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Sweet siblings with different faces: The mechanisms of FBP and F6P aldolase, transaldolase, transketolase and phosphoketolase revisited in light of recent structural data

Kai Tittmann *

Göttingen Center for Molecular Biosciences, Georg-August University Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany

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ABSTRACT

Nature has evolved different strategies for the reversible cleavage of ketose phosphosugars as essential metabolic reactions in all domains of life. Prominent examples are the Schiff-base forming class I FBP and F6P aldolase as well as transaldolase, which all exploit an active center lysine to reversibly cleave the C3–C4 bond of fructose-1,6-bisphosphate or fructose-6-phosphate to give two 3-carbon products (aldolase), or to shuttle 3-carbon units between various phosphosugars (transaldolase). In contrast, transketolase and phosphoketolase make use of the bioorganic cofactor thiamin diphosphate to cleave the preceding C2–C3 bond of ketose phosphates. While transketolase catalyzes the reversible transfer of 2-carbon ketol fragments in a reaction analogous to that of transaldolase, phosphoketolase forms acetyl phosphate as final product in a reaction that comprises ketol cleavage, dehydration and phosphorolysis. In this review, common and divergent catalytic principles of these enzymes will be discussed, mostly, but not exclusively, on the basis of crystallographic snapshots of catalysis. These studies in combination with mutagenesis and kinetic analysis not only delineated the stereochemical course of substrate binding and processing, but also identified key catalytic players acting at the various stages of the reaction. The structural basis for the different chemical fates and lifetimes of the central enamine intermediates in all five enzymes will be particularly discussed, in addition to the mechanisms of substrate cleavage, dehydration and ring-opening reactions of cyclic substrates. The observation of covalent enzymatic intermediates in hyperreactive conformations such as Schiff-bases with twisted double-bond linkages in transaldolase and physically distorted substrate–thiamin conjugates with elongated substrate bonds to be cleaved in transketolase, which probably epitomize a canonical feature of enzyme catalysis, will be also highlighted.

1. Enzymes, substrates, products and catalyzed net reactions

Phosphorylated ketose and aldose monosaccharides are essential metabolites in numerous cellular pathways across all species. They are an important source of energy, and further required for the biosynthesis of nucleic acids, amino acids and lipids, either as direct building blocks or as precursor molecules that are fed into different pathways. A fundamental reaction of carbohydrate metabolism is the (often reversible) C–C bond cleavage of ketose phosphates such as of D-fructose-1,6-bisphosphate (FBP), D-fructose-6-phosphate (F6P), D-xylulose-5-phosphate (X5P) and D-sedoheptulose-7-phosphate (S7P) into smaller units, which, in some instances, are then transferred to aldose phosphate acceptors (Scheme 1). In general, these ketose phosphates are susceptible to cleavage of either the C2–C3 bond (ketol cleavage), or of the adjacent C3–C4 bond (aldol cleavage) depending on the enzyme class involved (see below).

One of the best-studied examples of an enzyme breaking the carbon skeleton of ketose phosphates is the almost ubiquitously occurring fructose-1,6-bisphosphate aldolase (FBP aldolase), which converts the hexoketose FBP into the three-carbon ketose dihydroxyacetone phosphate (DHAP) and the three-carbon aldose glyceraldehyde-3-phosphate (G3P) by aldol cleavage of the C3–C4 bond as a key reaction of glycolysis (Scheme 1A) [1,2]. This reaction is essentially reversible, during cellular periods of gluconeogenesis, FBP aldolase catalyzes the aldol condensation of DHAP and G3P. Two major classes of FBP aldolases can be distinguished: class I (higher eukaryotes), which utilizes a lysine and operates via covalent Schiff-base conjugates, and class II (bacteria, fungi, algae),

* Fax: +49 551 395749.
E-mail address: ktittma@gwdg.de

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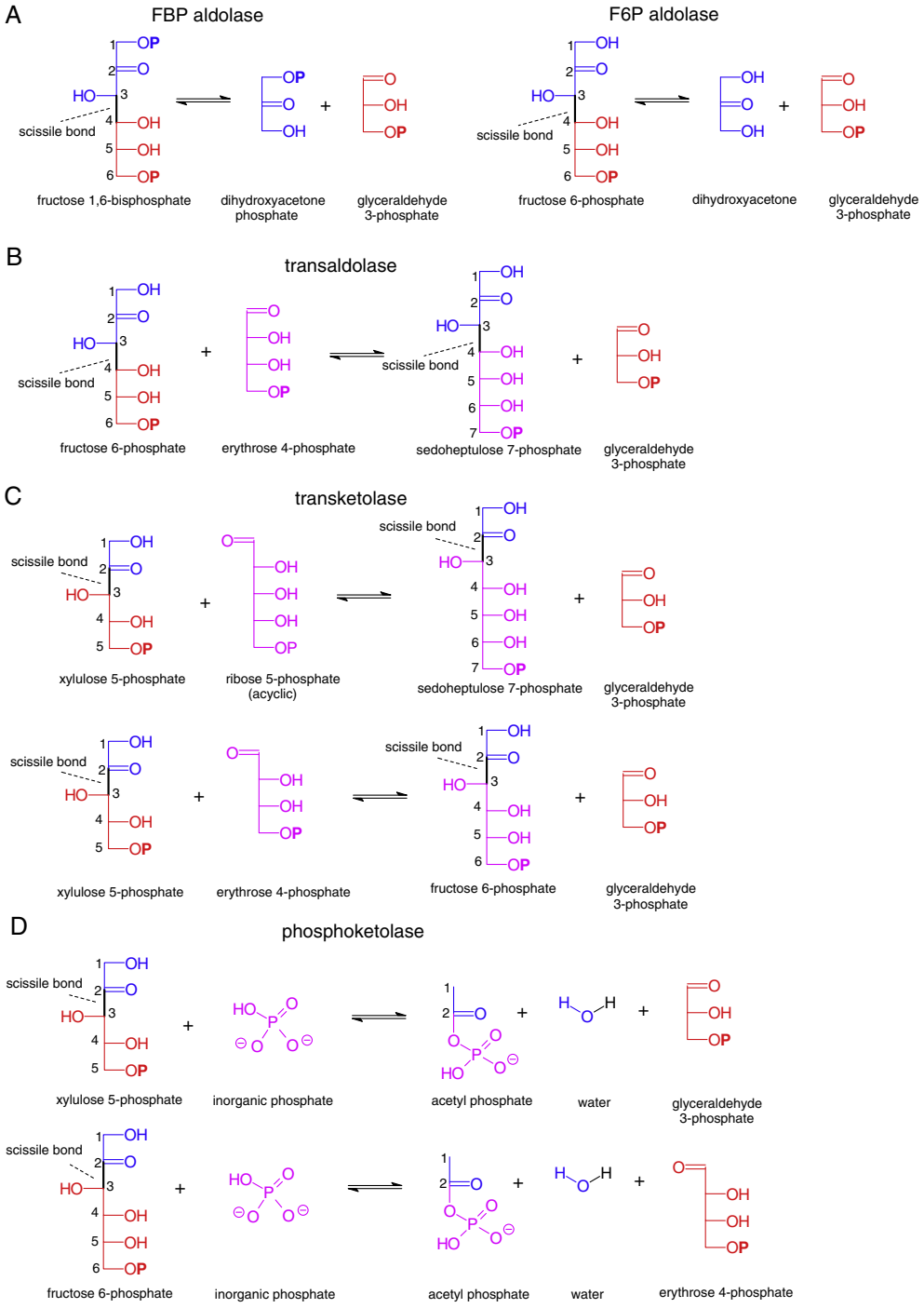


Scheme 1. Catalyzed reactions and physiological substrates of FBP and F6P aldolase (A), transaldolase (B), transketolase (C) and phosphoketolase (D).

which requires a divalent metal ion as cofactor for enzymatic activity [3,4]. In the context of this review, we will focus on class I FBP aldolase with the enzyme from rabbit muscle serving as a prototypical example. In addition, we will discuss the structure and putative mechanism of the recently discovered F6P aldolase from *Escherichia coli* (FSA), which is seemingly unique in its ability to reversibly cleave ketose F6P into dihydroxyacetone (DHA) and G3P (Scheme 1A) [5]. While its aldolase activity could be demonstrated in vitro, its metabolic function *in vivo* is not fully clear yet.

Transaldolase, which also belongs to the Schiff-base forming class I aldolase family, is an almost ubiquitous enzyme that catalyzes the reversible transfer of a three-carbon dihydroxyacetone unit from ketose F6P (after C3–C4 aldol cleavage) to the C1 position of acceptor aldose D-erythrose-4-phosphate (E4P) yielding the elongated S7P ketose and aldose G3P as products (Scheme 1B) [6–8]. Transaldolase thus establishes, together with transketolase that transfers two-carbon dihydroxyethyl units (see below), a reversible link between glycolysis and the pentose phosphate pathway. The latter pathway serves manifold purposes in cellular metabolism as it e.g. supplies D-ribose-5-phosphate (R5P) as a building block for the synthesis of nucleic acids and E4P as precursor for biosynthesis of aromatic amino acids (shikimate pathway), and further generates NADPH for reductive biosyntheses (fatty acids, cholesterol) and sustaining the glutathione level. Unlike FBP aldolase, transaldolase retains a three-carbon unit of its ketose substrates after initial C3–C4 cleavage bound in a sufficiently

stable form to allow departure of the first aldose product followed by subsequent binding of and aldol condensation with a newly arrived aldose acceptor [7].

Transketolase is a key enzyme both in the Calvin cycle of photosynthesis as well as in the nonoxidative branch of the pentose phosphate pathway, where it acts in tandem with transaldolase and fulfills multiple important metabolic functions as outlined above [9–11]. It requires thiamin diphosphate (ThDP) and a divalent metal ion (often Ca^{2+}) as essential cofactors, and catalyzes the reversible transfer of 2-carbon dihydroxyethyl units from donor ketose X5P to the C1 position of alternative acceptor aldoses R5P or E4P affording either S7P or F6P plus aldose G3P as products (Scheme 1C). In contrast to aldolase and transaldolase, transketolase cleaves the C2–C3 bond of its ketose substrates in a ketol cleavage reaction with ThDP as catalyst, a reaction that is in some aspects similar to the classic aldol cleavage (it is therefore often referred to as aldol-like). Akin to transaldolase, a portion of the ketose substrates remains covalently bound to the enzyme–ThDP complex in a stable form after substrate cleavage for eventual transfer to an alternative aldose acceptor.

The closely related enzyme phosphoketolase shares the same cofactor set (ThDP, divalent metal ion), and catalyzes a reaction that is in part identical with that of transketolase (Scheme 1D) [12,13]. Initially, phosphoketolase covalently binds the ketose substrate X5P (and/or F6P in some cases) followed by C2–C3 bond cleavage and liberation of G3P (or E4P) as product. The resultant covalent intermediate on the enzyme subsequently undergoes dehydration and phosphorolytic cleavage to give high-energy metabolite acetyl phosphate as product, which in turn is used to produce ATP via acetate kinase. Phosphoketolase is a key enzyme in heterofermentative bacteria and in the pentose catabolism of various microbes such a *Bifidobacteria* and some *Lactobacillus* strains.

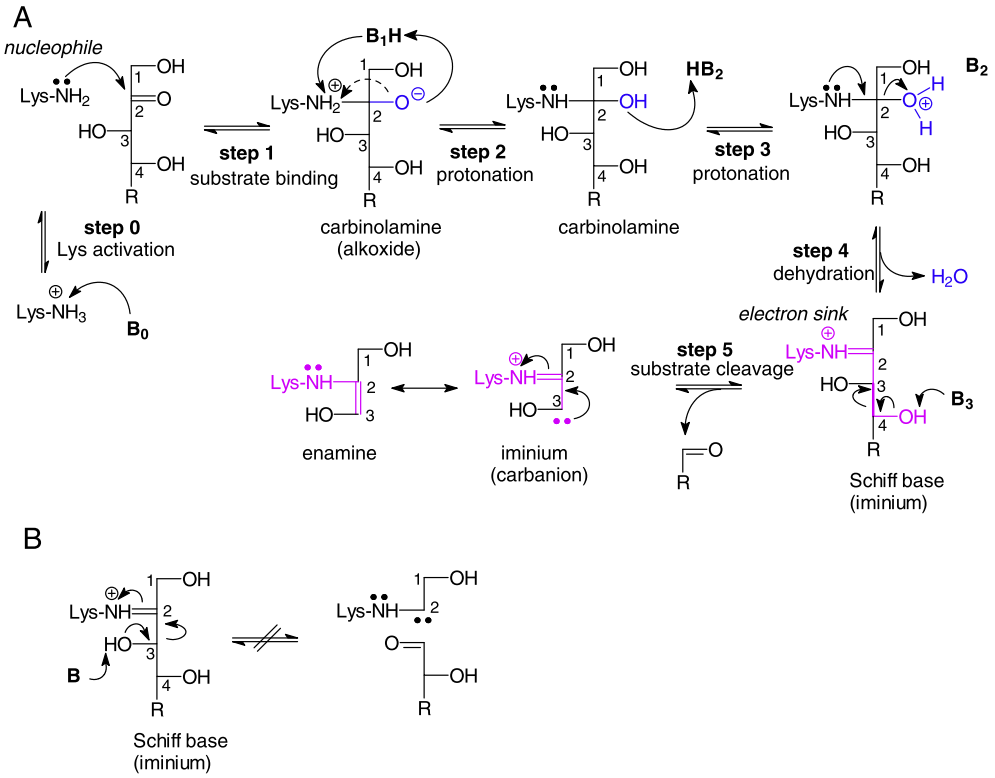
2. Chemical basis of the different bond fission specificities in Schiff-base forming and thiamin-dependent enzymes: aldol versus ketol cleavage

The different bond fission specificities of ketose phosphates in Schiff-base forming aldolases/transaldolases (C3–C4 aldol cleavage) and thiamin-dependent enzymes (C2–C3 ketol cleavage) are "by design". In Schiff-base forming enzymes (Scheme 2A), the ε-amino group of an active center lysine acts initially as a nucleophile to attack the 2-keto carbon of a given ketose affording a covalent tetrahedral intermediate (step 1), the carbinolamine [14,15]. In order to function as nucleophile, the lysine must either exist as the uncharged amine, or, if protonated, be subject to activation through action of a base provided by the enzyme (B_0 in step 0). The formed dipolar carbinolamine alkoxide is then protonated with Nε of the engaged lysine presumably serving as a proton source in either a direct εN–O transfer, or mediated by an interjacent enzyme acid–base catalyst in a concerted mechanism (B_1H in step 2). The C2–OH group of the charge-neutral carbinolamine is then protonated (B_2H in step 3), followed by dehydration and concomitant formation of the Schiff-base iminium, in which the substrate is held in double-bond C–N linkage with the positively charged nitrogen of the lysine. The pK_a for protonated imines is typically in the range of 7–8, so these highly electrophilic species do exist in substantial part at physiological pH. Deprotonation of the substrate C4–OH group by base catalysis (B_3 in step 4) leads to heterolytic cleavage of the scissile substrate C3–C4 bond and liberation of an aldose product. The resultant covalent three-carbon intermediate is resonance stabilized and may adopt an enamine or iminium–carbanion state. A potential C2–C3 bond cleavage initiated by ionization of the C3–OH group of the substrate is an unlikely scenario (Scheme 2B) as the incipient C2-centered free electron pair cannot be stabilized through conjugation as in case of a C3-centered carbanion. Taken together, the hallmarks of enzymatic Schiff-base chemistry employed by aldolases and transaldolases involves polarity inversion of the nucleophilic amine by dehydration and generation of a highly electrophilic iminium species, of which the positively charged nitrogen acts as an electron sink for stabilization of the C3-centered carbanion through delocalization [14]. The reaction involves multiple proton transfers, both acid as well as base catalysis is required at different stages of the reaction.

