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Contributed Mini Review

Conformational change of organic cofactor PLP is essential for catalysis in PLP-dependent enzymes

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Pyridoxal 5′-phosphate (PLP)-dependent enzymes are ubiquitous, catalyzing various biochemical reactions of approximately 4% of all classified enzymatic activities. They transform amines and amino acids into important metabolites or signaling molecules and are important drug targets in many diseases. In the crystal structures of PLP-dependent enzymes, organic cofactor PLP showed diverse conformations depending on the catalytic step. The conformational change of PLP is essential in the catalytic mechanism. In the study, we review the sophisticated catalytic mechanism of PLP, especially in transaldimination reactions. Most drugs targeting PLP-dependent enzymes make a covalent bond to PLP with the transaldimination reaction. A detailed understanding of organic cofactor PLP will help develop a new drug against PLP-dependent enzymes. [BMB Reports 2022; 55(9): 439-446]

ORGANIC COFACTOR PLP

Thousands of cellular biochemical reactions are mainly carried out by enzymes, which are proteins with or without organic or inorganic cofactors (1, 2). Although cofactors provide complementary functions to proteins through their diverse non-amino acid structural motifs, they are believed to be rigid, passive, and too simple to have direct roles in catalysis. It is reported that catalysis is presumably performed by the proteinaceous component of enzymes, which have myriad of sequential differences and exhibit frequent conformational changes. However, evidences have suggested that pyridoxal 5′-phosphate (PLP) is representative of a group of catalytic cofactors possessing diverse catalytic roles with concomitant conformational changes.

PLP is the active form of vitamin B6. Approximate 0.5-1.5% of all genetic products in the bacteria, archaea, and eukaryota kingdoms require PLP for their catalytic activities (3). PLP-dependent enzymes catalyze diverse biochemical reactions, including transaminations, β- or γ-carbon eliminations, decarboxylations, aldol condensations, and racemizations (4-6). Since the discovery of PLP in the 1930s (7), PLP-dependent enzymes have been actively studied for their myriad of catalytic activities, which synthesize key metabolites and signaling molecules. PLP-dependent enzymes participate in essential metabolic pathways, making them potential drug targets (8-10).

In PLP-dependent enzymes, PLP directly interacts with and catalyzes reactions with substrates and also reacts with drugs targeting PLP-dependent enzymes (11-16). The high reactivity of PLP, characterized by its complicated intermediate structures with substrates, contributes to the diversity of catalytic reactions performed by PLP-dependent enzymes. Among the catalytic steps performed by PLP, transaldimination (switching of the internal aldimine to external aldimine and vice versa) is particularly conserved and essential.

Current drugs targeting PLP-dependent enzymes target mostly the transaldimination step of PLP and understanding the detailed mechanism of transaldimination will be helpful for developing new drugs. In this study, we reviewed the catalytic mechanism of PLP-dependent enzymes, with a especial focus on transaldimination of PLP, the so-called enzyme-like conformational changes of PLP. It is expected that the sophisticated mechanism of PLP will provide valuable information for understanding PLP-dependent enzymes.

HIGH CATALYTIC REACTIVITY OF PLP

PLP consists of a central pyridine ring with four different chemical groups (methyl, hydroxyl, formyl, and phosphomethyl) attached sequentially at the 2′ to 5′ positions (Fig. 1). The pyridine ring has conjugated double bonds and a nitrogen atom with a pair of non-bonded electrons, which facilitate electron movement within PLP (4). The electron movement can be extended to Schiff-base linkage with substrates or reaction intermediates, as well as across the pyridine ring plane. PLP works as an efficient electron sink to pull down the excess electrons of substrate reaction intermediates via its quinonoid structure (17, 18). The planarity of the Schiff base

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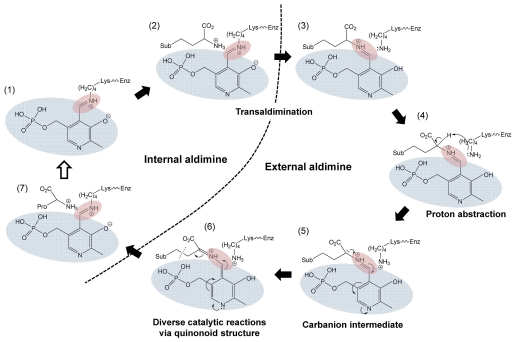


