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Section: Metabolism

Critical Review:

Biological functions of thiamine derivatives: Focus on non-coenzyme roles

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Abbreviations

AThTP, adenosine thiamine triphosphate; CSF, cerebrospinal fluid; ThDP, thiamine diphosphate; ThMP, thiamine monophosphate; ThTP, thiamine triphosphate; ThTPase, thiamine triphosphatase.

Abstract

Introduction

Thiamine (vitamin B1) is mainly known for its diphosphorylated derivatives (ThDP), an essential coenzyme in energy metabolism. However non-coenzyme roles have been suggested for this vitamin for many years. Such roles have remained hypothetical, but recent data from various sources have shed a new light on this hypothesis. First, the existence of other phosphorylated thiamine derivatives, most prominently thiamine triphosphate (ThTP) and adenosine thiamine triphosphate (AThTP) can reach significant levels in *E. coli*, respectively during amino acid starvation and energy stress. Though much less is known about these compounds in animals, mammalian cells contain a highly specific soluble thiamine triphosphatase controlling cytosolic ThTP concentrations. Second, there is now growing evidence in favour of the existence of thiamine-binding proteins with specific roles in the nervous system, possibly in the regulation of in neurotransmitter release. Thiamine and some of its synthetic precursors with higher bioavailability have beneficial effects in several models of Alzheimer's disease and may be beneficial for patients suffering from Alzheimer's or Parkinson's diseases. These effects might be related to non-coenzyme roles of thiamine, possibly involving thiamine-binding proteins.

Conclusion

A hundred years ago, the discovery of thiamine opened the way to the vitamin era of biochemistry, leading to the discovery of the importance of pyruvate oxidation in energy metabolism. This vitamin still has not revealed all of its secrets at a time when metabolomics is emerging as a new powerful tool to refine our knowledge of cellular reactions.

Introduction

Like other B vitamins, thiamine (vitamin B1, Fig. 1A) is an indispensable molecule for all known organisms. This is mainly because, in mammalian cells, its diphosphorylated form, thiamine diphosphate (ThDP), is the coenzyme for five key metabolic enzymes (Fig.1B)[1]; the most important being mitochondrial pyruvate and oxoglutarate dehydrogenase complexes as well as the cytosolic transketolase. Therefore, it is generally believed that thiamine deficiency leads to decreased oxidative metabolism, which eventually causes cell death. In animals, the brain heavily relies on oxidative metabolism for the synthesis of ATP, making this organ particularly sensitive to thiamine deficiency. In humans, nutritional thiamine deficiency leads to beriberi, a polyneuritic condition, rapidly reversed after thiamine administration. In alcoholics, but also in children, thiamine deficiency can lead to typical selective diencephalic brain lesions[2] generally referred to as Wernicke-Korsakoff syndrome. The reason why some brain regions are more sensitive to thiamine deficiency remains unknown[3] and it was suggested that this selective vulnerability could be due to a coenzyme-independent role of thiamine or one of its derivatives[4].

Indeed, in addition to ThDP and free thiamine, several other phosphorylated and adenylated derivatives are observed (Fig. 2): thiamine monophosphate (ThMP), thiamine triphosphate (ThTP), adenosine thiamine triphosphate (AThTP) and adenosine thiamine diphosphate[5,6]. The existence of such forms in many living cells would suggest that they also have some biological role(s). It is indeed worth wondering why the diphosphorylated form of thiamine is the coenzyme, when the monophosphorylated form would do just as well, as is the case for pyrodoxal-phosphate for instance. It is indeed true that the diphosphate contributes to the binding energy to apoenzymes, but the catalytic properties of thiamine solely rely on the thiazolium ring able to lose a proton and form a reactive ylide (Fig. 1C). Ylide formation is not influenced by the presence of phosphate groups on the hydroxyethyl arm and there is no obvious advantage to use ThDP (rather than ThMP or ThTP) as coenzyme.

A recent study emphasizes beneficial effects of benfotiamine (a thiamine precursor) in a transgenic mouse model of Alzheimer's disease, though only levels of unphosphorylated thiamine were increased in the brain of the animals. Levels of thiamine phosphorylated derivatives, including ThDP were unaffected[7]. Moreover, it was recently suggested that the antinociceptive effects of thiamine in humans and animals could be mediated by the non-phosphorylated form of the vitamin[8], raising the possibility that free thiamine has pharmacological effects independent of ThDP.

Nearly 20 years ago, we have reviewed data concerning a possible non-coenzyme role of thiamine or its derivatives, particularly in relation to nerve function[9]. Here, we want to critically examine the new data that have been obtained since then.