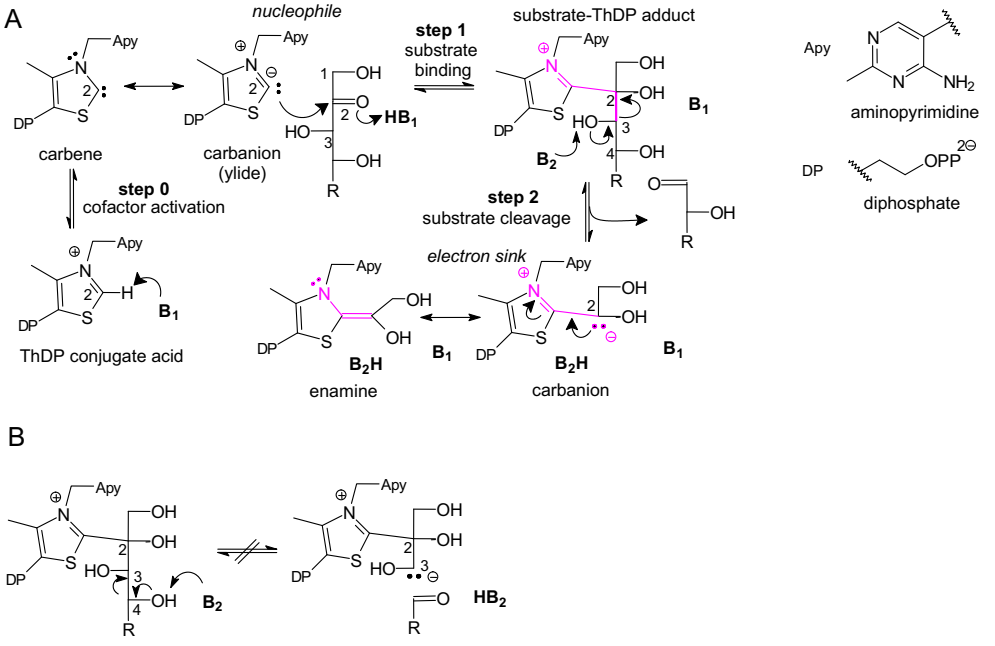
In contrast to the required polarity inversion from a nucleophilic amine to an electrophilic iminium Schiff base in aldolases/transaldolase enzymes, the vitamin B_1-derived cofactor thiamin diphosphate (ThDP) acting in transketolase/phosphoketolase is a "hybrid nucleophilic–electrophilic catalyst" (Scheme 3). The main catalytic site of ThDP is the thiazolium heterocycle with acidic carbon 2, the nucleophilic center, and the neighboring, positively charged ring nitrogen as a built-in electrophilic center [16,17]. This highlights a major difference between Schiff-base forming enzymes and ThDP enzymes, and further explains the different bond fission specificities; whereas Nε of the lysine is both point nucleophile and electrophile, the nucleophilic and electrophilic centers in ThDP are not identical but in neighboring (α) position. The catalytic cycle of ThDP enzymes typically commences with formation of the reactive C2 carbanion or carbene (B_1 in step 0), recent studies suggested that binding of the substrate to the enzyme triggers this initial reaction, and leads to substantial accumulation of the conjugate base of ThDP, which is not observed in the resting state of ThDP enzymes [18–21]. This implies a remarkable pK_a suppression of $\geqslant$12 pH units in view of the known pK_a [18–20] of C2–H of free thiamin [22]. Next, the carbanion/carbene adds to the C2 keto function of the ketose substrate affording a covalent tetrahedral substrate–cofactor conjugate, presumably with concomitant protonation of the substrate carbonyl (B_1H in step 1). It is well established that the second heterocycle of ThDP, the aminopyrimidine, acts as an acid–base catalyst in steps 0 and 1 [17,18,23–25]. Subsequently, ionization of the substrate C3–OH is followed by breakdown of the C2–C3 bond and liberation of an aldose product (B_2 in step 2). The resultant two-carbon dihydroxyethyl-ThDP intermediate with a substrate C2-centered carbanion is resonance-stabilized through conjugation into the thiazolium ring, where the ring nitrogen acts as an effective electron sink and facilitates stabilization of the enamine contributor [26]. A potential ThDP-promoted C3–C4 bond cleavage as catalyzed by aldolases/transaldolases is impossible as this would lead to a C3-centered, localized carbanion without any delocalization (Scheme 3B).

3. Chemical basis of the different reaction specificities in aldolase versus transaldolase and transketolase versus phosphoketolase: the different fates of the central enamine intermediates

As outlined in the previous chapter, the chemical pathways in FBP/F6P aldolases and transaldolases both comprise formation of a covalent lysine-substrate Schiff-base iminium intermediate, which is cleaved to give an aldose as product and a three-carbon substrate fragment in covalent linkage with the active lysine (see Scheme 2A). This intermediate may adopt an enamine-type, charge-neutral structure with a double bond between C2 and C3 of the substrate, or, an iminium–carbanion zwitterion state characterized by a double bond between Nε and C2 of the substrate. In aldolases, this intermediate is stereospecifically protonated at carbon 3 by an acid–base catalyst (B_4H in step 6), and the resultant conjugate acid (termed the C3 Schiff-base iminium) undergoes

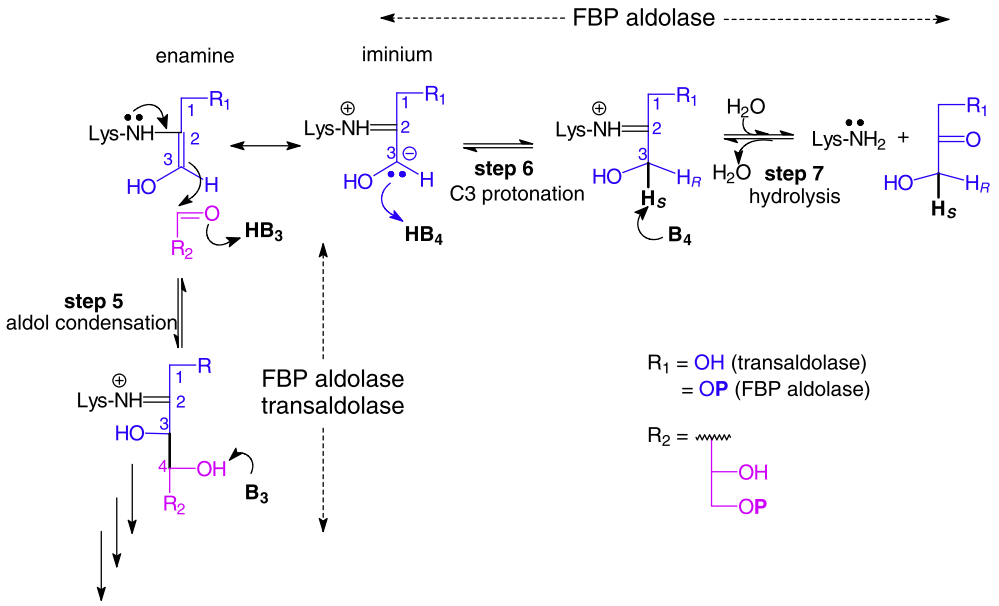


Scheme 2. Mechanism of substrate binding and aldol cleavage of class I aldolases highlighting key intermediates and elementary steps of catalysis (see text) (A). An alternative cleavage of the C2–C3 bond is chemically unlikely (B).



Scheme 3. Mechanism of substrate binding and ketol cleavage of ThDP-dependent enzymes highlighting key intermediates and elementary steps of catalysis (see text) (A). An alternative cleavage of the C3–C4 bond is chemically unlikely (B).

hydrolysis (step 7) leading to the liberation of either DHAP (FBP aldolase) or DHA (F6P aldolase) (Scheme 4) [27]. It is obvious that transaldolase must somehow suppress protonation of C3 by stabilizing the enamine contributor. Aldolases, on the other hand, must fulfill the complicated task to catalyze both protonation of the (enamine–carbanion) and subsequent DHAP/DHA release as well as aldol condensation with acceptor aldose G3P for FBP/F6P synthesis in the reverse reaction in a delicately balanced manner to allow flux through the C3 enamine–carbanion in both directions. This requires a fine-tuned catalytic machinery in which either two acid–base catalysts (B_3, B_4) are engaged in the different proton transfers, or, alternatively, that one residue acts as an acid and as



Scheme 4. Different chemical fates of the C3 enamine–carbanion in FBP aldolase and transaldolase showing key intermediates and elementary steps of catalysis.

base in different reaction steps ($B_3 = B_4$). In transaldolase, acid catalyst HB_4 should be absent or held in a position (in case $B_3 = B_4$), where it can interact with C4–OH but not protonate C3.

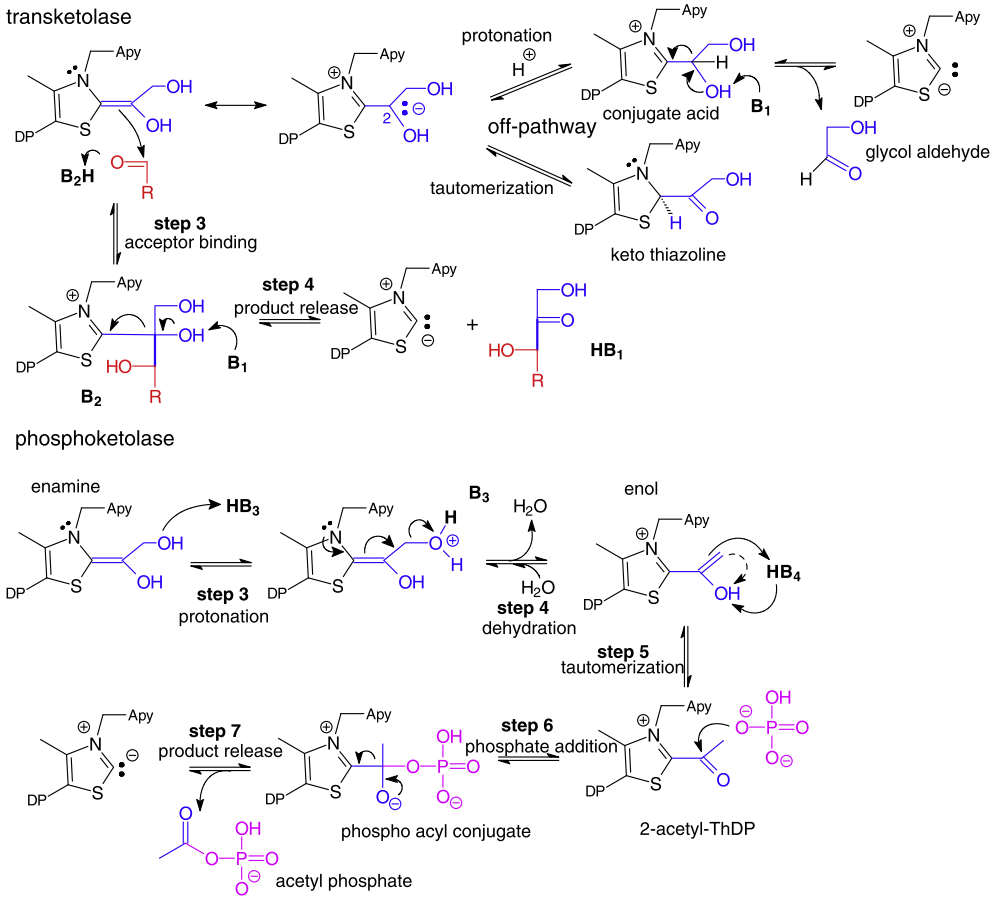
Similar to aldolase and transaldolase, the two ThDP-dependent enzymes transketolase and phosphoketolase both form a central resonance-stabilized enamine–carbanion intermediate, which is the branching point of catalysis for the two pathways (Scheme 5). Obviously, once the enamine–carbanion has been formed, both enzymes must avoid/suppress an off-pathway (*i*) protonation of the C2-centered carbanion with subsequent liberation of an aldehyde product as catalyzed by e.g. ThDP decarboxylases, and (*ii*) a tautomerization of the enamine, which is also an enol, to the corresponding keto-thiazoline as recently observed for a ThDP oxidase and in chemical models [17,28–30]. While transketolase kinetically stabilizes the enamine, allowing carboligation with a newly arrived aldose acceptor (step 3) and release of the thereby formed ketose product (step 4) [31], the enamine in phosphoketolase undergoes acid-catalyzed protonation at the 1-OH position (B_3H in step 3) followed by dehydration and formation of an enol intermediate (step 4) [32]. The enol is then subject to enol → keto tautomerization yielding 2-acetyl-ThDP (step 5). Due to poor frontier orbital overlap, this 1,3-H shift requires assistance by an enzyme acid–base catalyst or from solvent. Next, inorganic phosphate nucleophilically attacks the keto carbon of 2-acetyl-ThDP with transient formation of a covalent phospho conjugate (step 6) before acetyl phosphate is expelled to restart the catalytic cycle (step 7). While acid–base catalysts B_1 and B_2 would seem to have similar roles in the mechanisms of transketolase and phosphoketolase for substrate binding (B_1) and cleavage (B_2), one would expect at least one additional acid–base catalyst at the active site of phosphoketolase, B_3, required for dehydration of the dihydroxyethyl-ThDP enamine. Another catalyst would presumably be needed to facilitate keto–enol tautomerization of the transiently formed enol species.

Taken together, the different chemical fates of the enamine intermediates in aldolase and transaldolase must (at least in part) be due to the existence of an aldolase-specific acid HB_4 with a (i) suitable position relative to C3 of the intermediate and further possessing (ii) an appropriate pK_a facilitating protonation of the three-carbon (enamine–carbanion) intermediate. In addition, a suitably placed positive charge could help to stabilize the developing negative charge at atom C3 in the transition state of protonation. The required stabilization of the enamine in transaldolase can be achieved by placing all the acid–base catalysts involved in the early steps of catalysis such as in substrate binding, dehydration and substrate cleavage in such a way that they cannot protonate C3 once the enamine–carbanion has been formed. Another means to stabilize the enamine could be a H-bonding interaction of an active site residue with the Nε lone pair of the enamine (which is impossible at the carbanion–iminium stage), or by sterically favoring a co-planar substituent conformation of the 3-OH relative to the C2–C3 double bond.

