UMPOLUNG IN REACTIONS CATALYZED BY THIAMINE PYROPHOSPHATE DEPENDENT ENZYMES

Umpolung en reacciones catalizadas por enzimas dependientes de pirofosfato de tiamina

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Abstract

The temporal exchange of the electrophilic/nucleophilic character of an atom by chemical manipulation is known in organic chemistry as umpolung. This inversion of polarity allows the exploration of new synthetic possibilities which cannot be achieved from the normal reactivity of functional groups. It is not only a useful synthetic tool, but it is also utilized by certain enzymes during biochemical reactions in living organisms. Thiamine pyrophosphate dependent enzymes, such as pyruvate decarboxylase, pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, branched-chain α-keto acids dehydrogenase and transketolase, provide clear examples of umpolung in cellular reactions that fulfill different purposes. In this review, after a discussion regarding the meaning of the term umpolung found in the chemical literature, the reaction mechanisms and the biochemical meaning of transformations carried out by the aforementioned enzymes are analyzed.

Keywords: dehydrogenase; decarboxylase; thiamine pyrophosphate; transketolase; umpolung.

Resumen

El intercambio temporal del carácter electrofilico/nucleofílico de un átomo mediante manipulación química, es conocido con el vocablo alemán de umpolung. Esta inversión de polaridad permite explorar nuevas posibilidades sintéticas que no pueden llevarse a cabo partiendo de la reactividad normal de los grupos funcionales. Se trata de una herramienta sintética útil, que también es utilizada por ciertas enzimas durante reacciones bioquímicas que tienen lugar en las células. Las enzimas dependientes del pirofosfato de tiamina, como la piruvato decarboxilasa, la piruvato deshidrogenasa, la α-cetoglutarato deshidrogenasa, la deshidrogenasa de α-cetoácidos de cadena ramificada y la transcetolasa, proporcionan ejemplos claros de umpolung en reacciones celulares. En esta revisión, tras una discusión sobre el significado del término umpolung encontrado en la literatura química, se analizan los mecanismos de reacción y el significado bioquímico de las transformaciones llevadas a cabo por las enzimas mencionadas.

Palabras clave: deshidrogenasa; decarboxilasa; pirofosfato de tiamina; transcetolasa; umpolung.
1. Introduction

Multiple reactions in cellular metabolism consist in C-C bond formation or breakage. During glycolysis, the retro-aldol reaction catalyzed by aldolase, in which the fructose-1,6-diphosphate splits in two phosphorylated trioses, represents an example of C-C bond cleavage.

Usually, the linkage of carbons occurs between atoms with opposite polarities (electrophile/nucleophile). This can be observed in reactions such as the Claisen condensation between acetyl-CoA and oxaloacetate (OAA), catalyzed by citrate synthase during the Krebs cycle. In this reaction, both the carbonyl group and the enolate keep their typical reactivity, them being electron acceptor and electron donor, respectively (Nelson, Cox & Lehninger, 2013). Figure 1 shows the enolate of acetyl-CoA (1) reacting with the carbonyl group of the OAA (2) to form a new C-C bond in the citrate molecule (3).

![Figure 1. Nucleophilic attack of enolate to oxaloacetate (OAA) in citrate synthase reaction mechanism.](image)

**Note:** Acetyl-CoA (1), OAA (2), citrate (3), coenzyme A (4). (a): acceptor (electrophile); (d): donor (nucleophile). Dashed arrows represent the involvement of several steps. The Citroyl-CoA intermediate thioester formation has been omitted.

The formation of C-C bonds between carbons sharing the same polarity is also possible. Nevertheless, it requires the reversal of the electron donor/acceptor character of one of the atoms, a phenomenon known as umpolung. The carbonyl group is intrinsically an acceptor, and therefore reacts with electron donors and not with another electron deficient carbons. Examples of reactions between carbonyl groups to form a C-C bond are found in metabolic pathways such as the Calvin cycle and in the non-oxidative phase of the pentose phosphate. In the latter, as shown in Figure 2, a C-2 fragment must be transferred from a ketopentose (4) to an aldopentose (5), resulting in a ketoheptose (6) and a triose (7). This reaction is catalyzed by the transketolase, a thiamine pyrophosphate (TPP) dependent enzyme which belongs to a diverse group of biocatalysts involved in the rupture and/or formation of C-C bonds (Mosbacher, Mueller & Schulz, 2005).
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The objective of this review is to illustrate how the inversion of the original polarity of the carbonyl group in certain cellular metabolites takes place inside the active site of TPP-dependent enzymes. Additionally, the biochemical meaning of these inversions is analyzed.

