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PROLIDASE: ROLE IN HEALTH AND DISEASE

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SUMMARY

The enzyme prolidase (EC 3.4.13.9), encoded by the *PEPD* gene, is a member of the MMP family, hydrolyzes the imidodipeptides at the C-terminal proline or hydroxyproline. Hence, prolidase is critical for the turnover of proline and hydroxyproline-rich proteins, such as collagen. Collagen metabolism is essential for matrix regeneration during cellular proliferation and therefore plays a crucial role in several physiological processes such as wound healing, inflammation, angiogenesis, cell proliferation, and carcinogenesis. However, as a ligand, PEPD can bind directly to the epidermal growth factor receptor (EGFR, epidermal growth factor receptor 2 (HER2)) and regulate cellular metabolism. In addition, alterations in prolidase enzyme activity have been documented in numerous pathological conditions, making prolidase a useful biochemical marker to measure disease severity. The purpose of this review is to provide and highlight an emerging role of prolidase in health and diseases at cellular level.

Keywords: Prolidase, collagen, wound healing, prolidase deficiency, regulation

INTRODUCTION

Prolidase is the only dipeptidase known to catalyze the hydrolysis of a dipeptide containing a C-terminal proline or hydroxyproline into constituent amino acids. Prolidase has many aliases, including peptidase D (PEPD), Xaa-Pro dipeptidase, X-Pro dipeptidase, proline dipeptidase, or imidodipeptides. Prolidase is present in all three domains of life - archaea, bacteria, and

eukaryotes. The enzymatic activity of prolidase releases proline or hydroxyproline in the final stages of the catabolism of endogenous and dietary proteins. The most studied substrate of prolidase are imidodipeptides that are generated during breakdown of collagen, a predominant component of the extracellular matrix (ECM). Collagen is the most abundant structural protein in the human body, attributing to approximately 33% of total protein mass. Proline and hydroxyproline constitute over 20% of collagen therefore; collagen degradation generates a considerable amount of dipeptides containing proline or hydroxyproline. Prolidase plays an essential role in ECM remodeling by facilitating the intracellular digestion of these dipeptides and promoting the recycling of proline for collagen synthesis[1,2] Thus, prolidase is required in several physiological and pathological processes such as wound healing, inflammation, angiogenesis, cell proliferation and carcinogenesis. The presence of proline in numerous biomolecules such as neuroactive peptides or growth factors prevents unexpected proteolysis in order to maintain their biological activity[3,4]. However, there are many factors responsible for the degradation of peptides containing proline at the C-terminus. The purpose of this review is to provide an overview of the latest knowledge of significance of prolidase in health and diseases at the cellular level.

1. PROLIDASE:STRUCTURE, GENERAL PROPERTIES AND MECHANISM OF ACTION:

Prolidase belongs to the family of metallopeptidases dependent on divalent cations that enable its catalytic activity. PEPD is known as X-Pro dipeptidase, proline dipeptidase, imidodipeptidase, and peptidase D. From a molecular point of view, prolidase is encoded by the *PEPD* gene located on the long arm of

chromosome 19 at locus 13.11. It has been observed that the gene structure has 15 exons[5]. Point mutations in this gene are responsible for the lack or reduction of the enzymatic activity, and thus causing prolidase deficiency. It is known that the *PEPD* gene has 29 point mutations that result in a reduction or complete loss of the enzymatic activity. Out of these, eight point mutations are of clinical significance[6].

Prolidase can exist in three isoforms depending on the transcriptomic variant. Isoform 1 is the product of the longest transcript, while prolidase isoform 2 is shortened by an

Internal segment from 184 to 224 nucleotide. Because of alternative splicing, isoform 3 is deprived of a nucleotide fragment from 68 to 131 nucleotides**[7].** It is known that in eukaryotes, prolidase undergoes post-translational modifications such as glycosylation and phosphorylation. The analysis of the carbohydrate content in the prolidase structure showed that it constitutes 0.5% of the total protein mass**[8].** This report presents prolidase as a glycoprotein; however, further research is needed to assess the binding sites of carbohydrate groups and evaluate whether glycosylation affects biological properties of prolidase. Indirectly, it has been demonstrated that glycosylation does not influence the catalytic activity of the enzyme**[9]**. Another study showed that N-glycosylation can occur at N13 and N172 sites, and O-glycosylation—at position T458 in the amino acid chain**[10]**.

