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Thiamin Function, Metabolism, Uptake, and Transport

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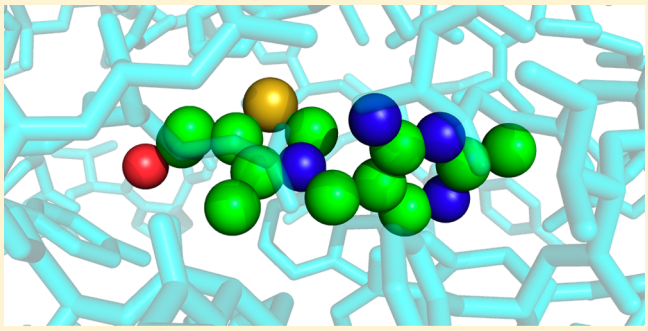
ABSTRACT: Vitamins are crucial components in the diet of animals and many other living organisms. One of these essential nutrients, thiamin, is known to be involved in several cell functions, including energy metabolism and the degradation of sugars and carbon skeletons. Other roles that are connected to this vitamin are neuronal communication, immune system activation, signaling and maintenance processes in cells and tissues, and cell−membrane dynamics. Because of the key functions of thiamin, uptake and transport through the body are crucial. Its uptake route is relatively complex, encompassing a variety of protein families, including the solute carrier anion transporters, the alkaline phosphatase transport system, and the human extraneuronal monoamine transporter family, some of which are multispecific proteins. There are two known structures of protein (subunits) involved in thiamin uptake in prokaryotes. Binding of thiamin to these proteins is strongly guided by electrostatic interactions. The lack of structural information about thiamin binding proteins for higher organisms remains a bottleneck for understanding the uptake process of thiamin in atomic detail. This review includes recent data on thiamin metabolism, related deficiencies and pathologies, and the latest findings on thiamin binding transporters.



I. INTRODUCTION

1. Background. Thiamin (Figure 1) (2-{3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-4-methylthiazol-5-yl}ethanol), known as vitamin B_1, is an essential compound for all living multicellular organisms that was first mentioned in Chinese medical literature 4000 years ago.[1] Thiamin is composed of a thiazole ring and a pyrimidine group, which together make up the sulfur-containing structure of two rings joined by a methylene group (Figure 1). Thiamin is synthesized in bacteria,[2] fungi,[3] and plants[4,5] and is essential in all diets in mammals.[6] It is a crucial molecule indirectly involved in several metabolic steps of the electron transport chain, contributing to the extraction of energy from carbohydrate sources,[7,8] where it acts as a prosthetic group or coenzyme in the conversion of glucose to ATP and in catalyzing the conversion of several carbon skeletons in the Krebs cycle.[9] In these biochemical pathways, thiamin is used as a cofactor in thiamin pyrophosphate-dependent enzymes and participates in the decarboxylation of α-ketoglutarate.[10−12] Thiamin functions as a catalyst in the pentose and hexose monophosphate pathways, as well.[10−12] The respective pathways are dependent on its integration in thiamin diphosphate enzymes. This depicts crucial redox mechanisms that exist in virtually all organisms[13] where the general thiamin-dependent enzymatic activities can be grouped into four different metabolic classes.

In the first class of energy metabolism, the diphosphorylated thiamin type, thiamin diphosphate [TPP (Figure 1)], has a role in the pyruvate dehydrogenase multienzyme complex. Here, the integrated TPP moiety receives electrons in the redox processes conducted by the multienzyme complex, which catalyzes the conversion of pyruvate to acetyl-CoA, where NAD^+ is the oxidizing agent.[12] In the second system of thiamin-dependent carbon-metabolizing enzymes, the pyruvate ferredoxin oxidoreductase system causes the conversion of pyruvate to acetyl-CoA, using three iron clusters (four iron ions chelated by sulfur atoms) during catalysis to oxidize to the substrate CoA in concert with thiamin diphosphate,[14] where bound TPP is a direct acceptor of the two electrons oxidized from pyruvate.[11] In the third energy metabolism system, TPP is a cofactor in the acetyl phosphate-producing pyruvate oxidases, where organisms like the Lactobacillae, including *Lactobacillus plantarum*, apply this multienzyme system, as they are unable to synthesize heme groups for oxidative phosphorylation.[12] In these enzymes, the incorporated TPP interacts with Mg^{2+} and FAD as cofactors to produce acetyl phosphate.[15] TPP is found also in a fourth case of energy-metabolizing enzymes in *Escherichia coli*, in which the phosphate-independent pyruvate oxidases co-bind ubiquinone-8 and convert pyruvate to acetate via the reduction of ubiquinone-8 where TPP acts as a cofactor.[16] In addition to being a central component in energy metabolism, thiamin pyrophosphate is also vital in several other functions, such as in proteins performing oxidative decarboxylation,[17] activating conduction channels in

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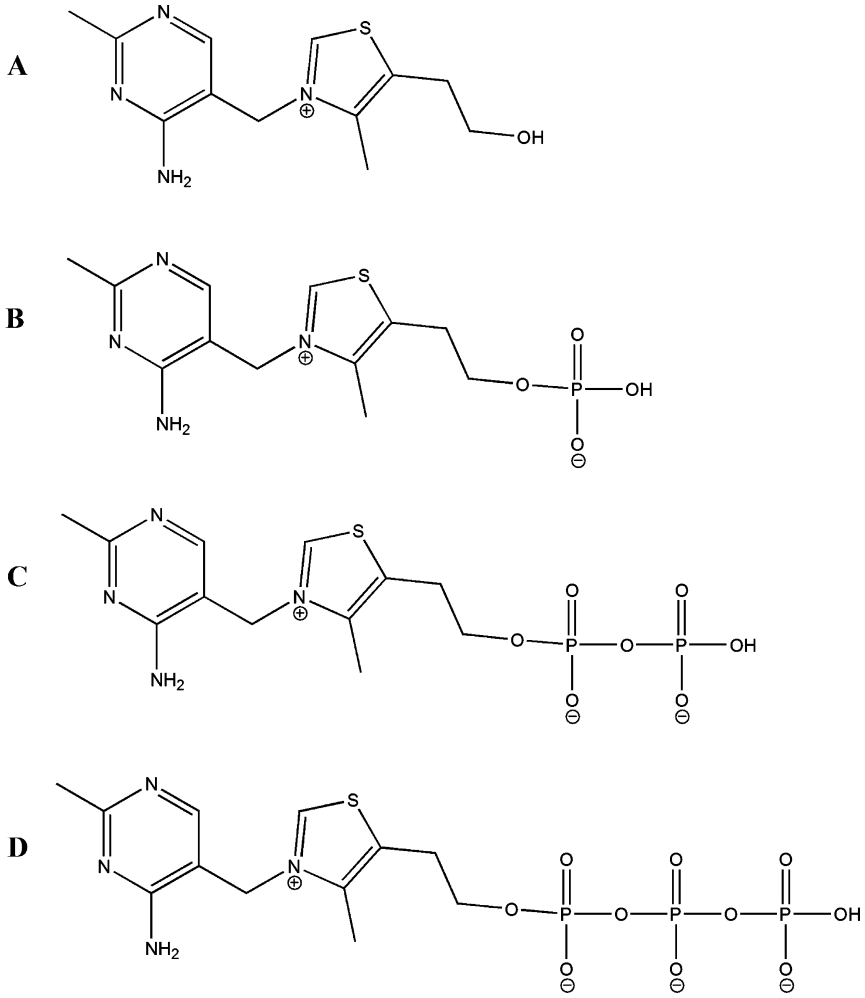

