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Separating Thermodynamics from Kinetics – A New Understanding of the Transketolase Reaction

Stefan R. Marsden[a], Lorina Gjonaj[a], Stephen J. Eustace[a] and Ulf Hanefeld*[a]

Transketolase catalyzes asymmetric C-C bond formation of two highly polar compounds. Over the last 30 years, the reaction has unanimously been described in literature as irreversible due to the concomitant release of CO_2 when using lithium hydroxypyruvate (LiHPA) as substrate. When following the reaction over a longer period of time however, it was now revealed to be initially kinetically controlled. For the non-natural conversion of synthetically more interesting apolar substrates, a complete change of active site polarity is counterintuitively not necessary. Docking studies revealed water and hydrogen bond networks to be essential in substrate binding, thus allowing aliphatic aldehydes to be converted in the charged active site of transketolase.

Introduction

Transketolase (TK, E.C. 2.2.1.1) is a Mg^{2+} and thiamine diphosphate (ThDP) dependent enzyme which naturally catalyzes the conversion of glycolysis derived metabolites into carbohydrates utilized for nucleotide synthesis and the production of essential aromatic amino acids via the Shikimate pathway.[1] The overall reaction comprises of the reversible transfer of a C_2-ketol group and an asymmetric C-C bond formation. This makes the reaction interesting for synthetic applications. A multitude of enzymatic strategies have been developed in order to address the substantial importance of asymmetric C-C bond formation in organic synthesis, of which many rely on decarboxylation as driving force for the C_2-ketol transfer.[2-5]

Scheme 1: Natural TK reaction.

Scheme 2: Synthetic TK reaction.

In order to obtain an improved understanding of the TK catalyzed reaction, two points will be addressed: hydroxypyruvate (HPA) is currently utilized as the ketol donor of choice, because the considerable change in Gibb's Free Energy, which follows from the liberation of CO_2, results in an equilibrium constant entirely in favor of the product. For this reason, the TK catalyzed reaction with LiHPA and decarboxylation driven reactions in general are traditionally described as *irreversible* in literature.[2-10] Indeed, the first *S. cerevisiae* TK catalyzed synthesis of L-erythrulose was performed with LiHPA to ensure it to be irreversible.[11-13] Yet in 2004, the coupling of two molecules of glycolaldehyde to L-erythrulose was reported.[14] In combination with the reversibility of the natural TK catalyzed reactions, this renders an irreversible product formation unlikely from a mechanistic point of view. In recognition of the extensive use of decarboxylation in contemporary C-C bond formation strategies, a correct understanding of the actual impact of decarboxylation on the overall reaction is thus of great importance. In particular since this synthetically very powerful decarboxylation has the disadvantage of a poor atom economy. Secondly, although aliphatic substrates were successfully converted, it remains yet to be fully understood how this is possible. With phosphorylated polyols as typical substrates, TKs are naturally not disposed towards aliphatic substrates. Nevertheless, *E. coli* TK has successfully been engineered by single-point mutations to convert a variety of aromatic and aliphatic aldehydes.[6,7] This is surprising, since the mutations introduced in *E. coli* TK do not render the active site highly lipophilic.[6]

S. cerevisiae TK shares 47% sequence identity with *E. coli* TK and the aligned crystal structures (1QGD and 1TRK) have an RMSD of 0.81 indicating extensive structural homology. Due to its facile heterologous overexpression in *E. coli*, *S. cerevisiae* TK was chosen as model enzyme to representatively investigate both the actual impact of decarboxylation in asymmetric C-C bond synthesis and the cause of enhanced activity towards aliphatic aldehydes previously observed for single-point mutations.[6,7]

Results and Discussion

Figure 1: compounds overview.

The *E. coli* TK mutants D469E and D469T described earlier remarkably showed that highly polar or even charged amino acids improved enzyme activity towards aliphatic aldehydes.[6] This is in contrast to our results that non-phosphorylated substrates are better converted by TK mutants of reduced polarity (R528K, R528Q, R528K/S527T and R528Q/S527T).[15-16] Therefore, the equivalent

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mutations D477E and D477T were created in *S. cerevisiae* TK to allow for a direct comparison and an improved understanding. Preparative reactions (Table 1) were in line with those reported for *E. coli* TK. Again, mutant D477E was surprisingly identified as the best catalyst for the conversion of aliphatic aldehydes. While synthetically relevant, these data however do not allow the evaluation of the catalytic activity of the separate mutants.

Table 1: Isolated yields, enantiomeric excess.

Analysis of the Michaelis-Menten parameters confirmed the results obtained preparatively. Mutant D477E was found the most successful one in the conversion of aliphatic aldehydes **1a** and **2a** showing an enhanced activity of 50 to 100 fold compared to the WT. While mutations at position R528, which natively binds to the phosphate group in phosphorylated substrates[15-16] and the incorporation of a group mutation strategy[17] did enhance enzyme activity, the improvements were only minor compared to the effect of mutation D477E.