PEPD phosphorylation, confirming that this post-translational modification increases PEPD enzymatic activity. Ysrayl et al. 2019[11], provided further evidence supporting the phenomenon of prolidase phosphorylation. They observed that cocaine stimulates prolidase phosphorylation and increases its enzymatic activity. They also demonstrated that phosphorylation of prolidase depends on the iNOS pathway, which inhibits the level of phosphorylated protein, which is consistent with the previous study[12].

Structurally, human prolidase is a homodimer consisting of two subunits, 493 amino acids (AA) each[13]. The molecular weight of one subunit is 58 kDa[9]. Both subunits are comprised of the N- and C-terminal domains. The carboxyl-

terminal domain (185–493 AA) shares the structure with peptidases from the 'pita-bread' family (e.g., aminopeptidase P, methionine aminopeptidase, and creatinase)[14]. In this domain, there is an active center in which the Mn2+ ion is necessary for its enzymatic activity. The substrate (Gly-Pro) binds to the active center in the C-terminal domain, while the N-terminal domain (1–185 AA) remains less closely linked to the substrate. The disulphide bond between Cys158A and Cys158B links the monomers. Notably, this disulphide bridge is only present in the inactive enzyme-substrate complex[15]. The divalent cations: Zn2+, Mg2+, Ca2+, Co2+ can be also present as cofactors required for PEPD enzymatic activity. However, prolidase activity is decreased if one of these cations is located in the active center. Under these conditions, the activity of prolidase drops below 30%[16].

Prolidase belongs to the group of hydrolases; therefore, the enzymatic reaction catalyzed by PEPD requires H_2O. Wilk et al, 2017[13] established the mechanism of the reaction catalyzed by recombinant human prolidase. First, water binds to Mn^{2+}ions in the active center of prolidase, which leads to the formation of a hydroxyl ion. Then, Gly-Pro binds to the active center, resulting in a change of the enzyme conformation. Approaching His255, it coordinates the -COOH group in the substrate. The C=O and -NH_2 groups in Gly-Pro are stabilized by manganese ions, providing a positive charge at the carbon atom. A nucleophilic hydroxyl ion attack is followed by a break in the peptide bond, and the products (Gly, Pro) leave the active center. First, Gly is released followed by proline, and then the enzyme returns to its original conformation. The last step involves replenishing water and restoring the enzymatic activity of prolidase.

PEPD exhibits the highest specificity for Gly-Pro in the *trans* conformation[17]. Although prolidase has the highest catalytic activity against Gly-Pro[9], it also hydrolyses other C-terminal proline-containing dipeptides such as Ala-Pro, Phe-Pro, Met-Pro, Val-Pro, and Leu-Pro[10]. Unlike many proteases, prolidase is present in the cytosol[9]. However, it is not known why prolidase occurs in that subcellular location. PEPD is abundantly expressed in enterocytes, where it