Fig. 1. A representative general catalytic cycle of PLP-dependent enzymes as an example of β-elimination. (1) In the internal aldimine structure of PLP, PLP is Schiff-base linked with the active site Lys residue. The blue shade represents the PLP molecule, and the orange shade represents the internal Schiff-base linkage between PLP and the active site Lys. (2) Substrate of an amino compound is bound at the active site. (3) The external aldimine structure of PLP is formed with Schiff-base linked substrate by forward transaldimination reaction. (4) Proton abstraction on the Cα carbon of substrate is performed by the active site Lys. (5) Resulting carbanion intermediate, showing non-bonded electrons at the Cα carbon, is formed. (6) The β-elimination cleavage, shown in the dotted line, is achieved with elaborated electron movements via the quinonoid intermediate structure. (7) The internal aldimine structure is restored with a released product by reverse transaldimination reaction.

linkage with the PLP pyridine ring is affected by the extent of the π-bond electron conjugation. The non-bonded electron pairs of the nitrogen atoms in the Schiff base linkage and the pyridine ring contribute to the reactivity of PLP by switching its π-bond conjugating status, enabling diverse catalytic reactions on the substrate. The PLP molecule also has a different critical reactive OH group attached to the pyridine ring, which increases its reactivity. The central catalytic properties of PLP are based on the conjugated π-bond system, which could be expendable with substrates; the Schiff-base linkage, which could switch between single and double bonds; and the additional catalytically active motif of the hydroxyl group in the pyridine ring.

PLP-DEPENDENT ENZYMES AS DRUG TARGETS

The main substrates of PLP-dependent enzymes are amino compounds, such as, amines and amino acids (Table 1) (16). Their metabolites are frequently involved in many important signaling pathways. For instance, GABA-aminotransferase is a PLP-dependent enzyme that degrades Gamma-aminobutyric acid (GABA), a key inhibitory neurotransmitter in the mammalian central nervous system, into succinic semialdehyde. A low concentration of GABA in the brain is directly related to epilepsy, Parkinson's disease, and Alzheimer's disease (19) and as such GABA-aminotransferase is an important drug target for the management of these diseases. Relatedly, kynurenine aminotransferase is another PLP-dependent enzyme involved in the metabolism of neuroactive compounds. Kynurenine aminotransferase catalyzes the degradation of tryptophan to kynurenine catabolic intermediates, such as, kynurenic acid, 3-hydroxykynurenine, and quinolinic acid, which are neuroactive and related to human neurodegenerative disorders (20, 21), making kynurenine aminotransferase a valid drug target. The major human neurotransmitters dopamine and serotonin are generated from L-DOPA and L-5-HTP, respectively, by L-3,4-dihydroxyphenylalanine (DOPA) decarboxylase, a PLP-dependent enzyme (22). DOPA decarboxylase degrades L-DOPA within the peripheral nervous system, making it a target in the management of Parkinson's disease (23).

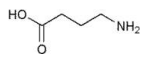
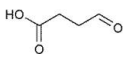
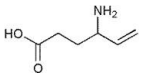
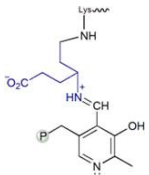
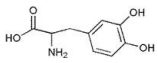
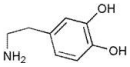
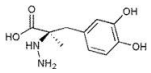
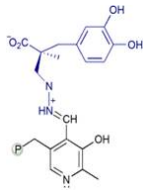
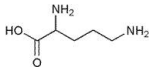
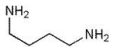
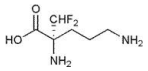
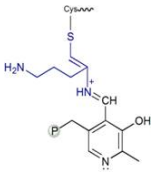
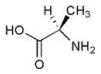
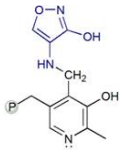
In addition to central nervous system diseases, PLP-dependent enzymes are closely related to other disorders. Ornithine decarboxylase, converting L-ornithine to diamine putrescine, is a PLP-dependent enzyme and a drug target. The biosynthesis of polyamines, such as, diamine putrescine is essential for cell proliferation and differentiation, as well as tumor development (24), making ornithine decarboxylase a valid therapeutic target for the management of cancer. Histidine is catalyzed to histamine by the PLP-dependent enzyme histidine decarboxylase, which is a therapeutic target for inflammatory and immune system diseases, several neurological and neuroendocrine disorders, and osteoporosis (25). D-alanine, essential for bacterial cell wall synthesis, is synthesized from L-alanine by the PLP-dependent enzyme alanine racemase which is a target for the development of antibiotic, such as, for the treatment of tuberculosis (26).

