14,15].

2. ThDP-dependent enzymes and their applications

2.1. Transketolase

The principal reactions of TKT (EC 2.2.1.1) are depicted in Scheme 2. In general, an active glycolaldehyde group (α,β-dihydroxyethyl group) is transferred from a ketose donor (e.g., **2**, **4**, or **6**) to an α-hydroxyaldehyde acceptor (e.g., **1**, **3**, or **5**). TKT has attracted much interest as catalyst for chemoenzymatic reactions. The enzyme therefore has been purified from spinach, yeast, or from recombinant *Escherichia coli* strains carrying the homologous *tktA* gene, and is now available in sufficient amounts to perform preparative scale reactions (see Refs. [8,14]). Although the usual donor/acceptor pairs stem from the pentose-phosphate pathway of sugar metabolism, a wide variety of nonphosphorylated 2-hydroxyaldehydes **7** (Table 1) are accepted at reasonable

Table 1
Substrate range of TKTs from different organisms

Acceptor substrate	Source organism		
	E. coli	*Yeast*	*Spinach*
R_1–CHOH–CHO			
H	+	+	+
CH_3	+	+	
CH_2OH	+	+	+
CH_2F		+	
CH_2N_3	+	+	
CH_2CN		+	
CH_2NO_2	+		
CH_2SH		+	
CH_2OCH_3		+	
CH_2SCH_3		+	
$CH_2SCH_2CH_3$		+	
CH_2CH_3		+	
CH_2CH_2OH		+	
CHOH-CH_3	+		+
D-*erythro*-$CHOHCH_2OH$	+	+	
D-*threo*-$CHOHCH_2OH$	+	+	
$CH_2CH_2CH_3$		+	
CH_2CH_2CHO		+	
$CH_2CH_2CH_2CHO$	+		
$COH(CH_3)_2$	+		
$C(CH_3)_3$		+	
$CH{=}CH_2$	+	+	
R_1–CHOH–CHO			
$C{\equiv}CH$	+		
$CH_2CH{=}CH_2$	+	+	
(*S*)-CHOH–$CH{=}CH_2$		+	
(*R*)-CHOH–$CH{=}CH_2$		+	
D-*ribo*-$(CHOH)_2CH_2OH$	+	+	+
D-*gluco*-$(CHOH)_3CH_2OH$	+	+	
D-*gluco*-$(CHOH)_3CH_2OPO_3^{2-}$	+	+	
$CH(S_2C_3H_6)$ **21**			+
$CH_2CH(S_2C_3H_6)$ **21**			+
C_6H_5	+		
$CH_2C_6H_5$	+		
$CH_2OCH_2C_6H_5$	+	+	
R_2–CHO			
H	+		
CH_3	+	+	+
CH_2OCH_3			+
CH_2SCH_3			+
$CH(OCH_3)_2$		+	
CH_2CH_3	+		
CH_2CH_2OH	+		
$COCH_3$	+		
$CH_2CH_2SCH_3$			+
$CH_2CH_2CH_3$		+	
$CH_2CHOHCH_3$		+	
$CH_2CHOHCH_2OH$		+	
$CH{=}CHCH_3$	+		
$CH_2C_6H_5$	+		

Notes to Table 1:
Substrate range of TKT (adapted from compilation in Ref. [14]). α-hydroxyaldehydes (R_1–CHOH–CO) as acceptor substrates with various residues R_1 are given in the upper panel; aldehydes without an α-hydroxy group (R_2–CHO) and varying residues R_2 are given in the lower panel.
+ = Reported as substrate.

rates [7,8,14,19] to yield ketoses with a 3*S*, 4*R*-configuration **9**. As only hydroxyaldehydes with the configuration 2*R* are accepted by TKT, L-hydroxyaldehydes **10** can be obtained as 'spin-off' through kinetic resolution with good yields [20–22]. Aldehydes lacking an OH-group at C2 (e.g., **11**) are also transformed by TKT leading to the product **12** [23–25] (Table 1), albeit with a significantly lower rate than the hydroxylated acceptors [26]. In contrast to the TKTs from spinach and yeast [23], no conversion of aromatic aldehydes as e.g., benzaldehyde or hydroxybenzaldehydes could be detected with purified *E. coli* TKT [19]. It should be pointed out, however, that so far no structures of the reaction products from conversions of aromatic or heterocyclic substrates using TKT have been shown yet. An earlier report claimed that the only nonaldehyde substrate 4-chloronitrosobenzene **13** was also used as acceptor [27], but again the structure of the postulated product **14** has not been shown either.