Figure 1. Structures of thiamin forms at neutral pH.[168] (A) Thiamin ion (T^+), which is found in various food sources and is essential for all animals. Thiamin derivatives: (B) monophosphate (TMP), (C) pyrophosphate (TPP), and (D) triphosphate (TTP).

neuroblastomal cells,[18] and also functioning in the intestinal lumen as phosphate ester forms, as reported in a recent study.[19]

2. Thiamin's Role in the Brain and Nervous Tissue. Thiamin is pivotal in many enzymatic processes involved in brain development, brain function, maintenance, and interneuronal communication.[20,21] Its role in these contexts is in the generation of acetylcholine.[22] Thiamin is in cationic form (T^+) at physiological pH (Figure 1) and acts on the nerve-action potential, affecting membrane conductance and neuronal signal transmission[23−28] and stimulating the function and activity of the cerebellum and spinal cord.[29,30] T^+ is also active in the conduction of nerve signals through synapses and has been suggested to serve as a shuttle molecule across biological membranes, given its positive charge.[23] Thiamin is also involved in nerve tissue repair and nerve signal modulation, by virtue of its recently proven role in the modulation of thermal hyperalgesia.[31,32] Regulation of nerve signal transmission has been demonstrated in mouse models,[33] furthermore emphasizing the role of thiamin in neuronal activity. Thiamin furthermore reduces the concentrations of RNS (reactive nitrogen species) but not ROS (reactive oxygen species) in neurons during neurotoxicological events.[34,35]

For transfer across membranes, T^+ is phosphorylated extracellularly to the neutral form thiamin monophosphate (TMP) (Figure 1) and transported to the cytosol with TMP-specific transporters,[36] although it has also been hypothesized that passive diffusion of the original cation form (T^+) across proton channels is a viable route of transport, as well.[37] The phosphorylation of the cationic form to the neutral TMP is a critical prerequisite for the transport across membranes, particularly in the case of proliferating neurons[38] and in blood cells. The monophosphorylated form of thiamin, TMP, is an otherwise inactive form of thiamin and can be considered to represent a "thiamin-supplying potential" located outside the cell that serves as a substrate for the generation of active thiamine forms (Figure 1). The diphosphorylated form, TPP, participates in several metabolic functions (as described above in energy metabolism) and is the most abundant form of thiamin in the body (>80%).[19,39] TPP may be converted further to thiamin triphosphate [TTP (Figure 1)] in some cases, depending on the localization and function in the cell.[36] TTP encompasses 5−10% of the thiamin concentration in the body[18,36,38,40] and plays a role in membrane conductance properties through activation of chloride channels[18] and functions as a phosphate group donor in phosphorylation reactions in energy metabolism.[27,28,41] In conclusion, thiamin functions predominantly in one of its phosphorylated forms, mainly TTP and TPP,[6,17,24,26,28,30] while the main role of T^+ is being an antioxidant.[34,35,43,44]

3. Thiamin in the Immune System. Thiamin is involved in several functions in the immune system, through its regulation

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Table 1. Biochemical Functions of Thiamin in Various Cellular Environments

type	localization	function	comments	refs
prosthetic group	cytosol	maintain the pH balance in the cytosol	component of oxido-reductases and dehydrogenases	7, 8, 40
antioxidant	cytosol, ECM	antioxidative function	regulator of ROS and RNS levels	34, 35
antioxidant	cytosol	genetic regulator	regulator of peroxidase function during antioxidative reactions	34, 35
prosthetic group	neuronal cytosol	maintain intraneuronal activity	generates acetylcholine	22
free molecule (T^+, TMP, TTP, TPP)	all cells	phosphate source	acceptor and donor of phosphate groups in all cells	19, 39
organic cation (T^+)	brain and nerve tissue	charge supplier	a positively charged molecule that is used to equilibrate the net charge of cells, via the action of ion channels	37, 85
organic cation (T^+)	nervous tissue, brain	nerve signaling factor	conductance modulator during neuronal activity	23−28

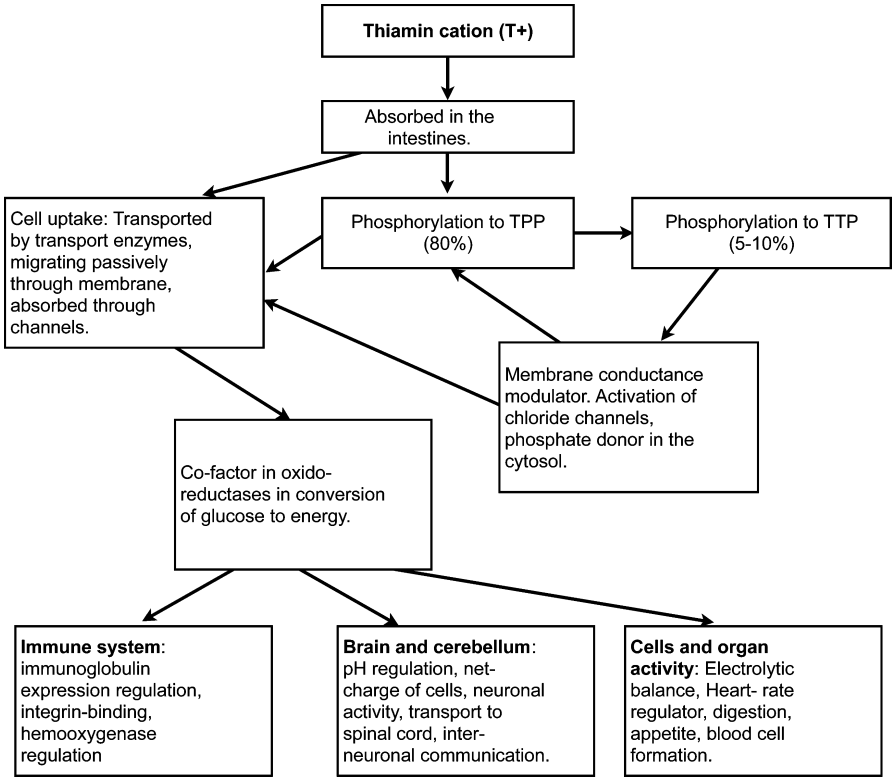

Figure 2. Physiological functions of thiamin in tissues and organs.