Thiamine derivatives other than thiamine diphosphate

Thiamine is transported into mammalian cells by specific transporters and immediately phosphorylated to ThDP by cytosolic thiamine pyrophosphokinase (Fig. 2). ThDP can then be phosphorylated to ThTP or transformed to adenylated derivatives. However, the most obvious fate for cytosolic free ThDP is hydrolysis to ThMP, which is recycled to thiamine. No specific enzymes have been identified for the latter reactions and there is no known role for ThMP. Intracellular ThMP levels are generally much lower than ThDP levels. However, ThMP seems to be excreted, probably by a process involving the reduced folate carrier (RFC1 or SLC19A1)[10], a transporter closely related to thiamine transporters, and it is present in extracellular fluids such as blood plasma, cerebrospinal fluid and breast milk.

The case of thiamine triphosphate

ThTP is a particularly intriguing molecule. It is found in nearly all organisms and is the only known triphosphorylated compound that is not a nucleotide. With two phosphoanhydride bonds, it is an energy-rich compound and as such it has been shown to be able to phosphorylate proteins[11], though it is not clear whether such phosphorylation is of physiological significance. While ThTP seems to be constitutively synthesized in animal cells, in *E. coli* it accumulates only in the absence of amino acids and therefore could be a signalling molecule involved in the adaptation to amino acid starvation[12]. While it was long thought that ThTP is synthesized by a ATP:ThDP phosphotransferase, the existence of such a mechanism has never been unambiguously demonstrated. It appears now that two ATP-synthesizing mechanisms may be diverted towards the synthesis of ThTP: adenylate kinase isoform 1 (AK1) (ThDP + ADP ⇆ ThTP + AMP)[13] and F_oF_1-ATP synthase by a chemiosmotic mechanism (ThDP + P_i → ThTP)[14-16] in intact *E. coli* cells and isolated brain mitochondria. Interestingly both mechanisms are conserved from bacteria to mammals. However, while the synthesis by adenylate kinase seems to be constitutive and is probably merely a side-reaction, the synthesis by F_oF_1-ATP synthase is strongly dependent on metabolic conditions. While on one hand there is presently no evidence for a specific enzyme involved in ThTP synthesis, on the other hand mammalian cells contain a highly specific thiamine triphosphatase (ThTPase)[17-19]. This 25-kDa cytoplasmic protein is a highly efficient ThTPase ubiquitously expressed in adult mammalian tissues. However, it seems to be most

abundant in highly differentiated cells while it is hardly detectable in cultured cells, suggesting that the expression of this enzyme is linked to the degree of cellular differentiation[20,21].

It was suggested that ThTPase is a repair enzyme whose role is to remove potentially toxic ThTP produced as a by-product of the above-mentioned reactions[22]. However, in those organisms where 25-kDa ThTPase is absent (chicken) or catalytically inefficient (fish, pig), cytosolic ThTP indeed accumulates and, in skeletal muscles and electric organs its levels can even exceed those of ThDP, but without apparent toxic effect[21]. It is possible that ThTP has mainly a mitochondrial role, *i. e.*, intramitochondrial ThTP synthesized by F_oF_1-ATP synthase is the physiologically relevant pool, while cytosolic ThTP synthesized by adenylate kinase would be only a by-product of this enzyme activity. In this respect cytosolic ThTP concentrations might just reflect the abundance of AK1 in the absence of 25-kDa ThTPase.

Adenylated thiamine derivatives

AThTP (or thiaminylated ATP, Fig. 2) was first discovered in *E. coli*, where it accumulates in response to carbon starvation or uncoupling[5,23]. While other B vitamins have long been known to form coenzymes by combination with adenylate (riboflavin in FAD, nicotinic acid in NAD^+, pantothenic acid in CoA for instance) this was the first time that such a condensation product was demonstrated for thiamine. AThTP exists in small amounts in animals and plants (mainly in roots) but also in many cultured mammalian cells[21]. AThTP was shown to be an inhibitor of poly(ADP-ribose) polymerase-1 *in vitro*[24]. Moreover, small amounts of AThDP were also discovered in various organisms[6].

Thiamine-binding proteins

We refer here to proteins that specifically bind thiamine or one of its phosphorylated derivatives, but the bound thiamine compound is not supposed to act as a coenzyme. Likewise, we shall not consider enzymes using thiamine derivatives as substrates (*i.e.* enzymes involved in the metabolism of phosphoryl derivatives, see Fig. 2) nor thiamine transporters.