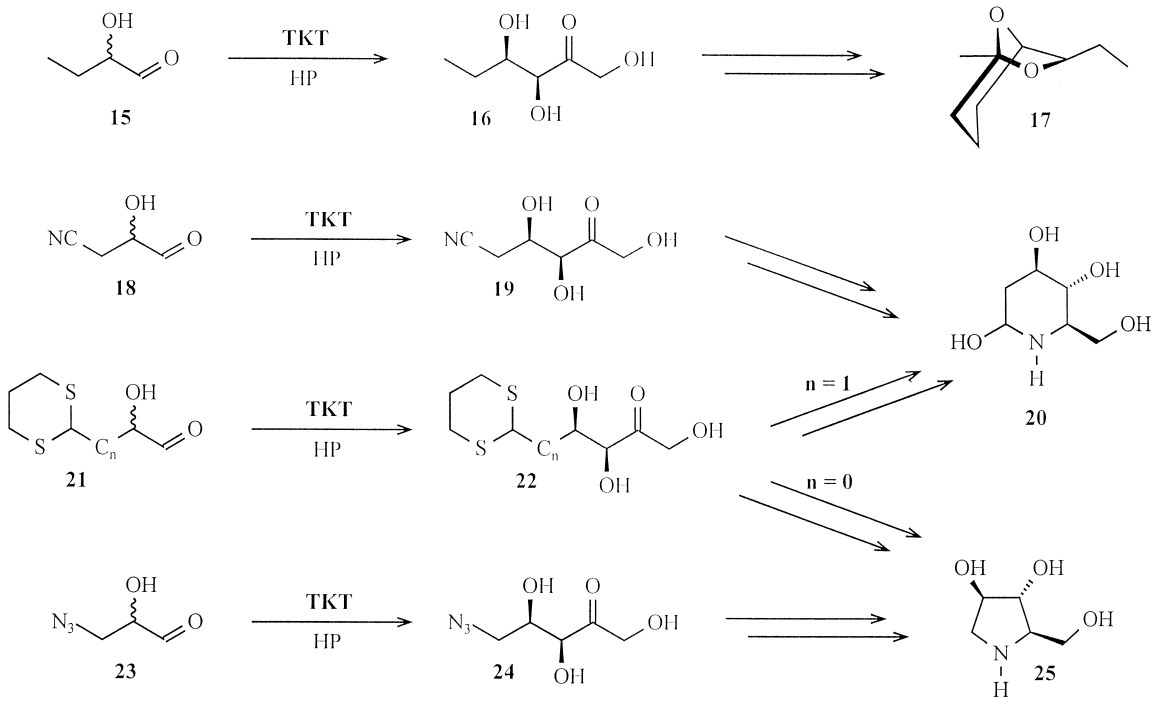
The use of hydroxypyruvate **8** as a donor substrate is of synthetic significance as it allows a practically irreversible product formation with carbon dioxide leaving the assay. Hydroxypyruvate **8** is accepted by the TKTs from spinach, yeast and *E. coli* [28–31]. For synthetic purposes the *E. coli* TKT has a certain advantage over the enzymes from spinach and yeast, because the conversion of **8** with a rate of 60 U/mg [19] is significantly higher than the rates of 2 U/mg and 9 U/mg reported for the spinach and yeast enzymes [23,32]. ^{13}C-labelled **8**, which was generated from DL-serine by the action of D-amino acid oxidase, has been used to synthesize the labelled sugar [1, 2-^{13}C2]-xylulose with spinach TKT [33]. Using TKT, valuable natural compounds were synthesized easily (Scheme 3), whereas the chemical synthesis of these chiral substances afforded multi-step syntheses with complex techniques involving introduction and removal of protective groups. Starting from a racemic mixture of **15** the chiral product **16** was obtained from the conversion with yeast TKT and used for the chemical synthesis of the beetle pheromone *α-exo*-brevicomin **17** [34]. The enantioselective reaction of TKT was further used for the chemoenzymatic synthesis of the glycosidase inhibitors fagomine **20**, and 1,4-dideoxy-1,4-imino-D-arabinitol **25**. Starting points for the syntheses were the racemic mixtures of **18** and **23**, respectively, which were converted to the chiral products **19** and **24** [35,36]. A similar strategy to gain precursors for the synthesis of **20** and **25** was used by Hecquet et al. [37]. The precursors **22** were obtained from the TKT conversion of the racemic substrates **21** in the protected dithiane form. In all described syntheses, TKT generated two asymmetric centers at carbons C3 and C4 and therefore the use of a chiral starting compound could be avoided.

TKT generates the same stereochemistry in its reaction products as fructose-1,6-bisphosphate aldolase (EC 4.1.2.13). The commercially available aldolase from rabbit muscle (RAMA) has a broad substrate spectrum [1–8] and has been used for the chemoenzymatic synthesis of a variety of natural compounds [8]. However, Kobori et al. [22] highlighted several advantages of TKT over the classical RAMA reactions.

(1) Resolution of racemic aldehyde mixtures using TKT allows the use of these less expensive starting materials for the synthesis of enantiomerically pure products.

(2) Unphosphorylated sugar compounds are formed directly with TKT whereas RAMA utilizes DHAP **33** and hence the resulting product has to be dephosphorylated to obtain the desired end product.