and activation of immune cells and proteins of the immune system. Here, thiamin is closely involved with hemin-dependent oxygenases, whose action in the immune system affects the release of the specific members of the ICAM (intracellular adhesion molecule) proteins.[45] ICAMs bind integrins during immunological reactions,[46] which in turn affect T-cell activity and other immune system cells during immunological responses.[47] Thiamin furthermore plays a vital part in the immune system's reactivity, by its role in the expression of immunoglobulins and the CD40L-mediated immune and inflammatory responses in brain cells.[48−50] It exerts antioxidative effects on neutrophil cells, by protecting the sulfhydryl groups on the cell surfaces from oxidation,[43] and promotes the motility of the cell through its antioxidative role via an unknown mechanism.[44] Thiamin also plays a protective role on macrophages by suppressing the oxidative stress-induced activation of NF-κB (necrosis factor), which induces macrophages to release a variety of inflammatory markers like cytokines, chemokines, growth factors, and immune-responsive proteins.[51] The action of thiamin in regulating NF-κB expression in tumors has also recently been demonstrated, where it has been shown to have an inhibitory effect on the growth of tumors in rat models.[52]

Thiamin is also an anti-inflammatory factor preventing recurring inflammation and regulating the expression of inflammatory agents.[53] Interestingly, thiamin has an important effect on the activity of the p53 suppressor protein, a key protein in the cell cycle switch mechanisms that operates during proliferation, apoptosis, and cell death.[54] Thiamin has been shown recently to inhibit p53 intracellular activity, during re-replication and apoptosis.[55,56] A role for thiamin in inflammatory responses has been demonstrated in the context of immune cell reactivity in the brain.[57] Also, in other biological kingdoms, thiamin plays a role as an activator of disease resistance,[58] by triggering the defense responses of the plant immune system through Ca^{2+}-dependent intercellular signaling.[59] In yeast species such as *Schizosaccharomyces pombe*, thiamin-specific promoters are present in the DNA for the expression of antigens

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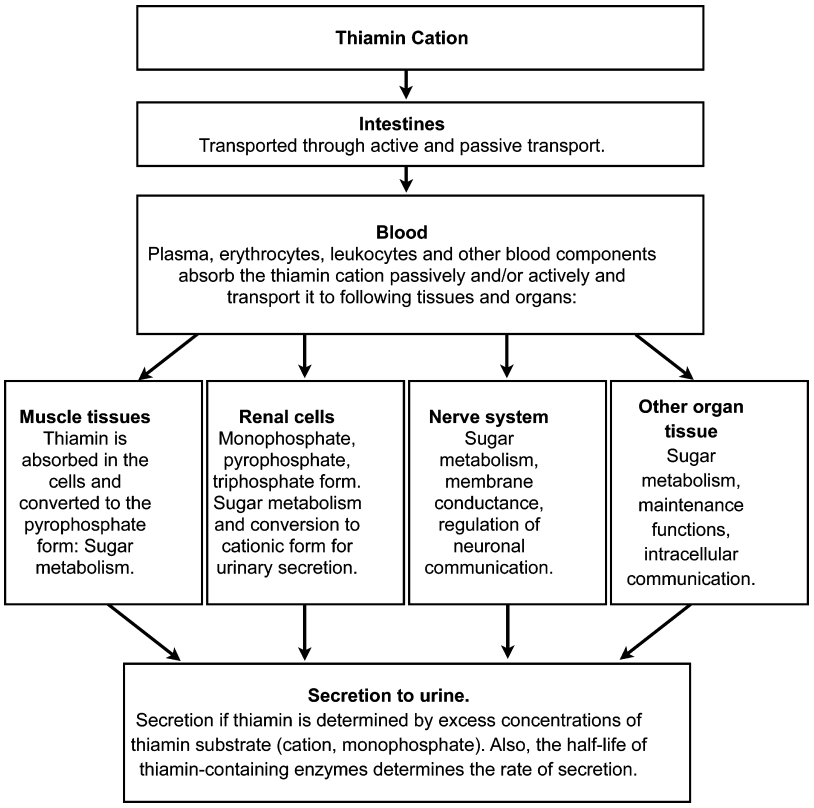

Figure 3. Physiological fate of thiamin in the body.

against viral infections, where thiamin is the sole activator and inducer for antigen production during immune reactions.[60]

4. Thiamin Pathologies. The deficiency of thiamin leads to a series of conditions in animals related to complications with nerve and brain system development, energy metabolism, digestion, cell proliferation, and cell cycle activity in many processes in the body. Through its suggested role in the regulation of lactic acid concentrations,[61] thiamin maintains the cell activity and metabolism necessary for organ function, tissue regeneration, and neuronal activity.[9,62] If a thiamin deficiency exists, the lactic acid concentration increases, leading to a decrease in pH and cell death.[40] The results of this pathology have several implications in humans and animals, including learning and memory deficits,[63,64] a lack of appetite,[65] bradycardia (changes in heart rate and blood pressure),[66] motor cortex activity and coordination defects,[67,68] and other neuromechanical defects.

Thiamin has a function in the uptake of serotonin, which in turns affects the activity of the cerebellum, the hypothalamus, and hippocampus.[69] Its deficiency leads to a series of neurochemical dysfunctions and specific neurodegenerative disorders.[70] Thiamin is involved in the uptake of GABA (γ-butyric acid), and a lack of this neurotransmitter leads to cell abnormalities affecting the cerebellum activity.[71] A further role for thiamin is in the maintenance of the structure and activity of cerebellar cells, which, if depleted for thiamin results in damage to the thalamic regions displaying clear lesions in the cortex, stem, cerebellar region, and hippocampus structures,[18,72,73] causing cognitive and motor impairment symptoms.[74] The deficiency of thiamin also causes detrimental effects on microglia brain cells by its weakened regulation of peroxidase enzymes, resulting in higher oxidative stress in neural systems and nervous tissues in affected animals.[75]

Cases of thiamin deficiency have been recorded throughout history. Thiamin was in effect rediscovered in the early 18th Century in western colonies, when native tribes showed symptoms of *beriberi,* or malnourishment conditions with vitamin deficiency symptoms.[76] More recently, mass deaths in wild birds in Sweden have been accredited to thiamin deficiency, where animals that were found paralyzed showed signs of recovery after injections with thiamin.[77] Similar cases were registered in pigeons in 1940.[78] Also, lake trout (*Salvelinus namaycush*) was affected by thiamin deficiency due to a diet based on Alewife (*Alosa pseudoharengus*) that contains a significant quantity of thiaminase, a thiamin-degrading enzyme.[79] After the Alewife population collapsed in Lakes Huron and Michigan, the lake trout recovered. These (alleged) cases of thiamin deficiency show potential connections to one another; however, the debate in the literature suggests that other causes, such as botulism, should be considered as an explanation for mass deaths, as well.[80−83]

Thiamin is a key indicator for the viability of species and is an indispensable factor for many processes in animals, plants, bacteria, and fungi. An overview of the vast array of functions of this vitamin is provided in Table 1 and Figures 2 and 3, with an emphasis on the biochemical functions, localization, and modes of action of thiamin.