Several proteins that specifically bind the unphosphorylated form of the vitamin have been described. Some are thiamine-storage proteins and they were characterized mainly in plant tissues. In mammals, a few thiamine-binding proteins have been described, but their possible roles remain unclear. Such a protein has been purified from rat erythrocytes[25]. It is a soluble

32 kDa protein binding unphosphorylated thiamine. It is not clear whether it also binds phosphate esters, or whether it is specific. The group of Yulia Parkhomenko in Kiev extensively studied thiamine-binding proteins from brain. By affinity chromatography (thiamine covalently bound to a Sepharose 4B matrix), they isolated a thiamine-binding protein from a synaptosomal acetone powder[26]. This 103 – 107 kDa protein also binds ThMP and ThTP and to a lesser extent ThDP. The same group later showed that the thiamine-binding activity is mainly associated with synaptic vesicles and synaptosomal membranes[26]. It was also claimed that this thiamine-binding proteins had ThTPase activity[27], but this has not yet been proven using a purified homogenous protein preparation. If this synaptosomal thiamine-binding protein is indeed a membrane-associated membrane protein, it could act as a presynaptic "thiamine receptor". There is some evidence that thiamine can act as a neuromodulator at some synapses, regulating neurotransmitter release (see next section). It is also worth pointing out that the antinociceptive effect of thiamine seems to require prostatic acid phosphatase, which could act as or be part of a thiamine receptor[8].

Synaptosomes prepared from *Torpedo* electric organ are enriched in thiamine and its phosphate esters, while synaptic vesicle are not, suggesting that they are localized in the axoplasm[28]. Another study suggested that thiamine is an integral component of synaptomal membranes[29]. A role of thiamine in mammalian neuromuscular transmission has also been suggested in other studies[30]. Taken together, all those data suggest that thiamine may have a specific, coenzyme-independent role in synapses. The existence of ThDP-binding proteins other than apoenzymes using ThDP as coenzyme has long been debated. Cooper and associates claimed that protein-bound ThDP, isolated from a soluble liver fraction, was the substrate for ThTP synthesis[31], but it was later shown that the only ThDP-binding protein in liver cytosol was transketolase[32]. In rat brain, Yoshioka et al. described the immunohistochemical localization of a 68-kDa ThDP-binding protein[33]. In this case too, the protein probably corresponds to transketolase as the molecular mass is about the same.

Thiamine in neurotransmitter release

A specific neuroactive role of thiamine in relation to nerve excitability has been postulated as early as the 1940s and these data have been previously reviewed[9]. While there is presently no convincing evidence that thiamine has physiologically relevant effects on axonal conductance, it has been reported consistently that thiamine (and/or some of its phosphate esters) facilitates neurotransmission in various preparations, probably by potentiation of the release of the neurotransmitters acetylcholine[28,34,35], dopamine[36] and noradrenaline[35]. Here,

we are exclusively interested in direct (rapid) effects on neurotransmission, as in chronic experiments (for instance after administration of thiamine for several weeks in animals) it is very difficult to discriminate between putative coenzyme-independent and coenzyme-dependent effects: for instance, increased pyruvate dehydrogenase activity could lead to increased acetyl-CoA production which in turn could increase acetylcholine synthesis.

In addition to thiamine, several thiamine antimetabolites, the most widely used being pyrithiamine and oxythiamine (Fig. 3) have been tested. These structural analogues of thiamine are called antimetabolites, as when administered to animals they produce signs of thiamine deficiency, pyrithiamine acting primarily centrally and oxythiamine acting peripherally as it presumably does not cross the blood-brain barrier. Both compounds competitively inhibit thiamine transport[37] and ThDP synthesis by thiamine pyrophosphokinase[38,39] (though pyrithiamine is more effective).

The fact that they are antimetabolites does not preclude the possibility that they may also act as thiamine agonists when thiamine acts as a non-coenzyme modulator. Indeed, oxythiamine stimulates potassium-evoked acetylcholine release in the presence of Ca^{2+} in isolated brain slices [40].