The different pathways in transketolase and phosphoketolase seem to result from the existence of a phosphoketolase-specific acid–base catalyst engaged in the dehydration of the 1-OH group of the common enamine intermediate. Notably, both enzymes must effectively suppress an off-pathway protonation at C2 (substrate) and stabilize the enamine–carbanion (pK_a in thiamin models 18–20!) at least kinetically (and/or thermodynamically) [17,33].

4. Structural basis of FBP aldolase action

Although the crystal structure of FBP aldolase from rabbit muscle as the prototypical and most intensively studied representative of the class I family has been solved as early as in the 1980s shedding light on the active site architecture and consequently on the positioning of the Schiff-base forming Lys229 residue and its immediate vicinity [2], the exact chemical mechanism, in particular the identity of amino acid residues involved in the different elementary (proton transfer) steps of catalysis (steps 1–7, see Schemes 2 and 4), remained unclear as the active site is lined with numerous amino acid residues that could potentially act as acid–base catalysts including two additional lysines (Lys107, Lys146) and three acidic residues (Asp33, Glu187, Glu189). Also, the mode of action of the C-terminal residue Tyr363 that had been implicated as being important for reversible protonation of the C3 enamine–carbanion intermediate (step 6, see Scheme 4) remained obscure due to conformational mobility in the crystal structure [34,35]. Due to this lack of structural information, the exact role of the C-terminal tyrosine, which is not conserved in class I FBP



Scheme 5. Different chemical fates of the dihydroxyethyl-ThDP enamine intermediate in transketolase (top panel) and phosphoketolase (bottom panel) with key intermediates and elementary steps of catalysis invoked.

aldolases throughout all domains of life, remained to be defined. It was postulated that it promotes the alignment and or attachment of DHAP by transitory binding to the active site and a direct catalytic (as acid–base) or a more indirect role by reorganizing the hydrogen bonding network.

Structural analysis of genuine covalent (Schiff-base) reaction intermediates in class I FBP aldolase turned out to be a very difficult and challenging task. It was initially observed that soaking of aldolase crystals with substrate FBP or DHAP produced Michaelis complexes but these were not undergoing covalent catalysis *in crystallo* [36,37]. A major breakthrough for understanding the FBP aldolase mechanism was then the structural analysis of the chemically trapped, borohydride-reduced DHAP Schiff-base in rabbit muscle aldolase, which pinpointed a key catalytic role of Glu187 for proton transfers in early steps of catalysis, in particular for dehydration of the neutral carbinolamine, for which Glu187 was suggested to act as acid (step 3 in Scheme 2) [38]. This structural snapshot of catalysis was further suggesting that the conserved Asp33 residue might act as a base in the deprotonation of the substrate 4-OH at the Schiff-base iminium state in the course of substrate cleavage (step 5 in Scheme 2). Mutagenesis and detailed kinetic analysis, however, implicated Glu187 to not only act as an acid–base catalyst for substrate binding and dehydration of the carbinolamine but to also carry out deprotonation of the substrate 4-OH during substrate cleavage [39]. The latter functional assignments, which were in part in conflict with the proposed mechanism at the time, were then corroborated by elaborate structural analysis of key intermediates including a carbinolamine mimic, the FBP Schiff-base iminium and the C3 Schiff-base iminium after FBP cleavage [27,40]. First, soaking of rabbit muscle FBP aldolase under newly established conditions allowed the structural characterization of the enzyme in complex with the competitive inhibitor D-mannitol-1,6-bisphosphate (MBP), which bears high resemblance with the native substrate FBP. The only difference between the two molecules is the replacement of the 2-keto function of FBP by a H–C–OH group in (*R*)-configuration in case of MBP, hence this molecule mimics the carbinolamine state, although it does not engage in covalent binding to Lys229 (Fig. 1A). The 2-OH of MBP makes intimate contact with the side chain of Glu187 as would the 2-OH group of the genuine carbinolamine thus supporting the proposed role of Glu187 to act as an acid for dehydration. The novel crystallization-soaking conditions further allowed trapping of the genuine FBP Schiff-base intermediate for the first time, here, the ionizable C4–OH of the substrate is held in place by H-bonding interactions with Glu187 and Lys146, whereas Asp33, previously suspected to deprotonate C4–OH hydrogen bonds to C3–OH and the two lysine residues Lys146 and Lys107 (Fig. 1B). In conjunction with mutagenesis and kinetic data, a mechanism of substrate cleavage was proposed, according to which Glu187 pulls off the proton from the substrate C4–OH with the presumably positively charged Lys146 helping to stabilize the developing negative charge at O4. The atoms Lys229-Nε, C2, C3, C4, O4 and the Glu187 carboxylate Os form a chair-like conformation and as such suggest a pericyclic transition state as observed in aldol condensation reactions in enolate models [41–43]. Atom C4 of the FBP Schiff-base is out-of-plane relative to the Schiff-base double bond as required for the transition state of C3–C4 bond cleavage. The catalytic role(s) of Asp33 would seem (i) to stabilize

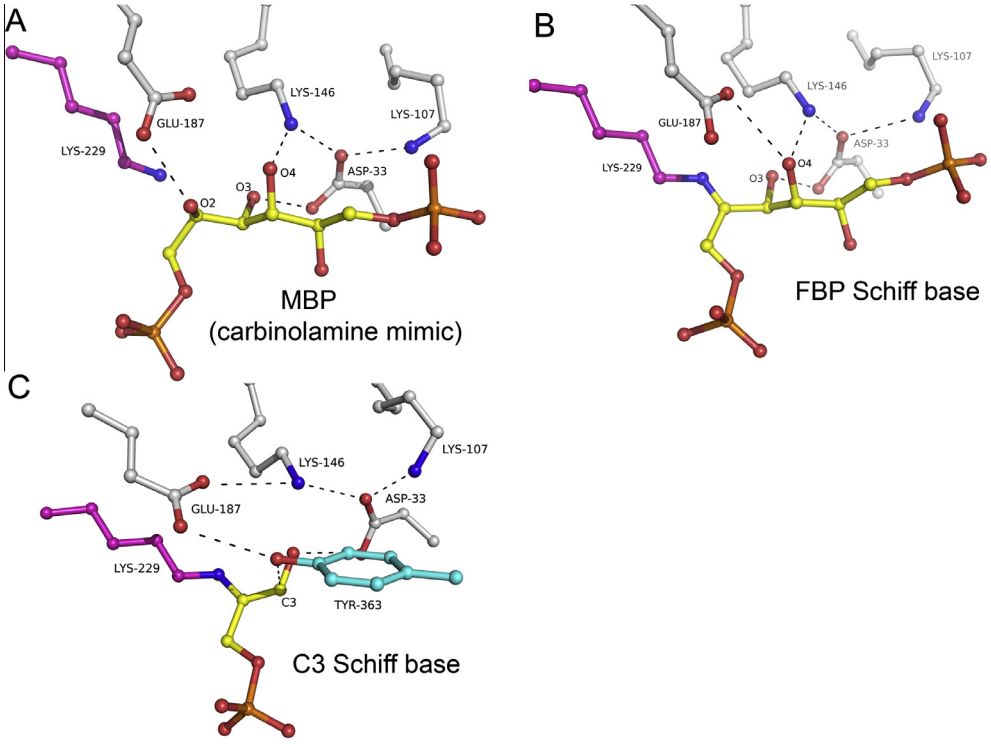


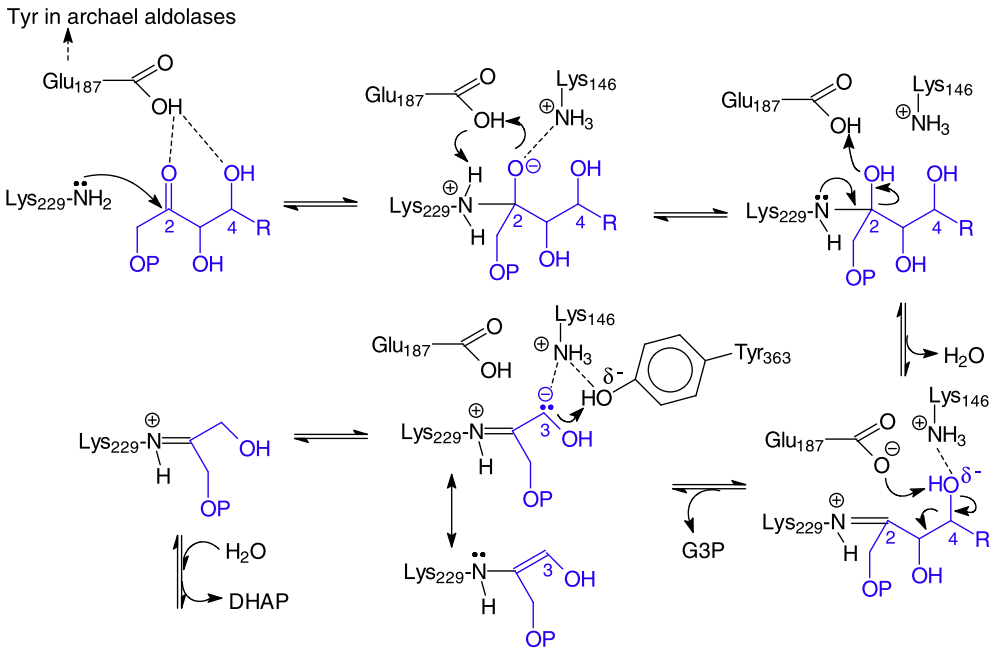
Fig. 1. Snapshots of catalysis in FBP aldolase showing key intermediates and selected active site residues (see text). Hydrogen-bonding interactions are highlighted. Note that for panel C, residue Tyr363 is not from wild-type FBP aldolase but from the superposed variant Lys146Met (see text).

the cation form of Lys146, (ii) to keep Lys146 in proper place for it to interact with O4 and (iii) to serve as a stereoselectivity filter for the substrate skeleton with its stereocenters C3, C4 and C5. The essential catalytic function of Tyr363 for reversible and stereospecific protonation at atom C3 of the C3 carbanion–enamine was finally underscored in structural studies, in which the DHAP Schiff-base conjugate was trapped in the crystal (Fig. 1C) [27]. While the C-terminal part of the enzyme is too flexible to allow it be traced in the crystal structure of wild-type FBP aldolase, it was clearly detectable with Tyr363 accommodating close to C3 of the intermediate in variant Lys146Met, which is defective in deprotonation of the C3 iminium Schiff base. The structure suggests that Tyr363 is the acid–base catalyst responsible for the aldolase-specific reversible protonation of C3, while a potential role of Lys146 might be to stabilize the negative charge of the conjugate base of Tyr363, the phenolate anion, and to reduce the configurational entropy of the C-terminus.

Taken together, the structural and functional studies suggest that residue Glu187 exhibits a multifunctional role in FBP aldolase catalysis and acts as an acid–base in the course of substrate binding, dehydration and substrate cleavage (Scheme 6). The "mobile catalyst" Tyr363 is facilitating reversible protonation of the C3 enamine–carbanion intermediate, and Lys146 serves to stabilize the developing negative charge in various transition states as e.g. in the course of substrate cleavage (at O4) and (de-) protonation of the C3 iminium (at C3). It is interesting to note that this Glu is strictly conserved in eukaryotic class I FBP aldolases but absent in the corresponding orthologs from archaea, here, a Tyr side chain is found in a similar position as Glu187, and thus suggests a mechanism, according to which the Tyr side chain carries out the numerous proton transfers as do Glu187 and Tyr363 in rabbit muscle FBP aldolase [44–46]. In support of this proposal, the genuine FBP-derived carbinolamine intermediate could be trapped *in crystallo* and structurally characterized in an enzyme variant, where the catalytic Tyr had been replaced by a Phe [45]. In related aldolases acting on other substrates the active site contains either a Glu (KDPG aldolase) or a Lys-water dyad (DERA) as sole acid–base catalyst [47,48]. Noteworthy, a Glu residue in transaldolase seems to exhibit a similar multifunctional role as Glu187 in FBP aldolase, although it relays, at least in the course of substrate cleavage, proton transfers through an interjacent catalytic water (see Section 6).

5. Structural basis of fructose-6-phosphate aldolase (FSA) action

Soon after the discovery of FSA and of its innate enzymatic aldolase activity and specificity towards F6P as substrate, the crystal structure could be solved and allowed direct comparison of the active site with that of FBP aldolase [5,49]. It turned out that FSA bears high structural resemblance with transaldolase, whereas there exist numerous distinct differences when compared with FBP aldolases from eukaryotic organisms such as from rabbit muscle. Most notably, as highlighted below in an active site portray of FSA (Fig. 2) shown in identical perspective as before for FBP aldolase (see Fig. 1), the multifunctional Glu is replaced by a glutamine (Gln59) and the neighboring Lys by an asparagine (Asn28). In addition, Gln59 is not as closely situated to the substrate locale as Glu187 in case of FBP aldolase. Gln59 engages in a hydrogen-bond with a catalytic water molecule sitting atop the reactive Lys85, which is further held in place by H-bond interactions with Thr109 and Tyr131. An aspartate (Asp6) is found in almost identical position as Asp33 in FBP aldolase. In the resting state structure, a covalent modification of the reactive Lys85 was observed; the electron density maps suggested it to be the carbinolamine formed with glyceraldehyde, which was formed in the crystallization mixture (oxidation of glycerol). To date, no direct structural information is available for genuine reaction intermediates in FSA, however, modeling of the F6P carbinolamine and of the F6P Schiff-base using the structural information from FBP aldolase and transaldolase (see below) suggests a mechanism [40,50],



Scheme 6. Suggested mechanism of class I FBP aldolase. Numbering refers to the rabbit muscle enzyme.