DRUGS TARGETING PLP-DEPENDENT ENZYMES

Most drugs on the market that target PLP-dependent enzymes are suicidal inhibitors that make a direct covalent bond with PLP (Table 1) (11-15). To make irreversible covalent bonds with the active site of target proteins, drugs usually contain unique core structures responsible for their high reactivity. For example, penicillin has the core structure of a β-lactam ring, which binds the target transpeptidase (27). Aspirin, an antipyretic, analgesic, and anti-inflammatory drug, has a phenolic ester group that delivers its acetyl group to human cyclooxygenase-1 (28). Sarin, a nerve agent, has a phosphonofluoridate group, which inactivates acetylcholinesterase (29).

In PLP-dependent enzymes, the high reactivity is provided by PLP itself rather than the drug compounds. All PLP-dependent drugs contain a substrate amino group, and the transaldimination reaction between the substrates and PLP is highly reactive and essential in all PLP-dependent enzymes. The current PLP-dependent enzyme-targeting drugs mimic the PLP-attacking amino group of a substrate. At the same time, other parts of the compounds were modified to specifically and tightly fit into the active site of each specific target enzyme. Accordingly, the unique structure of PLP is essential for the mechanism by which specific inhibitors or drugs distinguish PLP-dependent enzymes from other unrelated proteins.

Table 1. Drugs against PLP-dependent enzymes in market

Substrate	Product	Drug	Inhibition mechanism	Diseases	Traget enzyme (catalytic activity)	Reference
4-aminobutanoate 	Succinate semialdehyde 	Vigabatrin 		Epilepsy	GABA aminotransferase (transaminase)	(14, 29, 30)
L-DOPA 	Dopamine 	Carbidopa 		Parkinson's disease, hypertension	DOPA decarboxylase (decarboxylase)	(11, 33, 34)
Ornithine 	Putrescine 	Eflornithine 		African trypanosomiasis, malaria	Ornithine decaroxylase (decarboxylase)	(36)
L-alanine 	D-alanine 	Seromycine 		Tuberoculosis	Alanine racemase (racemase)	(13, 25)

One good example of this strategy is the anti-epilepsy drug vigabatrin (14, 30), a GABA-aminotransferase substrate analog, which mimics the nucleophilic attack of GABA on PLP to switch Schiff-base linkage from internal to external aldimination (Supplementary Fig. 1). Compared to the GABA substrate, vigabatrin has a unique vinyl group attached at its Cγ position. The vinyl group reacts with the active site Lys side chain in the middle of the catalysis. As a result, vigabatrin becomes covalently linked to both PLP and enzyme, and works as a suicide inhibitor against GABA-aminotransferase. Unfortunately, vigabatrin has the severe side effect of irreversible loss of the peripheral visual field, which limits its current use (31). The Parkinson's disease drugs carbidopa (11) and benserazide (32) highlight another crucial problem in current PLP-dependent enzyme targeting strategies, specificity. Both drugs are powerful irreversible inhibitors of DOPA decarboxylase, but non-selectively bind to other PLP-dependent enzymes, such as, kynurenine hydrolase, and can even bind to PLP alone, which could result in the reduced synthesis of nicotinamide coenzymes, niacin deficiency, and pellagra (33-35). Other drugs of eflornithine and seromycine targeting PLP-dependent enzymes in malaria and tuberculosis show similar issues (13, 36).

In addition to the aforementioned diseases and drugs, PLP-dependent enzymes are involved in many more illnesses due to their versatile and ubiquitous functions. PLP-dependent enzymes also synthesize a wide variety of small bioactive molecules, which could be used as therapeutic agents. These small bioactive molecules have even been pursued to be used as cancer drugs (37, 38).

