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# Redesigning thiamin synthesis: Prospects and potential payoffs

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ABSTRACT

Thiamin is essential for plant growth but is short-lived in vivo and energetically very costly to produce – a combination that makes thiamin biosynthesis a prime target for improvement by redesign. Thiamin consists of thiazole and pyrimidine moieties. Its high biosynthetic cost stems from use of the suicide enzyme THI4 to form the thiazole and the near-suicide enzyme THIC to form the pyrimidine. These energetic costs lower biomass yield potential and are likely compounded by environmental stresses that destroy thiamin and hence increase the rate at which it must be made. The energy costs could be slashed by refactoring the thiamin biosynthesis pathway to eliminate the suicidal THI4 and THIC reactions. To substantiate this design concept, we first document the energetic costs of the THI4 and THIC steps in the pathway and explain how cutting these costs could substantially increase crop biomass and grain yields. We then show that a refactored pathway must produce thiamin itself rather than a stripped-down analog because the thiamin molecule cannot be simplified without losing biological activity. Lastly, we consider possible energy-efficient alternatives to the inefficient natural THI4- and THIC-mediated steps.

## 1. Introduction

Synthetic biology (SynBio) concepts and tools are enabling metabolic engineering to radically reconfigure metabolic networks and to create entirely novel pathways that do not exist in nature [1–3]. The first-generation targets for SynBio-enabled engineering of plant primary metabolism have been the redesign of photosynthesis [4–7] and photorespiration [7,8], reflecting the importance of these processes to carbon gain and their well-understood inefficiencies. The processes that cause loss of fixed carbon via respiration have not been SynBio targets even though respiration consumes roughly half of the net carbon fixed by crops and is therefore a massive drain on productivity [9–12]. This lack of SynBio attention to respiration may be partly due to the historical neglect of respiratory carbon losses [12] but it is also because no specific candidate enzymes and pathways for improvement have been identified. This article identifies two enzymes of thiamin biosynthesis as such candidates, and shows how redesigning thiamin biosynthesis could increase crop productivity.

To make the case for redesign, Sections 2 and 3 explain how thiamin is metabolically crucial but fragile, and extremely energetically expensive to replace by the natural biosynthesis pathway – a highly unfavorable combination from the productivity standpoint. Section 4 indicates how much crop biomass production could increase if an energy-efficient designed biosynthesis pathway replaced the natural one. Section 5 then explores whether thiamin can be structurally simplified to make it easier to biosynthesize, and concludes that unfortunately it cannot. Section 6 considers alternative, energy-efficient routes to the thiazole and pyrimidine precursors of thiamin.

## 2. Metabolic physiology of thiamin

### 2.1. Thiamin is indispensable but unstable

Thiamin consists of thiazole and pyrimidine moieties (Fig. 1A). It is essential to plants because its active form, thiamin diphosphate (ThDP), is the cofactor for at least eight enzymes in core pathways of primary metabolism (Fig. 1B and C). These enzymes, which differ greatly in abundance (Fig. 1B), bind almost all the cellular ThDP so that very little

*Abbreviations:* MVT, 4-methyl-5-vinylthiazole; ThDP, thiamin diphosphate; THZ, 4-methyl-5-β-hydroxyethylthiazole; SynBio, synthetic biology

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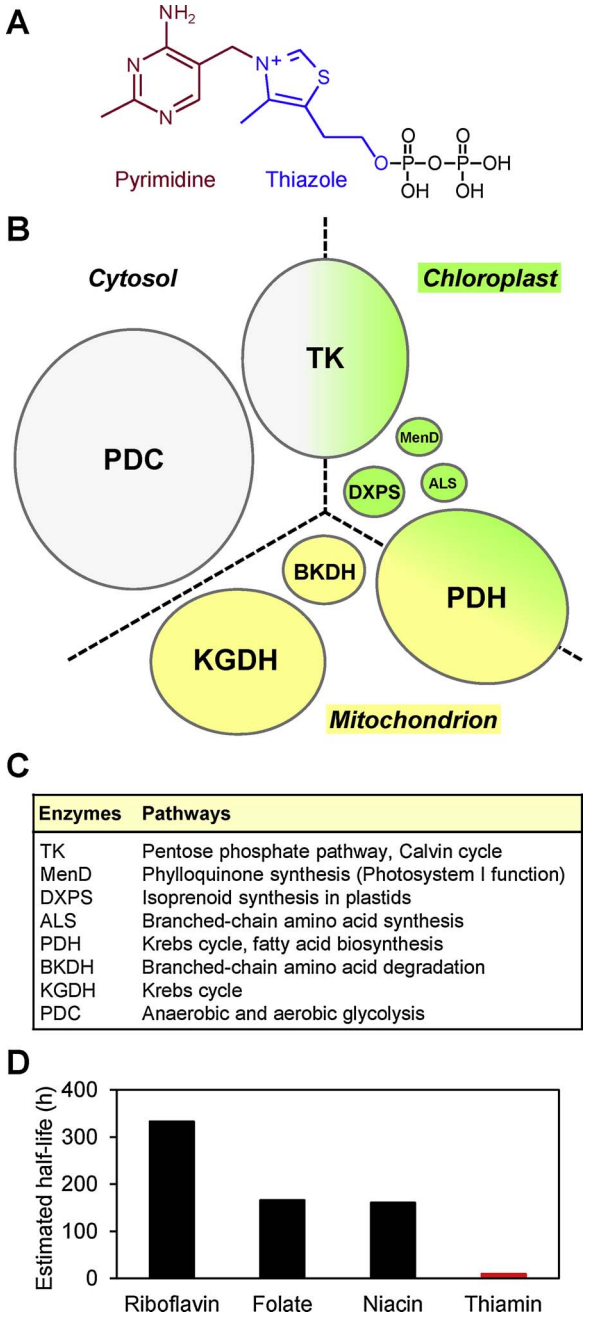