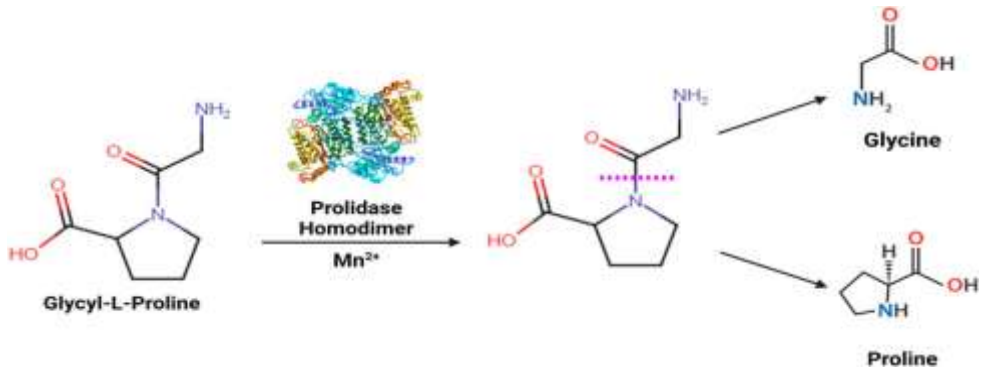
is probably involved in the hydrolysis of dietary proline-containing dipeptides**[18].** Regarding the tissue specificity of prolidase, the highest level of prolidase mRNA expression is observed in the kidneys, small intestine, and duodenum[19], while high prolidase activity has been reported in erythrocytes and human skin fibroblasts[20]. showed that platelet-rich plasma is an important source of prolidase.

The key source of substrate (Gly-Pro) are proteins rich in amino acid sequences containing C-terminal proline or hydroxyproline, e.g., collagen[21], complement component C1q[22], dietary proteins[23], and many biomolecules such as substance plasminogen, oxytocin, vasopressin, and angiotensin[24]. Out of these, collagen is the most abundant source of Gly-Pro. Its molecule comprises three polypeptide chains in which the Gly-X-Y triplet is commonly repeated. Mostly X and Y are occupied by proline and hydroxyproline, respectively[25]. Thus, a significant role of PEPD is reflected in extracellular matrix (ECM) remodeling as collagen constitutes its structural protein**[26,27].** Collagen degradation is initiated by metalloproteinases[28]. Then, the proteolysis of collagen breakdown products by cathepsins and peptidases takes place in lysosomes. However, they cannot degrade di- and tripeptides containing C-terminal proline or hydroxyproline. Prolidase acting in the cytosol releases free amino acids from dipeptides[29]. This process is impaired in prolidase deficiency, manifested by skin lesions resulting from disturbed collagen metabolism. Another symptom of PD is immunodeficiency, which is probably a consequence of impairment in the C1q complement component built from Gly-Pro repeat[22]. Prolidase also plays a role in the deactivation of C-terminal proline-containing neuropeptides, which could be related to mental disorders in patients with PD. Hui et al, 1980[30] observed increased enzymatic activity in the brain tissue. In the central nervous system (CNS), prolidase is responsible for proline delivery. Out of symptoms of prolidase deficiency, mental retardation may occur because of a low level of proline as a neurotransmitter. However, there have been reports in which an increase of proline in CNS results in increased glutamate concentration, leading to neuronal death due to excessive stimulation of N-

methyl-D-aspartate (NMDA) receptors. This mechanism could explain the relationship between increased prolidase activity and the pathogenesis of some neurological disorders[31].

The human prolidase enzyme, encoded by the *PEPD* gene, has three known isoforms which are generated through alternative splicing of the mRNA transcript. These isoforms have different protein lengths and potentially different functional characteristics: Isoform-1: This is the longest and most studied isoform, consisting of 493 amino acids. It is the most abundant in human plasma and exhibits the highest activity with substrates like Gly-Pro and Ala-Pro. It has a molecular mass of approximately 56 kDa. Isoform-2: This variant is a shorter protein of 452 amino acids, lacking two alternate in-frame exons in the central coding region. It has a molecular mass of about 95 kDa in some reports and displays a higher affinity for Met-Pro compared to Gly-Pro.Isoform-3; This is the shortest variant, translated into a 429 amino acid protein, also due to missing a different segment of the coding region through alternative splicing [32]

Research suggests that prolidase deficiency in patients primarily involves a defect in Isoform 1, while the activity of Isoform 2 remains largely unaffected. The precise differences in subcellular localization or physiological roles of all three variants are still a subject of ongoing research. **[33]**