These results suggest a coenzyme-independent effect of thiamine on neurotransmitter release, affecting at least three different neurotransmitters (acetylcholine[28,34,35], dopamine[36] and noradrenaline[35]) in different preparations ranging from fish electric organ to mammalian brain. This suggests a rather conserved mechanism. Conversely, thiamine deficiency leads to synaptic vesicle dysfunction with decreased release of dopamine[41], glutamate[42] or acetylcholine[43]. Moreover, episodes of pyrithiamine-induced thiamine deficiency in the rat lead to a significant reduction in phosphosynapsin I[44]. Interestingly, in these experiments, the animals were treated with thiamine after appearance of thiamine deficiency symptoms (loss of righting reflex and seizures) for three weeks before sacrifice, suggesting that the reduction of phosphosynapsin cannot be readily reversed by thiamine treatment and is an epigenetic phenomenon. It can indeed not be explained by a decrease in ThDP-dependent enzyme activities, as brain thiamine and ThDP levels have presumably been restored. It is thought that phosphorylation of synapsin I leads to a detachment of synapsin from the synaptic vesicles allowing their fusion with the presynaptic membrane and neurotransmitter release. An interesting hypothesis would be that thiamine, directly or indirectly, acts on synapsin I thereby promoting neurotransmitter release. This effect could be antagonized by pyrithiamine.

Thiamine in stress, diabetes and neurodegenerative diseases

In many instances, beneficial and probiotic effects of thiamine (and/or pharmaceutical preparations of thiamine precursors with higher bioavailability) have been demonstrated. In these cases, we are most likely dealing with pharmacological effects as therapeutic (superphysiological) doses were used. Indeed, under laboratory conditions, either animals or cultured cells are generally in a thiamine-rich environment: animal chows as well as cell culture media are enriched in vitamins.

According to some reports, thiamine increases disease resistance in plants[45,46]. Moreover, intracellular thiamine and thiamine phosphate pools are regulated by various stress conditions in *Z. mays* and *A. thaliana* seedlings; it was suggested that thiamine is a signalling molecule important for the adaptation to various stress conditions[47,48]. Interestingly, such a signalling role is assigned to unphosphorylated thiamine in plants, while it should be assigned to ThTP and AThTP in *E. coli*[5,6,12] (see above). Note that in *Arabidopsis* leaves, ThTP accumulates during withering[49]. Protective effects of thiamine have also been described in mammalian cells: thiamine protects retinal neurons against glutamate toxicity[50] and promotes the survival of hippocampal neurons in high cell density culture[51].

Thiamine requires specific transporters to enter cells[52]. As the rate of transport by these transporters is relatively slow, membrane transport is a rate-limiting step in thiamine homeostasis. For that reason, synthetic thiamine precursors were developed. These molecules are either relatively hydrophobic (sulbutiamine, fursultiamine) or are converted to a hydrophobic precursor (benfotiamine) allowing them to cross membranes relatively freely (Fig. 3). The general effect of these derivatives is to rapidly increase circulating thiamine to levels higher than those obtained by an equivalent dose of thiamine. It must be emphasized than none of these precursors has ever been demonstrated to reach the brain parenchyma. They are all converted to thiamine either during the passage from intestine to blood or in the liver. As the blood-brain barrier strongly limits thiamine uptake by the brain (thiamine entry could be limited by a self-exchange), no important increase in brain thiamine levels are observed even with these derivatives[7,53-55]. It would therefore be interesting to synthesize derivatives that have a half-life sufficiently long to reach significant blood levels to cross the blood-brain barrier.

Nonetheless, thiamine and/or thiamine precursors have been shown to have beneficial effects in diabetes and an animal model of Alzheimer's disease[7,56,57]. One study has shown improved cognitive functions and a striking decrease in charge of ß-amyloid plaques in a mouse model of Alzheimer's disease[58]. This study, however, needs confirmation.

A relationship between thiamine and Parkinson's disease has recently been suggested[59,60]. It had previously been shown that free thiamine levels are decreased in the cerebrospinal fluid of patients with Parkinson's disease compared to control patients[61]. Moreover, a very recent preliminary clinical study reported the beneficial effects on thiamine treatment (100 - 200 mg daily doses of parenteral thiamine) on a limited number of patients[62]. This again needs confirmation.

Concluding remarks

Thiamine, by the number of its derivatives, is certainly one of the most diverse B vitamins. By virtue of the role of ThDP as coenzyme of several key enzymes it is involved in nearly all aspects of cell metabolism: energy production, ribose and nucleic acid synthesis, lipid biosynthesis and neurotransmitter synthesis to name only the most important. Therefore thiamine is particularly important for the nervous system, which is highly sensitive to thiamine deficiency. However, the existence of potential non-coenzyme roles, summarized in Figure 4 remains a puzzling issue. First, the existence of triphosphorylated derivatives, unable to replace the coenzyme ThDP, is highly suggestive of such roles. ThTP and AThTP may be involved in some signalling processes under specific conditions of cellular stress. Second, thiamine itself, possibly through specific thiamine-binding proteins, may regulate processes such as neurotransmitter release and, in plants, protect against disease and stress. Though there is still no direct evidence for a physiologically important non-coenzyme role of thiamine, in view of the potential therapeutic interest of thiamine in Alzheimer's and Parkinson's diseases, this may become a key issue in the future.