PLP-dependent enzymes share the invariably conserved catalytic mechanism involving carbanionic intermediates of substrates, stabilized by PLP cofactor (3). To accommodate the amino group of substrates close to the PLP cofactor for the conserved catalytic mechanism, the overall geometry of the active site and surrounding substrate-binding pocket should also be conserved. In addition, the high chemical reactivity of PLP allows the catalytic promiscuity of PLP-dependent enzymes, implying that the enzyme can catalyze different chemical reactions (39). Therefore, relatively low specificity and high reactivity of PLP are critical issues for developing drugs against PLP-dependent enzymes.

GENERALLY CONSERVED CATALYTIC MECHANISM OF PLP-DEPENDENT ENZYMES

For the development of efficient inhibitors and drugs, a comprehensive understanding of the target enzymatic mechanism is essential (4). Considering that diverse catalytic reactions are

performed by PLP-dependent enzymes, the overall catalytic steps of PLP-dependent enzymes are well conserved, except for the carbanion formation step (Fig. 1 and Supplementary Fig. 2). As the amino group of a substrate approaches PLP within the active site, it replaces the Schiff-base linkage between PLP and the catalytic Lys in the native internal aldimine structure. Usually, a nearby residue (including the previously PLP-linked catalytic Lys), which is a catalytic base, subtracts a proton from the resulting PLP-linked substrate, changing it into a carbanion intermediate. However, carbanion formation is also possible by the decarboxylation of the external aldimine substrate (22) or the attack of another cofactor like tetrahydrofolate (40). The resulting non-bonded electrons of the carbanion intermediate are stabilized by the elaborated electron movements through the extended conjugated π-bond system of PLP and the bound substrate via the quinonoid structure. The electron-rich intermediates and their resonance structures provide the catalytic power to enable > 140 different enzymatic reactions, according to the neighboring active site geometry of each PLP-dependent enzyme and bound substrate. The free amino group of the active site Lys then re-attacks PLP to restore an internal aldimine structure and release a product.

The diverse catalytic activities of PLP-dependent enzymes are possible because of the high reactivity of PLP. The chemical structures of substrates, their tendency to form a conjugated π-bond with PLP in the external aldimine structure, and the potent catalytic residues close to PLP work together to activate substrates into carbanion intermediates for diverse biochemical reactions (Supplementary Fig. 2). For example, in GABA aminotransferase the active site Lys deprotonates the Cγ atom of GABA in the external aldimine structure, which is stabilized by the extended conjugated π-bond system and eventually leads to amino group transfer (15). In DOPA decarboxylase, the Cα decarboxylation generates a carbanion intermediate, which is also stabilized by the extended conjugated π-bond system of PLP (22). Alanine racemase has a different catalytic base on the opposite side of the typical catalytic Lys, based on the central PLP plane. The other base deprotonates the substrate Cα carbon at the opposite side of the PLP plane for its stereospecific racemase reaction, which is immediately stabilized by the same extended conjugated π-bond system (41). In serine hydroxymethyltransferase, the Cβ carbon of the serine side chain can be directly attacked by the N5 atom of tetrahydrofolate, which results in retro-aldol cleavage between the Cα and Cβ carbons, with the help of the quinonoid form of PLP (42, 43). Accordingly, the structure and catalytic property of PLP sustain the reactivity of PLP-dependent enzymes, while nearby residues determine the specificity of PLP-dependent enzymes.

CONFORMATIONAL CHANGES OF PLP IN CATALYSIS

PLP cofactor conformational changes during catalysis have been reported in various biochemical studies (37, 38, 44-49). Crystallographic snapshots of serine dehydratase activity of a PLP-dependent enzyme, XometC from *Xanthomonas oryzae* pv. *oryzae*, revealed a sophisticated catalytic mechanism involving the PLP cofactor (50). The enzyme provided the structural information, thus eliminating possible artifacts from different enzyme sequences, crystallization conditions, and crystal packing. In particular, the conformational change of PLP, which alters the dihedral angle rotation, simultaneously coordinates three different catalytic events, namely, attracting nucleophilic attack on PLP by the substrate, deprotonation of the attacking substrate amino group, and transfer of a proton from the substrate to the catalytic Lys (Fig. 2A). During transaldimination reaction, all the main catalytic properties work together at the same time.