2. GENERAL PROPERTIES OF PROLIDASE

Prolidase (peptidase D, PEPD) is a ubiquitously expressed cytosolic metallopeptidase with key enzymatic and non-enzymatic regulatory

properties. Its primary enzymatic role is the unique ability to cleave imino- or imidodipeptides that have a proline or hydroxyproline residue at the C-terminal end [34]

2.1 Enzymatic Properties :

i. **Substrate Specificity**: Prolidase is the *only* enzyme known to be able to hydrolyze a peptide bond where the C-terminus (carboxyl end) is a proline or hydroxyproline. Its most specific and preferred substrate in humans is glycyl-proline (Gly-Pro), which is abundant in collagen breakdown products.

ii. **Cofactor Requirement**: It is a metal-dependent enzyme that requires divalent metal cations, primarily manganese (Mn2+Mn raised to the 2 plus power Mn2+), for its catalytic activity. The active site in the human enzyme contains a bimetallic center where two manganese ions are bridged by a hydroxide ion during catalysis.

iii. **Optimum Activity**: The enzyme's optimal activity occurs at a neutral pH (around 7.5-8.0) and a temperature of approximately 37°C to 50°C, aligning with physiological conditions.

iv. **Physiological Role**: Prolidase plays a critical role in protein metabolism and collagen recycling, particularly in the final, rate-limiting step of collagen degradation. This process releases free proline, which is then recycled for new collagen synthesis, making it essential for tissue repair, wound healing, and cell proliferation.

2.2 Non-Enzymatic Regulatory Properties

In addition to its enzymatic functions, recent studies have revealed novel, non-catalytic roles in cellular regulation as under mentioned :

a. **Receptor Ligand**: Extracellular prolidase can act as a ligand for the epidermal growth factor receptor (EGFR/ErbB1) and the human epidermal growth factor receptor 2 (HER2/ErbB2), activating downstream signaling pathways involved in cell growth and proliferation.

b. **Tumor Suppressor Regulation**: Intracellular prolidase binds to the p53 tumor suppressor protein and regulates its activity, controlling cell cycle and apoptosis.

c. **Immune Response**: It is involved in the maturation of the type I interferon receptor (IFNAR1), which is crucial for the immune response against viral infections. Dysfunction of prolidase, typically due to mutations in the *PEPD* gene, causes the rare autosomal recessive disorderprolidase deficiency, characterized by severe symptoms such as chronic skin ulcers, recurrent infections, and developmental delays. [35-36]

3. ***Biological Significance of Prolidase:*** Prolidase activity at the cellular level is controlled by the several mechanisms. Stimulation of the β1-integrin receptor by interaction type I collagen leading to leading to the auto-phosphorylation of FAK (Focal Adhesion Kinase) and its subsequent interaction with Grb2 and Src, activates a signaling cascade. The signal is further transmitted through SoS, Ras, and Raf pathway to the ERK1/2 kinases and results in transcription of genes involved in cell growth regulation as well as proliferation and collagen biosynthesis/extracellular matrix (ECM) remodeling. The key steps in this pathway are: a) Type I collagen binds to the β1-integrin receptor on the cell surface. b) The binding of Type I collagen to β1-integrin receptor induces the autophosphorylation of FAK at Tyr397, which activates the kinase. c) The phosphorylated FAK then creates docking sites, allowing it to associate with the Src family kinases and the adaptor protein Grb2 (Growth factor receptor-bound protein 2), often via Shc proteins. d) The FAK/Src/Grb2 complex leads to the activation of the Ras/Raf/ERK1/2 (extracellular signal-regulated kinase) mitogen-activated protein (MAP) kinase pathway. e) This signal cascade ultimately results in the increased expression and activity of prolidase (also known as PEPD). The resulting increase in prolidase activity provides free proline and hydroxyproline, which are essential substrates for new collagen

synthesis, thus linking integrin signaling to ECM production and tissue repair processes like wound healing.[37,38,39]