2. Umpolung

Umpolung is a German word, used since the mid-twentieth century (Wittig, Davis & Koenig, 1951; Seebach & Corey, 1975), that refers to any process by which the nucleophilic (electron donor) or electrophilic (electron acceptor) character of an atom in a molecule is exchanged (Seebach, 1979). This inversion of the atoms inherent polarity allows the carrying out of reactions that are not possible from the original reactivity of its functional group (Eymur, Göllü & Tanyeli, 2013). The Corey-Seebach reaction is a classic example of umpolung in which the inversion of the normal reactivity of a carbonyl group (electrophile) is produced. This allows the carbonyl carbon to react with electrophiles as an acyl anion, exhibiting the opposite chemical behavior of its natural reactivity and being very useful in organic synthesis (Corey & Seebach, 1965).

As shown in Figure 3, in the Corey-Seebach reaction the treatment of 1,3-dithianes (9) with n-butyl-lithium leads to a carbanion (10) with an excellent nucleophilic reactivity (Corey & Seebach, 1965). The carbanions thus formed can be used in multiple reactions in which a new bond is formed between two originally electrophilic carbons.

Figure 2. A step in the non-oxidative phase of the pentose phosphate pathway.

Note: Xylulose-5-phosphate (4), ribose-5-phosphate (5), sedoheptulose-7-phosphate (6), glyceraldehyde-3-phosphate (7). TK: transketolase, (a): acceptor.

Figure 3. The Corey-Seebach reaction.

Note: Treatment of aldehyde (8) with 1,3-propanedithiol yields a 1,3-dithiane (9). The carbanion obtained by treatment of (9) with n-butyl-lithium reacts with an electrophile (E+) to form a new carbon-carbon bond (11). The removal of the dithiane protecting group leads to a ketone (12). (a): acceptor; (d): donor.
The Corey-Seebach reaction has been widely used in natural product synthesis (Yus, Nájera & Foubelo, 2003). Figure 4 shows the conjugate addition of a lithiated dithiane derived from (14) to 2-furanone; this sequence of reactions has been employed in (±)-podorhizol, (±)-isopodophyllotoxone (Ziegler & Schwartz, 1978) and in arylnaphtalene lignans synthesis (González, Pérez & Trujillo, 1978; Boluda, López, Pérez & Trujillo, 2005). Figure 5 shows the strategy used by Noda and Watanabe in the enantiospecific synthesis of the (R)- and (S)-enantiomers of a flavanone. The nucleophilic addition of the carbanion of a dithiane (17) to a chiral epoxide forms a diol (18) that under Mitsunobu cyclization yields the synthetic target (19) (Noda & Watanabe, 2002). The dithianes have also been used to obtain more complex natural products as spongistatins (Smith et al., 2001).

**Figure 4.** Conjugate addition of a lithiated dithiane derived from (14) to a 2-furanone.

**Note:** Conjugate addition of the aryl dithiane anion of compound (14) to 2-furanone yielding the corresponding enolate, which leads to the compound (15) by protonation. Further steps lead to (±)-isopodophyllotoxone (16).

**Figure 5.** Strategy used in the enantiospecific synthesis of the (R)- and (S)-enantiomers of a flavanone.

**Note:** Treatment of dithiane (17) with n-BuLi and addition of a derived carbanion to (+)-(R)- and (-)-(S)-styrene oxide. The resulting diol (18) forms a flavanone (19) through Mitsunobu cyclization.

Organometallic reagents such as Grignard reagents or alkyl-lithium and phosphorus ylides shown in Figure 6 provide clear examples of *umpolung* by atom exchange (Arndt, Kunde & Mahrwald, 2016).
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Figure 6. Umpolung by atom exchange.

Note: Obtention of a Grignard reagent (21), an alkyl-lithium compound (22) and a phosphorus ylide (23) from an haloalkane (20). In the three cases, the polarity of the carbon atom directly linked to the halogen atom in the haloalkane is changed from acceptor to donor. (a): electron acceptor (electrophile); (d): electron donor (nucleophile); (X): halogen atom; (R): alkyl group.