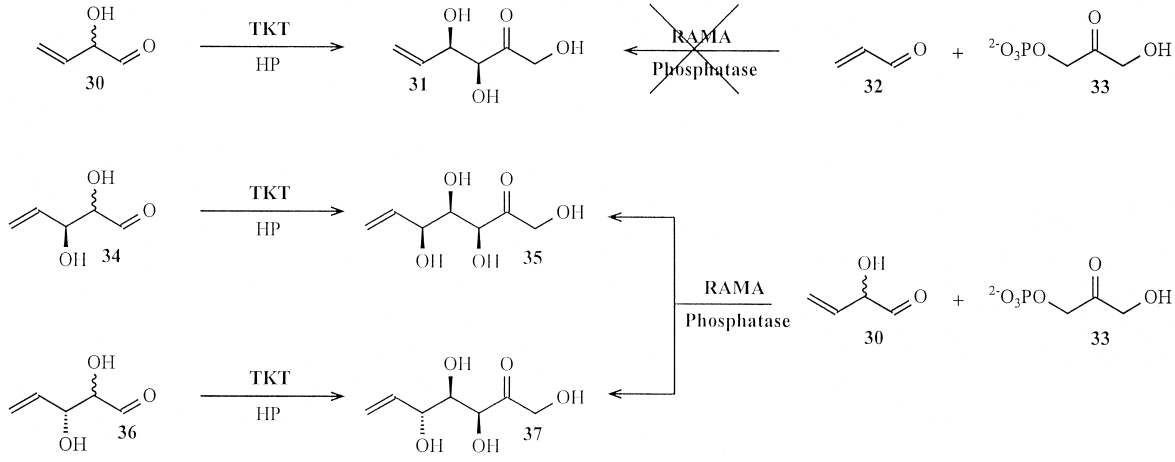
(3) Some products are exclusively obtained through TKT (Scheme 4), e.g., **31** is generated by TKT from **30**, whereas the corresponding **32** does not serve as a substrate for the RAMA-catalyzed condensation with **33**. The compounds **35** and **37** are synthesized with TKT from the aldehydes **34** and **36** which can easily be synthesized individually by chemical means [22]. In contrast, the RAMA catalyzed conversion of **30** followed by phosphatase treatment would lead to a mixture of the diastereomers **35** and **37**.



Scheme 3. TKT as catalyst in the chemoenzymatic synthesis of the pheromone *α-exo*-brevicomin (**17**) and of the glycosidase inhibitors fagomine (**20**) and 1,4-dideoxy-1,4-imino-D-arabinitol (**25**), adapted from Ref. [14] and references therein. HP = hydroxypyruvate as donor of C2 unit.

2.2. Dihydroxyacetone synthase (formaldehyde TKT)

DHAS (alternatively named formaldehyde TKT; EC 2.2.1.3) is found in methylotrophic yeast species and performs the C2-transfer from xylulose-5-phosphate **2** to formaldehyde **39**. DHAS shows high sequence similarity to TKT and can also use hydroxypyruvate **8** as C2-donor. DHAS has been used, a.o., for the preparation

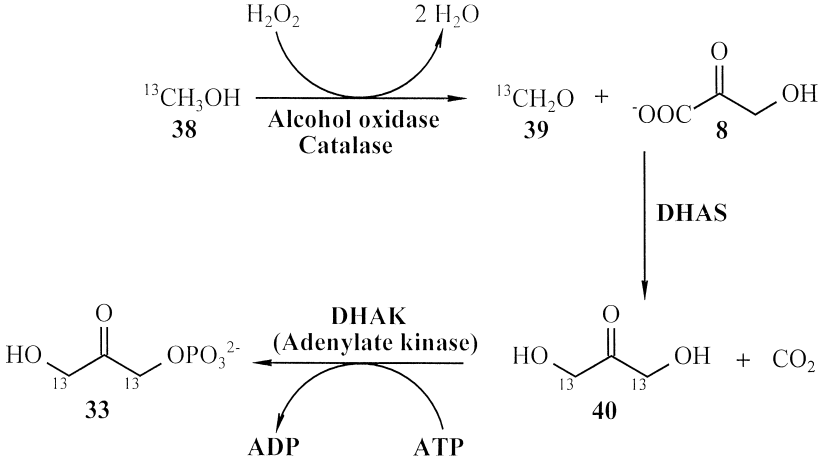


Scheme 4. The catalytic potential of TKT in comparison to fructose-1,6-diphosphate RAMA [22]; adapted from Ref. [14]. HP = hydroxypyruvate.

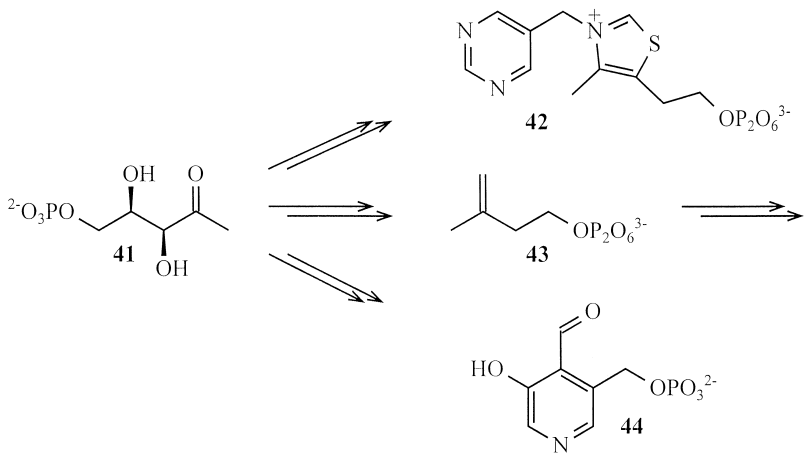
of labelled [1, 3-^{13}C]dihydroxyacetone **40** and [1, 3-^{13}C]-dihydroxyacetonephosphate **33** (Scheme 5) [38]. Using alcohol oxidase and catalase as auxiliary enzymes, ^{13}C-labelled **38** was transformed to formaldehyde **39** which served as acceptor to yield **40** with concomitant release of CO_2. A coupled reaction with dihydroxyacetone kinase and adenylate kinase enabled the synthesis of **33**, which can be used as a precursor for the generation of labelled sugars with DHAP-dependent aldolases as RAMA [38].