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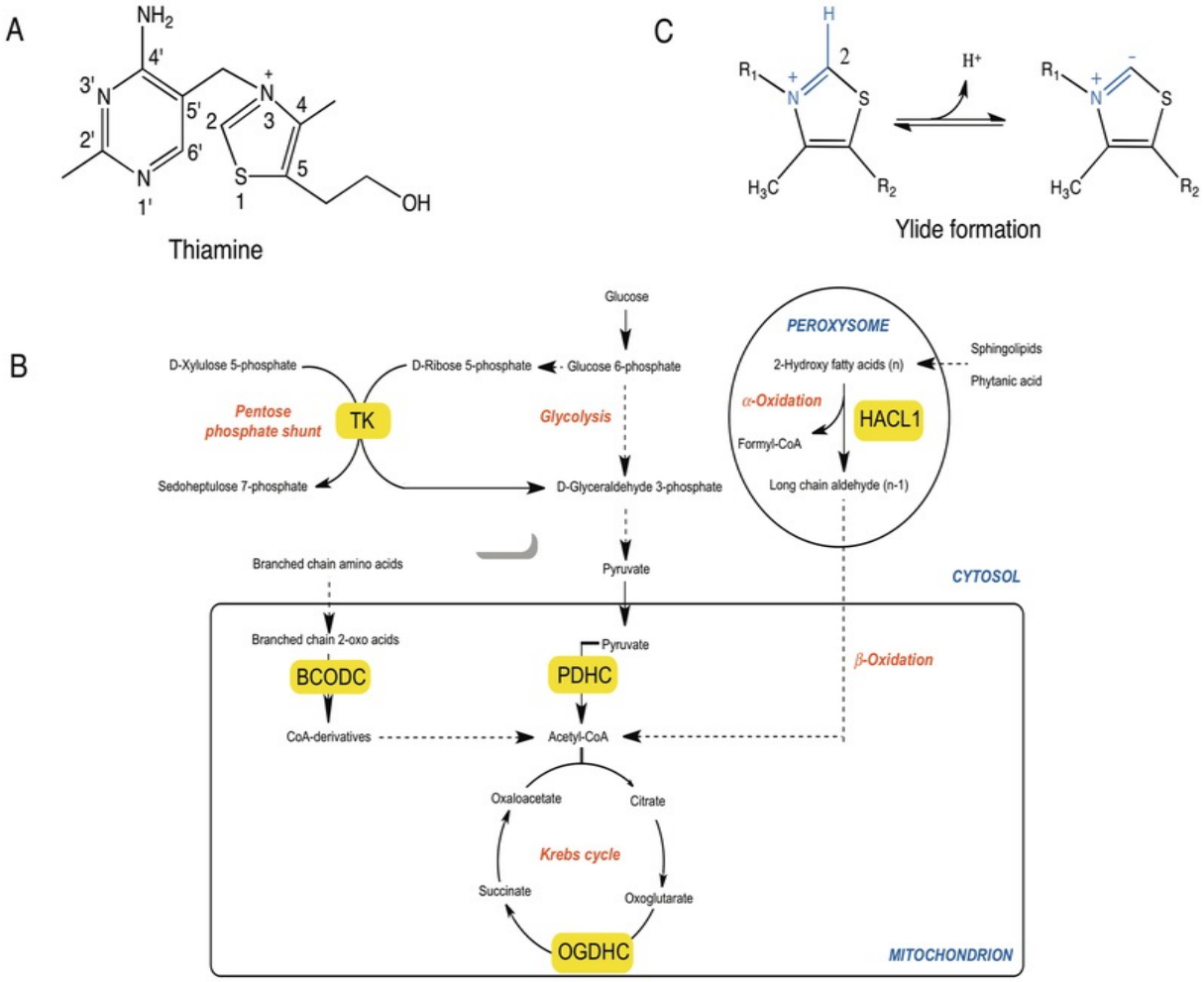
Legend to Figures

Figure 1: Thiamine diphosphate as a coenzyme. (A) Structural formula of thiamine with both heterocycles numbered according to the usual conventions. (B) Enzyme-catalysed proton loss at the C_2 of the thiazolium ring and ylide formation are at the molecular basis of the catalytic properties of thiamine. (C) ThDP-dependent enzymes in a mammalian cell and subcellular localization (TK, transketolase, PDHC, pyruvate dehydrogenase complex, OGDHC, oxoglutarate dehydrogenase complex, BCODC, branched chain 2-oxo acid dehydrogenase complex, HACL1, 2-hydroxyacyl-CoA lyase 1) (modified from [1]).