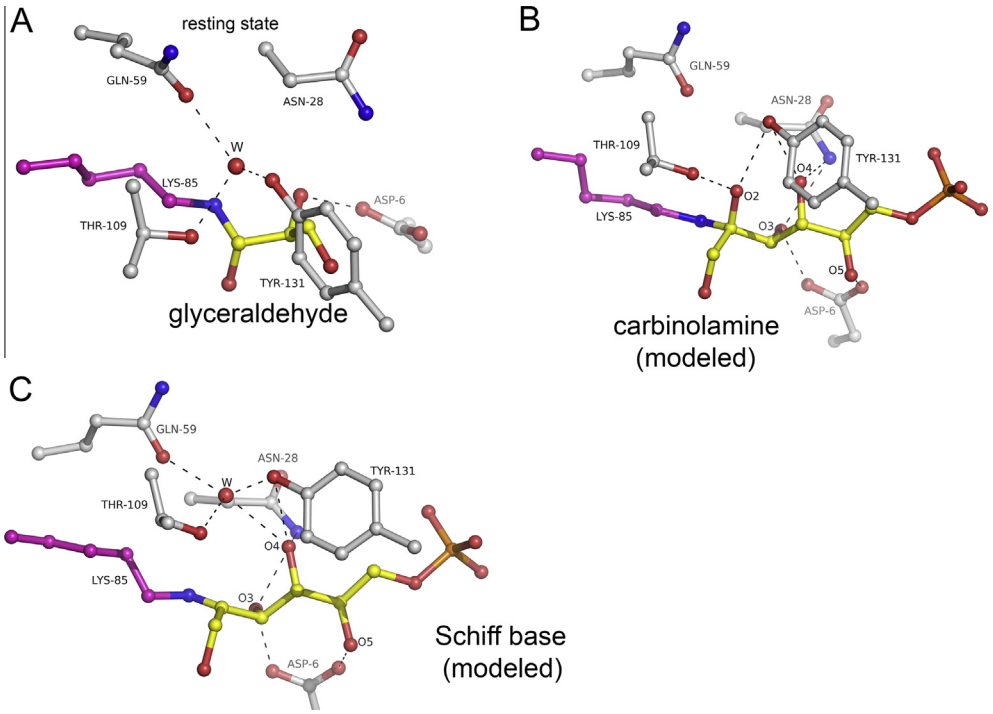


Fig. 2. Snapshots of catalysis in *E. coli* F6P aldolase (FSA) showing key intermediates and selected active site residues (see text). Hydrogen-bonding interactions are highlighted. Note that the carbinolamine formed upon addition of substrate F6P (panel B) and the F6P Schiff base (panel C) were modeled into the active site using information from the structurally related transaldolase (50).

where Tyr131 exhibits a multifunctional role as both acid and base in all key steps of catalysis such as dehydration (Tyr131 as acid), substrate cleavage (Tyr131 as base) and protonation of the enamine (Tyr131 as acid) as its side chain OH group is very likely contacting both the 2-OH & 4-OH groups as well as C3 of the formed intermediates. Interestingly, its spatial position relative to the intermediate is different from that of Glu in eukaryotic aldolases and that of the corresponding catalytic Tyr in archaeal orthologs as it is pointing towards the substrate locale from the opposite structural face. It accommodates close to where the mobile, catalytically important C-terminal Tyr363 in FBP aldolase binds to (compare Fig. 1C).

6. Structural basis of transaldolase action

The structure of transaldolase in the resting state was determined in the 1990s and showcased that transaldolase differs from other class I aldolases in that the $(\beta,\alpha)_8$ TIM barrel fold has

undergone a circular permutation [51]. Subsequently, structural analysis of a stable, borohydride-reduced C3-lysine Schiff-base conjugate (εN-ß-glyceryl lysine) derived upon reaction of transaldolase with native substrate F6P in the presence of an auxiliary enzymatic system provided first important insights into the reaction mechanism as well as common and divergent active site features compared to class I aldolases (Fig. 3C) [52]. Most notably, transaldolases contain a Glu residue in a similar position as the multifunctional Glu187 in FBP aldolases, although here, it is engaged in catalysis by relaying protons through a catalytic water that is further firmly held in place by a conserved Thr residue. An additional conserved feature in both enzyme families is the existence of an aspartate, which is apparently required for properly aligning the substrate and intermediates. While a lysine (Lys143) is located in the immediate vicinity of this aspartate in case of FBP aldolase, one observes a conserved Asn residue in transaldolases instead. The catalytic tyrosine in F6P aldolase (FSA) is replaced by a Phe, which occupies virtually the identical position at the active site of transaldolase as the Tyr in FSA. The early structural observations on the borohydride reduced C3-Schiff base trapped in transaldolase led to the proposal that the conserved aspartate might act as a base that deprotonates the C4–OH in the course of substrate cleavage [8,52]. Later structural analysis of genuine F6P and S7P Schiff-base conjugates in TAL from *Thermoplasma acidophilum* revealed, however, that the catalytic Glu residue (Glu60 in *T. acidophilum* TAL, Glu96 in *E. coli* TAL) likely catalyzes deprotonation of C4–OH with the catalytic water acting as a proton relay, while the aspartate (Asp6) serves to hold the intermediate in place, and forms hydrogen bonds with both 3-OH and 5-OH suggesting it to be ionized at this stage [50]. The Asn residue (Asn28) interacts with 3-OH and 4-OH and is likely to stabilize the developing negative charge at O4 in the course of substrate cleavage by donating a hydrogen bond. The geometry of the Schiff base is comparable to that observed in FBP aldolase with a chair-like conformation of atoms Lys86-Nε, C2, C3, C4, O4 and O of the catalytic water favoring a pericyclic transition state. Also, conforming with the required orbital alignments of C3–C4 bond cleavage, atom C4 is out of the Schiff-base plane as before discussed for FBP aldolase. Modeling of the carbinolamine into the active site of transaldolase relying on the existing structural information about the F6P and S7P Schiff base intermediates and invoking a least-motion mechanism as in FBP aldolase suggests that the conserved Glu residue in transaldolase also catalyzes the dehydration of the carbinolamine by protonating 2-OH, and thus exhibits a similar multifunctional role in the transaldolase reaction as Glu187 in FBP aldolase with key roles for substrate binding, dehydration and substrate cleavage. A mechanism consistent with the available structural information is shown in Scheme 7. It is not fully clear yet, whether or not the catalytic water is present at all stages of the catalytic cycle, it cannot be excluded that it is pushed out of the active site in the course of carbinolamine formation. It is clear though that it is kept in striking distance (~3.6 Å relative to C2) to attack the iminium Schiff base for eventual hydrolysis and release of the ketose. Recent structural analysis of F6P and S7P Michaelis complexes of TAL, in which the catalytic lysine had been replaced by a methionine, indicated that the substrates apparently bind in a multistep mechanism and undergo structural rearrangements while approaching the site of action [53]. In the same study, structures of F6P and S7P Schiff bases confirmed the previously suggested functional roles of active site residues. Interestingly, replacement of the active site Phe by a Tyr as observed in FSA conferred aldolase activity to transaldolase supporting the aforementioned proposal that the Tyr is a catalytic key residue in the FSA mechanism [54].

7. Commonalities and differences between aldolases and transaldolases

A comparison of the suggested, structure-guided chemical mechanisms of FBP and F6P aldolases versus that of transaldolase

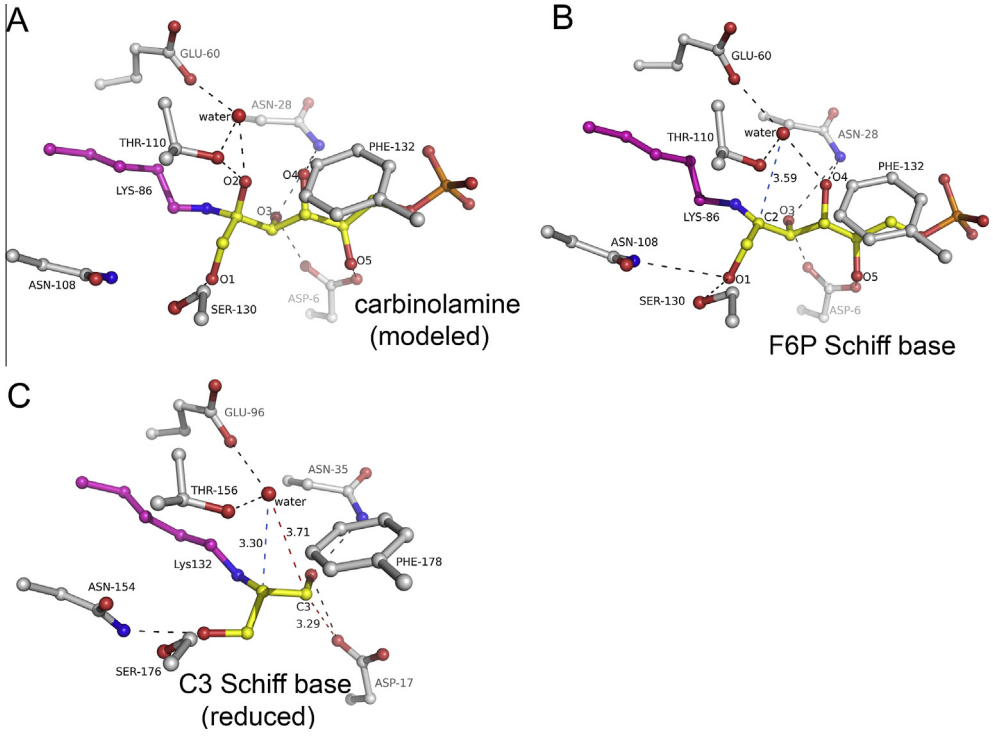
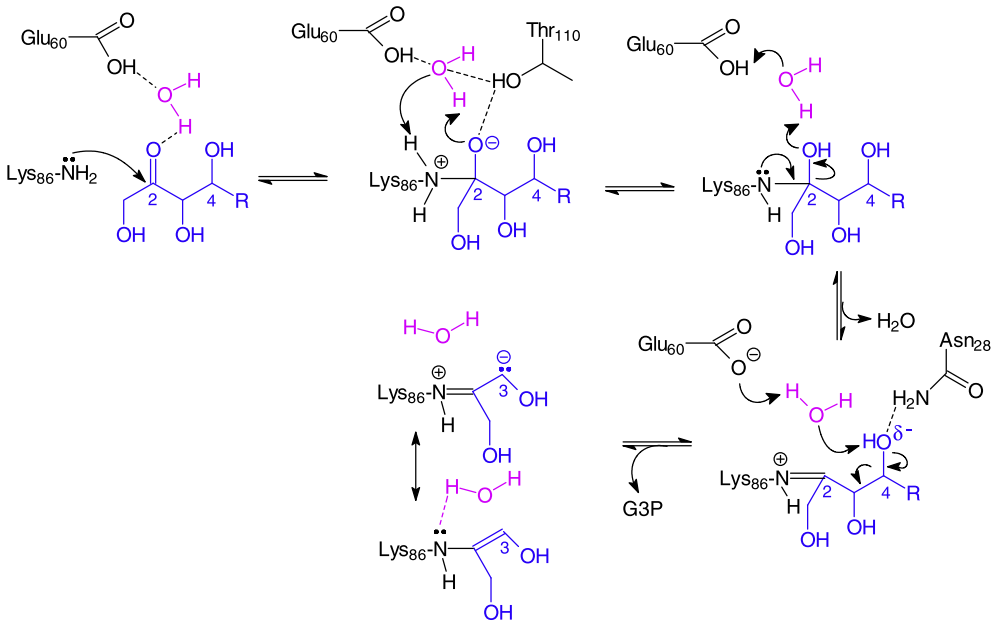


Fig. 3. Snapshots of transaldolase catalysis showing key intermediates and selected active site residues (see text). Hydrogen-bonding interactions are highlighted. Note that the carbinolamine (panel A) was modeled into the active site assuming a least-motion mechanism of dehydration. Panel A and B show transaldolase from *Thermoplasma acidophilum*, while the borohydride reduced C3 Schiff base (C) is shown in the *E. coli* enzyme (50, 52).



Scheme 7. Suggested mechanism of transaldolase. Numbering refers to the *T. acidophilum* enzyme.

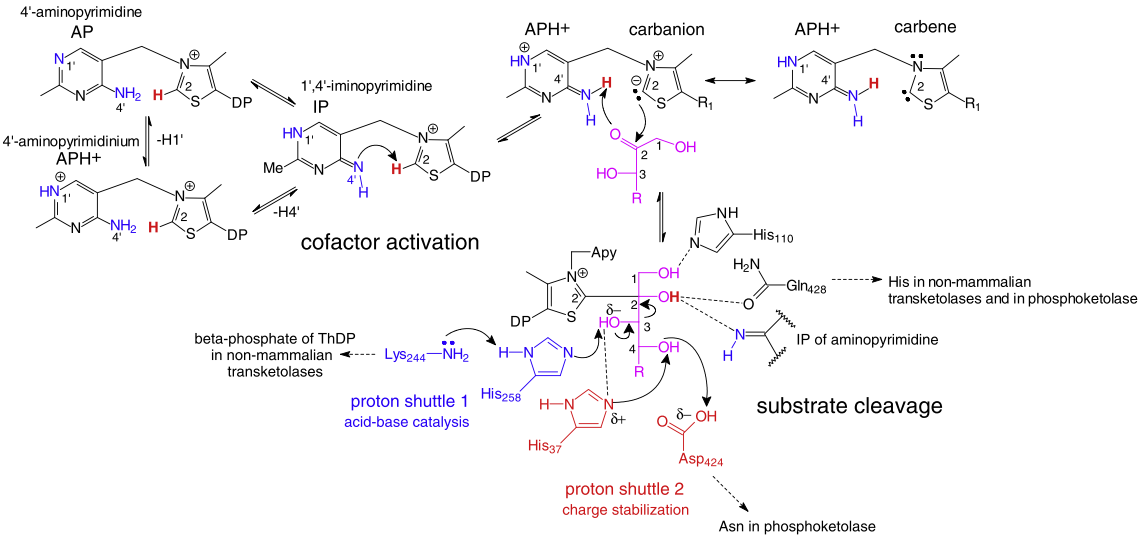
showcases common and specific traits of the different enzymes with immediate implications for the mechanism. The numerous proton transfers occurring along the pathway in both enzyme families (Schemes 2–5) are in most instances catalyzed by a single amino acid residue that alternates between acid and base. The structural, mutagenesis and kinetic studies identified this residue to be a Glu in transaldolase, or a Tyr in FSA and archaeal FBP aldolases, respectively. A notable exception in this context is FBP aldolase from higher eukaryotes, which exploits two acid–base catalysts. Here, a multifunctional Glu catalyzes most of the proton transfers except for the reversible protonation of the C3 enamine–carbanion, which is facilitated by the mobile Tyr of the C-terminus. As for the spatial positions relative to the intermediates, these acid–base catalysts can be grouped into two classes: they are (*i*) either located at the bottom of the active site and co-align with the side chain of the reactive lysine pointing outwards with respect to the substrate channel (Glu in eukaryotic FBP aldolase, Tyr in archaeal FBP aldolase, Glu in transaldolase) or, (*ii*) they are located at the opposite face at the active site pointing to the inside towards the reactive lysine (mobile Tyr in eukaryotic FBP aldolase, Tyr in FSA). In transaldolases, proton transfers are relayed through a catalytic water, which remains bound atop the Schiff bases and seems to be key for the stability of the C3 enamine, probably through hydrogen-bonding to Nε of the lysine. Another intriguing structural observation in this context is that the C3 atom of the enamine in transaldolase (see Fig. 3C) is slightly too far away from the catalytic water (~3.7 Å) to easily accept a proton from the Glu-water dyad. This would explain, as to why the enamine is so stable in transaldolase although the multifunctional Glu is very likely protonated at this stage of the reaction and could potentially serve as a source of protons. As expected, the Glu and Tyr acid–base catalysts in the FBP and F6P aldolases are suitably positioned to contact the substrate/intermediate atoms as required for their function. In all three enzymes, an Asp is found in similar positions at the active site and helps aligning the substrate and intermediates through hydrogen-bonding interactions with the 3-OH and 5-OH groups suggesting that it is ionized. Although it is not directly engaged in acid–base catalysis as previously thought it orients neighboring residues with dedicated catalytic roles. Developing charges at substrate/intermediate atoms are stabilized by properly placing either a positively charged Lys (FBP aldolase) or an Asn (FSA and transaldolase), which seems to function as a hydrogen-bond donor to the O4 atom of the Schiff bases. The structural similarity between FSA and transaldolase is striking, it seems that replacement of just two residues (Glu → Gln, Phe → Tyr) in an otherwise mostly conserved active site transformed transaldolase into an efficient F6P aldolase.