i. Prolidase activity, regulated by an IGF-1R-dependent pathway, which boosts cell growth, proliferation, and collagen synthesis by increasing available proline and activating signaling cascades like PI3K/Akt/mTOR and ERK1/2, crucial for tissue repair, especially in wound healing. The main steps of the pathway are; i) Insulin like Growth Factor-1 (IGF-1) bind to its receptor (IGF-1R) on the cell surface. ii) The bind of IGF-1 to IGF-1R activates downstream signaling pathways such as PI3K/Akt/mTOR and ERK1/2 pathways, which are central to cell growth and proliferation. iii) The activated pathways increase prolidase activity, often through cross-talk with integrin receptors. iv) Prolidase then breaks down collagen fragments (dipeptides with C-terminal proline/hydroxyproline) to release free proline. v) This released proline becomes a vital substrate for new collagen production, supporting tissue repair and remodeling[40,41,42,43]. Prokop et al,2013[44] proposed that prolidase activity is controlled by the interplay between the Insulin-like Growth Factor-1 Receptor (IGF-1R) and β1-integrin receptors, where both activate the ERK1/2 and PI3K/Akt/mTOR signaling pathways, ultimately influencing prolidase function, often in collagen synthesis and cell growth. This suggests prolidase isn't just a passive enzyme but is deeply integrated into growth factor signaling networks, with crosstalk between these receptors modulating its activity, crucial for processes like wound healing and tissue remodeling.[45]

Prolidase activity plays a significant role in regulating angiogenesis. It primarily promotes the process by influencing key signaling pathways and providing essential components for the formation of new blood vessels. Prolidase regulates angiogenesis through both its enzymatic activity and a non-enzymatic function as a cell surface receptor ligand. a) Enzymatic Activity & HIF-1α/VEGF Pathway**:** Prolidase breaks down collagen-derived dipeptides into free proline and hydroxyproline. These free amino

acids inhibit the degradation of the crucial transcription factor that is hypoxia-inducible factor 1-alpha (HIF-1α). The stabilized HIF-1α then accumulates and promotes the transcription of pro-angiogenic genes; most notably vascular endothelial growth factor (VEGF).VEGF is a key signaling molecule that stimulates the formation of new blood vessels (revascularization) in response to tissue damage or hypoxia. **b)** Non-Enzymatic Activity & Growth Factor Receptors**:** When prolidase released into the extracellular space (e.g., from damaged cells), can act as a ligand for the epidermal growth factor receptor (EGFR) and HER2 receptors on the cell surface. This binding activates downstream signaling pathways, such as the ERK and PI3K/Akt/mTOR pathways, which are involved in cell proliferation, migration, and survival—all critical components of angiogenesis·[45-50]

4. Clinical Significance *of Prolidase:*

The importance of prolidase in angiogenesis is highlighted in conditions where its activity is altered.

4.1 Prolidase Deficiency (PD), a rare genetic disorder is characterized by defective wound healing and chronic skin ulcers. Postmortem examinations of patients reveal issues with blood vessel formation (angiopathy), strongly suggesting that a deficiency in functional prolidase leads to impaired angiogenesis [32].

4.2 **Cancer**: Increased prolidase activity is often observed in various cancers, where it is associated with enhanced tumor growth, invasion, and metastasis, partly by promoting angiogenesis that supplies the tumor with blood and nutrients. The products of prolidase activity, namely proline and hydroxyproline, have been shown to inhibit the degradation and promote the accumulation of the Hypoxia-inducible factor 1 alpha (HIF-1α) transcription factor through the Von Hippel–Lindau (VHL)-dependent proteasome pathway. *Under normal oxygen conditions (normoxia);* a specific proline residues (Pro402 and Pro564) on the HIF-1α protein are

hydroxylated by prolyl hydroxylase domain (PHD) enzymes. This hydroxylation allows the VHL tumor suppressor protein to bind to HIF-1α, leading to its ubiquitination and rapid degradation by the proteasome.[51, 52]