Table 2: Michaelis-Menten parameters.

In silico docking studies

Figure 2: docked substrate in the model active site.

With an observed improvement of 50 to 100 fold in $k_{cat}K_M^{-1}$ for the conversion of substrates **1a** and **2a** with D477E by only a single-point mutation, the mutation D477E was introduced *in silico* into the corresponding crystal structure 1GPU[18] in order to investigate the resulting changes in the active site. The obtained model was energy minimized before docking of substrates **1a-4a** into the active site using YASARA.[19] The model showed that extension of the carbon chain by mutating aspartate to glutamate newly enabled hydrogen bond interactions between the glutamate carboxylate and the substrate carbonyl groups bridged by a molecule of coordinated water at 1.7 Å each. In this manner, the substrate is correctly aligned towards the cofactor and the forming oxyanion is stabilized by charge delocalization during nucleophilic attack. This interaction was correctly predicted by the model for the converted substrates **1a-3a** and not predicted for the unconverted substrate **4a** (fig. 2 and fig. S5-S8 ESI). In combination with preparative and kinetic data the docking studies illustrate, that correct substrate orientation towards the activated cofactor (improving not only k_{cat}, but potentially also K_M) is of greater importance for catalysis than an increase based solely on substrate affinity (improving only K_M). This would also explain why the introduction of an isoleucine into the equivalent position in the TK of *Geobacillus stearothermophilus* did not lead to such large rate improvements.[20]

Mechanistic reflections

Scheme 3: proposed mechanism for carbanion formation.

For the synthesis of L-erythrulose from glycolaldehyde and LiHPA as substrates in aqueous solution under standard conditions, the total change in Gibb's Free Energy Δ_rG^0 amounts to -264.5 kJ/mol (L-erythrulose, S18 ESI), largely due to the contribution of decarboxylation. Overall, this would correspond to an equilibrium constant of $K_{eq} = 10^{46}$ in favor of the product. In 2004, the one-substrate TK catalyzed reaction coupling two molecules of glycolaldehyde to L-erythrulose was reported[14] and in strong contrast to the decarboxylation driven reaction, an equilibrium constant of $K_{eq} = 5.0$ was calculated from the change in Gibb's Free Energy, ($\Delta_rG^0 = 4.0$ kJ/mol L-erythrulose in aqueous solution under standard conditions, S18 ESI). Supported by the reversibility of the natural reactions, the one-substrate reaction should therefore be a true equilibrium reaction. In the proposed mechanism for TK catalyzed reactions with lithium hydroxypyruvate, the thermodynamically irreversible decarboxylation of LiHPA effects the direct formation of the carbanion on the activated ketol. For the one-substrate reaction however, the activated carbanion must be formed by catalytic deprotonation from residue His481 as alternative to decarboxylation, generating the activated intermediate at a lower rate in comparison to its generation by decarboxylation. At the stage of the activated ketol bearing the carbanion, the enzyme can no longer distinguish whether it was formed via a reaction pathway involving decarboxylation, or via catalytic deprotonation. The information about the thermodynamic driving force of decarboxylation is therefore already lost prior to the actual product formation. These mechanistic reflections consequently suggest, that TK catalyzed synthesis reactions are reversible via the mechanism of the one-substrate reaction, splitting the product back into one molecule of the respective acceptor aldehyde and one molecule of glycolaldehyde. The thermodynamic contribution of decarboxylation therefore should not affect the position of the overall equilibrium (compare scheme 3) and argue against an irreversible product formation. In conclusion, it should thus be possible to avoid the release of CO_2 and to improve the atom economy of the reaction.

Equilibrium analysis

Scheme 4: reaction schemes equilibrium analysis.

In order to experimentally confirm the reversibility of the TK catalyzed product formation suggested by the mechanistic reflections, L-erythrulose was synthesized both via the one-substrate reaction coupling two molecules of glycolaldehyde and via the conversion of glycolaldehyde with LiHPA to afford the product L-erythrulose in 100 mM concentration for complete conversion using WT *S. cerevisiae* TK. The reactions were performed in sealed NMR tubes allowing for direct measurements of the product erythrulose[21] (fig. S40 ESI). The substrates were not followed due to complete consumption within 30 minutes in the case of LiHPA and due to the issue of oligomerization

and hydration of glycolaldehyde in aqueous solution.[22] Both reactions were followed over an extended period of time. In line with the results earlier published,[14] L-erythrulose formation was observed. The one-substrate reaction proceeds relatively rapidly (fig. 3A) but is limited to less than 30% yield due to the thermodynamic equilibrium of the reaction (fig. 3B).