8. Structural basis of transketolase action

Transketolase was the first ThDP-dependent enzyme, the crystal structure of which had been determined [55]. The structure showcased that the enzyme-bound ThDP cofactor adopts, as predicted by biochemical studies using cofactor analogs [23], the canonical *V*-conformation, in which the exocyclic 4′-amino group of the co-catalytic aminopyrimidine heterocycle of ThDP is juxtaposed relative to the reactive C2–H of the thiazolium [56]. It was then shown for transketolase and many other ThDP enzymes that the aminopyrimidine portion acts as a built-in acid–base catalyst at the early stages of the reaction, and likely facilitates (*i*) formation of the carbanion–carbene species by deprotonation of the thiazolium C2 (step 0 in Scheme 3), and (*ii*) eventually returns the abstracted proton to the substrate carbonyl in the course of covalent substrate binding (step1 in Scheme 3) [18,25,28,57]. Spectroscopic analysis of enzyme-bound ThDP indicated that the aminopyrimidine exists in three different forms: (*i*) as the classic amino form, (*ii*) as the 1′,4′-imino tautomer that acts as base, and (*iii*) as the aminopyrimidinium cation as the corresponding conjugate acid of the imino tautomer (Scheme 8) [24,58,59].

Structural analysis of transketolase from various organisms highlighted that the active site is highly conserved containing an Asp and an array of His residues engaged in H-bonding interactions with the substrate OH groups, plus a set of two Arg and a Ser, which bind to the phosphate portion of the substrate [55,60]. In transketolase from mammals, one of the otherwise strictly conserved His residues in the immediate binding vicinity of the substrate carbonyl and of the 4′-amino group of ThDP is replaced by a Gln [9,61].

High-resolution structures of different key intermediates of the pathway including covalent substrate–ThDP conjugates



Scheme 8. Suggested mechanism of cofactor activation and substrate cleavage in transketolase (see text). Numbering refers to human transketolase. Note that a similar mechanism can be envisaged for phosphoketolase except that the Asp belonging to proton shuttle 2 is replaced by an Asn suggesting that the equivalent residue of His37 in phosphoketolase is permanently protonated to stabilize the evolving negative charge at O3 of the substrate-ThDP intermediate during substrate cleavage.

(X5P-ThDP, F6P-ThDP in *E. coli* transketolase, X5P-ThDP, F6P-ThDP, S7P-ThDP in human transketolase) and the central DHE-ThDP enamine (yeast transketolase) have been determined by cryocrystallography [31,62,63]. Structural analysis of the covalent X5P-ThDP conjugate in human transketolase (Fig. 4A) exemplary showcases that the scissile C2–C3 bond of the intermediate is oriented perpendicular relative to the thiazolium ring plane suggesting a "maximum-overlap" mechanism, which appears to be a salient feature of ThDP enzymes [17]. Notably, small deviations from the optimal perpendicular orientation result in greatly diminished bond cleavage rates [64]. The ionizable 3-OH group of the intermediate forms H-bonds with two histidines (His37, His258) and with a glutamine (Gln189, this residue is unique in mammalian transketolases). Both His residues in transketolase are crucially important for catalysis as their individual mutations render the enzyme almost completely inactive [65,66]. It is not fully clear yet how exactly these two residues act in concert to facilitate deprotonation of the substrate 3-OH. It would seem reasonable to assume that one of the two His is not protonated and acts as base, while the other His teams up in protonated form to stabilize, by virtue of its positive charge, the developing negative charge at atom O3 of the intermediate in the course of substrate cleavage. Mutagenesis and kinetic studies on yeast transketolase favored the equivalent residue of His258 to be likely involved in acid–base chemistry as the most deleterious effects on catalysis were observed upon mutation of this particular residue, while the equivalent of His37 was suggested to have roles in substrate binding and catalysis indicated by increased K_M-values for ketose substrates and diminished k_{cat} (not to the extent as observed for the other His) in variants [65,66]. Interestingly, His258 in human transketolase is held in place by Lys224 and suggests the possibility that these two residues form a catalytic dyad as proton shuttle (Scheme 8). A similar role can be envisioned for the beta-phosphate of bound ThDP in case of non-mammalian transketolase as one of the beta-phosphate oxygen atoms is found in almost identical position as the Nε of Lys224 in human transketolase. On the other hand, His37 and the conserved Asp424 both form hydrogen bonds with the 4-OH group of the intermediate. At the very high resolution <1 Å accomplished in case of human TK, the structure suggests Asp424 to be protonated at this catalytic stage (at least not fully ionized) (Fig. 4A). This would in turn imply that His37 is not protonated and acts as hydrogen-bond acceptor. Intriguingly, the conserved Asp not only bears an important function for binding the substrate, it further possesses a catalytic role as substitutions

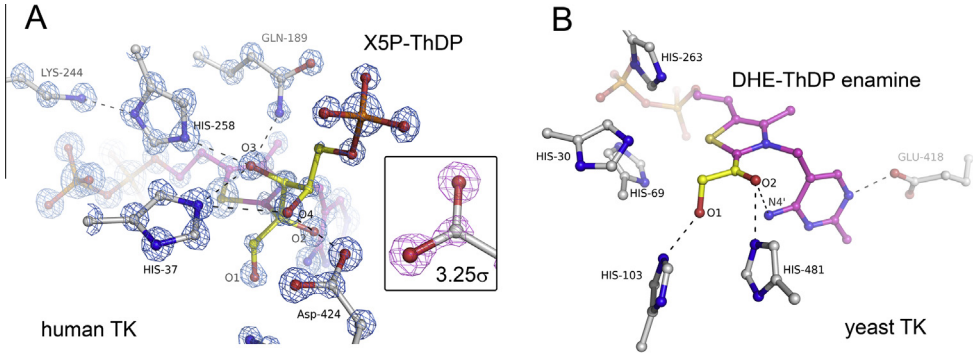


Fig. 4. Snapshots of catalysis in transketolase. (A) Structure of the X5P-ThDP intermediate at the active site of human transketolase showing selected amino acid residues and indicating hydrogen-bonding interactions with atoms O3 and O4 of the intermediate. Note that the electron density map around residue Asp424 is suggesting that it is not (fully) ionized with the upwards pointing oxygen likely being protonated. (B) Structure of the dihydroxyethyl-ThDP enamine in yeast transketolase showing selected amino acid residues and hydrogen-bonding interactions of the intermediate. Note that His481 interacting with O2 of the intermediate is a Gln in mammalian transketolase. The observed conformation of the intermediate renders a hydrogen-bonding interaction between O2 and N4′ unlikely.

of this residue completely abolish enzyme activity [10,67]. A putative, at this point speculative function of the Asp could be to relay a proton through O4 of the intermediate onto His37 in a concerted proton transfer mechanism (Scheme 8). The thereby formed positively charged His37 could then effectively promote the development of negative charge at O3 and as such reduce the barrier for deprotonation of the 3-OH group by the His-Lys (mammalian transketolase) or His-(β)-phosphate (non-mammalian transketolase) catalytic dyads. Despite recent progress in understanding the mechanism of substrate cleavage in transketolase, the details of the mechanism, in particular the seemingly concerted action of two proton relays, remain to be established in future studies. It is highly likely though that the reversible proton transfer onto the substrate carbonyl in transketolase is catalyzed by the aminopyrimidine of ThDP as the 2-OH group of X5P-ThDP is interacting with N4′ of ThDP. Proper alignment of the arriving substrate for covalent binding to occur is ensured by a hydrogen-bonding interaction of the substrate carbonyl with either a His (non-mammalian TK) or a Gln (mammalian TK) residue.

Structural analysis of the dihydroxyethyl-ThDP (DHE-ThDP) enamine–carbanion, which is formed upon substrate cleavage (see Scheme 3) was accomplished for yeast transketolase by using the artificial substrate ß-hydroxypyruvate rather than a ketose phosphate for soaking experiments [62]. Due to quasi-irreversible decarboxylation of the substrate analog on the enzyme, the enamine–carbanion could be accumulated to high occupancy in the crystal. The intermediate exhibits a planar conformation suggesting stabilization of the enamine contributor in *E*-configuration. The 2-OH group forms a hydrogen bond with His481 (this residue is a Gln in mammalian transketolases), while 1-OH engages in a hydrogen bond with His103, which is strictly conserved in all transketolases. Interestingly, the observed conformation of the intermediate suggests that a hydrogen bond interaction between 2-OH and N4′ of the aminopyrimidine is geometrically not very favorable. This could explain as to why the enamine in transketolase is not tautomerizing to the corresponding keto-thiazoline as observed in a related ThDP oxidase, where the aminopyrimidine is thought to facilitate transfer of a proton from 2-OH to C2 of the thiazolium [29]. It seems that a putative tautomerization pathway in transketolase with proton transfer from 2-OH of the enamine to C2 of the thiazolium with N4′ acting as facilitator is blocked due to unfavorable geometry of the transfer pathway.

Besides structural analysis of covalent reaction intermediates, structures of transketolase in non-covalent complex with aldose acceptors E4P (yeast transketolase) and R5P (*E. coli* transketolase) were solved [31,60]. These proved to be very informative for understanding how acceptors are properly aligned for eventual reaction with the enamine intermediate. The mechanistic implications for how ring opening of the predominantly cyclic R5P aldose acceptor is accomplished will be discussed in a separate chapter (see Section 12).

9. Structural basis of phosphoketolase action

The crystal structures of phosphoketolase from different organisms (*Bifidobacterium breve, Bifidobacterium longum*) have been solved only very recently [68,69], although its catalytic activity had been demonstrated as early as in 1950s [12,13]. In addition to the resting state structures, structural analysis further included these of the *B. breve* enzyme (*i*) in complex with the dihydroxyethyl-ThDP (DHE-ThDP) enamine–carbanion intermediate, (*ii*) in complex with acetyl-ThDP (Ac-ThDP) resulting from dehydration of the DHE-ThDP enamine–carbanion, and (*iii*) in noncovalent complex with substrate phosphate [68]. So far, there is no structural information available for a phosphoketolase with a bound covalent substrate–ThDP conjugate (X5P-ThDP, F6P-ThDP) as accomplished in case of transketolase or with product acetyl phosphate.

Modeling of the X5P-ThDP intermediate as observed in transketolase into the active site of phosphoketolase highlights the high degree of structural similarity between the two enzymes (Fig. 5A). Phosphoketolase contains a similar array of conserved His residues (His64, His97, His142, His320, His553), which are all engaged in hydrogen-bonding interactions with the intermediate 1-OH, 2-OH, 3-OH and 4-OH groups. Akin to human transketolase, a presumptive His-Lys catalytic dyad (His320, Lys300) might act as a proton shuttle catalyzing ionization of the substrate 3-OH group (see Scheme 8), although it cannot be ultimately ruled out that His64 accomplishes this reaction. A notable difference between phosphoketolase and transketolase is the replacement of the strictly conserved Asp in transketolase by an Asn (Asn549) in phosphoketolase. While this excludes a potential involvement of this residue in proton transfers as suggested for transketolase, the introduction of the uncharged Asn is seemingly required to allow for facile binding of the negatively charged inorganic phosphate, which enters the catalytic cycle as substrate at a later stage. This would in turn imply that His64 is protonated by itself to effectively stabilize the developing charge at O3 of the intermediate in the course of substrate cleavage initiated by deprotonation of 3-OH by the Lys300-His320 dyad. Another comprehensible difference between the two enzymes concerns a phosphoketolase-specific Tyr (Tyr501), which is involved in binding of inorganic phosphate, while there is a Phe located at almost identical spatial position in transketolase.

The structural analysis of the DHE-ThDP intermediate in phosphoketolase (Fig. 5B) interestingly revealed that the intermediate is not adopting a planar, enamine-type conformation as observed in case of transketolase. Instead, the electron density around the intermediate suggests it to be tetrahedral, i.e. protonated at atom C2. While the observation of the conjugate acid of the enamine–carbanion is at first sight counterintuitive as it is not on the pathway and could reflect a crystallographic 'artifact', a transient protonation might facilitate reactant separation that is departure of the G3P or E4P aldose product formed upon substrate cleavage from the enamine–carbanion. Such a trapping of the carbanion by protonation is considerably effective in ensuring irreversible substrate cleavage by avoiding internal return of the electrophilic aldose as actually required for the mode of action of phosphoketolase. A similar problem has been addressed for ThDP-promoted decarboxylation reactions [17,70,71]. A water molecule (water 2) is sitting atop the DHE-ThDP intermediate and firmly held in place by interactions with His320 and His64. It is not unreasonable to suggest that the enamine is kinetically accessible through action of the His-Lys dyad and the water (deprotonation of C2 of DHE-ThDP) in order to permit dehydration of the 1-OH substrate group.

The 1-OH leaving group of DHE-ThDP is coordinated by H-bonding interactions with His142, the backbone carbonyl of Gly155 and a water molecule (water 1) that itself is further contacting His97 and the backbone carbonyl of Gly154. The structure would suggest that His142 likely acts as the acid that protonates 1-OH of the DHE-ThDP enamine in the course of dehydration as it is suitably positioned, however, mutagenesis and kinetic studies rather favored a mechanism according to which His97 relays a proton through water 1 [68]. Very remarkably, both His residues (His142 and His97) are conserved in phosphoketolases and transketolases, and are located at almost identical positions relative to the common DHE-ThDP intermediate, hence it is not fully clear why phosphoketolase catalyzes a dehydration and transketolase does not.