The umpolung is a synthetic tool which includes the reversion of the normal reactivity of amines (donors) by replacing one or more of their hydrogens with good leaving groups. This strategy provides another method of obtaining amines from organometallic compounds such as Grignard reagents (RMgBr), as an alternative to the alkylation of ammonia and primary amines, which takes advantage of the normal reactivity of the amines (Figure 7) (Erdik & Ay, 1989). Another example of amine umpolung utilizes N-haloamines and α-halonitroalkanes, as an acyl donor, for obtaining amides (Shen, Makley & Johnston, 2010).

Figure 7. Electrophilic amination of a Grignard reagent via umpolung of the amino group.

Note: Normal reactivity of ammonia (electron donor) is reversed by the introduction of a leaving group (Z), allowing electrophilic amination of carbanion (R:MgBr).

As shown in Figure 8, other umpolung examples include the inversion of the inherent reactivity of alkenes, umpolung by atom exchange and umpolung by addition of carbon fragments.
| Entrance | Normal polarity | Reversed polarity |
|----------|----------------|------------------|
| 1        | ![Normal polarity](example1) | ![Reversed polarity](example2) |
| 2        | ![Normal polarity](example3) | ![Reversed polarity](example4) |
| 3        | ![Normal polarity](example5) | ![Reversed polarity](example6) |
| 4        | ![Normal polarity](example7) | ![Reversed polarity](example8) |
| 5        | ![Normal polarity](example9) | ![Reversed polarity](example10) |

**Figure 8. Umpolung examples.**

**Note:** Alkene epoxidation (entrance 1), bromonium ion formation (entrance 2). Umpolung by atom exchange: α-position halogenation of carbonyl compound (entrance 3) and halogenation of 1-alkynes (entrance 4). Cyanohydrins (entrance 5), an umpolung by addition of carbon fragments. (a): electron acceptor, (d): electron donor.

The term *umpolung* has also been used in a broader sense to indicate a change in the reactivity of a compound and not to refer to a particular atom. In 2010 Guérard, Sabot, Beaulieu, Giroux, & Canesi utilized the expression “Aromatic ring umpolung” to refer to the formation of a highly electrophilic phenoxenium ion which reacts with nucleophiles. These acceptor species were formed from phenols by treating them with an oxidizing agent such as diacetoxyiodobenzene (DIB) (Guérard et al., 2010). Additionally, in 2013 the inversion of the normal polarity in phloroglucinol (1,3,5-trihydroxybenzene) was reported. This compound is expected to behave as a nucleophile in an aromatic electrophilic substitution reaction (Figure 9) but reacts as an electrophile under certain conditions. Phloroglucinol (24) reacts as an electron donor under typical Friedel-Crafts reaction conditions with benzoyl chloride in nitrobenzene to form phlorobenzophenone (25), although with low yield. Nevertheless, when reacting with toluene and no solvent or with chlorobenzene, it behaves as an electrophile forming 4’-methylbiphenyl-3,5-diol (26) with an 89% yield. This “umpolung” example observed in phloroglucinol allows a simple way to obtain 5-aryl-substituted resorcinols (Gulyás, Boluda, Westermann & Wessjohann, 2013).
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Figure 9. Phloroglucinol  umpolung.

Note: Synthesis of phlorobenzophenone (25) and 4′-methylbiphenyl-3,5-diol (26) from phloroglucinol (24) through an “umpolung” (Gulyás et al., 2013).

This  umpolung lato sensu has also been used to describe the aldol reaction mechanism carried out by aldolases. This enzymatic transformation involves the formation of an enamine (aldolases type I) or an enolate (aldolases type II) in the active site of the enzyme. Then the donor performs a nucleophilic addition to an acceptor (Figure 10) (Faber, 2018). However, the abstraction of a hydrogen from the α-position by the enzyme should not be considered strictly an umpolung example, since that α-carbon is a donor (Arndt et al., 2016) and no inversion of polarity takes place; the enzyme only takes advantage of the normal polarity of the molecule, increasing its reactivity. Nevertheless, an umpolung stricto sensu, as defined by Seebach in 1979, can be observed in the mechanism of enzymatic action of TPP dependent enzymes.