**Fig. 1.** Thiamin diphosphate as a critical but short-lived cofactor.
(A) The structure of thiamin diphosphate, indicating its pyrimidine and thiazole moieties. (B) Plant enzymes that use thiamin diphosphate as a cofactor and their subcellular locations. The sizes of the ovals are roughly proportional to the abundances of the proteins reported in the PaxDb database [25]. TK, transketolase (EC 2.2.1.1); MenD, 2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase (EC:2.2.1.9); DXPS, 1-deoxyxylulose-5-phosphate synthase (EC 2.2.1.7); ALS, acetolactate synthase (EC 2.2.1.6); PDH, pyruvate dehydrogenase complex (EC 1.2.4.1), BKDH, branched-chain keto acid dehydrogenase (EC 1.2.4.4); KGDH, α-ketoglutarate dihydrogenase complex (EC 1.2.4.2); PDC, pyruvate decarboxylase (EC 4.1.1.1). Note that TK and PDH are both in two compartments. (C) The main pathways in which the above enzymes participate. (D) Approximate estimates of average half-lives in plants of thiamin and other B vitamins/cofactors. The primary data sources for these estimates are: riboflavin [24] (assuming 12 light per day); folate [25]; niacin [26,27]; thiamin [14,28,29].

occurs in a free state [13]. ThDP is the main form of thiamin; thiamin itself and thiamin monophosphate usually make up < 20% of the total [14,15].

Thiamin is the least metabolically stable B vitamin, with a whole-body half-life in humans that is up to 100-fold shorter than other B vitamins [16–19]. A major cause of this metabolic instability is that – unlike other B vitamins – thiamin (as ThDP) can be irreversibly inactivated during catalysis [20]; the inactivation is due to an oxidative side-reaction that damages the thiazole moiety [21,22]. The pyrimidine moiety can likewise be inactivated by a hydrolytic deamination reaction [23]. Although half-lives of B vitamins in plants have been very little studied, average values of > 100 h can be estimated for riboflavin [24], folate [25], and niacin [26,27] in various tissues, versus ~10 h for thiamin in Arabidopsis leaves (Fig. 1D). This thiamin value is derived from the turnover rate and abundance of the biosynthetic enzyme THI4 [28,29] and from thiamin content [14] (see Section 3.2). The fast turnover of thiamin in plants is most probably further accelerated by abiotic stresses [30,31].

### 2.2. Thiamin is trafficked between tissues but not stored

Unlike the situation for most other B vitamins, certain plant cells and organs are not self-sufficient for thiamin or its pyrimidine and thiazole precursors and must therefore import them from elsewhere [15,32,33]. Examples include pea roots, which import either thiamin or both precursors [34], tomato roots, which import thiamin or the thiazole precursor [35], and maize root and shoot meristems, which also appear to import thiamin or thiazole [15]. Again unlike other B vitamins, no glycosides or other classical storage forms of thiamin or its precursors are known from plants [30].

### 2.3. Plants are at risk of thiamin deficiency and productivity loss

Metabolic lability and rapid turnover, the need for transport between sites of synthesis and utilization, and lack of storage capacity combine to make thiamin deficiency *a priori* likely, particularly in certain cells and in stress conditions, and experimental evidence supports this (reviewed in [30]). A key point is that even moderate thiamin deficiency severely impacts productivity, e.g. a 35% drop in ThDP level in maize cripples leaf development and leads to shoot abortion [33] and a 50% drop in Arabidopsis causes chlorosis and stunts growth [14]. The severity of these effects fits with the observations that (i) nearly all ThDP is enzyme-bound (Section 2.1) so that loss of ThDP means a proportionate loss of enzyme activity across multiple, central anabolic and catabolic pathways, and (ii) modest reductions in ThDP-dependent enzyme activities have large impacts [36,37]. ThDP-dependent enzymes (Fig. 1B) in plants thus operate precariously close to the deficiency zone.

## 3. Energetic costs of thiamin biosynthesis

### 3.1. Thiamin biosynthesis relies on suicide and near-suicide enzymes

The pyrimidine and thiazole moieties of thiamin are synthesized separately and coupled together in plastids to give thiamin monophosphate, which exits to the cytosol and is converted to ThDP [14,38–42] (Fig. 2A). The first two enzymes of this pathway are highly unusual. The thiazole synthesis enzyme THI4 is a suicide enzyme that catalyzes its reaction only once because the sulfur atom for the thiazole ring is taken from a cysteine residue in THI4 itself, causing irreversible inactivation [43]. The pyrimidine synthesis enzyme THIC is a near-suicide enzyme that uses a highly reactive 5′-deoxyadenosyl radical intermediate and self-inactivates after about five catalytic cycles [44]. The synthesis of one thiazole molecule therefore requires degradation, resynthesis, and plastid import of an entire THI4 protein, and synthesis of five pyrimidine molecules requires degradation, resynthesis, and import of a THIC protein. THI4 and THIC therefore turn over extremely fast; in both barley and Arabidopsis leaves, THI4 is the fastest-turning over protein and THIC is in the top ten [28,45] (Fig. 2B).