Figure 3:

When LiHPA was used as ketol donor, a fast and complete conversion was observed as expected[3-10,15-16,20] (fig. 3A). If this reaction was thermodynamically controlled by the release of CO_2 it should stop here. However, in line with a reversible reaction, a slow decline of L-erythrulose concentration was subsequently observed ultimately coinciding with the equilibrium concentration of the one-substrate reaction at K_{eq}=29.1±0.6 mM. The synthesis reaction was thus shown to benefit from a kinetic effect enabling high yields at the beginning of the reaction. The reverse reaction causing thermodynamic equilibration to occur over a time course of several weeks then shifted the product distribution; in line with the outcome of the one-substrate reaction (fig. 3B). In order to confirm that the observed equilibration indeed was enzyme catalyzed, another portion of LiHPA was added at the end. Retained enzymatic activity was observed (fig. 3B, inset), while control reactions without enzyme showed no conversion.

The representative formation of L-erythrulose from glycolaldehyde and LiHPA was thus shown to be initially kinetically controlled contrary to all earlier assumptions about the thermodynamic driving force of CO_2 release. The proposed reaction mechanisms depicted in scheme 3 suggest these findings to generally hold true for all TK catalyzed reactions with HPA. Following the example of the pyruvate decarboxylase catalyzed synthesis of (*R*)-phenylacetylcarbinol with acetaldehyde replacing the traditional donor substrate pyruvate,[23] the development of novel strategies which do not rely on decarboxylation is of commercial relevance. To do so, a correct understanding of decarboxylation is of utmost importance. In syntheses where aldehydes other than glycolaldehyde are used as acceptors, formation of the desired product will be competing with the one-substrate reaction. Active site engineering as pioneered by Pohl for a range of ThDP dependent enzymes could ensure that glycolaldehyde will be the donor molecule in mixed carbo ligation reactions.[24]

Conclusions

Creating novel interactions between an active site residue and a desired substrate should include a network of hydrogen bonds.[25-29] As was shown, this is an effective strategy to increase the substrate's affinity towards the active site, although a polarity based analysis would suggest the opposite. This alternative approach for the rational mutagenesis of TKs towards hydrophobic substrates was demonstrated. While decarboxylation driven C-C bond formation reactions traditionally are misinterpreted in literature as irreversible, mechanistic reflections and experimental evidence unambiguously showed the reaction to initially be under kinetic control. In the context of man-made climate change, we thus have to extensively re-evaluate our choice of donor substrates and the utilization of decarboxylation strategies in synthetic applications.

Experimental Section

Materials

Chemicals and solvents were obtained as reagent grade from Sigma-Aldrich. Aldehydes were freshly distilled and their purity confirmed by ^{1}H NMR before usage. Petrolether (b.p. 40-60°C) was freshly distilled before usage. Lithium hydroxypyruvate was obtained both commercially and synthesized as previously described.[30]

Methods

Reaction progress was monitored by TLC (TLC Silica gel 60 F_{254}, Merck) using UV light and a potassium permanganate stain for visualisation. NMR spectra were recorded using an Agilent 400 MHz (^{1}H, 9.4 Tesla) spectrometer operating at 399.67 MHz for ^{1}H at 298K and were subsequently interpreted using MNOVA. A benzene-D_6 NMR insert capillary (Sigma-Aldrich) was used for external locking during water suppression experiments using the PRESAT-PURGE pulse sequence in sealed Wilmad® screw-cap NMR tubes (Sigma Aldrich). Spectra were recorded using a recycle delay of 2 seconds and 64 repetitions. Preparative scale bioconversions were carried out in an Excella E24 Incubator Shaker (New Brunswick Scientific).

Preparation of cell free extract. The cell pellet containing the respective mutant TK was resuspended in sodium phosphate buffer (5 mM, pH = 7.0, 10 mL/g cell pellet). A protease inhibitor (PMSF, 200 µL, 0.1M in EtOH) was added to each sample. Lysosyme was added at 20 mg/g cell pellet and a spatula tip of DNAse was added to each sample and incubated on ice for 30 minutes. The cells were broken using a sonifier 250 (Branson) and the cell debris removed by centrifugation.

Enzyme purification. The cell pellet was resuspended in binding buffer (5 mM sodium phosphate, pH = 7.4, 20 mM imidazole) and incubated with PMSF, lysozyme and DNAse as previously described. The cells were subsequently broken using a cell disrupter (Constant Systems Ltd, 1.8 kbar), the cell debris removed by centrifugation and the cell free extract filtered (0.45 µm). Affinity chromatography was performed on a NGC Quest 10 system (Biorad) using XK16/20 columns (GE Healthcare Life Sciences) packed with 10 mL Ni-sepharose 6 FF resin (GE Healthcare Life Sciences). For full details see ESI.