The structure of the post-dehydration intermediate was obtained by soaking phosphoketolase crystals with substrate F6P in the absence of phosphate thereby stalling the catalytic cycle

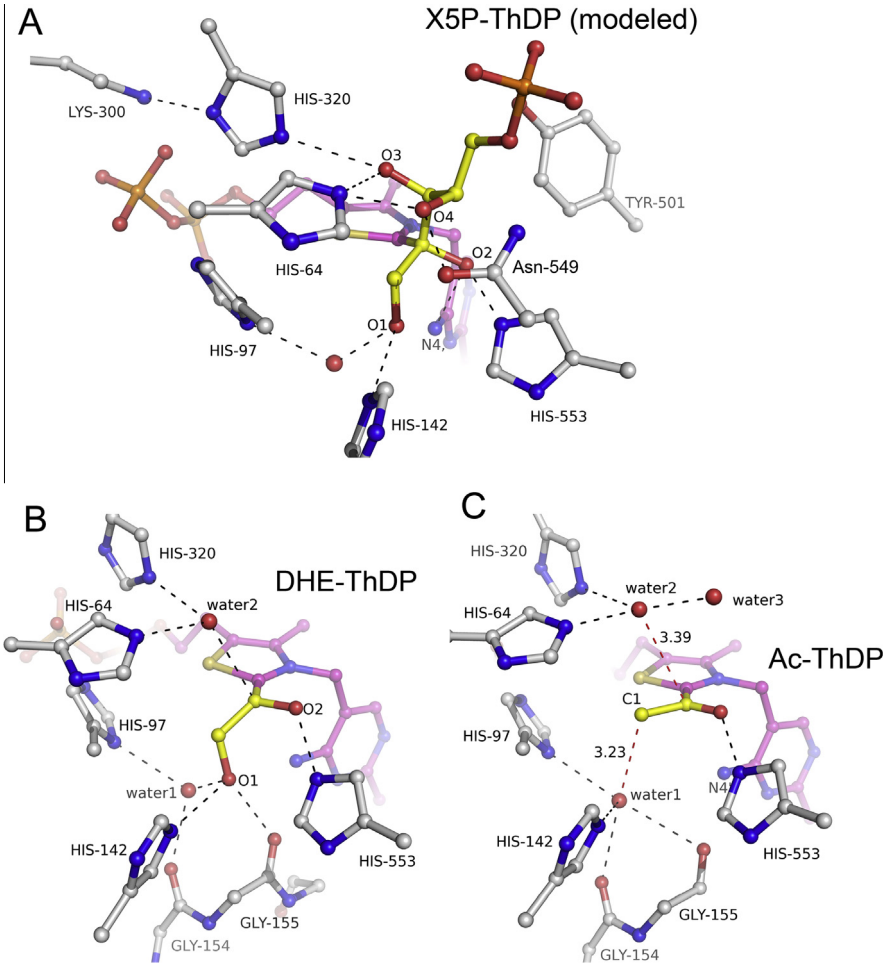


Fig. 5. Snapshots of catalysis in phosphoketolase showing reaction intermediates, selected amino acid residues, solvent molecules and indicating hydrogen-bonding interactions. (A) Structure of the covalent X5P-ThDP adduct modeled into the active site of *B. breve* phosphoketolase. Deprotonation of the intermediate 3-OH as required for substrate cleavage could be either catalyzed by the Lys300-His320 dyad, or, alternatively, by His64. (B) Structural analysis of the DHE-ThDP intermediate trapped in *B. breve* phosphoketolase suggests that either His142 or His97 (through water 1) is acting as an acid catalyzing protonation of O1 in the course of dehydration. (C) Structure of the post-dehydration intermediate suggested to be Ac-ThDP (see text). Note the presence of structurally closely located water molecules, which could be engaged in off-pathway hydrolysis of the intermediate.

right before phosphorolysis (Fig. 5C). The electron density around the intermediate that exhibited a branched triatomic substrate portion was trigonal planar, and the chemical state trapped *in crystallo* was hence assigned to correspond to the Ac-ThDP intermediate. On the other hand, at the obtained resolution of ~1.9 Å it is almost impossible to differentiate between Ac-ThDP and the previously formed enol (see Scheme 5) as both species are at this resolution structurally indistinguishable, so the identity of the intermediate is not clear. The oxygen of the intermediate, which could be either a carbonyl O (Ac-ThDP) or an OH (enol) is contacting residue His553. A single water molecule (water 1) is found to interact with His142, His97 and the backbone carbonyls of Gly154 and Gly155. Remarkably, there are now two water molecules detectable atop the thiazolium (water 2 and 3) rather than just one as observed prior to dehydration (see Fig. 5B). It is currently completely unclear, how tautomerization of the enol to the corresponding keto (Ac-ThDP) is catalyzed by phosphoketolase. Computational studies suggested key roles of His553, His97 and His142 in this process but further experimental studies will be required to gain further insights into the mechanism of keto–enol tautomerism [72]. It is clear though that facile formation of Ac-ThDP in the absence of acyl acceptor phosphate can potentially uncouple the phosphoketolase reaction due to off-pathway hydrolysis as observed in related enzymes [73,74].

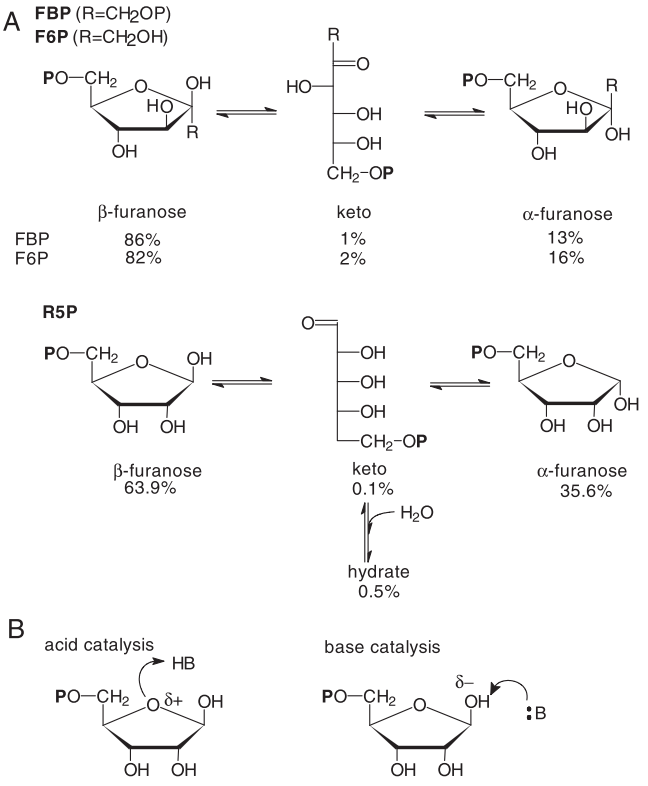
10. Commonalities and differences between transketolase and phosphoketolase

The active sites of transketolase and phosphoketolase share an exceptional degree of structural similarity such that the basis for the different chemical pathways catalyzed by the two enzymes remains in part enigmatic. In both transketolase and phosphoketolase, the cofactor ThDP adopts the canonical *V* conformation and it seems likely that the mechanism of cofactor activation and carbonyl addition of ketose substrates to the cofactor carbanion–carbene is conserved among the two enzymes with a crucial role of the co-catalytic aminopyrimidine heterocycle in proton transfers (deprotonation of C2 of ThDP, subsequent protonation of the substrate carbonyl) (see Scheme 3 and 8). Moreover, substrate cleavage, that is rupture of the C2–C3 bond of the covalent substrate–ThDP conjugates seems to proceed similarly in both enzymes. The scissile C2–C3 bond of the intermediate is oriented perpendicular relative to the thiazolium ring plane, in agreement with the predicted "maximum-overlap" mechanism, according to which the incipient electron pair developing in the transition state of substrate cleavage is effectively delocalized into the thiazolium ring [17,75]. The ionizable 3-OH group of the intermediate hydrogen-bonds to two strictly conserved His residues in each enzyme (His258, His37 in human TK; His320, His64 in *B. breve* PK), which

act in tandem to deprotonate 3-OH of the intermediate. There is no clear evidence yet, which of the two residues catalyzes deprotonation of 3-OH as a bona fide acid–base catalyst, and which one serves to properly align the intermediate and to provide charge stabilization of the evolving negative charge at O3, however, steady-state kinetic analysis of variants from yeast transketolase would favor His258 (TK) and by analogy His320 (PK), respectively, to act as a base [11,65,66]. Remarkably, in both transketolase and phosphoketolase, this "acid–base" His residue is held in place by an interaction with either a Lys or with the beta-phosphate of the cofactor diphosphate portion. This suggests a role of the latter for either structurally aligning the His side chain, or even for acting catalytically as part of a functional proton shuttle dyad with the His residue. The second of the two His residues bearing a crucial role for substrate cleavage (His37 in human TK, His64 in PK) seems to be required for aligning the intermediate and to provide electrostatic stabilization of the developing negative charge at O3, either by itself (PK) or by forming a proton shuttle with a strictly conserved and catalytically important Asp residue (TK) (see Scheme 8). The different chemical fates of the DHE-ThDP enamine–carbanion in the two enzymes cannot be straightforwardly rationalized solely on the basis of structural information. The structural and kinetic data consistently suggest that the enamine is kinetically stabilized on transketolase and is not subject to dehydration as in case of phosphoketolase. Despite the strikingly different reactivities of the DHE-ThDP enamine–carbanion, the micro-binding pocket of the intermediate 1-OH leaving group and the amino acid residues contacting it are highly similar although not completely identical in both enzymes. Subtle differences in the positions of residues His142, water 1 and the coordinating backbone were detected and these could potentially account for optimized proton transfer onto 1-OH [68], although different pK_a values of the involved His residues could also play a major role due to slightly different microenvironments. The structure of the DHE-ThDP intermediate trapped in PK along with kinetic and mutagenesis data suggested that either His142 or His 97 (through water 1) protonate 1-OH in the course of dehydration. The mechanism of the subsequently occuring enol → keto tautomerization of the formed enol to give Ac-ThDP remains completely enigmatic. His553, His97 and His142 are potential candidates to facilitate tautomerization, however, our current understanding of the PK mechanism is far from being as good as in case of transketolase. From a standpoint of efficiency of throughput, chemical coupling of tautomerization and acyl transfer to phosphate while at the same time avoiding off-pathway hydrolysis of Ac-ThDP, one could envisage a scenario in which binding of substrate phosphate triggers tautomerization and as such ensures generation of a highly reactive keto species only once the acyl acceptor has arrived at the reaction site.

11. Structural basis of ring-opening reactions

Many of the physiological ketose and aldose substrates the title enzymes of this review are acting on, including FBP, F6P, S7P and R5P, are predominantly occurring in a ring-cyclic form under physiological conditions (Scheme 9) [76]. A notable exception in this regard is X5P along with the short-chain sugars E4P, G3P, DHAP and DHA, all of which exclusively exist in acyclic forms. Since the prevalent cyclic forms of the sugars mentioned above are catalytically not competent as opposed to their acyclic but only marginally accumulated keto counterparts, it is questionable whether enzymes specifically select for and bind the reactive ring-open forms, or whether they carry out acid–base catalysis at the active site to promote ring-opening after binding of the cyclic forms. As the proportion of the reactive keto forms is considerably small accounting for just ⩽2% at equilibrium in all cases (FBP: 1%, F6P: 2%, R5P 0.1%, see Scheme 9A) [76] compared to the preponderant



Scheme 9. (A) Equilibrium compositions of FBP, F6P and R5P. (B) Mechanisms of acid-catalyzed and base-catalyzed ring-opening of the β-furanose form of R5P. Acid and base catalysis are likely to occur concertedly in enzymic ring-opening reactions.

α- and β-anomeric furanose forms, it seems more likely that the enzymes bind the cyclic forms, either promiscuously both anomers, or, alternatively, specifically one of those, and catalyze a ring-opening of these *in situ*. In fact, pre-steady state kinetics evidenced in case of FBP aldolase that there is a catalytic impact of the enzyme on ring-opening of substrate FBP [77]. Earlier work devoted to the analysis of anomer specificity of FBP aldolase highlighted that the muscle and liver enzyme acts on the ß-anomer, while the yeast enzyme seems to utilize both anomers of the substrate [78–80]. The anomeric specificities of the other enzymes (FSA, transaldolase, transketolase, phosphoketolase) for substrate F6P remain to be examined in detail. The means by which ring-opening could potentially be accomplished at the active site is acid–base catalysis, more specifically, protonation of the furanose ring O (acid catalysis) and/or deprotonation of the anomeric OH group (base catalysis) (Scheme 9B). Notably, ring-opening of phosphorylated sugars proceeds substantially faster when compared to corresponding non-phosphorylated forms. Kinetic analysis of non-enzymic anomerization reactions support a mechanism, where the phosphate portion(s) of the phosphosugars acts as an intramolecular acid–base catalyst [76,81].

Structural analysis of FBP aldolase co-crystallized with FBP revealed that the acyclic, linear substrate form was stabilized on the enzyme supporting the notion of enzyme-promoted ring-opening as proof-of-principle [36,37]. A similar observation was made in case of transaldolase, where a variant was studied, in which the catalytic Lys had been replaced by a Met [53]. Soaking of the obtained protein crystals with substrate F6P allowed structural characterization of the enzyme:substrate Michaelis complex. Here, too, the linear form of F6P was found to be accumulated at the active site demonstrating that there is no absolute requirement for the catalytic Lys to afford the reactive keto form of F6P, at least not in transaldolase.

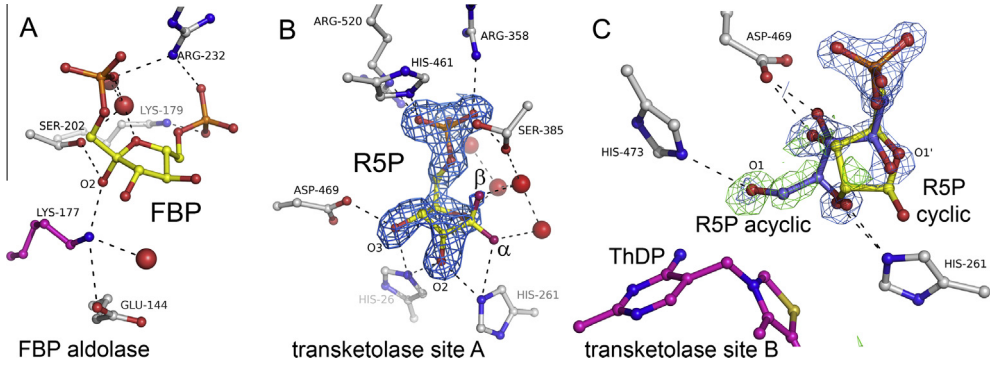


Fig. 6. Snapshots of ring-opening reactions in FBP aldolase and transketolase. (A) Structure of the β-anomer of FBP trapped at the active site of *Thermoproteus tenax* FBP aldolase variant Tyr146Phe/ Trp144Glu showing selected amino acid residues, solvent molecules and H-bonding interactions. (B) Structure of acceptor aldose R5P bound at active site A of *E. coli* transketolase showing selected amino acid residues, solvent molecules and H-bonding interactions. The electron density around R5P is contoured at 1σ. (C) Structure of acceptor aldose R5P bound at active site B of *E. coli* transketolase showing selected amino acid residues, solvent molecules and H-bonding interactions. The electron density maps around R5P are contoured at 1σ (blue) and 3σ (green, difference map), respectively. Note that ring-opening of R5P is accompanied by a flip-over of the formed 1-aldo function.