Figure 2: Thiamine derivatives observed in living organisms. (Adapted from [1,63]). ThDP is synthesized from thiamine and ATP by thiamine pyrophosphokinase (1). Hydrolysis of ThDP by thiamine pyrophosphatases (2) yields ThMP, which in turn can be hydrolysed to thiamine by thiamine monophosphatases (3). ThDP can be phosphorylated to ThTP by two mechanisms: mitochondrial F_oF_1-ATP synthase (4) and cytosolic adenylate kinase (5). ThTP can be hydrolysed to ThDP by a very specific cytosolic 25-kDa thiamine triphosphatase (6). ThDP can also be converted to AThTP by a ThDP adenylyl transferase (7). AThTP can be hydrolysed to ThDP and AMP by a putative AThTP hydrolase (8). AThDP has been shown to exist in prokaryotes and eukaryotes but its mechanism of synthesis has not yet been demonstrated *in vitro*.

Figure 3: Thiamine provitamins and antimetabolites. Fursultiamine (Thiamine tetrahydrofurfuryl disulfide) and sulbutiamine (O-isobutyrylthiamine disulfide) are disulfides while benfotiamine (S-benzoylthiamine O-monophosphate) is a thioester. The most common thiamine antimetabolites are oxythiamine and pyrithiamine.

Figure 4: Potential non-coenzyme roles of thiamine and its phosphorylated derivatives. For explanations see text.



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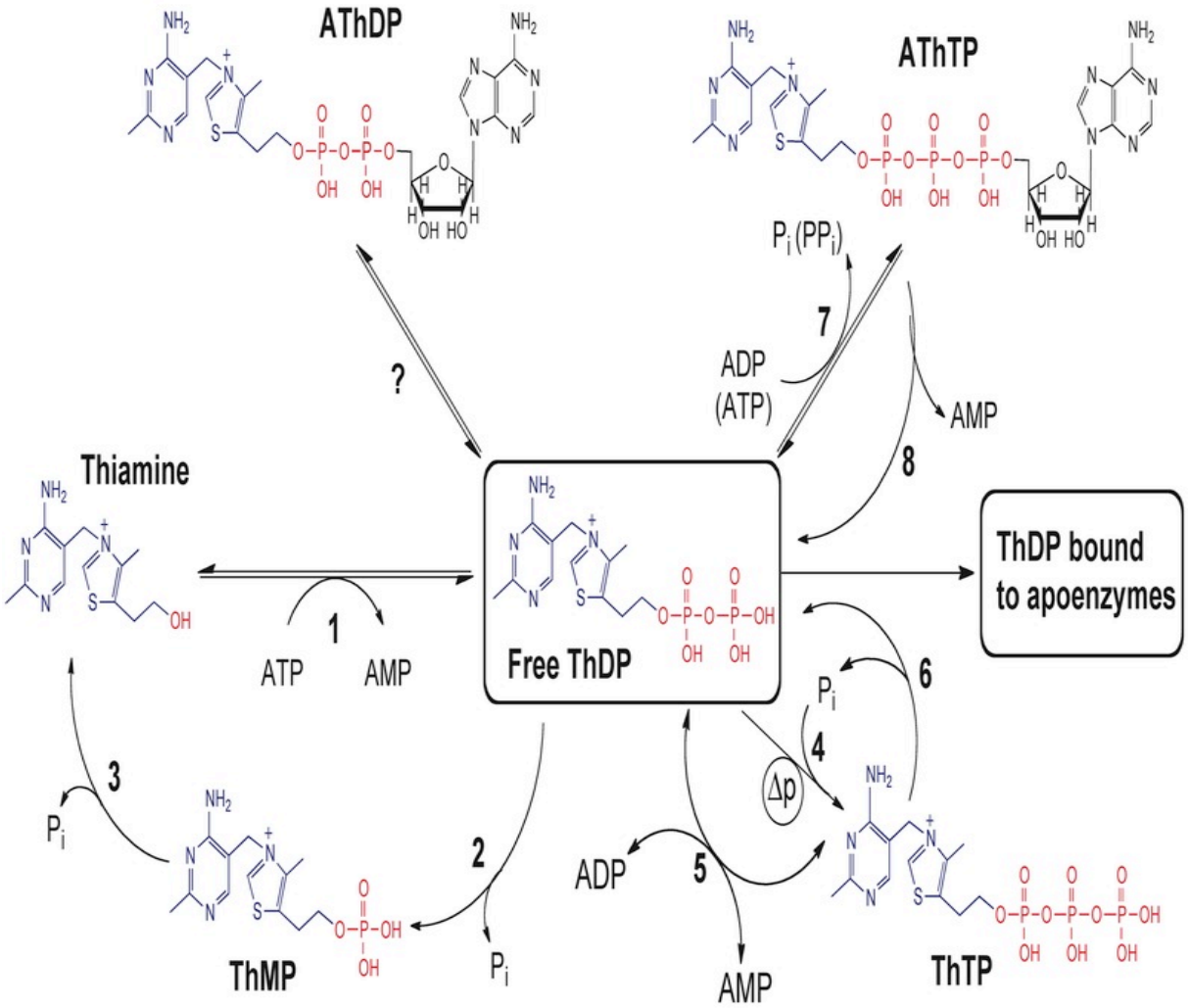