Synthesis of racemic standards. Racemic standards were synthesized according to a method previously described.[31] *N*-methylmorpholine (330 µL, 3.0 mmol, 1.0 eq.) was dissolved in water (40 mL) and the pH was adjusted to 8.0 using 10% HCl. LiHPA (330 mg, 3.0 mmol, 1.0 eq.) and the corresponding aldehyde (3.0 mmol, 1.0 eq.) were added and the reaction was stirred overnight at room temperature. Conversion was monitored by TLC (*n*-pentane / EtOAc 1:1). Silica powder was added, the water removed *in vacuo* and the crude product purified by flash chromatography (*n*-pentane / EtOAc 1:1). For full details see ESI.

Dibenzoylation of enantiomers. Dihydroxyketone (1.0 eq.) was dissolved in dry dichloromethane (10 mL) under N_2 atmosphere in a

flame dried round bottomed flask. Dry triethylamine (10.0 eq.) and benzoyl chloride (5.0 eq. per hydroxyl) were added and the reaction mixture was stirred for two hours at room temperature. It was quenched by addition of sat. $NaHCO_3$ (30 mL), the phases separated and the organic phase was washed (sat. $NaHCO_3$, 2x, 50 mL, then sat. NH_4Cl, 1x, 50 mL, then brine, 1x, 30 mL). The organic phase was dried over Na_2SO_4, the solvent was removed *in vacuo* and the crude product was purified by flash chromatography in the case of racemic standards (petrolether / EtOAc 10:1). Purification by flash was omitted in the determination of the enantiomeric excess. For full details see ESI.

Glycolaldehyde activity assay.[15] The volumetric activity of cell free extracts was determined by incubating 50 µL with the cofactors (25°C, 800 rpm, 20 min, ThDP: 5 mM, Mg^{2+}: 18 mM). LiHPA and glycolaldehyde were added to achieve final concentrations of 50 mM in 300 µL total reaction volume (5 mM sodium phosphate buffer, pH = 7.0). The reaction mixture was shaken (25°C, 800 rpm, 15 min), quenched by addition of TFA (300 µL, 0.2% v/v), the enzyme precipitated by centrifugation and analyzed by RP HPLC (R^2 = 0.998) to determine the volumetric activity. Due to considerably varying volumetric activities of cell free extracts the enzyme content was normalized to 20 U of activity based on a glycolaldehyde activity assay previously reported.[15]

Computational docking of glycolaldehyde into the corresponding mutant active sites with YASARA predicted comparable binding energies for all mutants. It was thus concluded that none of the mutations are likely to have introduced a major bias to an activity based analysis using glycolaldehyde as reference. Full details see ESI.

Preparative scale bioconversions. Cell free extract (20U based on the glycolaldehyde activity assay) was incubated with its cofactors (20 min, room temperature, 5 mM sodium phosphate buffer, pH = 7.0, ThDP: 18 mM and Mg^{2+}: 5 mM). LiHPA (110 mg, 1.0 mmol, 1.0 eq.) and the corresponding aldehyde (1.0 mmol, 1.0 eq.) were added and the reaction volume was adjusted to 10 mL. The reaction was carried out in a sealed flask overnight (25°C, 200 rpm). The product was extracted with MTBE (2x, 40 mL) and the solvent was removed *in vacuo*.

Chiral separation. Enantiomers were derivatized by dibenzoylation and chiral separation was performed on a Shimadzu LC-20AD prominence system equipped with a Chiralpak AD-H column (0.46x25 cm, Daicel) using *n*-heptane / *i*-PrOH 97:3 as mobile phase (35°C, 1 mL/min).

Analytical quantitation.[15] Dihydroxyketone product concentrations were determined by RP HPLC on a Shimadzu LC-20AD prominence system equipped with an IC-Sep Coregel 87H3 column (0.4x25 cm, Transgenomic). The absorbance was followed at 210 nm using 0.1% (v/v) aqueous trifluoroacetic acid (TFA) pH = 2.5 as mobile phase (60°C, 0.8 mL/min).

Determining Michaelis-Menten parameters. Individual reaction times were initially determined to measure the parameters under credible initial rate conditions (<20% conversion). The buffered reaction mixture (300 µL, 5 mM sodium phosphate, pH = 7.0) containing holotransketolase (50 µg / 337 pmol, 1 mM ThDP, 4 mM Mg^{2+}), LiHPA (100 mM) and the corresponding aldehyde at varied concentrations (5 – 150 mM) were incubated (25°C, 500 rpm) in duplicate. The reactions were quenched by 1:1 addition of 0.2% (v/v) TFA, the enzyme was precipitated by centrifugation and the supernatant was subjected to RP HPLC analysis. A Michaelis-Menten type non-linear fit was obtained from the Excel built-in solver successively minimizing the sum of the squared errors between measured and fitted data points converging towards values for K_M and v_{max}. For full details see ESI.