4.3 Effect of Proline and Hydroxyproline: Prolidase breaks down imidodipeptides, releasing free proline and hydroxyproline within the cell.An increase in the intracellular concentration of proline and, particularly, hydroxyproline leads to the inhibition of the prolyl hydroxylase (PHD) enzymes.Because the PHDs are inhibited, the critical proline residues on HIF-1α are not hydroxylated.The unhydroxylated HIF-1α cannot be properly recognized and bound by the VHL protein-E3 ubiquitin ligase complex.Consequently, HIF-1α escapes VHL-mediated ubiquitination and proteasomal degradation, leading to its stabilization and accumulation even under normoxic conditions. This process results in increased expression of HIF-1-dependent target genes, such as vascular endothelial growth factor (VEGF) and glucose transporter-1 (Glut-1), which are involved in angiogenesis and cell survival pathways, particularly in cancer cells. Hence hydroxyproline is the key signal that triggers HIF-1α destruction via the VHL/proteasome pathway, helping cells respond to oxygen levels[32, 42,53].

Prolidase and estrogen receptors (ERs) interact complexly to influence HIF-1α, particularly in breast cancer; prolidase activity, linked to collagen breakdown, can stabilize HIF-1α by providing proline substrate, while estrogen signaling, especially via ERα, can modulate prolidase activity and directly affect HIF-1α levels, sometimes promoting its accumulation or, conversely, inhibiting it depending on cell type and signaling context, suggesting they're key players in cancer progression. Prolidase Affects HIF-1α: Prolidase breaks down collagen-derived dipeptides (Gly-Pro) to release free proline, which is then used for collagen synthesis or other pathways.When prolidase activity increases (e.g., from ECM breakdown), it provides more proline, which can accumulate and inhibit the degradation of HIF-1α, leading to its accumulation and

activation of HIF-1α-dependent genes like VEGF (angiogenesis) and Glut-1 (glucose metabolism). On the other hand Estrogen Receptors (ERs) InfluenceERα. In ERα-positive cells (like MCF-7)), estradiol (estrogen) can upregulate prolidase activity, but also, paradoxically, prolactin in the presence of estrogen might inhibit prolidase, leading to decreased HIF-1α. ERα is implicated in regulating HIF-1α levels in certain contexts, with studies suggesting it might downregulate HIF-1α in some scenarios or, through specific pathways (like GPER), increase it.In cells with only ERβ (like MDA-MB-231), the effects are different, suggesting ERα plays a crucial role in the estrogen-prolidase-HIF-1α interplay. This Interplay (interaction) isn't straightforward; it's a "crosstalk" where ERs can influence prolidase activity, and prolidase activity influences HIF-1α, which in turn can affect ER expression. The outcome (increased or decreased HIF-1α) depends heavily on the cell type (ER-positive vs. ER-negative), the specific ER subtype, and the presence of other factors like prolactin.In essence, prolidase provides the fuel (proline) for HIF-1α stabilization, while estrogen receptor signaling fine-tunes prolidase activity, creating a complex regulatory loop relevant to cancer biology[54].

The study by Surażyński et al.,2010[55], published in 2008, demonstrated that overexpression of human prolidase (PEPD) in RKO cells leads to an increase in intracellular concentrations of proline (Pro) and hydroxyproline (Hyp). This, in turn, stabilizes the hypoxia-inducible factor-1 alpha (HIF-1α) and increases the expression of HIF-1-dependent gene products, including vascular endothelial growth factor (VEGF) and glucose transporter-1 (Glut-1). The key findings and mechanisms indicated by the study are: Higher levels of these amino acids in the cell contribute to the described effects.Proline and hydroxyproline stabilize HIF-1α by inhibiting its degradation. The increased and stabilized nuclear HIF-1α then induces the expression of target genes like VEGF and Glut-1.This mechanism is particularly relevant in cancers characterized by elevated PEPD expression, where it contributes to increased angiogenesis (via VEGF) and glycolysis

