

Protein Disulfide Isomerase: Structure, Mechanism of Oxidative Protein Folding and Multiple Functional Roles

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Abbreviations

ER: Endoplasmic reticulum;

ERAD: Endoplasmic Reticulum Associated Degradation;

MTP: Microsomal Triglyceridtransfer Protein;

PACMA: Propynoic Acid Carbamoyl Methyl Amides;

PDI: Protein Disulfide Isomerase;

P4H: Prolyl-4-Hydroxylase;

PPI: Peptidyl Prolyl Isomerase;

UPR: Unfolded Protein Response;

ABSTRACT

Protein disulfide isomerase (PDI) is a member of the thioredoxin superfamily of redox proteins. Originally, PDI was identified in the lumen of the endoplasmic reticulum and subsequently detected abundantly in many other tissues and account for 0.8% of total cellular protein. PDI consists of four tandem thioredoxin-like domains *a*, *b*, *b'*, and *a'* plus a C-terminal extension which are arranged into a U-shape structure. PDI has three catalytic activities including, thiol-disulfide oxidoreductase, disulfide isomerase and redox-dependent chaperone. Now the functions ascribed to PDI have evolved significantly because recent studies have shown that it has detrimental as well as protective effects in diseases states. Keeping the above views in mind, in this review we have discussed the structure of PDI, its catalytic and chaperone activity and its role in various diseases states.

Key words: PDI; Protein disulfide isomerase; Chaperone; Oxidoreductase; Isomerase; Thioredoxin

INTRODUCTION

Protein folding is a complex process in which the neighboring and distant amino acid residues of a polypeptide chain cling to each other to give rise to the native structure in which protein exhibits distinct biological activity. The main processes that govern protein folding have been mainly deduced from the *in vitro* studies. Thus, *in vitro* refolding is considered as a good model to understand the mechanism by which the polypeptide chain acquires unique three dimensional structure in the cell^[1]. However, *in vivo* conditions of protein folding differs from that *in vitro* because the environment *in vivo* (or intracellular) is highly crowded containing about 300-400 mg/ mL of macromolecules^[2]. The crowded environment provides many opportunities for improper associations among protein molecules leading to aggregation. To overcome these hurdles, cell has evolved molecular chaperones and enzymes peptidyl prolyl isomerase (PPI) and protein disulfide isomerase (PDI) for preventing mis-associations and aggregation among protein molecules and catalyzing proper folding reactions respectively.

Protein disulfide isomerase (PDI) is a major ER protein that functions as a molecular chaperone and a folding enzyme by

catalyzing the formation, cleavage, and rearrangement of the disulfide bonds in unfolded or misfolded proteins^[3-6]. PDI was first isolated from rat liver in 1963 by the pioneer of protein folding; Christian B. Anfinsen^[7]. The enzyme is resident in the endoplasmic reticulum (ER) of eukaryotic cells, where many folding factors and protein chaperones are present. It is also found abundantly in many other tissues and account for 0.8% of total cellular protein^[8]. PDI is induced during endoplasmic reticulum (ER) stress^[3] and possess chaperone activity that binds misfolded protein and assists proper folding reaction. PDI is not only a catalyst of the formation of disulphide bonds, but also catalyses folding steps which involve significant conformational change in the folding protein^[9]. PDI is also a subunit of two well characterized ER enzymes, prolyl-4-hydroxylase (P4H)^[10], and microsomal triglyceride transfer protein (MTP)^[11].

The structural and functional studies on PDI from various species, including human^[12-16] yeast^[17,18], and thermophilic fungus^[19-21] have been reported by many researchers. The data showed that PDI consists of four tandem thioredoxin-like domains *a*, *b*, *b'*, and *a'* plus a C-terminal extension^[3-5,22], which are arranged into a U-shape structure^[16-18]. Among the four domains, *a* and *a'* domains possess a catalytic CXXC motif, whereas *b* and *b'* domains lack this active site motif and activity. Accumulating evidences suggest that the *b'* and *a'* domains of PDI are primarily responsible for the substrate recognition^[5,20,23]. In particular, mutational and cross-linking analyses indicate that the *b'* domain represent peptide binding site in PDI^[23]. The *a'* domain is oxidized by the flavoprotein Ero1 and thereby acts as a disulfide donor for the PDI substrates^[24,25].

The PDI plays an important role in the cell. This was first recognized in yeast, where PDI was found to be essential for cellular viability^[26]. Currently, no viable PDI knockout strain has been reported in rodents. This further validates the importance of PDI in normal cellular physiology^[27]. Further, protein disulfide isomerase ameliorates β -cell dysfunction in pancreatic islets overexpressing human islet amyloid polypeptide. Thus, it has protective role in Type II diabetes^[28]. Recent studies have convincingly demonstrated that PDI also plays an important role in both the physiology and pathophysiology of disease states including diabetes^[29], cardiovascular diseases^[30]. In particular, PDI serves a critical role in thrombus formation, which is underlying cause of myocardial infarction and stroke. Quercetin-3-rutinoside, a flavonol highly abundant in common foods, inhibits PDI and blocks thrombus formation both *in vitro* and *in vivo*^[31]. It also plays important roles in cancer^[32], neurodegenerative conditions^[33] and mediates entry of pathogens in infectious diseases^[34].

Keeping the above views in mind, in this review we have discussed PDI structure, catalysis of disulfide bond formation and isomerization and its role as chaperone and in pathophysiology^[35].