2.3. 1-deoxyxylulose 5-phosphate synthase

In a number of organisms, 1-deoxyxylulose 5-phosphate **41** has been identified with ^{13}C-incorporation experiments as the precursor of a nonmevalonate pathway leading to isopentenyl diphosphate **43** (Scheme 6) the precursor of isoprenoids in some bacteria and in plant chloroplasts [39–42]. Additionally, **41** has been known as precursor in the biosynthesis of ThDP **42** and pyridoxal phosphate **44** in bacteria (refs. in Refs. [43,44]). Formation of 1-deoxy-D-*threo*-pentulose has recently been reinvestigated in one of the authors' laboratory [42]. We detected a novel enzyme in *E. coli* (DXS) which is ThDP-dependent and catalyzes the formation of 1-deoxyxylulose from pyruvate and glyceraldehyde, or of 1-deoxyxylulose 5-phosphate from pyruvate and glyceraldehyde-3-phosphate [42]. DXS is related to TKTs and the E1 subunit of the pyruvate dehydrogenase complex but constitutes a novel group of carboligases [42]. The enzyme was successfully utilized for the enzymatic synthesis of 700 mg **41** (in its barium salt form) in a one-pot multi enzyme synthesis (Scheme 7) [45]. Starting point was the RAMA-catalyzed cleavage of **45** to yield **33** and **3** in equal amounts. **3** was converted irreversibly to **41** and CO_2 using **28** as the donor substrate and DXS as catalyst. An almost complete conversion of **45** to **41** was achieved with triosephosphate isomerase (TPI), which converted **33** and **3** reversibly. NMR-analysis of the product confirmed that the D-*threo*-pentulose was formed with a high enantiomeric excess. Since the understanding of the pathways leading to **42**, **43** and **44** are at an early stage, large amounts of 1-deoxyxylulose 5-phosphate will be needed. Compared to the rather complicated chemical synthesis of the chiral compounds 1-deoxyxylulose and 1-deoxyxylulose 5-phosphate [45–47] the enzymatic route to 1-deoxyxylulose or its 5-phosphate certainly has advantages. With the use of labelled pyruvate, the large scale enzymatic synthesis of labelled



Scheme 5. The use of DHAS in a multi-enzyme synthesis of ^{13}C-labelled dihydroxyacetone and its corresponding phosphate, starting from methanol [38]; adapted from Ref. [14]. DHAK = dihydroxyacetone kinase, DHAS = dihydroxyacetone synthase.



Scheme 6. 1-deoxyxylulose 5-phosphate (**41**) as precursor of three biosynthetic pathways eventually leading to thiamine diphosphate (**42**), isopentenyl diphosphate (**43**), and pyridoxal phosphate (**44**) [42]; adapted from Ref. [14].

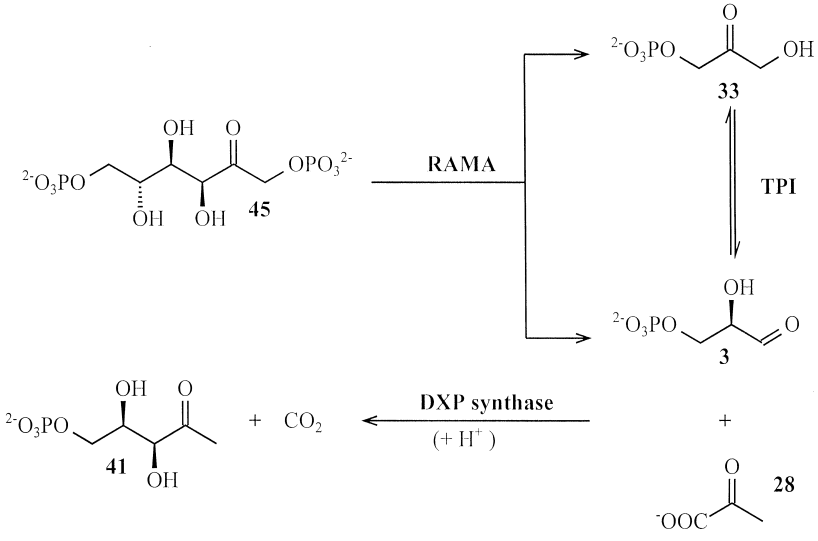
1-deoxyxylulose 5-phosphate for incorporation experiments should be feasible.

A similar carboligase reaction leading to 1-deoxy-ketose compounds had been reported earlier for ThDP-dependent pyruvate dehydrogenase complexes from various microorganisms. Interestingly, the E1 subunit of pyruvate dehydrogenase complexes from bacteria is related both to TKTs and to the newly detected DXS [42].

Three types of reactions were shown for E1 subunit of *Bacillus subtilis* [48], i.e.,

(I) pyruvate + aldose → CO_2 + 1-deoxy-ketose

(II) acetoin + aldose → acetaldehyde + 1-deoxy-ketose



Scheme 7. The synthesis of 1-deoxyxylulose 5-phosphate (**41**) starting from fructose-1,6-bisphosphate (**45**) and pyruvate (**28**) with a multi-enzyme system containing RAMA, TPI and DXP [42,45]; adapted from Ref. [14].