Equilibrium analysis by NMR.[21,32] The benzene signal (s, 7.15 ppm) from a NMR insert capillary was used as reference and its integral (including ^{13}C satellites) was normalised to 1000. The erythrulose concentration was followed by its characteristic peaks 4.61 (1H, d, $^2J_{HH}$ 19.6 Hz), 4.52 (1H, d, $^2J_{HH}$ 19.6 Hz). L(+) erythrulose was obtained in the highest quality commercially available (Sigma-Aldrich) and the calibration curve was corrected mathematically for a purity of 85%. Enzyme (WT TK, 200 µg, 1.35 nmol) was incubated with its cofactors (25°C, 20 min, ThDP: 5 mM, Mg^{2+}: 18 mM, 5 mM sodium phosphate buffer pH = 7.0). LiHPA driven conversion: glycolaldehyde and LiHPA were added to achieve final concentrations of 100 mM each and the reaction volume was adjusted to 500 µL. One-substrate reaction: glycolaldehyde was added to achieve a final concentration of 200 mM and the reaction volume was adjusted to 500 µL.

Computational docking studies. *In silico* docking studies were carried out with YASARA (Version 16.2.18) using the crystal structures 1TRK (free ThDP cofactor) and 1GPU (containing the activated ketol) for *S. cerevisiae* TK and 1QGD for *E. coli* TK. The simulation box was defined at 10 Å around the thiamine C2 in 1TRK and around the ylid anion in 1GPU. The substrates were energy minimized with ChemBio3D Ultra 12.0 (Cambridgesoft) using MM2 energy minimization. The mutation D477E was introduced into 1GPU and the model was subsequently energy minimized using YASARA before docking. For full details see ESI.

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Keywords: Transketolase • Thermodynamic control • Kinetic control • water network • aldehyde

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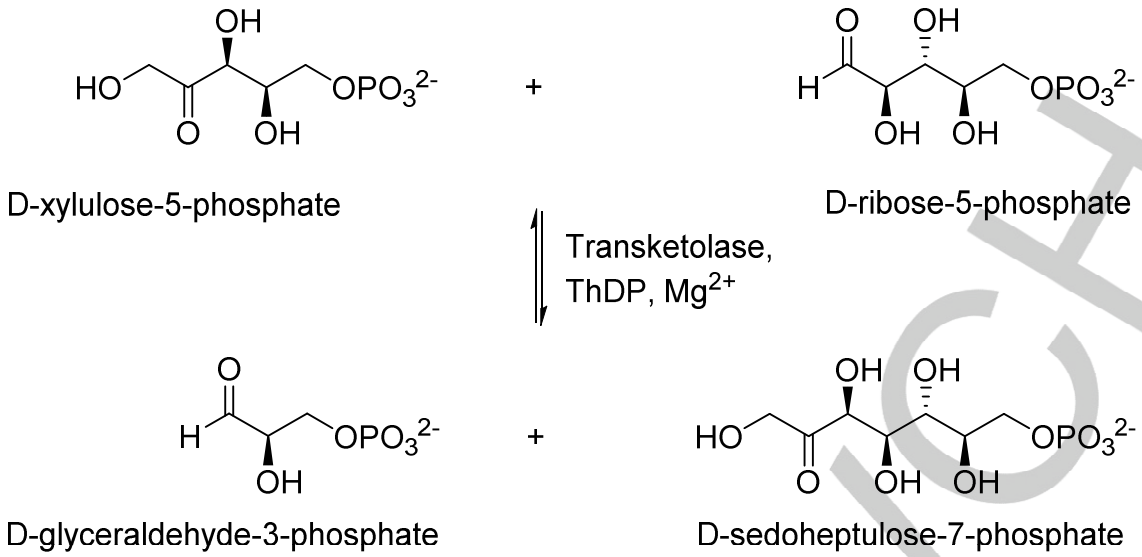

Scheme 1. Natural transketolase catalyzed reaction.

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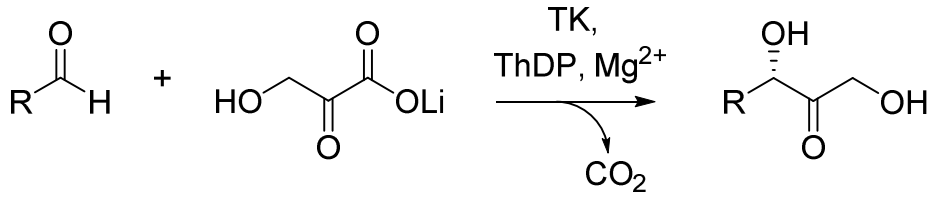

Scheme 2. Use of LiHPA as a ketol donor in TK catalyzed synthetic applications.