Figure 10. Enzymatic reaction mechanism of type I aldolases.

Note: After a Schiff base formation (27), deprotonation by the enzyme leads to an enamine (donor) (28) that reacts with a carbonyl compound (acceptor) to form an aldol (29) (Faber, 2018). R₁ = H, OH, NH₂. (a): electron acceptor; (d): electron donor.
3. Thiamine pyrophosphate (TPP)

Thiamine pyrophosphate (TPP) (Figure 11) is a vitamin B1 derived coenzyme (Jong, Meng, Dent & Hekimi, 2004) used by a diverse collection of enzymes that catalyze the formation and rupture of carbon-carbon bonds adjacent to a carbonyl group. Additionally, the TPP is an allosteric regulator of enzymes in cellular metabolism and has a relevant role in the response to several environmental factors such as pathogens and oxidative stress (Tylicki, Lotorowski, Siemieniuk & Ratkiewicz, 2018). According to the NCBI PubChem Database (2019), the chemical structure of TPP consists of a methylamino pyrimidine ring linked through a methylene bridge to a methylthiazolium ring. A pyrophosphate group is attached to its hydroxyethyl side chain (Figure 11). This methylthiazolium ring of TPP behaves as an electron sink during reactions and stabilizes what would otherwise be an unstable acyl carbanion intermediate in the form of an enamine (Agyei-Owusu & Leeper, 2009; Nelson et al., 2013).

As shown in Figure 11, the thiazolium ring proton (C-2) is slightly acidic (pKa=18) and its deprotonation results in the formation of an ylide with a nucleophilic carbene as a resonance form, as proposed by Breslow in 1958. The aminopyrimidine ring participates actively in the TPP ionization (Figure 11). The deprotonated form of the imino tautomer (31) of 4'-aminopyridinium (30) acts as an internal base accepting the proton of the thiazolium ring (Agyei-Owusu & Leeper, 2009; Balakrishnan et al., 2012).

The carbene stabilized by adjacent heteroatoms (Hanson, 1987; Prier & Arnold, 2015; Heidari, Howe & Kluger, 2016) reacts as a strong nucleophile, by addition with the carbonyl group of a metabolite (Broderick, 2001; Amyes & Richard, 2017).

Figure 11. Thiamine pyrophosphate ionization forming an ylide in resonance with a carbene.

Note: The aminopyrimidine ring participates as base in an intramolecular proton exchange reaction.
In absence of the apoenzyme, TPP decarboxylates pyruvic acid at room temperature at pH 8.4 (Mizuhara, Tamura & Arata, 1951; Breslow, 1958). Therefore, promoting the non-enzymatic decarboxylation of α-keto acids seems to be an inherent ability of this coenzyme. Furthermore, other thiazolium compounds share this catalytic capacity and mimic the TPP enzymatic reactions (Breslow, 1958; Coopper, Ginos & Meister, 1983; Nemeria, Chakraborty, Balakrishnan & Jordan, 2009).

4. TPP dependent dehydrogenases: PDHC, KGDHC and BCKDHC

Pyruvate dehydrogenase complex (PDHC), α-ketoglutarate dehydrogenase complex (KGDHC), and branched-chain α-keto acid dehydrogenase complex (BCKDHC) are mitochondrial enzymatic complexes composed by multiple copies of three enzymes and five coenzymes. Their basic structure has been preserved during evolution and allows the transformation of their specific α-keto acids, into their corresponding thioesters through a decarboxylation reaction (Lonsdale, 2006; Nelson et al., 2013), while also catalyzing the reduction of nicotinamide adenine dinucleotide (NAD⁺) to NADH (Fries, Jung & Perham, 2003).

PDHC oxidizes pyruvate to acetyl coenzyme A (acetyl CoA), linking glycolysis and the Krebs cycle (Figure 12) (Tylicki et al., 2018); KGDHC catalyzes the oxidative decarboxylation of α-ketoglutarate to form succinyl-CoA in the tricarboxylic acid cycle; and the BCKDHC produces the initial and irreversible step in the catabolism of branched-chain amino acids (valine, leucine and isoleucine) to form the respective thioesters with coenzyme A (Wynn, Daviel, Mengl & Chuang, 1996). After the transamination of these amino acids to their corresponding α-keto acids, this enzymatic complex is responsible for catalyzing the oxidative decarboxylation of the branched-chain α-keto acids (Figure 12) (Johnson, Yang & Patel, 2000; Wynn et al., 2004).