II. THIAMIN TRANSPORT

1. Uptake, Distribution, and Metabolism. The first study of thiamin uptake and transport showed that thiamin is actively transported across the blood−brain barrier by carrier proteins.[84] A selective binding action of the transporter proteins, accompanied by a simultaneous passive diffusion of T^+ across the membrane, was demonstrated, and the transport of thiamin across the brain−cell membrane was found to proceed at a rate

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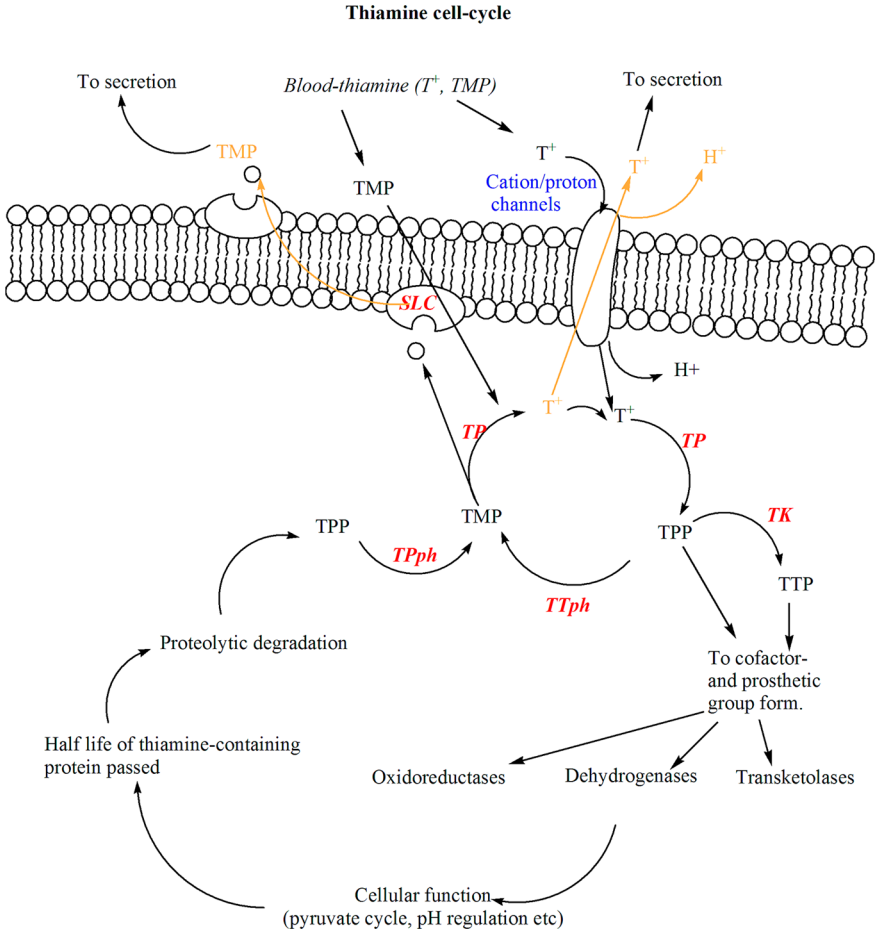

Figure 4. Thiamin cycle. From the inactive cation (T^+) to the functional cofactor (TPP, TMP, and prosthetic group). Labels and arrows in orange indicate secretion paths. Enzymes labeled in red, bold, italic type in abbreviated format: TP, thiamine pyrophosphokinase; TK, thiamine kinase; TPph, thiamin pyrophosphatase; TTph, thiamin triphosphatase; SLC, solute carrier transporters. Enzymes are described in refs 42, 94, 97, 105, 108, and 169−172.

similar to that of the uptake of amino acids in cells and to be interdependent with calcium concentration.[84] These findings suggest a potential role of T^+ ions in the exchange of net charges from the cytosol to the extracellular matrix, as suggested previously.[85] Further studies showed that thiamin was absorbed and distributed to organs through a mechanism that is very similar to Na^+ uptake, and that the process is connected to the electrolytic balance of Na^+ and K^+ ions.[38] Also, T^+ levels affect the activity of ATPase channel proteins, which have been suggested to be potential transporters of thiamin.[62,86] Other groups that studied the relationship between transmembrane channels and thiamin reported results that indicate that thiamin transport occurs simultaneously with proton transport and that both are inhibited equally by selective inhibitors.[37] A recent review reports, however, that the uptake of vitamin B is more complex and depends also on multispecific vitamin transporters,[1] which are described in detail below.

2. Intestinal Uptake and Distribution through the Blood. In animals, the primary site for uptake of thiamin in the body is at the intestinal walls where it is absorbed as T^+ and transferred to the blood.[42] This transfer to the blood encompasses erythrocytes (~75% of blood thiamin content), plasma (~10% of blood thiamin content), leukocytes (~15%), and platelets.[87] The active and passive uptake of T^+ in the blood is independent of temperature, pH, and sodium concentration and is poorly inhibited by a range of other substrates.[88] This transport is exerted by organic cation transporter proteins (OCTs) and the alkaline phosphatase transport system (ALP), which are multispecific proteins with affinity for organic ions.[10,89] Some of the OCTs specifically bind thiamin, in particular OCT1 and OCT3,[90−93] which take up thiamin in the intestines within a 50-fold concentration range (10−500 μM)[88] and release it to the blood, for distribution to organs and tissues. The distribution of thiamin to the body from the intestines occurs within a time frame of 1−2 h in humans in the two main forms of thiamin, T^+ and TMP,[94,95] with half-lives of 96 and 300 min, respectively.[94] Approximately 53% of thiamin is eventually secreted to the urine,[39] while 47% is localized and processed within 6−12 h in humans and distributed from the blood to the tissues and organs.[87,94]

The thiamin concentration determines the type of uptake mechanism in the intestines. At high concentrations ($[T^+] > 2$ μM), thiamin passes through the intestinal membranes by spontaneous diffusion.[96,97] The spontaneous passage of T^+ proceeds through an exchange with protons, which allows T^+ to migrate through proton channels driven by a polarization of the net charge inside and outside the cell[97] (Figure 4). When the intestinal concentration of T^+ is <2 μM, however, T^+ is actively transported by OCTs[90−93] and ALPs.[89] Recent studies show, however, that both active transport and passive transport of T^+ occur simultaneously in the same tissues and organs.[19,98] For other forms of thiamin, TPP, and TTP (which are normally not