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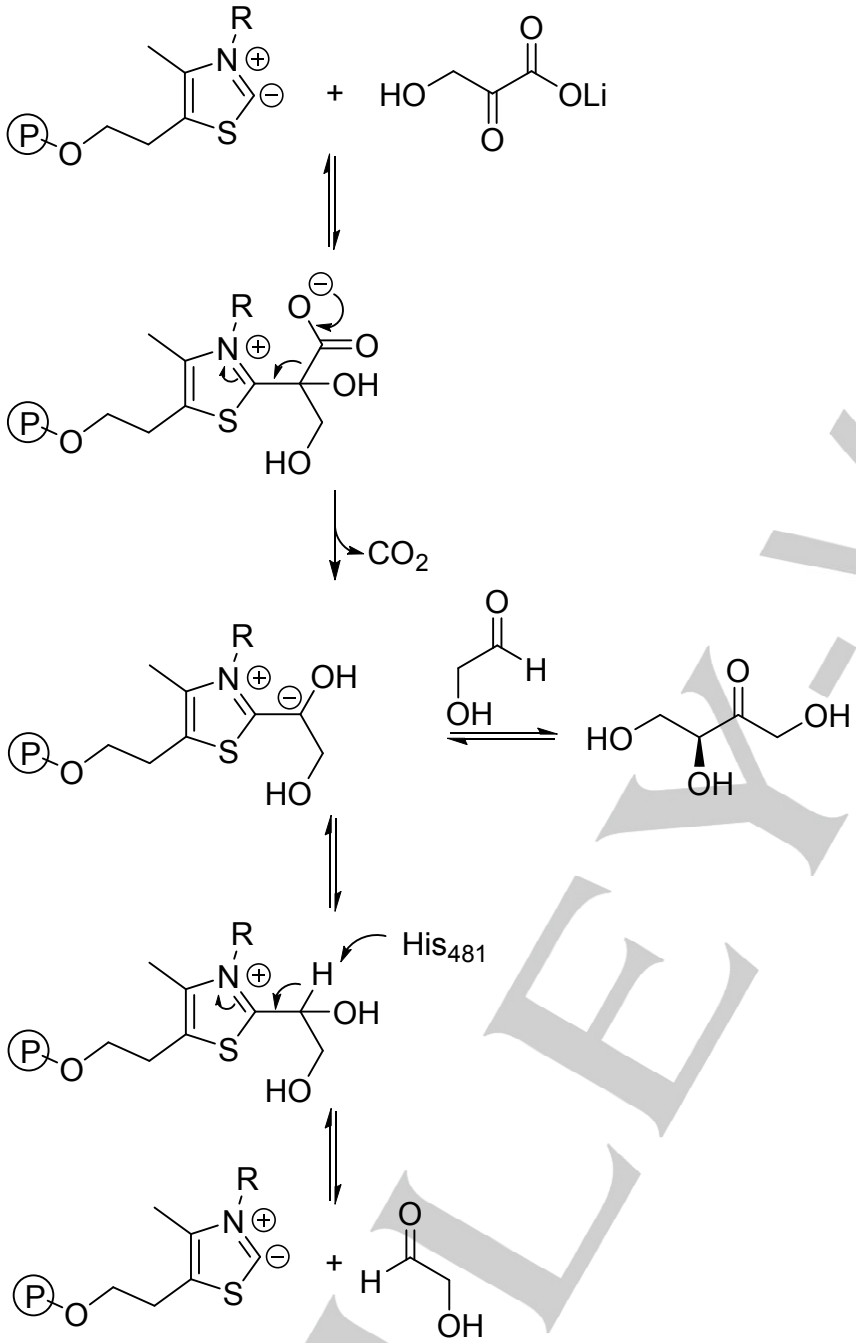

Scheme 3. Proposed mechanism for the formation of the activated ketol bearing the carbanion by either decarboxylation (top) or catalytic deprotonation (bottom).

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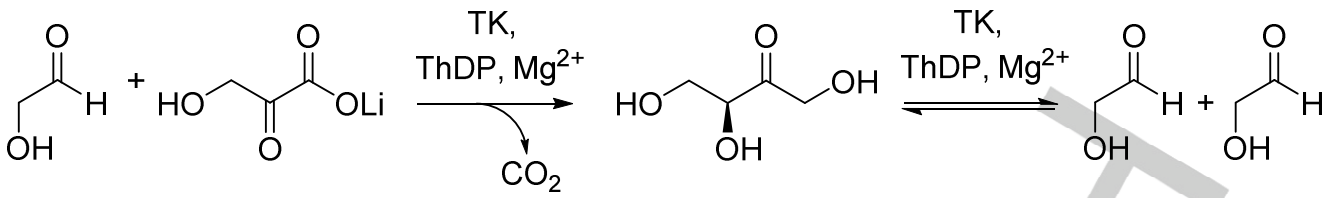

Scheme 4. Decarboxylation driven reaction (left) and one-substrate reaction (right) for the TK catalyzed synthesis of L-erythrulose.