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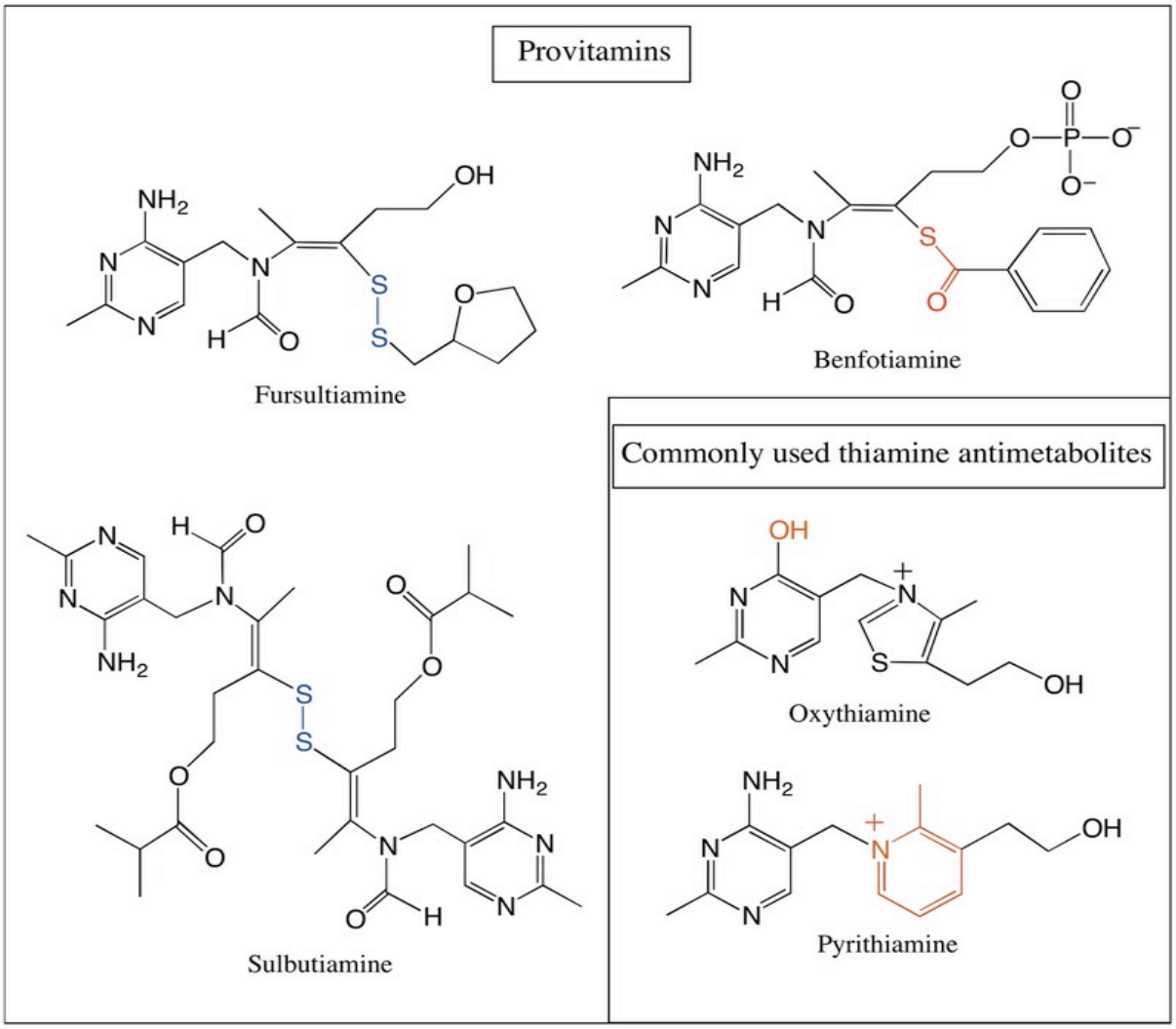