(III) methylacetoin + aldose → acetone + 1-deoxy-ketose

Purified pyruvate dehydrogenase E1 subunit (EC 1.2.4.1) both from *B. subtilis* and *E. coli* were shown to perform reaction (I), while reactions (II) and (III) were attributed to partially purified acetoin dehydrogenase, another ThDP-relative of pyruvate dehydrogenase. Among the aldoses which were used as acceptor compounds were glycolaldehyde, D- and L-glyceraldehyde, D-erythrose and D-threose. Products were 1-deoxy-erythrulose, 1-deoxy-D-*threo*-pentulose, 1-deoxy-L-*threo*-pentulose, 1-deoxy-D-fructose, and 1-deoxy-D-sorbose plus 1-deoxy-D-tagatose when D-threose was the acceptor [49,50].

2.4. α-Keto acid decarboxylases

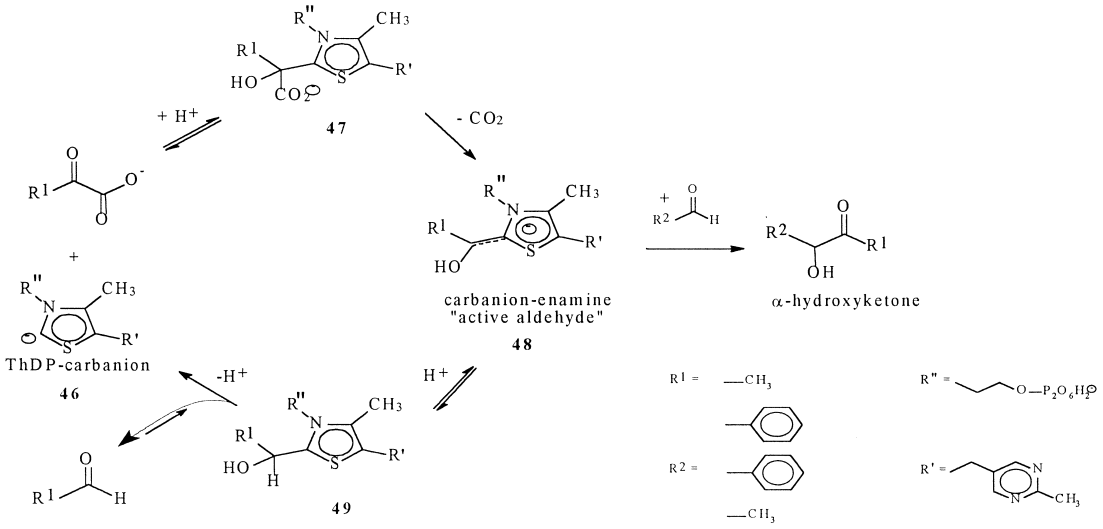
2.4.1. Pyruvate decarboxylase

PDC (E.C. 4.1.1.1.) is the first enzyme of the branched glycolytic pathway that, together with alcohol dehydrogenase, converts pyruvate to ethanol. Genes of PDCs have been isolated from various yeasts, fungi, plants, and from the bacterium *Zymomonas mobilis* [51].

Despite of the large number of available PDC-genes, only the enzymes from *Saccharomyces* sp. and *Z. mobilis* have been intensively studied with respect to the reaction mechanism and the substrate spectra of both, the decarboxylase and carboligase reaction (for a review, see Refs. [15,51]). The three-dimensional structures have been determined from *Saccharomyces uvarum* and *S. cerevisiae* (PDC*S.u.* [52], PDC*S.c.* [53]) in the nonactivated state. Recently, Lu et al. [54] published the X-ray-structure of pyruvamide-activated PDC*S.c.*

2.4.2. Benzoylformate decarboxylase

BFD (E.C. 4.1.1.7.) is a component of the mandelate pathway, which allows bacteria to utilize (*R*)-mandelic acid as a sole carbon source by converting it to benzoic acid, which is then metabolized by the β-ketoadipate pathway and TCA cycle. BFD-activity has been found in *Pseudomonas putida* [55], *Pseudomonas aeruginosa* [56], and *Acinetobacter calcoaceticus* [57]. The enzyme from *P. putida* has been cloned [58], and the crystal structure is under investigation [59,60]. Like PDC, BFD is a tetrameric enzyme of similar size (240 kDa). The X-ray data [60] suggest, that the structure of BFD*Ps.p.* is more compact than that obtained from PDC from yeast in the nonactivated state [52,53].



Scheme 8. Reaction path of enzymatic α-keto acid decarboxylation and formation of α-hydroxyketones by PDC and BFD.

BFD was found to exhibit a synthetical potential to form α-hydroxyketones similar to PDCs [61–65].