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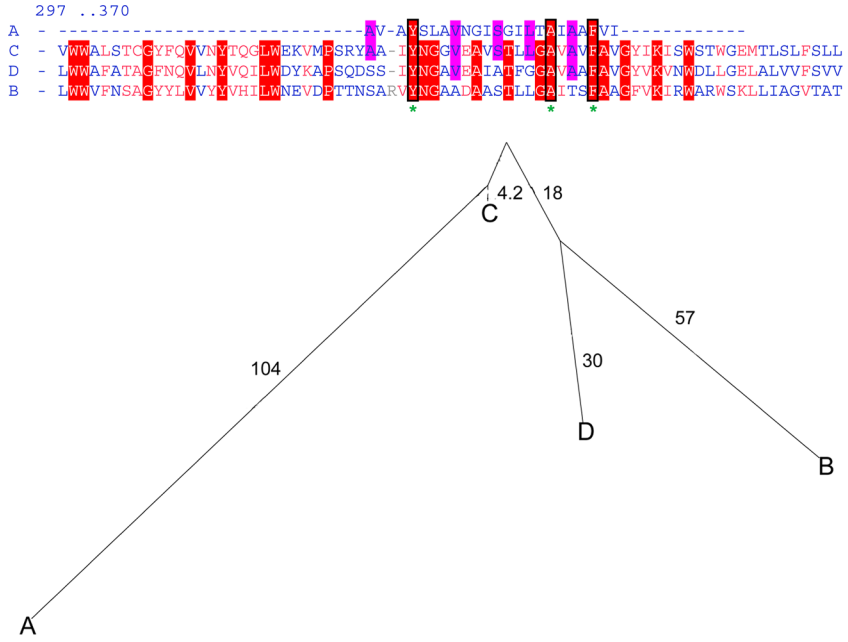

Figure 5. Fragment from the phylogenetic and multiple-sequence analysis of *L. lactis* ECF thiamin binding protein ThiT (PDB entry 3RLB) (A), SLC19A1 (GI: 13111762) (B), ThTr1 (GI: 27734719) (C), and ThTr2 (GI: 13376856) (D). The multiple-sequence alignment shows that the sequence of the thiamin binding motif from the crystal structure ThiT[126,127] contains a conserved region with the three other thiamin transporters. This thiamin binding motif is most similar with the ThTr1 transporter, which is present in all tissues and organs in the body and transports the thiamin cation across the membrane. Interestingly, ThTr2, which has a specific expression pattern in the intestines, has a more distant phylogenetic relationship with respect to ThiT and ThTr1. Considering phylogentic equivalents (numbers adjacent to lines), ThTr1 is more similar in its thiamin binding motif to SLC19A1 than to ThTr2. The phylogenetic analysis was performed with the AllAll multiple-sequence analyzer at ETH-Zurich.[173]

available extracellularly[95,99]), the transfer across membranes is conducted after conversion back to the TMP and T^+ forms.[94,99] Once delivered to the cytosol, thiamin is metabolized to all three forms of thiamin (Figure 1) in the tissues and organs, as well as the cationic form[100,101] (Figure 4).

3. Cross-Membrane Transport to the Cytosol. The first studies of routes of transport to the cytosol[98] suggested that the delivery of thiamin from the blood to cells in tissues and organs required pyrophosphokinase proteins (T^+ pyrophosphokinase),[102] which facilitate the extracellular phosphorylation to TMP before delivery to the cytosol.[10] Earlier studies argued that thiamin was transferred to the cytosol via phosphokinase transporters[99] (Figure 4). However, more recent work provides greater details about the routes of transport of thiamin to the cytosol from the extracellular matrix. New families of proteins have been mapped in the human genome projects,[103,104] and in particular, three distinct thiamin-specific transporter families were found.[89,105,106] The first group consists of the solute carrier family thiamin-specific transporters (SLC19 proteins) that are localized in various tissues, with high levels of expression in muscle and heart tissue,[105] and are selective for the various forms of thiamin (TMP, TPP, and TTP).[107−109] The second group of thiamin transporters consists of specific members of the human extraneuronal monoamine transporter proteins (hEMT, including the OCTs) that are active in the transport of amine forms of nutrients and neurotransmitters, including thiamin, to the neurons.[99] The third group consists of the ALPs,[89] which are metalloenzymes with affinity for various molecular signaling factors, such as neurotransmitters and ionic metabolites.[110] Few other transporter families are known to be involved in the transport of thiamin in mammalian cells;[111] however, the SLC21 organic anion transporters are known to transport anionic forms of folates across the membranes (and phosphorylated thiamin forms are frequently negatively charged during metabolism).[112] Conclusively, a combination of organic cation transporters, the alkaline phosphatase transport system, pyrophosphokinases, and solute carrier proteins, conduct the total transport of thiamin from the intestines to the cytosol. These proteins are described in detail in the following sections.

III. THIAMIN-TRANSPORTING PROTEINS

1. SLC19 Thiamin-Specific Transporters. Thiamin and folates are transported by a subgroup of the SLC (solute carrier proteins) transporter protein superfamily, named the SLC19 proteins.[111,113−116] The thiamin-specific members of the SLC19 proteins are SLC19A1, SLC19A2, and SLC19A3, known as the reduced folate carrier (RFC), thiamin transporter-1 (ThTr1), and thiamin transporter-2 (ThTr2), respectively.[117−121] The central difference between these enzymes is the localization and function. SLC19A2 and SLC19A3 transport T^+ across cell membranes; however, they do not bind folates (T^+ carries a positive charge at physiological pH, while folates carry negative charges). SLC19A2 and SLC19A3 are facilitated transporters belonging to the MFS (major facilitator superfamily).[122] The folate-specific enzyme SLC19A1 is a transporter of the TMP, TPP, and TTP forms of thiamin, and not T^+,[19,107,108] and functions as an anion exchanger.[119]

The cation carrier ThTr1 is localized in skeletal muscles[105] systemic tissues,[119] whereas ThTr2 is expressed in the intestines,[119] predominantly in the first section of the small intestine, the duodenum.[106] Their thiamin binding affinity is different, where the first has an influx constant K_t of 2.5 μM, while the second operates in the range of 25 nM,[119] showing that ThTr2 has a particularly high specificity for thiamin in the context of generic absorption in the intestines. Their difference in binding affinity seems to be related to their attributed tissues of

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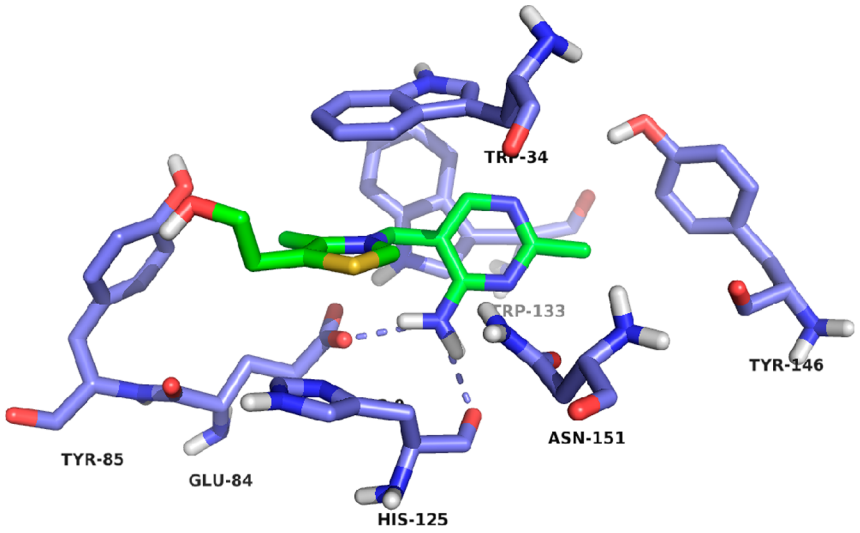

Figure 6. Binding pocket for the thiamin cation (green) from the crystal structure of ThiT protein from *L. lactis* (PDB entry 3RLB).