**Figure 12.** Linkage between glycolysis and branched-chain α-keto acid catabolism with the Krebs cycle.

**Note:** PHDC: Pyruvate dehydrogenase complex, KGDHC: α-ketoglutarate dehydrogenase complex. BCKDHC: branched-chain α-keto acid dehydrogenase complex. Val: valine, Leu: leucine, Ile: isoleucine. Dashed arrows represent the involvement of several steps.
As the PDH and KGDH complexes, the BCKDHC consists of three catalytic components: α-keto acid dehydrogenase (subunit E1), dihydrolipoyl transacylase (subunit E2) and dihydrolipoyl dehydrogenase (subunit E3) (Ævarsson et al., 2000; Wynn et al., 2004). The complex formed by these enzymes needs five cofactors to perform its function: FAD (flavin adenine dinucleotide), NAD+ (nicotinamide adenine dinucleotide), TPP (thiamine diphosphate), lipoic acid, and coenzyme A (Johnson et al., 2000).

The E1 component itself is a tetramer composed of two subunits (E1 alpha and E1 beta) with TPP as required coenzyme for the decarboxylation of branched-chain α-keto acids (Heffelfinger, Sewell & Danner, 1983; Li, Huo, Pulley & Liu, 2012). This conversion, illustrated in Figure 13, involves an umpolung. When the TPP binds to the enzyme, the deprotonation of the thiazolium ring at C2 (32) creates a nucleophilic ylide resonant with a carbene (33) (Tittmann et al., 2003). Once the branched-chain α-keto acid (34) reacts with the ylide/carbene, it forms a tetrahedral covalent intermediate (35). The subsequent decarboxylation of this substrate results in a stabilized carbanion (36) (Fiedler et al., 2002; Kluger, 1987). The carbanion is then transferred to the lipoamide (37), restoring the original polarity of the carbon atom (acceptor) (40). The addition-elimination sequence with coenzyme A acting as nucleophile (transthioesterification) forms a new thioester (acyl-CoA), releasing a branched acyl-CoA (41) and the dihydrolipoamide (42).

![Figure 13. Mechanism of enzymatic action of PDHC, KGDHC and BCKDHC.](image)

**Note:** The deprotonation of TPP leads to an ylide with a carbene as resonance structure that reacts with the carbonyl group of a specific α-keto acid. The thiazolium ring provides the possibility to decarboxylate the substrate. For these reactions the umpolung at the carbonyl group is a consequence of the loss of the carboxyl group as carbon dioxide. PDHC: \( R_3 = -H, R_4 = -H \); KGDHC: \( R_3 = -CH_2-CH_2-COOH, R_4 = -H \); BCKDHC: \( R_3 = \text{alkyl group}; R_4 = \text{alkyl group}; E = \text{enzyme} \).
5. Pyruvate decarboxylase

Yeast and all organisms that ferment glucose to ethanol use the TPP-dependent enzyme complex pyruvate decarboxylase (PDC) to catalyze the non-oxidative decarboxylation of pyruvate and produce acetaldehyde. This is then converted to ethanol by alcohol dehydrogenase (Hanson, 1987; Nelson et al., 2013) reoxidizing NADH to sustain glycolysis (Nelson et al., 2013; Tyllicki et al., 2018). Even though the mechanism of PDC is similar to that of PDH, KGDH and BCKDH, the enamine produced is protonated at the C2 α-position, releasing acetaldehyde (Eram & Ma, 2013).

![Umpolung in the mechanism of enzymatic action of pyruvate decarboxylase.](image)

**Figure 14.** Umpolung in the mechanism of enzymatic action of pyruvate decarboxylase.

*Note:* The resulting carbanion from the reaction of TPP with pyruvic acid and the non-oxidative decarboxylation (43), is protonated (44) and released from TPP as acetaldehyde (45). The latter is reduced to ethanol (46), allowing glycolysis to continue after the reoxidation of NADH.