Important molecular insights into the mechanism of ring-opening of FBP were obtained by structural analysis of a FBP aldolase double variant from *Thermoproteus tenax*, in which the (*i*) "omnifunctional" Tyr acid–base catalyst had been replaced by a Phe, and (*ii*) a Trp in direct vicinity of the reactive Lys was exchanged for a Glu [45]. Soaking of crystals of the double variant with FBP allowed for the first time the structural observation of a ring-cyclic form of FBP (Fig. 6A). The electron density maps were suggesting accumulation of the β-anomer of FBP, of which the anomeric OH group was H-bonding to the catalytic Lys177 and Ser202. The furanose ring oxygen contacts a water molecule that itself is interacting with the C1-phosphate portion of FBP. It was suggested that the catalytic Lys could be a key player of ring-opening in FBP aldolase as its detected engagement in a salt bridge with the introduced Glu144 might have shifted its pK_a in the variant and thus impaired its function as a base for deprotonation of the anomeric 2-OH group. The absence of a suitably placed enzyme-provided acid for protonation of the furanose ring oxygen indicates that the FBP C1 phosphate is likely promoting this reaction through an interjacent water in terms of "substrate-assisted catalysis".

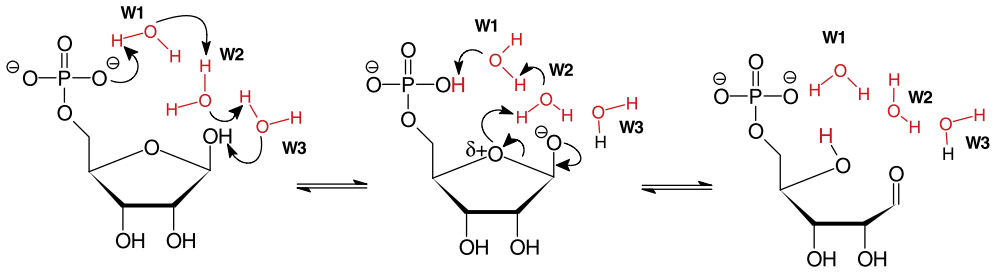
Structural analysis of *E. coli* transketolase in complex with R5P revealed interesting insights into the ring-opening mechanism of this aldose acceptor substrate in the wild-type enzyme [31]. Slightly different binding modes of R5P were detected in the two subunits belonging to the asymmetric unit. In one active site (Fig. 6B), the electron density was clearly suggesting accumulation of cyclic substrate, however, the density around the anomeric center was very diffuse suggesting that transketolase either binds the β- and the α-anomeric form equally well, or, alternatively, that it binds only one anomer but catalyzes an anomerization *in situ*. Very remarkably, there is no suitably positioned enzyme side chain that could easily serve to protonate the furanose ring O or to deprotonate the anomeric OH. His261, which H-bonds to the 2-OH group of R5P might also contact the 1-OH group of the α-anomer of R5P, but the observed angle is not ideal for a proton transfer. In the absence of an amino acid catalyst, how could ring-opening of R5P be achieved on the enzyme? Closer inspection of the binding cavity of R5P suggests that the phosphate portion of the substrate itself is likely involved in ring-opening as it is linked via a chain of water molecules with both the ring O and the anomeric OH group. The phosphate moiety could either act as an acid and/or base and relay proton transfers through the chain of water molecules in a concerted mechanism (Scheme 10). In the second active site (Fig. 6C), the electron density maps were suggesting that R5P has undergone partial ring-opening on the enzyme. A key interaction likely to stabilize the acyclic aldo form is a H-bond between His473 and the C1 aldo function of R5P. Upon ring-opening, the formed C1-aldo function flips from its position close to His261 to the opposite face relative to the neighboring 2-OH group and now contacts the side chain of His473. It was proposed that stabilization of this "near-attack conformer" of R5P could be catalytically important as the effective concentration of the reactive acyclic species is substantially raised at the site of action [82]. Its position would be perfectly suited to attack the DHE-ThDP enamine.

Taken together, the structural snapshots of cyclic phosphosugar substrates trapped in different enzymes (see above) suggest that the substrate phosphate moiety plays an important role in the ring-opening reaction, presumably serving as a bona fide acid–base catalyst in conjunction with solvent molecules that are acting as mediators for proton transfers in terms of "substrate-assisted catalysis" [83]. Whether a functional role of the catalytic Lys in ring-opening reactions in class I aldolases as suggested for FBP aldolase is a canonical feature of this enzyme family remains enigmatic [45]. The studies on transaldolase are clearly showing that ring opening and stabilization of the linear keto form of F6P can be accomplished even in the absence of the catalytic Lys [53]. In addition to the employed acid–base chemistry, a stabilization of the linear forms on the enzyme seems to result from exquisite interactions of the protein with the acyclic species as showcased for transketolase and formation of a near-attack conformer of R5P [31].

12. Observation of physically distorted and high-energy intermediate conformations in transaldolase and transketolase

While the main focus in the previous chapters was devoted to the identification of functional groups involved in elementary steps of catalysis with an emphasis on proton transfers, the stereochemical course of substrate processing and how the structures can be used to explain the different reaction specificities in the aldolase–transaldolase and transketolase–phosphoketolase families, we will now discuss fine-structural details of covalent reaction intermediates trapped at very high resolution, which proved to be very informative regarding more general principles of enzyme catalysis extending beyond specific aspects of the enzymes covered in this review.

Structural analysis of the genuine F6P and S7P Schiff-base intermediates in transaldolase from *T. acidophilum* at a resolution of



Scheme 10. Suggested ring-opening mechanism of acceptor aldose R5P at the active site of *E. coli* transketolase. The structure suggests "substrate-assisted catalysis" that is involvement of the phosphate moiety of R5P itself and several mediating water molecules in the process. Ring-opening can either occur stepwise or in concerted fashion.

around 1.6 Å suggested a slightly twisted (10–20° in the different subunits) Schiff-base double-bond linkage between Nε of the catalytic Lys and C2 of the substrate, that is the trigonal substituent planes around the two atoms are not co-planar as expected based on chemical rationale [50]. Although the extent of the twisting around the double bond cannot be determined with high accuracy at the given resolution, the calculated unbiased simulated-annealing electron density maps favored a twisted over a co-planar conformation based on R-factors and map correlation. In support of the proposed high-energy state of covalent Schiff-base conjugates is a recently published high-resolution structure (1.5 Å) of a transaldolase intermediate complex, where inhibitor arabinose-5-phosphate was found to be in Schiff-base linkage with the catalytic Lys [84]. Here, too, the substituent orientation around the Nε–C Schiff-base linkage is not coplanar but rather twisted by about 15° (Fig. 7A). Whether, and if so, to which extent the twisted conformation of the Schiff-base intermediates is catalytically productive in transaldolase remains to be studied in the future. It seems logical though that preservation of energy along the pathway i.e. generation of intermediates in hyper-reactive conformations is beneficial for efficient overall throughput in multi-step enzymatic reactions as the energy required to arrive at the next transition state is reduced [85].

The structures of the covalent X5P-ThDP, F6P-ThDP and S7P-ThDP intermediates in human transketolase could be determined at true atomic resolution (⩽1 Å) in each case, and thus permitted the calculation of the atomic positions of the intermediates with very high accuracy [63]. The covalent intermediates exhibit a remarkable out-of-plane distortion of over 20° for the bond linking sp^2-hybridized atom C2 of the aromatic thiazolium heterocycle of ThDP and atom C2x of the sugar substrate (Fig. 7B). In addition, the analysis of bond lengths of the intermediate revealed a specific bond elongation of the scissile substrate C2–C3 bond (1.61 ± 0.01 Å), while the other C–C bonds of the intermediate

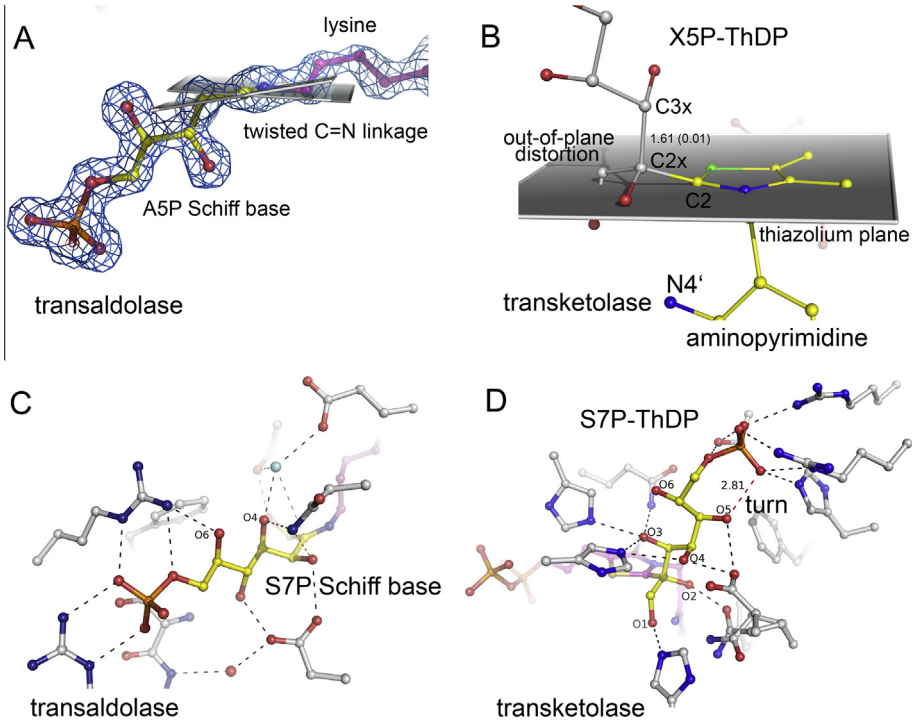


Fig. 7. High-energy conformations of reaction intermediates in transaldolase and transketolase catalysis (see text). (A) Structure of a Schiff-base intermediate in transaldolase formed between the catalytic lysine and inhibitor arabinose-5-phosphate with corresponding electron density map contoured at 1.5σ. Note the ~15° twisted substitutent arrangement around the C–N double bond linkage indicated by the individual trigonal planes around atom Nε and around the substrate carbon in linkage with Nε. (B) Structure of the covalent X5P-ThDP intermediate in human transketolase determined at a resolution of 0.97 Å. Note the out-of-plane distortion of the C2–C2x bond relative to the ring plane of the aromatic thiazolium heterocycle and the elongated scissile C2x–C3x bond of the substrate. (C) Structure of the S7P Schiff base in transaldolase. Note the co-parallel orientation of the 4-OH and 6-OH groups, which is not observed in alditol models. (D) Structure of the covalent S7P-ThDP intermediate bound to human transketolase. Note the local turn structure of S7P from 5-OH until the phosphate portion.

exhibited the expected normal bond lengths of around 1.51–1.53 Å. The observed bond lengthening of 0.1 Å corresponds to an increase in energy of the system as much as 30 kcal/mol [86], and showcases impressively that enzymes are able to channel substrate binding energy into the stabilization of intermediate conformations, in which the bond to be cleaved is selectively destabilized. The structures of human transketolase with authentic covalent intermediates and a chemically synthesized reactant-state analog suggested that the exocyclic 4′-amino group of the co-catalytic ThDP aminopyrimidine acts as a steric stressor that prevents full in-plane relaxation of the cofactor-substrate linkage. This highlights the synergistic opportunities that potentially reside in enzymatic cofactor catalysis as the cofactor 4′-amino group is not only a stressor but moreover an acid–base catalyst at various stages of the reaction. It is interesting to note that strain has been also suggested to contribute productively to catalysis in PLP enzymes and in the cofactor-free orotidylate-5-monophosphate decarboxylase [87,88].

Apart from the aforementioned physical distortions and strain, bond twists and bond elongations in covalent intermediates, a more general structural analysis of the adopted conformation of the sugar skeleton indicates in some instances generation of high-energy conformers. In case of the S7P Schiff-base conjugate bound to transaldolase, the 4-OH and 6-OH groups of the intermediate are oriented in co-parallel fashion (Fig. 7C). Such a conformation with a parallel orientation of C_n, C_{n+2} OH groups is essentially not detectable in alditol models due to intramolecular repulsion between the two groups [89]. Likewise, a rather unusual turn structure is observed for the S7P-ThDP conjugate in human transketolase encompassing 5-OH until the phosphate moiety, which suggests a high-energy situation, too (Fig. 7D). As these higher-energy conformations are particularly observed with the "long-chain" ketose S7P, one might speculate that the enzymes counterbalance the potentially increased binding energy of S7P due to the additional OH groups (F6P and X5P have less OH groups and consequently less productive interactions), avoiding overly-stable bound substrates/intermediates.

13. Emerging themes and outlook

In recent years, substantial progress has been made in our understanding of the aldolase, transaldolase, transketolase and phosphoketolase mechanisms. Structural analysis of the enzymes in the resting state and in complex with key intermediates conjointly with mutagenesis and kinetic data have unraveled exciting molecular details of these complex multi-step reactions, in some instances even fundamental principles of catalysis could be disclosed.

Despite major differences of the class I aldolase and ThDP enzyme superfamilies in terms of the underlying chemistry, overall scaffold and active site structure, the basic design principles of these enzyme catalysts are remarkably conserved. Both enzyme families exploit a "hybrid nucleophilic–electrophilic catalyst", which is either an active center Lys residue that interconverts between the nucleophilic amine and the electrophilic iminium Schiff base, or bioorganic cofactor ThDP, the reactive thiazolium heterocycle of which bears both a nucleophilic and an electrophilic center. Both families use a dedicated set of suitably positioned and pK_a-tuned acid–base catalysts for the numerous proton transfers along the pathway. While a Glu or a Tyr have evolved as multifunctional residues in class I aldolases and act alternately as acid and base in subsequently occuring proton transfers at different substrate positions, transketolase and phosphoketolase employ a set of strictly conserved His residues and the ThDP-supplied aminopyrimidine catalyst for the analogous protonation–deprotonation reactions. It is interesting to note here that the Glu/Tyr acid–base catalysts in (trans-)aldolases switch between a neutral and a negatively charged state, while the His in transketolase–phosphoketolase interchanges between being positively charged and neutral. The reason for why nature has dismissed His as a potential acid–base catalyst in class I aldolases is not obvious. It could be that a positively charged His residue is not as suited as a Glu or Tyr for the aldolase-common dehydration with a developing positive charge at the leaving group water.