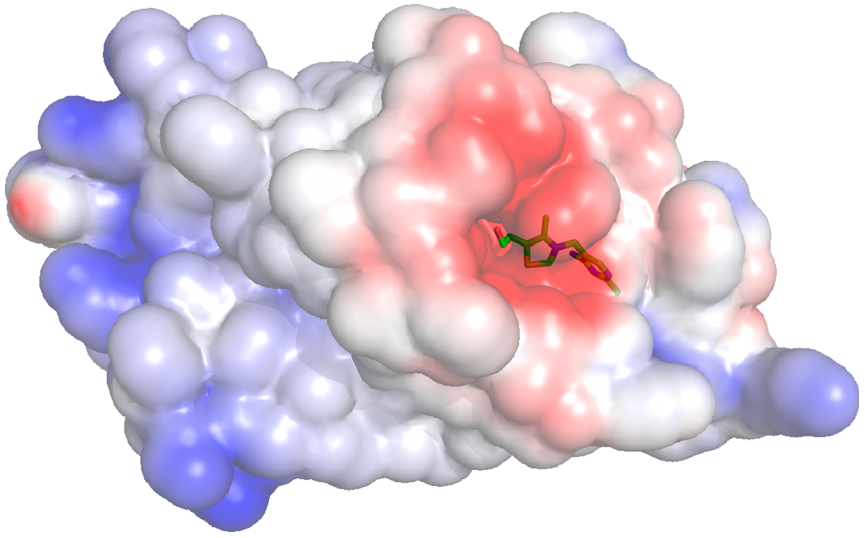

Figure 7. Molecular surface of the ThiT monomer from *L. lactis* (PDB entry 3RLB) with the thiamin analogue bound. The coloring is according to the electrostatic potential on the solvent accessible surface using the APBS interface[174,175] in PyMOL. It highlights the negative electrostatic potential in red (note that thiamin is positively charged).

expression, and ThTr2 is therefore the thiamin transporter in animals with direct contact to nutrients and ions from the diet.

2. Structural Features of Thiamin Binding Transporter Proteins. All three SLC19A proteins are transmembrane proteins of ~500 amino acids.[116] They share ~50% homology[105] (ThTr1 and ThTr2 are 48% identical, and both are ~40% identical to SLC19A1) and encompass 12 putative transmembrane regions of approximately 20 amino acids each and a large cytoplasmic loop between TM6 and TM7.[105,116] Regrettably, no three-dimensional atomic structures of any of the three SLC19A proteins are available, or of their binding domains, making structure-based studies[123−125] of binding of thiamin to the SLC19A proteins difficult.

Fortunately, one member of the ABC cassette transporter superfamily has been crystallized from the bacterium *Lactococcus lactis* with its atomic structure determined,[126] namely the ECF-type thiamin binding factor (ThiT). This structure corresponds to the S component of the complex, which is responsible for substrate binding and associates with the ECF module to form a functional ATP-dependent transporter.[126] The ABC cassette transporters make up a parental family of the SLC19 proteins, and phylogenetic analysis shows a distant but notable relationship with the three thiamin transporters of the SLC19A family (Figure 5). This analysis shows that the sequence of the crystal structure of ThiT and the three SLC19A proteins are particularly familiar in the segment between Ala143 and Ile165, which is the binding motif that binds thiamin at nanomolar affinity (12 nM),[127] a specificity that is similar to that of ThTr2 in animals and humans (25 nM). The binding of thiamin in ThiT is facilitated by several residues, where Glu84, His125, Trp133, Tyr146, and Asn151 (Figure 6) stabilize the pyrimidine moiety.[126] The phylogenetic analysis of the binding sites of SLC19A1, SLC19A2, SLC19A3, and ThiT (Figure 5) shows that in this shared region the Tyr146 residue is conserved in all four proteins. Furthermore, one can observe that Asn151 in ThiT is mutated to glutamic acid (Glu151) in the ThTr1 and ThTr2 transporters and to an aspartic acid in the SLC19A1 anion binder. This subsite interacts in the ThiT with N2 in the pyrimidine part of T^+ through a hydrogen bond (Figure 6). Two other residues of interest are Ala159 and Phe163, which are

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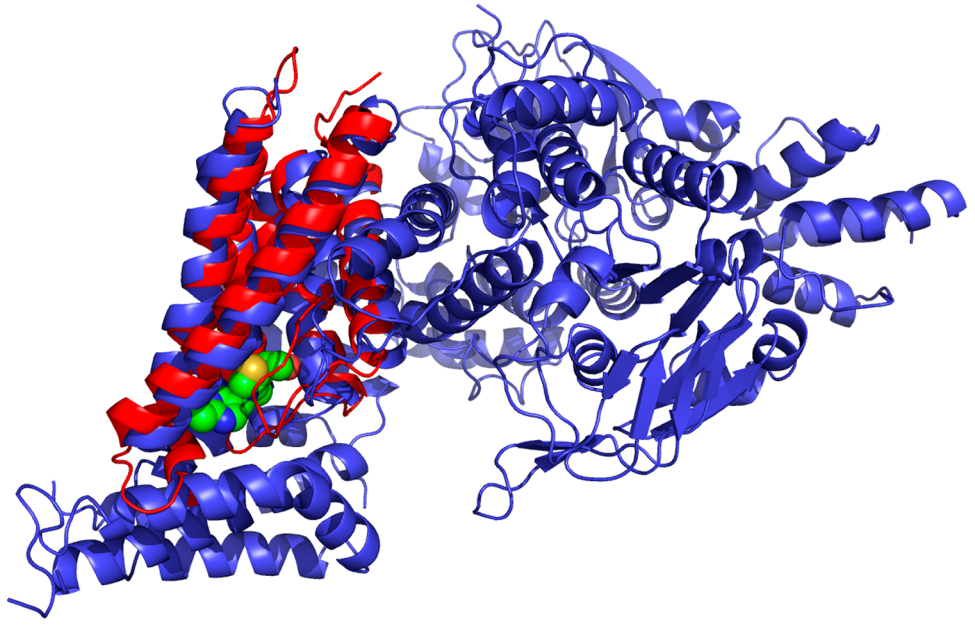

Figure 8. Superposition of thiamin binding proteins ThiT (PDB entry 3RLB, red) and the bacterial energy-coupling factor transporter (PDB entry 4HZU, blue) made using TopMatch.[176] Thiamin is shown in atomic representation.

conserved among all proteins. This putatively indicates structurally important sites for sustaining the architecture of the thiamin binding pocket relevant to thiamin binding transporters. Furthermore, Trp133, which is located outside the motif, is defined in the Trp-Trp-Ala motif for the human thiamin transporters (ThTr1 and ThTr2), while in the ThiT, this motif is Phe-Trp-Ser, displaying the conserved nature of Trp133 in all four proteins.