2.4.3. Mechanism of decarboxylation and carboligation

The capacity of ThDP to catalyze the decarboxylation of α-keto acids depends mainly on two properties of the thiazolium ring of ThDP: (a) its capacity to ionize to form a nucleophilic anion and thus bind to the α-carbonyl group of α-keto acids, and (b) its ability to stabilize the negative charge upon cleavage of the carbon dioxide [66]. The different steps which are relevant for thiamin-catalyzed decarboxylation and the formation of α-hydroxyketones are summarized in Scheme 8. The reaction cycle is started with activation of ThDP, **46**, by the enzyme. This initial deprotonation step has recently been elucidated for PDC *S.c.*, PDC *Z.m.* and TKT by Kern et al. [67]. Subsequently, the negatively charged C2-ThDP performs a nucleophilic attack on the α-carbonyl group of the keto acid. The resulting double negatively charged species is stabilized by proton transfer to the former carbonyl group to give **47**. Its decarboxylation results in the formation of an α-carbanion-enamine **48**. This step has been intensively studied with PDC from yeast using conjugated α-keto acids with strong electron-withdrawing substituents on the phenyl ring. PDC [68–70] and BFD [61,71] convert such compounds to an α-carbanion-enamine that is a visible chromophore with a discrete life-time and/or a competitive inhibitor of the enzymes, respectively.

The carbanionic intermediate is also known as 'active aldehyde' **48** [72] and is probably present in the mechanism of all ThDP-dependent enzymes, which decarboxylate α-keto acids as a first step. The main reaction path of α-keto acid decarboxylases is the generation of the respective aldehydes. Alternatively, the formation of α-hydroxyketones have been described for PDCs and BFD. It is important to mention that the last step of the reaction cycle is reversible in PDC [73], and probably also in BFD [64]. Thus, the 'active aldehyde' **48** may also be generated by addition of an aldehyde to ThDP to give **49** and subsequent deprotonation. The latter reaction path allows the formation of α-hydroxyketones from aldehydes as precursors.

2.4.4. Substrate spectra of the decarboxylation reaction

PDC from *Saccharomyces* sp. decarboxylates a very broad spectrum of α-keto acids. A survey is given in Table 2. Compared to PDC from yeast, the substrate spectra of PDC *Z.m.* and

Table 2
Comparison of the substrate spectra of PDC from yeast (*Saccharomyces* sp.), PDC from *Z. mobilis* and BFD from *P. putida* (according to Ref. [15])

α-Keto acid	Relative activity (%)		
	Enzyme		
	PDC yeast	PDC *Z.m.*	BFD *Ps.p.*
R–CO–COO⁻			
$-CH_3$	100	100	0
$-C_2H_5$	57	70	0
$-n\text{-}C_3H_7$	54	11	n.d.
$-n\text{-}C_4H_9$	49.5	< 1	n.d.
$(CH_3)_2CH-$	20.0	0	n.d.
cyclo-$C_6H_{11}-$	7.0	0	n.d.
$(CH_3)_2CH(CH_2)_2-$	19.6	0	n.d.
$(C_2H_5)(CH_3)-CH-$	51.3	0	n.d.
$(CH_3)_2CH-CH_2-$	5.8	0.3	n.d.
$(C_2H_5)_2-CH-$	8.0	n.d.	n.d.
$(n\text{-}C_3H_7)_2CH-$	3.0	n.d.	n.d.
$C_2H_5OOC(CH_2)_2-$	25.7	n.d.	n.d.
phenyl–CH_2-	n.d.	0	0
phenyl–	n.d.	0	100
R–phenyl–CO–COO⁻			
–H	100[a]	0	100
3-Br	57.9[a]	n.d.	n.d.
3-F	n.d.	n.d.	43
4-Br	134.0[a]	n.d.	n.d.
4-Cl	128.5[a]	n.d.	52
4-F	114.3[a]	n.d.	n.d.
4-CH_3	67[a]	n.d.	110
4-OH	n.d.	n.d.	100
4-OCH_3	44.3[a]	n.d.	23
4-C_2H_5	75.0[a]	n.d.	n.d.
4-$C(CH_3)_3$	0	n.d.	n.d.

[a] Relative activities of aromatic α-keto acids refer to benzoylformate.

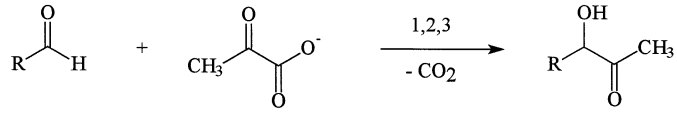
BFD*Ps.p.* are limited to unbranched aliphatic and aromatic substrates, respectively (Table 2). Besides pyruvate, only the C4 and C5-keto acids are substrates for PDC*Z.m.* [15,17]. BFD*Ps.p.* requires substrates with an aromatic ring directly connected to the α-carbonyl group. Phenyl pyruvate is not a substrate for BFD*Ps.p.* [55,62].

2.4.5. Carboligations mediated by PDCs

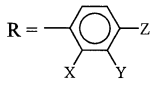
A survey of the α-hydroxy ketones that have been produced either by biotransformations or enzymatically are summarized in Tables 3 and 4. It has to be emphasized that most of the α-hydroxy ketones given in Tables 3 and 4 have been obtained in analytical scale only. Among these, the formation of (phenylacetyl carbinol) PAC **50** (Scheme 9) by biotransformation of benzaldehyde has been studied most intensively with regard to the improvement of yield and the stability of the microbial or enzymatic catalysts in presence of benzaldehyde (Scheme 1). Most of these investigations have been performed using yeasts of different species (sp.), mainly *Saccharomyces* sp. and *Candida* sp. The current fermentative process is largely limited by side-reactions due to various enzymes existing in living yeast cells and by instability of the cells in presence of benzaldehyde and the fermentative products (for reviews of yeast-mediated transformations, see Refs. [11,15]).