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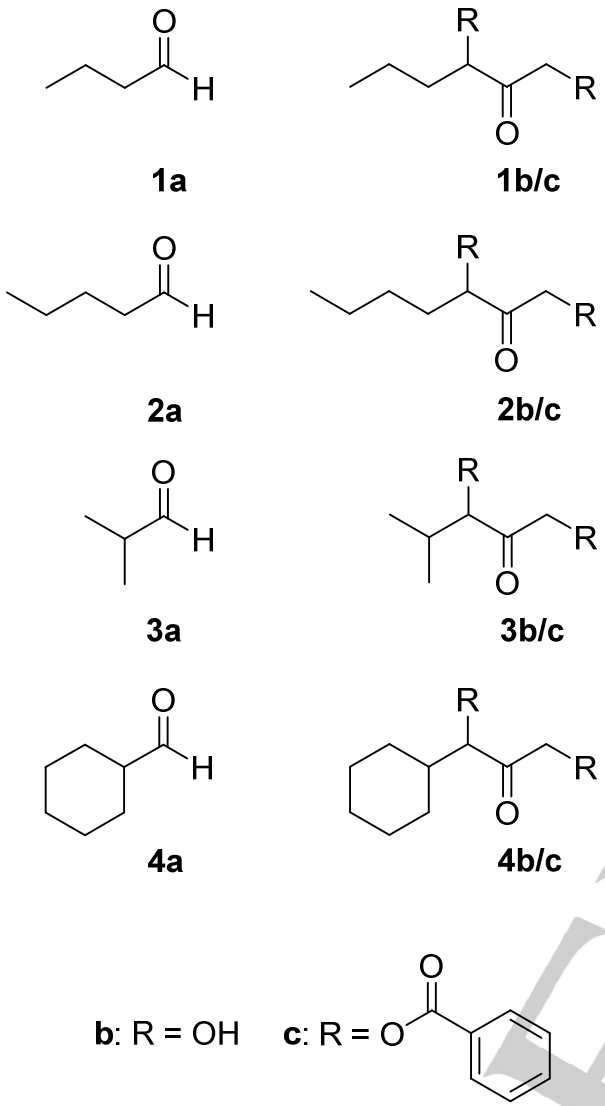

Figure 1. Overview of substrates (**a**), products (**b**) and derivatized products (**c**) required for chiral analysis. Products **1-3** (**b**) and (**c**) were obtained in the **3**-(*S*) configuration with TK. Products **4b/c** were not accessible enzymatically.

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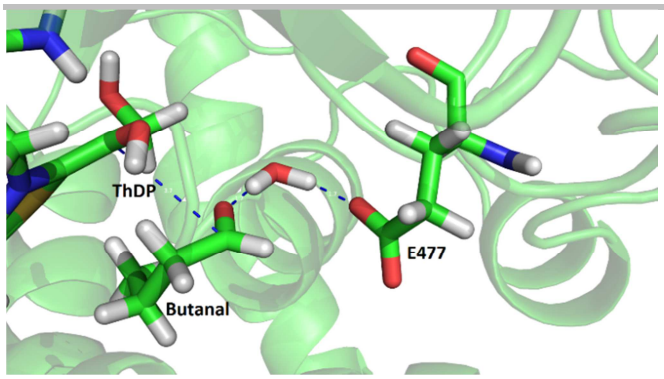

Figure 2. *in silico* docking of butanal into the energy minimized mutant active site D477E using YASARA.

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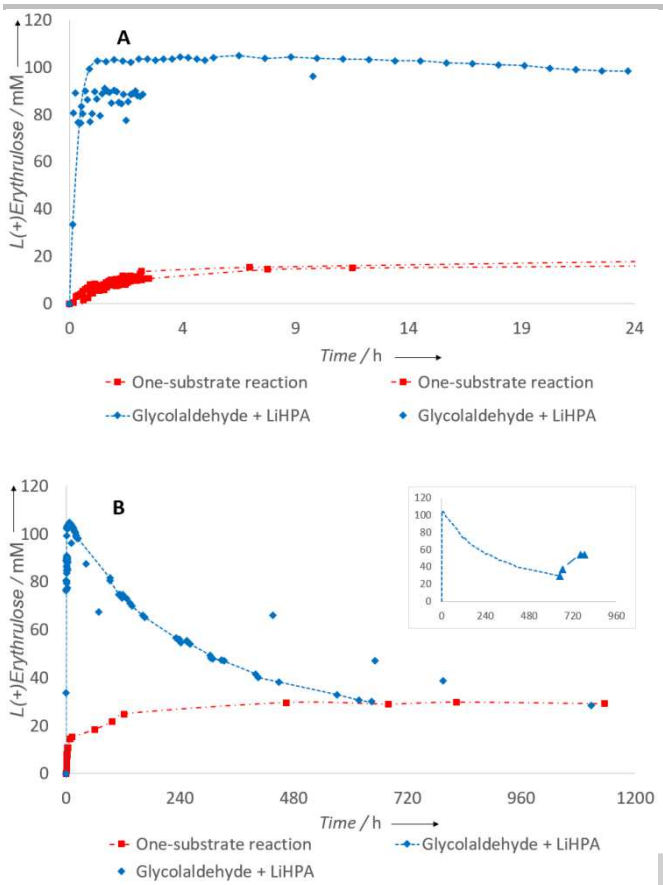