The dihedral angle rotation of PLP attracts nucleophilic attack

As the substrate approaches PLP in the active site, the amino group of the substrate pushes away the Schiff-base linked amino group of Lys because of the steric hindrance between both amino groups. The steric push on the Schiff-base linkage leads to a higher dihedral angle rotation of PLP, which shifts the electron cloud of the Schiff-base linkage from the PLP C atom to the N atom of Lys. The native internal aldimine structure has double-bond characteristics in the Schiff-base linkage, and its positive charge should be located on the N atom of

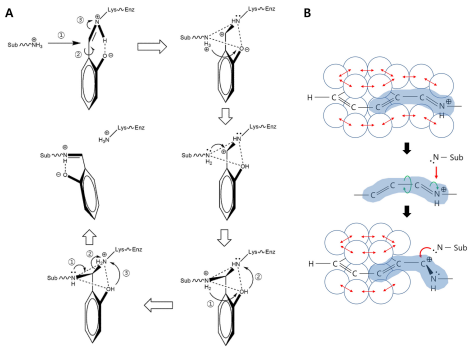


Fig. 2. Chemistry in transaldimination reactions. (A) Transaldimination reactions with the simplified models of PLP and an amino substrate compound. The structures from just prior to nucleophilic attack, via the *gem*-diamine, and after transaldimination completion are shown. Nucleophilic attack, conformational change, and electron movement are shown as arrows. When substrate amino group approaches to PLP in the native internal aldimine structure, the steric hindrance pushes Schiff-base linkage outside, which causes its dihedral angle rotation and shifts its double bond to single bond at the same time. The getting closed keto group in pyridine ring helps transaldimination by deprotonating the substrate amino group. The OH group can transfer a proton from substrate to Lys and helps transaldimination from internal aldimine to external aldimine. PLP pyridine ring tilts to substrate side to complete the transaldimination reaction. (B) Conjugated π-bond system of PLP at the nucleophilic attack in transaldimination reaction. The π-bonds and their conjugations are shown as blue balls and red arrows. The conformational change, the dihedral angle rotation, of PLP as a substrate approaches is illustrated in the shaded area.

Lys. The π-bond in the Schiff-base linkage is stabilized by conjugation with the π-bonds of the PLP pyridine ring in either its planar conformation or a lower dihedral rotation angle. When the dihedral angle rotates to a higher angle, the double bond of the Schiff-base linkage changes into a single bond (Fig. 2B). As a result, the previous positive charge on the N atom of Lys moves to the PLP C atom. The positive charge on the PLP C atom directly attracts a nucleophilic attack by the substrate amino group.

Hydroxyl group of PLP pyridine ring

The dihedral angle rotation moves the hydroxyl group of PLP closer to the substrate amino group and centers it between two amino groups N atoms, one from the attacking substrate and one from the leaving Lys. This hydroxyl group deprotonates the substrate amino group for nucleophilic attack on the positively charged PLP atom. At the same time, the hydroxyl group can deliver the plucked proton to the leaving amino group of Lys. The hydroxyl group of the pyridine ring exists as a tautomer of keto and enol forms via resonance structures (51), which can produce a H-bond with the N atom of Lys active site in the Schiff-base linkage at a lower dihedral angle of the internal aldimine structure or H-bonds with both N atoms of the substrate and Lys active site at a higher dihedral angle. The keto form is the active form for deprotonation, and the resulting enol form completes the concerted proton transfer to the leaving amino group. When the dihedral angle rotates from lower to higher angles, the hydroxyl group moves away from the N atom of the Schiff-base linked Lys. However, it obtains a new H-bond with the N atom of the substrate amino group. The dynamic resonance structures between keto and enol forms of the PLP hydroxyl group and the dihedral angle rotation of PLP favorably help the transaldimination reactions. After the transaldimination reaction is completed, the PLP cofactor tilts away from the Lys side in the internal aldimine structure to the substrate side in the external aldimine structure (52, 53). In summary, the full transaldimination process occurs within the substrate and PLP, without any electrons or protons being accepted by or released to any other molecules. The transaldimination mechanism of PLP might be applicable for all PLP-dependent enzymes with its essentiality for all their enzyme activities.