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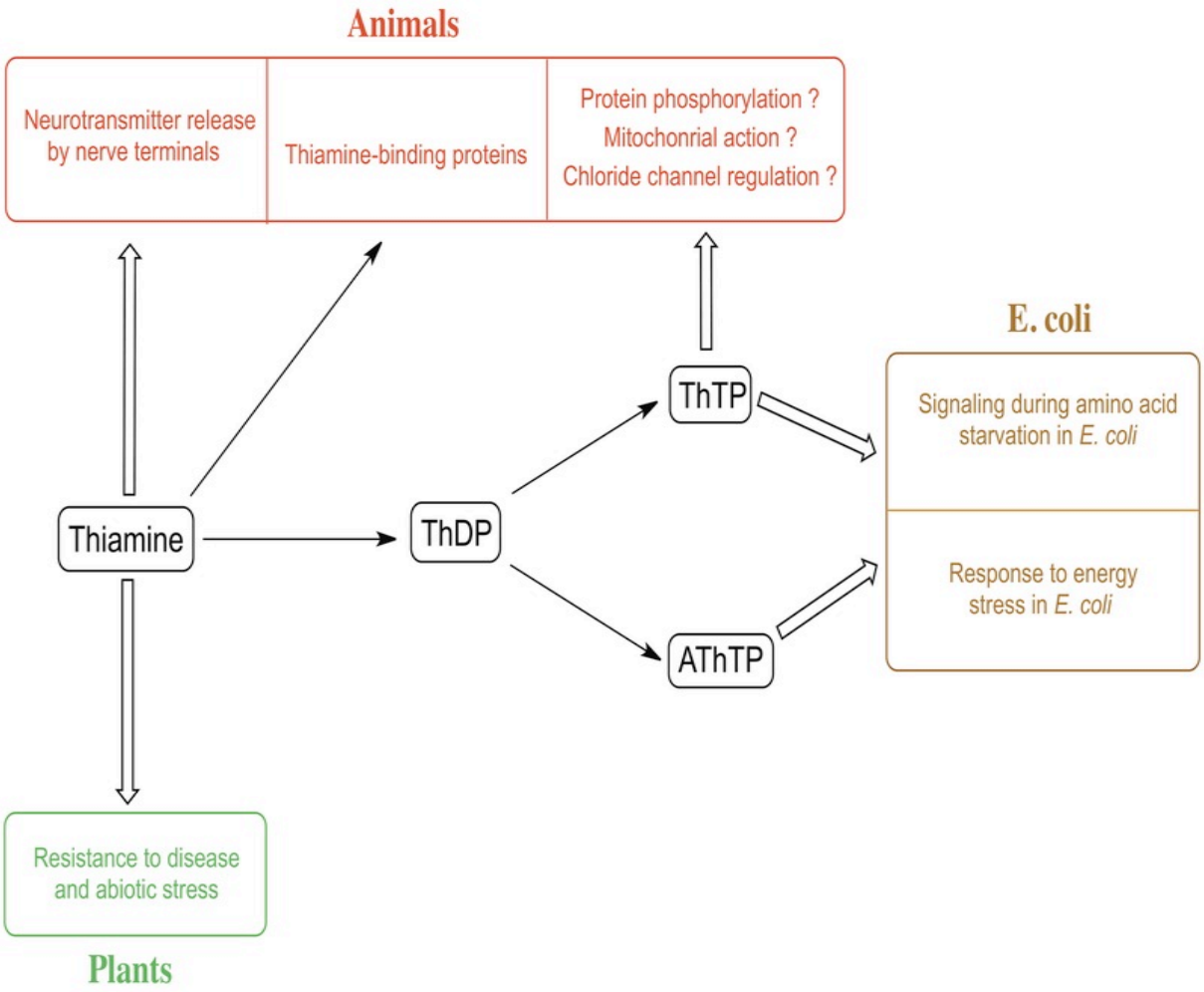

Figure 4: Figure 4.tif