Figure 3. TK reaction producing L-erythrulose followed by ^{1}H-NMR. 200 µg WT TK, ThDP: 5 mM, Mg^{2+}: 18 mM, 5 mM sodium phosphate buffer pH = 7.0. For the one substrate reaction (red): glycolaldehyde: 200 mM, for the decarboxylation driven reaction (blue): glycolaldehyde: 100 mM and LiHPA: 100 mM. A) Initial 24 hours showing complete conversion in the decarboxylation driven reaction; B) Extended time course showing equilibration of both reactions towards the equilibrium concentration of 29.1±0.6 mM for erythrulose. Inset: addition of LiHPA after 650 h showing retained enzyme activity (triangles).

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Table 1. Isolated product yields and enantiomeric excess (*ee*) of the (*S*) configured enantiomer.[a]

	WT	D477E	D477T	R528K	R528Q	R528K/ S527T	R528Q/ S527T
1b	11±8% (*84%*)	34±15% (*94%*)	8% (*n.d.*) [b]	10±8% (*81%*)	8±2% (*77%*)	8±3% (*73%*)	6±4% (*66%*)
2b	7% (*91%*)	61±13% (*90%*)	12±4% (*84%*)	6±4% (*82%*)	5±1% (*87%*)	6±1% (*68%*)	5±1 (*82%*)
3b	0% (*n.d.*) [b]	41±20% (*99%*)	n.d. [b] (*n.d.*) [b]	3±1% (*n.d.*) [b]	0% (*n.d.*) [b]	0% (*n.d.*) [b]	0% (*n.d.*) [b]
4b	0% (*n.d.*) [b]	0% (*n.d.*) [b]	n.d. [b] (*n.d.*) [b]	0% (*n.d.*) [b]	0% (*n.d.*) [b]	0% (*n.d.*) [b]	0% (*n.d.*) [b]

[a] 20U of *Sc*TK, 5 mM ThDP, 18 mM Mg^{2+},1 mmol LiHPA, 1 mmol aldehyde, 10 mL final volume in 5 mM sodium phosphate buffer, pH = 7.0, 25°C, 200 rpm, 18h
[b] not determined.

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Table 2. Michaelis-Menten parameters.[a]

		WT	D477E	D477T	R528K	R528Q	R528K/ S527T	R528Q/ S527T
1b	k_{cat}	1.2	42	0.5	0.8	1.5	1.9	0.8
	K_M	272	163	48	181	239	260	106
	$k_{cat}K_M^{-1}$	4.2	260	10	4.4	6.1	7.4	7.5
2b	k_{cat}	0.8	9.3	0.4	0.1	2.1	0.3	0.4
	K_M	327	40	43	16	611	67	42
	$k_{cat}K_M^{-1}$	2.4	233	9.9	6.9	3.5	4.2	8.2
3b	k_{cat}	0.4	0.6			0.3	0.3	
	K_M	150	66	n.d. [b]	n.d. [b]	99	86	n.d.
	$k_{cat}K_M^{-1}$	2.9	8.3			2.5	3.7	

[a] k_{cat} / s^{-1}, K_M / mM, $k_{cat}K_M^{-1}$ / $M^{-1}s^{-1}$. For errorbars see ESI fig S10-S12. Purified *Sc*TK: 50 µg, ThDP: 1 mM, Mg^{2+}: 4 mM, LiHPA: 100 mM, aldehyde: 5-150 mM, 5 mM sodium phosphate buffer, pH = 7.0, 25°C, 500 rpm. [b] not determined.

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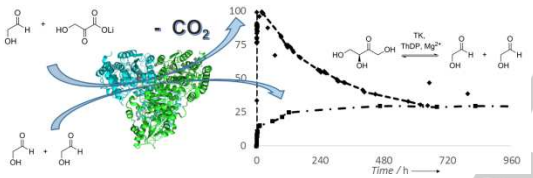

The initially kinetic control of the Transketoase catalyzed LiHPA reaction was until now mistakenly described as thermodynamic control. A new understanding of this effect and of the reasons why hydrophobic substrates are converted by an enzyme that naturally has a high affinity to hydrophilic substrates sheds new light on textbook biocatalysis.

*Stefan R. Marsden, Lorina Gjonaj, Stephen J. Eustace and Ulf Hanefeld**

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Separating Thermodynamics from Kinetics – A New Understanding of the Transketolase Reaction