2.4.5.1. Synthesis of (R)-phenylacetyl carbinol. The evaluation of isolated PDCs as catalysts for the synthesis of (*R*)-PAC has only recently been started with studies on PDC from *C. utilis* [74], *S. cerevisiae* and *Z. mobilis* [51,75–77]. Although Shin and Rogers [74] reported high yields of (*R*)-PAC using partially purified PDC from

Table 3
α-Hydroxyketones which have been obtained by decarboxylative incorporation of pyruvate using fermenting yeast (*Saccharomyces* sp.) (1), or isolated enzymes PDC*S.sp.* (2) and PDC*Z.m.* (3), respectively

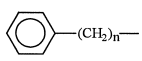
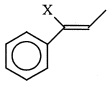
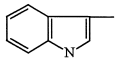
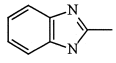


aliphatic α-hydroxyketones	$R = C_nH_{2n+1}$
Residue	catalyst
R = 1 – 12	1
R = 1,2	2
R = 1,2	3

aromatic α-hydroxyketones R =  (benzaldehyde derivatives)

Residue	catalyst
X, Y ,Z = -H	1 2 3
monosubstituted derivatives	
X, Y ,Z = -F, -Cl, -Br	1 2 3
X, Y ,Z = -OH, $-CH_3$, $-OCH_3$, $-CF_3$	1
Z = $-CH(CH_3)_2$, $-N(CH_3)_2$, $-C_6H_5$	1
disubstituted derivatives	
X,Y = -di-F	1,2
X,X' = -di-F	1,2

Table 3 (continued)

aromatic and heterocyclic α-hydroxyketones	
Residue	catalyst
R =  n = 1, 2	1
 X = H, -CH_3	1
 X = O, S	2 3
 X = O, S	1 2 3
	1
	1
	1

For more detailed information, see Ref. [15].

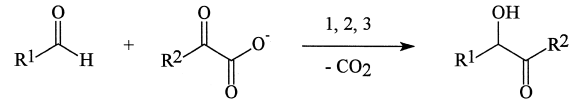
C. utilis in 8 h reaction time. It has to be mentioned that the purified yeast enzyme is only stable at 4°C making the application of the yeast enzyme inefficient for the technical production of (*R*)-PAC **50**. Similar results have been obtained with PDC from *S. cerevisiae* [51,75], which was shown to inactivate rapidly at 25°C ($t_{1/2} = 20$ h) and 30°C ($t_{1/2} = 10$ h), respectively. Additionally, the yeast enzyme is sensitive to benzaldehyde [17,78] and acetaldehyde [74].

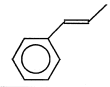
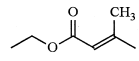
By contrast, the enzyme from *Z. mobilis* is significantly more stable at room temperature ($t_{1/2} \gg 100$ h). The high stability makes PDC*Z.m.*, especially against benzaldehyde, makes the enzyme well suited for the application in biotransformations. The lower carboligase activity of wild type-PDC*Z.m.* [17] was successfully enhanced by site-directed mutagenesis [51,75–77]. The amino acid residue tryptophan-392 is located at the dimer–dimer interface in the channel leading to the active center of PDC*Z.m.* Its exchange for alanine enhanced the carboligase activity by a factor of 3 to 4 [76,77]. This mutant enzyme PDC*W392A* was shown to be a useful catalyst for the synthesis of (*R*)-PAC, if the acetaldehyde which is continuously produced by decarboxylation of pyruvate is removed enzymatically [76,77]. However, the conformational stability of the mutant enzyme was reduced compared to the wild-type enzyme, due to the loss of hydrophobic stabilization at the dimer–dimer interface. Further mutagenesis studies yielded the mutants PDC*W392I* and PDC*W392M*, which are more stable and more active than PDC*W392A*. They catalyze the formation of (*R*)-PAC by a factor of 5 to 6 more efficiently than the wild-type enzyme [51].

2.4.6. Carboligations mediated by BFD

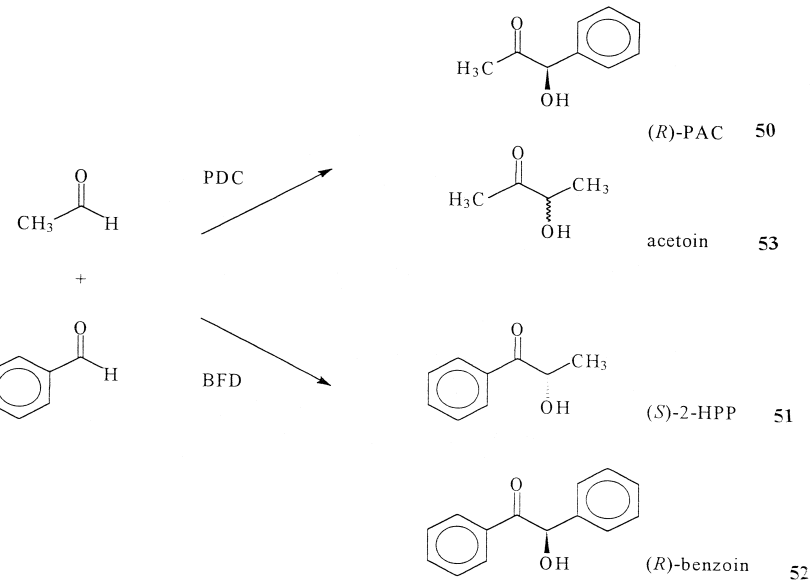
The formation of α-hydroxyketones by BFD*Ps.p.* was first described by Wilcocks et al. [64] using whole cells and cell extracts of *P. putida*, which formed (*S*)-2-hydroxypro-