Efficient electrostatic catalysis that is stabilization of developing charges in the different transition states [90] is accomplished by properly placed and charged residues such as Lys (aldolase) or His (transketolase, phosphoketolase), or by establishing H-bonding interactions with Asn side chains. In much broader context, the structural studies on the title enzymes nicely illustrate the synergistic concurrence of orbital steering, maximized overlap of orbitals, angular and bond strain, substrate-assisted catalysis and stabilization of near-attack conformers in addition to nucleophilic, electrophilic, electrostatic and acid–base catalysis as hallmarks of enzyme function [82,83,91–94].

Despite the enormous leaps forward in our understanding of the reaction mechanisms of the title enzymes made over the last years, there remain a lot of questions. Why are subsequent proton transfers carried out by a single omnifunctional residue in class I aldolase, while ThDP enzymes are seemingly using two independent sites? Why exactly is the central enamine in transaldolase and transketolase so amazingly stabilized on the enzyme compared to aldolases and phosphoketolase, respectively? Why is transketolase acting on ketose substrates X5P, F6P and S7P, while transaldolase just accepts F6P and S7P but not X5P? Is it a universal principle in these enzymes that the phosphate portion of the cyclic ketose and aldose substrates is engaged in ring-opening reactions (substrate-assisted catalysis)? Do the observed distortions, bond twists and elongations, the stabilization of high-energy conformers and of near-attack conformers contribute to catalysis and if so, to which extent? How does phosphoketolase catalyze keto–enol tautomerization while at the same time avoiding hydrolysis of the formed highly reactive acyl species? What is the role of water molecules in catalysis such as in transaldolase or for ring-opening of cyclic sugar substrates? Is there a link between chemical catalysis and the detected conformational flexibility of the enzyme catalysts [95–98]? The future answers to these question not only promise to provide fascinating novel insights into the complex enzymes' workings but also to inform strategies for the application and de novo design of these enzymes in biocatalysis as e.g. for C–C bond ligation reactions [99–103].

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References

[1] E. Grazi, B.L. Horecker, T. Cheng, Biochem. Biophys. Res. Commun. 7 (3) (1962) 250–253.
[2] J. Sygusch, D. Beaudry, M. Allaire, Proc. Natl. Acad. Sci. USA 84 (22) (1987) 7846–7850.
[3] J.J. Marsh, H.G. Lebherz, Trends Biochem. Sci. 17 (3) (1992) 110–113.
[4] S.J. Cooper et al., Structure 4 (11) (1996) 1303–1315.
[5] M. Schurmann, G.A. Sprenger, J. Biol. Chem. 276 (14) (2001) 11055–11061.
[6] B.L. Horecker, P.Z. Smyrniotis, J. Am. Chem. Soc. 75 (8) (1953) 2021–2022.
[7] B.L. Horecker, T. Cheng, S. Pontremoli, C. Ricci, Proc. Natl. Acad. Sci. USA 47 (12) (1961) 1949–1955.
[8] A.K. Samland, G.A. Sprenger, Int. J. Biochem. Cell Biol. 41 (7) (2009) 1482–1494.

[9] G. Schenk, R.G. Duggleby, P.F. Nixon, Int. J. Biochem. Cell Biol. 30 (12) (1998) 1297–1318.
[10] G. Schneider, Y. Lindqvist, Biochim. Biophys. Acta – Protein Struct. Mol. Enzymol. 1385 (2) (1998) 387–398.
[11] K. Tittmann, G. Wille, J. Mol. Catal. B – Enzymatic 61 (1–2) (2009) 93–99.
[12] E.C. Heath, J. Hurwitz, B.L. Horecker, A. Ginsburg, J. Biol. Chem. 231 (2) (1958) 1009–1029.
[13] M. Schramm, V. Klybas, E. Racker, J. Biol. Chem. 233 (6) (1958) 1283–1288.
[14] E. Grazi, O. Tchola, P.T. Rowley, B.L. Horecker, T. Cheng, Biochem. Biophys. Res. Commun. 9 (1–2) (1962) 38–43.
[15] J.C. Speck, P.T. Rowley, B.L. Horecker, J. Am. Chem. Soc. 85 (7) (1963) 1012–1013.
[16] R. Breslow, J. Am. Chem. Soc. 79 (7) (1957) 1762–1763.
[17] R. Kluger, K. Tittmann, Chem. Rev. 108 (6) (2008) 1797–1833.
[18] D. Kern et al., Science 275 (5296) (1997) 67–70.
[19] G. Hübner et al., Biochim. Biophys. Acta – Protein Struct. Mol. Enzymol. 1385 (2) (1998) 221–228.
[20] A. Baakrishnan, S. Paramasivam, S. Chakraborty, T. Polenova, F. Jordan, J. Am. Chem. Soc. 134 (1) (2012) 665–672.
[21] D. Meyer, P. Neumann, R. Ficner, K. Tittmann, Nat. Chem. Biol. 9 (8) (2013) 488–490.
[22] M.W. Washabaugh, W.P. Jencks, Biochemistry 27 (14) (1988) 5044–5053.
[23] A. Schellenberger, Biochim. Biophys. Acta – Protein Struct. Mol. Enzymol. 1385 (2) (1998) 177–186.
[24] N.S. Nemeria, S. Chakraborty, A. Balakrishnan, F. Jordan, FEBS J. 276 (9) (2009) 2432–2446.
[25] G. Wille et al., Nat. Chem. Biol. 2 (6) (2006) 324–328.
[26] R. Breslow, J. Am. Chem. Soc. 80 (14) (1958) 3719–3726.
[27] M. St-Jean, J. Sygusch, J. Biol. Chem. 282 (42) (2007) 31028–31037.
[28] K. Tittmann et al., Biochemistry 42 (26) (2003) 7885–7891.
[29] D. Meyer et al., Proc. Natl. Acad. Sci. USA 109 (27) (2012) 10867–10872.
[30] A. Berkessel, S. Elfert, K. Etzenbach-Effers, J.H. Teles, Angew. Chem. – Int. Ed. 49 (39) (2010) 7120–7124.
[31] P. Asztalos et al., Biochemistry 46 (43) (2007) 12037–12052.
[32] A. Yevenes, P.A. Frey, Bioorg. Chem. 36 (1–3) (2008) 121–127.
[33] J.J. Mieyal, G. Bantle, R.G. Votaw, I.A. Rosner, H.Z. Sable, J. Biol. Chem. 246 (17) (1971) 5213–5219.
[34] E.R. Drechsler, P.D. Boyer, A.G. Kowalsky, J. Biol. Chem. 234 (10) (1959) 2627–2634.
[35] E. Hannappel, J.S. Macgregor, S. Davoust, B.L. Horecker, Arch. Biochem. Biophys. 214 (1) (1982) 293–298.
[36] K.H. Choi et al., Biochemistry 38 (39) (1999) 12655–12664.
[37] A. Dalby, Z. Dauter, J.A. Littlechild, Protein Sci. 8 (2) (1999) 291–297.
[38] K.H. Choi, J. Shi, C.E. Hopkins, D.R. Tolan, K.N. Allen, Biochemistry 40 (46) (2001) 13868–13875.
[39] A. Maurady, A. Zdanov, D. de Moissac, D. Beaudry, J. Sygusch, J. Biol. Chem. 277 (11) (2002) 9474–9483.
[40] M. St-Jean, J. Lafrance-Vanasse, B. Liotard, J. Sygusch, J. Biol. Chem. 280 (29) (2005) 27262–27270.
[41] H.E. Zimmerman, M.D. Traxler, J. Am. Chem. Soc. 79 (8) (1957) 1920–1923.
[42] J.E. Dubois, J.F. Fort, Tetrahedron 28 (6) (1972) 1665–1675.
[43] J.E. Dubois, J.F. Fort, Tetrahedron 28 (6) (1972) 1653–1663.
[44] E. Lorentzen et al., J. Biol. Chem. 278 (47) (2003) 47253–47260.
[45] E. Lorentzen, B. Siebers, R. Hensel, E. Pohl, Biochemistry 44 (11) (2005) 4222–4229.
[46] J. Du, R.F. Say, W. Lu, G. Fuchs, O. Einsle, Nature 478 (7370) (2011) 534–537.
[47] J. Allard, P. Grochulski, J. Sygusch, Proc. Natl. Acad. Sci. USA 98 (7) (2001) 3679–3684.
[48] A. Heine et al., Science 294 (5541) (2001) 369–374.
[49] S. Thorell, M. Schurmann, G.A. Sprenger, G. Schneider, J. Mol. Biol. 319 (1) (2002) 161–171.
[50] A. Lehwess-Litzmann et al., Nat. Chem. Biol. 7 (10) (2011) 678–684.
[51] J. Jia et al., Structure 4 (6) (1996) 715–724.
[52] J. Jia, U. Schorken, Y. Lindqvist, G.A. Sprenger, G. Schneider, Protein Sci. 6 (1) (1997) 119–124.
[53] S.H. Light, G. Minasov, M.E. Duban, W.F. Anderson, Acta Crystall. Sect. D – Biol. Crystall. 70 (2014) 544–552.
[54] S. Schneider, T. Sandalova, G. Schneider, G.A. Sprenger, A.K. Samland, J. Biol. Chem. 283 (44) (2008) 30064–30072.
[55] Y. Lindqvist, G. Schneider, U. Ermler, M. Sundstrom, EMBO J. 11 (7) (1992) 2373–2379.
[56] Y.A. Muller et al., Structure 1 (2) (1993) 95–103.
[57] C. Wikner et al., J. Biol. Chem. 269 (51) (1994) 32144–32150.
[58] N. Nemeria et al., Biochemistry 43 (21) (2004) 6565–6575.
[59] N. Nemeria et al., Proc. Natl. Acad. Sci. USA 104 (1) (2007) 78–82.
[60] U. Nilsson, L. Meshalkina, Y. Lindqvist, G. Schneider, J. Biol. Chem. 272 (3) (1997) 1864–1869.
[61] L. Mitschke et al., J. Biol. Chem. 285 (41) (2010) 31559–31570.
[62] E. Fiedler et al., Proc. Natl. Acad. Sci. USA 99 (2) (2002) 591–595.
[63] S. Lüdtke et al., Nat. Chem. 5 (9) (2013) 762–767.
[64] D. Meyer et al., Biochemistry 49 (37) (2010) 8197–8212.
[65] C. Wikner et al., Biochemistry 36 (50) (1997) 15643–15649.
[66] E. Fiedler et al., J. Biol. Chem. 276 (19) (2001) 16051–16058.
[67] U. Nilsson, L. Hecquet, T. Gefflaut, C. Guerard, G. Schneider, FEBS Lett. 424 (1–2) (1998) 49–52.
[68] R. Suzuki et al., J. Biol. Chem. 285 (44) (2010) 34279–34287.
[69] K. Takahashi et al., FEBS Lett. 584 (18) (2010) 3855–3861.
[70] GW. Howe, M. Bielecki, R. Kluger, J. Am. Chem. Soc. 134 (51) (2012) 20621–20623.
[71] R. Kluger, G.W. Howe, S.O.C. Mundle, Adv. Phys. Org. Chem. 47 (47) (2013) 85–128.
[72] J. Zhang, Y.J. Liu, Comput. Theor. Chem. 1025 (2013) 1–7.
[73] K. Tittmann, R. Golbik, S. Ghisla, G. Hubner, Biochemistry 39 (35) (2000) 10747–10754.
[74] K. Tittmann et al., Biochemistry 44 (40) (2005) 13291–13303.
[75] A. Turano et al., J. Am. Chem. Soc. 104 (11) (1982) 3089–3095.
[76] J. Pierce, A.S. Serianni, R. Barker, J. Am. Chem. Soc. 107 (8) (1985) 2448–2456.
[77] K.H. Choi, D.R. Tolan, J. Am. Chem. Soc. 126 (11) (2004) 3402–3403.
[78] K.J. Schray, R. Fishbein, W.P. Bullard, S.J. Benkovic, J. Biol. Chem. 250 (13) (1975) 4883–4887.
[79] K.J. Schray, S.J. Benkovic, Acc. Chem. Res. 11 (4) (1978) 136–141.
[80] K.J. Schray, E.E. Howell, J.M. Waud, S.J. Benkovic, B.A. Cunningham, Biochemistry 19 (12) (1980) 2593–2597.
[81] A.S. Serianni, J. Pierce, S.G. Huang, R. Barker, J. Am. Chem. Soc. 104 (15) (1982) 4037–4044.
[82] S. Hur, T.C. Bruice, Proc. Natl. Acad. Sci. USA 100 (21) (2003) 12015–12020.
[83] W. Dall'Acqua, P. Carter, Protein Sci. 9 (1) (2000) 1–9.
[84] S.H. Light, W.F. Anderson, J. Struct. Funct. Genom. 15 (1) (2014) 41–44.
[85] W.J. Albery, J.R. Knowles, Angew. Chem. – Int. Ed. English 16 (5) (1977) 285–293.
[86] A.A. Zavitsas, J. Phys. Chem. A 107 (6) (2003) 897–898.
[87] M. Fujihashi, K. Mito, E.F. Pai, K. Miki, J. Biol. Chem. 288 (13) (2013) 9011–9016.
[88] D. Milic et al., J. Am. Chem. Soc. 133 (41) (2011) 16468–16476.
[89] S.J. Angyal, R. Lefur, D. Gagnaire, Carbohydr. Res. 23 (1) (1972) 121–134.
[90] A. Warshel et al., Chem. Rev. 106 (8) (2006) 3210–3235.
[91] J.R. Knowles, Nature 350 (6314) (1991) 121–124.
[92] A.D. Mesecar, B.L. Stoddard, D.E. Koshland, Science 277 (5323) (1997) 202–206.
[93] T.C. Bruice, S.J. Benkovic, Biochemistry 39 (21) (2000) 6267–6274.
[94] S.J. Benkovic, S. Hammes-Schiffer, Science 301 (5637) (2003) 1196–1202.
[95] K.A. Henzler-Wildman et al., Nature 450 (7171) (2007) 913–916.
[96] E.Z. Eisenmesser et al., Nature 438 (7064) (2005) 117–121.
[97] G.G. Hammes, S.J. Benkovic, S. Hammes-Schiffer, Biochemistry 50 (48) (2011) 10422–10430.
[98] G. Bhabha et al., Science 332 (6026) (2011) 234–238.
[99] J.A. Castillo et al., Org. Lett. 8 (26) (2006) 6067–6070.
[100] S. Schneider et al., ChemBioChem 11 (5) (2010) 681–690.
[101] M.M. Muller, M.A. Windsor, W.C. Pomerantz, S.H. Gellman, D. Hilvert, Angew. Chem. – Int. Ed. 48 (5) (2009) 922–925.
[102] L. Giger et al., Nat. Chem. Biol. 9 (8) (2013) 494–498.
[103] M. Müller, G.A. Sprenger, M. Pohl, Curr. Opin. Chem. Biol. 17 (2) (2013) 261–270.