6. Transketolase

Transketolase (TK), also called glycolaldehyde transferase, is an enzyme that uses thiamine pyrophosphate (TPP) as its coenzyme and a divalent metal cation (Mg²⁺ or Ca²⁺) in order to perform the cleavage of carbon-carbon bonds and transfer a dihydroxyethyl unit from ketose donors to aldose acceptors (Schenk, Duggleby & Nixon, 1998; Fullam, Pojer, Bergfors, Jones & Cole, 2012). From an organic chemistry perspective, the latter seems not to be a trivial task, but a challenge for cellular chemistry due to its occurrence in an aqueous media and with phosphorylated and non-phosphorylated sugars as substrate (Miyamoto & Ohta, 2007).

The TK is involved in the Calvin cycle and non-oxidative phase of the pentose phosphate pathway. In both cases, the enzyme transfers two carbon fragments from ketosugars such as xylulose-5-phosphate or fructose-6-phosphate, to aldoses like ribose-5-phosphate or glyceraldehyde-3-phosphate in order to assemble precursors of other molecules in each route (Figure 15) (Schenk, Duggleby & Nixon, 1998; Miyamoto & Ohta, 2007; Lonsdale, 2006).
Figure 15. Transketolase in the non-oxidative phase of the pentose phosphate pathway.

Note: The nucleophilic attack of the ylide (47) to a ketopentose (48) leads to an intermediate (49). Cleavage of C2-C3 bond of 49 results in a stabilized carbanion (51) which performs a nucleophilic addition to an aldopentose (52) forming the second intermediate (53). The latter releases TPP as a leaving group to yield a ketoheptose (54).

The C-C bond formation catalyzed by TK requires an *umpolung stricto sensu*. The nucleophilic attack from ionized TPP to a carbonyl group of the ketosugar produces a tetrahedral intermediate. The polarity inversion occurs because of the cleavage of this intermediate releasing an aldehyde and the resulting stabilized carbanion which is then transferred to the aldose. After the cleavage, a ketone and the TPP are released (Schneider & Lindqvist, 1993).

### 7. Conclusions

Although the scope of the term *umpolung* is usually restricted to organic chemistry, it is certainly a tool available to living organisms. Inside the cell, some chemical reactions require the formation of an anomalous synthon, an acyl carbanion (Agyei-Owusu & Leeper 2009). Due to the inability of the 20 amino acid residues normally found in enzymes to
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stabilize this intermediate, the coenzyme TPP has evolved to assume this role (Hanson, 1987). The mechanism of the TPP dependent enzymes analyzed provide a clear example of *umpolung stricto sensu* in nature, although with very different biochemical meanings.

In the case of pyruvate decarboxylase (PDC), the *umpolung* at C2 appears as a result of the non-oxidative decarboxylation and it seems not to obey a biosynthetic strategy, as the masked acyl anion (enamine) is protonated yielding acetaldehyde. The latter is reduced to ethanol by NADH providing the required NAD\(^+\) to continue glycolysis.

Nevertheless, in other enzymes that carry out a decarboxylation reaction of an \(\alpha\)-keto acid, such as pyruvate dehydrogenase complex (PDHC) or the branched-chain amino acid dehydrogenase complex (BCKDH), the *umpolung* is produced once more as a consequence of the loss of carbon dioxide, but it is used to obtain an acyl-CoA and NADH. As shown in Figure 13, once the decarboxylation takes place, the normal reactivity of the carbonyl group (acceptor) is restored by transferring the carbanion to the lipoyl moiety forming a thioester. The recovering of the normal polarity of the carbonyl group in this intermediate is necessary to react with coenzyme A and to yield an acyl-CoA.

Unlike the cases described above, in reactions catalyzed by transketolase, the inversion of the normal reactivity of the carbonyl group of a monosaccharide, consequence of the rupture of a C-C bond in the sugar skeleton, is utilized to perform a new bond between two originally electrophilic carbons. This allows the rearrangement reactions in the non-oxidative phase of the pentose phosphate pathway and the Calvin cycle.

The fact that the TPP has catalytic activity *per se*, catalyzing the decarboxylation of \(\alpha\)-keto acids, suggests that this might be the primordial function of this coenzyme in the cells. Later on, during evolution, the ability of the coenzyme to generate masked and highly reactive acyl anions would be opportunistically used to achieve more complex catalytic challenges, as forming bonds between two originally electrophilic carbons, as in transketolase catalyzed reactions. Performing this *umpolung* in aqueous medium had to be a major problem, since the carbanion could be easily protonated by water.

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