Table 4
α-Hydroxyketones which have been obtained by decarboxylative introduction of various aliphatic α-keto acids using fermenting yeast (*Saccharomyces* sp.) (1), or isolated enzymes PDC*S.sp.* (2) and PDC*Z.m.* (3), respectively



R^1	R^2	catalyst
	-CH_3 -C_2H_5 -C_3H_7	1
	-CH_3 -C_2H_5	1
	-CH_3 -C_2H_5	1
-CH_3 and -C_2H_5	-CH_3 and -C_2H_5	1 2
-C_2H_5	-CH_3 and -C_2H_5	3

For more details, see Ref. [15].



Scheme 9. Reaction products of the biotransformation of pyruvate and benzaldehyde with PDC, and benzoylformate and acetaldehyde with BFD, respectively.

piophenone (2-HPP) **51**, a tautomer of PAC, from benzoylformate and acetaldehyde (Scheme 9) [64]. The enantiomeric excess of 2-HPP was determined by ^{1}H-NMR spectroscopy and was found to be 91–92%. Factors effecting 2-HPP formation by BFD*Ps.p.* have been investigated by Wilcocks and Ward [63]. They found a broad pH-optimum (pH 5–8) and temperature optimum (20–40°C). We have recently detected that BFD*Ps.p.* does also produce (*R*)-benzoin **52** with high enantiomeric excess.[2] Thus, BFD and PDC may complement each other as catalysts for organic synthesis. This is for instance demonstrated with the product spectrum which is accessible by carboligation of benzaldehyde and acetaldehyde with both enzymes in Scheme 9.

2.4.7. Stereo control

A detailed investigation of the carboligase reaction mediated by PDC from yeast, wheat germ and *Z. mobilis* revealed that the stereocontrol of this reaction is only strict with aromatic or heterocyclic aldehydes [11,18] as acylanion-acceptors, while the formation of acetoin **53** resulted in mixtures of the (*R*)- and (*S*)-enantiomer [75,80–83]. From modeling studies, the relevance of the side-chain of isoleucine 476 (PDC*S.c.*), which is conserved in all PDCs, for the stereo-control during the formation of aromatic α-hydroxyketones became likely, since this side-chain may protect one site of the carbanion-enamine **48** (Scheme 8) against the bulky aromatic cosubstrate [84,85].

A comparison of the sequences from PDC and BFD enzymes reveals that the highly conserved isoleucine residue is replaced by alanine in BFD. A difference which might result in different substrate specificities and/or altered stereospecificity. The role of this conserved Ile-residue in PDC*Z.m.* (Ile 472) was recently studied by Pohl et al. [75] using site-directed mutagenesis. An exchange of Ile 472 for Ala reduced the decarboxylase activity to about 30% of the wild type-activity concomitant with an eight-fold increase of the K_M value for pyruvate. The specific carboligase activity with respect to the formation of PAC was reduced to

[2] H. Iding, M. Pohl, unpublished results.

60%, and, most remarkably, the resulting PAC showed significant amounts of the (*S*)-enantiomer, reducing the enantiomeric excess of (*R*)-PAC to 60%. Additionally, the formation of PAC is connected with the production of significant amounts of the tautomeric product 2-HPP (Scheme 9), a by-product which is not observed with biotransformations using the wild-type enzyme or other mutant enzymes of *Z. mobilis*. An investigation of the substrate spectrum of the decarboxylase reaction confirmed the result that the conserved Ile-residue has a significant function for both the substrate- and the stereospecificity of PDC. In contrast to the wild-type enzyme, the mutant enzyme PDC *I472A* is capable of decarboxylating extended aliphatic as well as aromatic α-keto acids and α-keto butanoic acid is a better substrate for this mutant than pyruvate [15].

3. Conclusions and outlook

So far, mainly the ThDP-dependent enzymes PDC, TKT, and, partially, BFD have been exploited for chemoenzymatic syntheses. Through recombinant DNA technology, the enzymes are now abundantly available and therefore a closer look towards the synthetic potential of these enzymes will become an easy and rewarding task for chemists and biotechnologists. This includes the approach to make ThDP-dependent enzymes even more interesting for chemoenzymatic syntheses through broadening of the substrate spectra using the tools of molecular biology. The 3D- structures of several ThDP-dependent enzymes have become available in recent years and already served to obtain a better understanding of the substrate binding and of the reaction mechanisms. Amino acid exchanges in the substrate channel of TKT have been described already [26,86–89] and were used to alter active sites of the respective enzymes from different organisms. Further insights will be available when more people start to use these interesting enzymes.

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