(via Glut-1) in response to hypoxia, promoting tumor growth, metastasis, and survival.[54,55]

TGF-β (Transforming Growth Factor-beta) signaling interacts with proline metabolism, with the enzyme prolidase and its products (proline/hydroxyproline) influencing TGF-β receptors and downstream pathways like Akt/mTOR, suggesting proline-dependent signals link prolidase activity to cell growth, ECM remodeling, and fibrosis. Prolidase activity generates proline, which activates Akt/mTOR, boosting TGF-β signaling, while inhibiting prolidase reduces these signals, showing a vital feedback loop affecting tissue repair and fibrotic diseases. Key Findings on Prolidase & TGF-β Signaling: Proline and hydroxyproline (products of prolidase) increase TGF-β1 and its receptor expression and activate the pro-growth Akt/mTOR pathway, indicating they act as messengers. TGF-β signaling, particularly via KLF6/Sp1 transcription factors, can increase prolidase (PEPD) gene expression, boosting collagen synthesis. Prolidase activity regulates TGF-β receptor-dependent functions through the mTOR pathway; blocking mTOR also reduces prolidase activity, showing a interconnected system.This interplay is crucial for cell proliferation, growth, and migration, and it's implicated in fibrotic diseases where excessive TGF-β leads to abnormal extracellular matrix (ECM) deposition [32 56,57].

Mechanistic target of rapamycin (mTOR) is a key element that explains the relationship between prolidase activity and the status of the PI3K/Akt/mTOR pathway; In this proposed mechanism, a specific component known as the prolidase-dependent signaling pathway may directly influence the activation state of the mTOR complex. Prolidase activity has been shown to modulate the availability of the amino acid proline, which in turn acts as a signaling molecule that can regulate key nodes within the larger PI3K/Akt/mTOR pathway, thus impacting cellular processes like growth, proliferation, and survival. The precise molecular interactions, however, can be complex and may vary depending on the specific cellular context or condition being studied[57].

5. OTHER USES OF PROLIDASE:

Organophosphorus acid anhydrolases (OPAA) are enzymes that hydrolyze highly toxic compounds such as acetylcholinesterase inhibiting compounds, chemical warfare G-type nerve agents, and pesticides. OPAA-2 from Alteromonas sp. strain JD6.5 EcoRI-lZAPII chromosomal library showed sequence homology with E. coli aminopeptidase P and human prolidase. OPAA-2 also demonstrates prolidase-like activity by cleaving leu pro and ala-pro, suggesting an evolutionary relationship. Though OPAA performs this activity, it hydrolyzes P-F and P-O bonds while prolidase hydrolyzes C-N. Further study suggested that Alteromonas haloplanktis OPAA is a type of prolidase. Alteromonas prolidase can hydrolyze G-type nerve agents and DFP, soman, sarin, and cyanide containing tabun, therefore is used as an antidote for poisoning[58,59,60]. Native and recombinant human prolidase also catalyzes these nerve agents with A252R or P365R substitution mutation of the enzyme, improving the catalytic activity against organophosphorus compounds[61,62] .

CONCLUSION: Prolidase is an enzyme that catalyzes dipeptides containing a C-terminal proline or hydroxyproline. These dipeptides are produced primarily during collagen turnover, thus making prolidase mediated catalysis as the rate-limiting step in collagen biosynthesis. The generated pool of proline is recycled during collagen synthesis and turnover. This process is important in several physiological and pathological processes. Therefore, alterations in prolidase activity are associated with not only prolidase deficiency, but also with several pathological conditions. Prolidase activity is vital for wound healing and prolidase deficiency is associated with poor wound healing in animals like mice and humans. Although prolidase is essential in various processes, fundamental research on the enzyme is limited. Thus, for a better understanding of the regulation of prolidase both during normal and diseased conditions will help identify novel pathways that can be exploited for possible therapeutic application, especially in patients suffering from chronic unhealing wounds.

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