Preference for catalytic direction through PLP conformation

In serine dehydratase, the dihedral angle of PLP influences the progress of the catalytic step in the catalytic cycle of the enzyme mechanism (50). The PLP dihedral angles of external aldimine structures are smaller when the conjugated π-bonds extend between PLP and Schiff-base linked substrate intermediates (Supplementary Fig. 3). A more planar dihedral angle inhibits nucleophilic attacks on PLP. On the contrary, the proton abstraction on a substrate (by a catalytic base) to form a carbanion intermediate is preferred, thereby causing the consecutive reactions to move forward rather than backward (Fig. 1).

Although understanding the transition-state structure of an enzyme is important for studying its catalytic mechanism and designing mechanism-based inhibitors (54), it is usually difficult to capture the transition-state or reaction intermediate structure of PLP-dependent enzymes. The multiple structures of PLP-dependent enzyme in complex with a substrate or multiple substrate intermediates at the same time, have not yet been determined. One of the few examples includes serine hydroxylmethyltransferase from *Bacillus Stearothermophilus*. Both native (only PLP-bound) and external aldimine structures with bound substrate were determined in eight different forms, including wild-type and mutant enzymes (55). In those structures, the dihedral angles of the external aldimine structures, which have more extensive π–bond conjugations, were approximately 20° lower than the dihedral angle in the native structure, which corroborates with the described catalytic roles of PLP, showing conformational changes during catalysis (50).

The dynamic conformational changes of PLP facilitate nucleophilic attack of a substrate on PLP, perform the catalytic acid/base roles for concerted proton transfer, and dictate the catalytic direction. It was believed that these mechanisms were controlled by amino acids at the active site. In our opinion, the general conformational changes and their effects on catalysis might be well conserved in all PLP-dependent enzymes.

OTHER CONJUGATED π–BOND SYSTEMS FOR ENZYME CATALYSIS

The conjugated π–bond system is an important conserved characteristic among organic cofactors: the π–bond conjugation stores high energy to enable or accelerate enzyme catalysis. In respiration and photosynthesis, the essential redox reactions that lead to the production of the universal cellular chemical energy, ATP, are catalyzed by the coupled oxidized and reduced forms of NAD^+/NADH and $NADP^+$/NADPH cofactors (56, 57). NADH has a distorted hexagon ring structure as compared to the almost perfect regular hexagon nicotinamide ring of NAD^+, although the only difference is the two electrons and the proton at the terminus of the nicotinamide ring (Supplementary Fig. 4) (58-62). The distorted ring structure destabilizes the π-bonding of the electrons in the ring, which causes NADH to lose two electrons and a proton to another molecule so that it can gain the low energy symmetric ring conformation of NAD^+ again. The conformational change between NAD^+ and NADH is more subtle than that between ATP and ADP/Pi, but reduced NADH stores almost twice as much chemical energy (61.8 kJ/mol compared with 30.5 kJ/mol for ATP hydrolysis) (63). In PLP, the similar conformational changes related to its conjugated π–bond system enable diverse catalytic reactions. Unfortunately, it is not as easy to observe the conformational changes of PLP as it is for the coupled cofactors of NAD^+/NADH.

CONCLUDING REMARKS

PLP-dependent enzymes are important drug targets. Many drugs targeting PLP-dependent enzymes are currently on the market for the management of many diseases and most of them affect PLP and its transaldimination. Although its unique catalytic property and high reactivity make PLP cofactor a good target for specific inhibitors or drugs, currently available drugs still have side effects because they can non-specifically and irreversibly bind other PLP-dependent enzymes, thereby impeding essential cellular functions of some PLP-dependent enzymes. Although PLP-dependent enzymes have been studied for 80 years, their catalytic mechanisms have not yet been fully understood. The PLP cofactor was shown to directly perform catalytic activities through a mechanism including active conformational changes similar to amino acids in proteins, thus implying that cofactors might be active key molecules rather than passive helpers in enzyme catalysis. Although protein is the main constituent forming the overall scaffold and substrate-binding pocket in enzymes, a better understanding of the reaction chemistry of PLP cofactor is crucial for developing selective and reversible drugs targeting PLP-dependent enzymes.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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