Tyr85 is another crucial subsite in ThiT, which is involved in binding the hydroxyl group in the thiazole part.[127] This residue is part of the Leu-Glu-*Tyr*-Leu-Val motif, and the corresponding motifs in the human thiamin transport 1 and 2 proteins are Leu-Asn-*Tyr*-Val-Gln for ThTr2 and Val-Asn-*Tyr*-Thr-Gln for ThTr1 (Figure 6), suggesting this as another subsite involved in binding thiamin. Trp34 in ThiT was predicted to be "shielding" the thiazole moiety,[126] but it is not found in the SLC19A proteins in the alignment. In Figure 7, the surface of ThiT is plotted to show the binding pocket in molecular surface, colored by electrostatic potential. This figure, interestingly, displays a large negative (red) patch functioning to attract the positively charged thiamin substrate (T^+).

Interestingly, a recent study by Majsnerowska et al. using electron paramagnetic resonance spectroscopy experiments and molecular dynamic simulations indicated that extracellular loop 1−2, where Trp34 is located, undergoes a conformational change and is involved in thiamin binding.[128] These results suggest that extracellular loop 1−2 functions as a lid on the thiamin binding site to allow and/or occlude the access of substrate from the extracellular side of the membrane to the binding site.[128] Further MD simulation of the binding process of thiamin to ThiT might help in improving our understanding of the role of extracellular loop 1−2 in more detail and the functions of the crucial residues Ala159 and Phe163 identified in the sequence alignment. Figure 7 strongly suggests that the binding process is guided by electrostatics.

As for other group II ECF transporters, the transport of thiamin by ThiT (S component) is driven by the energizing module (EcfT and two NBDs, EcfA and EcfA′), which binds and hydrolyzes ATP to ADP for the rotation of the axis of the domain, resulting in the transfer of the thiamine molecule across the membrane.[127] A quaternary nucleotide-free ECF transporter from the *Lactobacillus brevis* crystal structure reported recently suggests that the S component lies horizontally coaxially with the membrane while bound to the T component.[129] This result suggests that the S component undergoes a rotation in the membrane induced by the interaction with its substrate. Although the level of homology of five kinds of known S components is <20%, their root-mean-square deviations are <3.5 Å[130] and the energizing modules of *L. brevis* and *L. lactis* share a high level of sequence homology.[129] We therefore propose that the transport mechanism of thiamin in bacterial systems could be a useful model for studying thiamin transport in mammalian systems.

The loop in the ECF S component, corresponding to extracellular loop 1−2 in ThiT, is in contact with both the membrane and the ECF T component based on the ECF crystal structure described by Wang et al.,[129] emphasizing the importance of the membrane environment to the uptake of thiamin that was found by EPR spectroscopy.[128] The energizing module domain and the cytoplasmic domain of ThiT cannot be identified in the ThTr1 and ThTr2 proteins. Although the binding site is semiconserved among the aligned candidates (see the alignment in Figure 5 and binding site structure in Figure 6), there is a significant difference in the primary structure. The analysis by Zhang shows that the structural differences are minor,[130] however, and the known structures can directly overlap (Figure 8). Given the similarity between mammalian sequences around the binding site with ThiT and the structural overlap between ThiT and the energy-coupling factor transporter, it seems possible that the binding motifs in eukaryotic transporters are similar to the bacterial ones.

3. Human Extraneuronal Monoamine Transporter Proteins (hEMTs). The human extraneuronal monoamine transporters make up another group of proteins related to thiamin transport, which transport monoamine forms of various neurotransmitters through the body, with particular expression

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in the brain and the nervous tissue.[131] The neurotransmitter transporters are membrane-bound channel proteins, with several transmembrane regions[132,133] that perform a crucial function in intercellular communication by transporting organic cations to and from the cytosol, equilibrating the net electric charge of the cells (electrogenic transporters).[134]

A subfamily of the EMTs that are relevant to thiamine transport consists of the non-neuronal transporters, members of the solute carrier protein 22 superfamily (SLC22A), which are expressed in most tissues and organs, such as kidney, liver, intestines, and glands.[135,136] The non-neuronal transporters include two subfamilies, the OCT1, OCT2, OCT3, and OCT6 transporters (organic cation transporters) and the newly discovered OCT variants (OCTN1 and OCTN2).[133,137−140] These members are ∼550 amino acids in length and have high levels of sequence homology, with the highest degree of conservation among sequences of the OCTN transporters. Both subfamilies OCT and OCTN have 12 transmembrane domains and a minimum of one exodomain and one endodomain for substrate binding and release.[137−140]

The organic cation transporters (OCTs) are polyspecific proteins that bind various substrates simultaneously along with thiamine, such as tyramine, nor-adrenalinine, dopamine, or histamine, and these proteins are expressed in various tissues.[131,137,138,140,141] The binding affinities of OCTs are quite different from one another, and the transport efficiencies differ depending on the substrate, as well. Gründemann et al.[142] estimated the transport efficiency of OCT1 using an approach based on the concentration equivalents from an intracellular water space of 6.7 μL/mg of transporter protein[143] in addition to including a transfection efficiency of 100% for the positive control, at equilibrium a 15-fold accumulation of MPP^+ (1-methyl-4-phenylpyridinium) relative to medium. OCT1 binds, for instance, creatinine with a 10% efficiency and phenformin with a 70% efficiency, while OCT2 binds adrenaline with a 100% efficiency and seretonin with a 5% efficiency.[131,142] The specificity of the members of the second subfamily, OCTNs, is less known, although OCTN2 has been shown to bind and transport carnitine in the intestines.[133,140]

OCT1 and OCT3 are two OCTs found particularly in the intestines[91−93,144] and bind thiamine, tyramine, dopamine, clonidine, progesterone, and other organic cations of natural as well as synthetic origin.[88] The striking feature of OCTs is that they can bind several substrates simultaneously through the antiporter mechanism,[88,145] in agreement with Ugolevs theory, where "polysubstrate digestion-ingredients potentiate or inhibit each other's transport".[146] Conversely, the antiporter mechanism is the transport of multiple cations bound through a trans-stimulation mechanism where one cation increases the binding affinity of the second cation; e.g., serotonin enhances thiamin binding.[88,145] In this process, some substrates are selectively bound in the presence of Na^+ ions (e.g., carnitine), while others (thiamin) do not require Na^+ during transport.[133,140,147] The multiple-binding mode of OCTs and OCTNs has been confirmed by Wu and colleagues, who have shown the presence of several binding sites in OCTN2 through mutational studies.[140] The transport of thiamin by OCTs and OCTNs is thus more complex than that in thiamine transporter proteins (see the previous section) and includes the trans-stimulating mechanism as determined for serotonin and other neurotransmitters and hormones.[147] Interestingly, a series of compounds are known to inhibit and prevent the transport of thiamin, by competitive inhibition.[88] The mechanism of inhibition is, however, not completely understood as some compounds inhibit uptake (such as progesterone) while others stimulate the uptake of thiamin (such as dopamine) through their simultaneous binding to OCTs and OCTNs.[88]

The exact OCT transporter responsible for transporting thiamin is not known, although it is hypothesized to be OCT3,[88] while in other studies,[148] the OCT member responsible for transporting thiamin has been suggested to be a member of another subfamily of the OCTs, named hMATE2-K, a toxin extrusion protein expressed in kidney and placenta. This protein, which is closely related to the OCTs,[132] is a multitoxin carrier with a broad specificity.[148]

The structural features of the OCTs are poorly mapped. A crystal structure of lactose permease from *E. coli*[149] (PDB entry 2V8N) has been used earlier as a structural homologue of OCT1;[133] however, the mode of binding to thiamine is ambiguous, given that the modeled structures are only fold-recognized and the binding site has not been confirmed for thiamine by any other means such as homology modeling. The OCTs are therefore currently poor candidates for computational studies of thiamin binding, although they present a highly important protein family for structural biology studies by means of X-ray crystallography or nuclear magnetic resonance spectroscopy.

4. Alkaline/Acid Phosphatase Transport System. A third group of proteins that plays a role in thiamin transport are the alkaline/acid phosphatases (ALPs), a group of membrane-bound metalloenzymes that have various physiological functions in organs and tissues.[150,151] ALPs facilitate the transport of thiamin to the cytosol by dephosphorylating them to T^+.[152] ALPs are expressed in the intestines[89,153] in plasma membranes and are particularly active in exchange surfaces;[154] they are activated by fluctuating concentrations of thiamin.[88,155] ALPs are also expressed in the liver, kidney, bone, heart, intestine, and placenta[150] and are subclassified into two groups, the membrane-bound phosphatase transporters and the solute phosphatase transporters.[156,157] As they are involved in the import of metabolites into and export of metabolites from cells, ALPs also transport lipids and phosphates in the urinary tract and in the intestines[158−160] and play a role in thiamin-dependent detoxification mechanisms, as well.[161]

Three major types of ALPs found in animals are the placental ALPs, the intestinal ALPs, and the tissue ALPs. The intestinal ALP variants (iALPs), expressed solely in the intestines,[162] exert their enzymatic function on phosphorylated substrates, including thiamin.[158,163] Kinetic studies of iALPs show that the enzymatic activity of iALP is enhanced by other molecules,[155] indicating their ability to be cross-stimulated by other substrates, including peptides, lipids, alcohols, and hormones.[158,164−166] As shown in structural studies of ALPs, ALPs have two active sites, located some 30 Å from one another, and the enzyme functions as a dimer with six metal ions (Zn^{2+} and Mg^{2+})[164] (Figure 9).

The binding site of ALPs has not been related structurally to thiamin binding,[164,167] although expression studies show a thiamin phosphate processing function (see above). Nevertheless, the binding site of ALP encompasses Asp101, Ser102, Ala103, and the metal triplet, which consists of two Zn^{2+} ions and one Mg^{2+}.[164,167] The role of the Zn^{2+} ions is to coordinate the phosphate moiety of the substrate, which is processed by Lys328, Arg166, Asp101, and Ser102 during hydrolysis[164] (Figure 9). Unfortunately, no other structural features of other sequences are interconnected with this binding site. The features of binding

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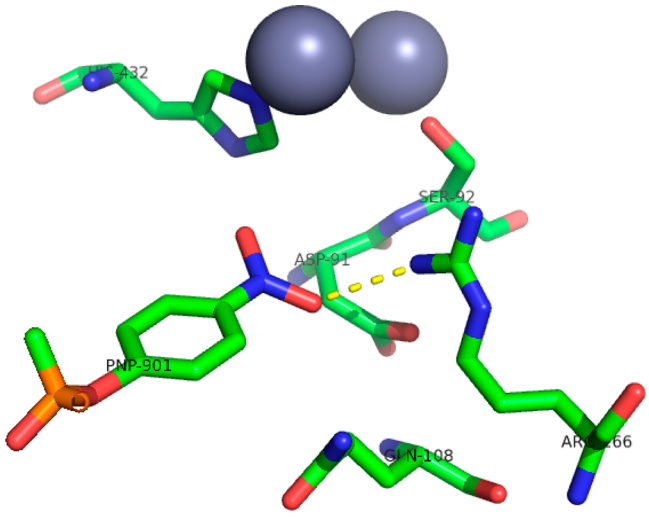

Figure 9. Binding site of alkaline phosphatase in complex with phosphoserine, a substrate analogue, and its two zinc ions.[167] Subsites for substrate binding are given by Arg166, Asp91 (101), and Ser92 (102) (PDB entry 1ZED).

of thiamin by ALPs therefore remain elusive, and further studies would be most helpful.

IV. CONCLUSIONS

Thiamin is an essential component in diets and a crucial vitamin for virtually all organisms. A deficiency of this vitamin causes increased levels of lactic acid and reduced concentrations of several cellular substrates that are generated from TPP-dependent enzymes. As a result, metabolic, neurological, and developmental problems may occur, encompassing brain, cerebellar, and neurological dysfunction. To understand the physiological role of this vitamin and other vitamins, the transport routes and mechanisms of thiamin need to be mapped. The major protein families involved in thiamin transport in these physiological functions are site-specific (e.g., specific OCTs and SLCA19 are expressed in the intestines) and encompass mainly the hEMTs, SLCA19, and ALPs, which are membrane proteins. So far, only bacterial proteins involved in thiamin binding and transport have been characterized structurally, making thiamin binding studies for mammalian systems challenging. However, there are sequence similarities between bacterial and eukaryotic proteins, in particular around the substrate binding site that displays a motif that is conserved among several members of the SLCA19 family and the bacterial ThiT protein. This suggests that further investigations of thiamin binding dynamics could be conducted using ThiT as a template. This review highlights furthermore that ALPs have also at least one member with potential structural features that can be used to understand thiamin binding at the structural level. OCT transporters are members of the hEMT superfamily that represent an elusive group of proteins with a broad specificity for ionic substrates. Structural biological investigations of OCTs would be highly interesting as OCTs use the antiporter mechanism for transporting thiamin across the intestinal membranes. A structural elucidation of this mechanism would significantly enhance the scientific understanding of thiamin uptake and transport. Many details of thiamin metabolism, uptake, and transport have yet to be unraveled, and hence, this represents an important field of research with direct relevance to diet, nutrition, and disease development in humans and animals.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

ALP, alkaline/acid phosphatase transport system; EPR, electron paramagnetic resonance; ECF, energy-coupling factor; hEMT, human extraneural monoamine transporter; ICAM, intracellular adhesion molecule; GABA, γ-butyric acid; MD, molecular dynamics; MFS, major facilitator family; NF-κB, necrosis factor; OCT, organic cation transporter; PDB, Protein Data Bank; RFC, folate carrier; RNS, reactive nitrogen species; ROS, reactive oxygen species; SLC, solute carrier transporter; T^+, thiamin cation; TK, thiamin kinase; TMP, thiamin monophosphate; TPP, thiamin pyrophosphate; TPPh, thiamin pyrophosphatase; TTP, thiamin triphosphate; TM, transmembrane; ThiT, thiamin binding factor; ThTr1, thiamin transporter-1; ThTr2, thiamin transporter-2.

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