PDI STRUCTURE

PDI is a soluble 55-kDa protein that contains the thioredoxin-like $\beta\alpha\beta\alpha\beta\alpha\beta\alpha\beta\alpha$ domain^[36]. Thioredoxins belong to a class of oxidoreductases that contain a dithiol-disulphide active site which participates in redox signaling^[37]. Besides this, 21 more family members have been studied for their structure and enzymatic properties^[38]. However, the enzymatic properties of these proteins differ in their redox potential hence substrate specificity^[39], the sequence of their active site and the pKa of the active site cysteine residues^[40]. They are primarily localized in the ER where they maintain an oxidative environment and thereby contribute to ER

homeostasis^[41]. Full length PDI contains 508 amino acid residues which are organized into four tandem domains namely *a*, *b*, *b'*, *a'* (Figure 1). The homologous *a* and *a'* domains share 47% similarity (Figure 1). The homologous *a* and *a'* domains share 47% similarity and contain the active site, CGHC motif^[42]. The active site cysteines interact with the thiol group of a newly synthesized substrate, thus mediating the formation and isomerization of protein disulphide bonds^[43]. The *b* and *b'* domains share 28% identity and they assist in the binding of protein substrates and lack the catalytically active cysteine residues^[44]. Recent findings indicate that the *b'* and *a'* domains provide substrate binding sites and undergo an open-closed domain rearrangement depending on the redox states of the *a'* domain^[45,46]. PDI also contains a x linker region and an acidic C-terminus containing a, KDEL-ER retrieval sequence^[47].

The first complete structure of yeast PDI has recently been determined^[17]. Although, the structures of individual domains of various PDIs have been solved over the past ten years, the recent structure of the yeast PDI now provides the first complete picture of how the individual pieces assemble together. The structure of each domain shows that N and C termini are flexible. But it was not understood, whether flexibility was due to an artifact or whether the domain termini are flexible in the intact protein. However, it is now clear that it is due to apparent flexibility in domain termini.

The structure of PDI is depicted in Figure 2. The figure shows that PDI comprises four thioredoxin-like domains (*a*, *b*, *b'* and *a'*) which are arranged in a twisted 'U' shape structure, in which the catalytic active motifs of domains *a* and *a'* face each other across the long sides of the U. Domains *b* and *b'* form a rigid base for the U-shaped molecule. The inner surfaces of domains *b* and *b'* form a hydrophobic region which is implicated in the substrate recognition. The C-terminal tail is located at the top of domain *a'* facing outwards from the U shape structure.

CATALYSIS OF DISULFIDE BOND FORMATION AND ISOMERIZATION

PDI contains two conserved active site motif (CGHC), where one

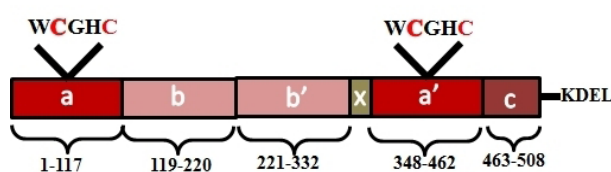


Figure 1 Domain organization of PDI. The linker region x (shown in mustard color) is responsible for the U shape structure of PDI. The C terminus is illustrated in brown, followed by an ER retrieval signal, KDEL.

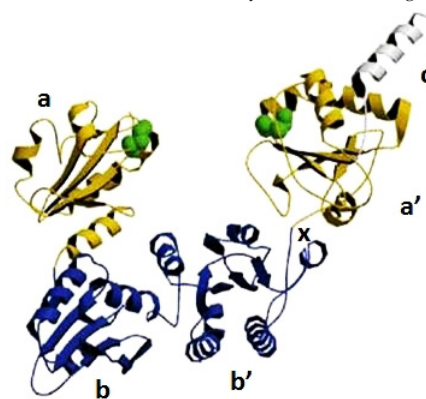


Figure 2 Ribbon diagram of PDI [2B5E]^[17] with the *a*, *b*, *b'*, *x* (linker region), *a'* domains, and the C-terminal.

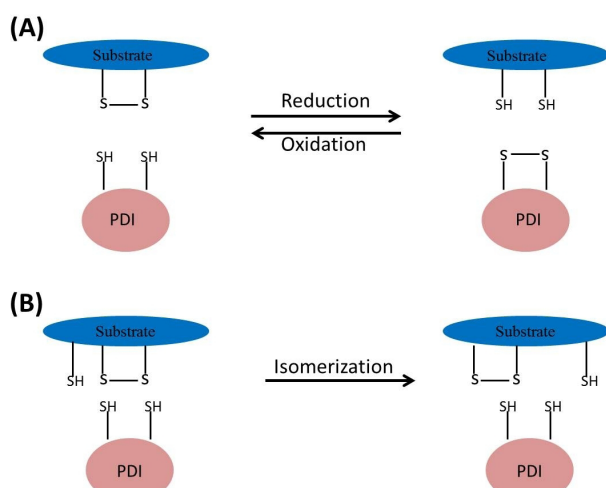


Figure 3 A. PDI-catalyzed disulfide bond formation. PDI-catalyzed disulfide bond formation occurs when oxidizing equivalents are transferred from the disulfide of the oxidized PDI active site to the reduced substrate, forming reduced PDI. Conversely, the reduction of a substrate disulfide oxidizes the PDI active site. B. PDI catalyzed disulfide isomerization in the protein substrate.

active site is situated near the N-terminus (a-domain) while the other near the C-terminus (*a'* domain)^[48-51]. Like other members of the family, these two cysteines can either form an intramolecular disulfide (oxidized PDI) or exist in the dithiol form (reduced PDI). In the oxidase activity, PDI inserts disulfides into protein substrates, which occur by pairing of two substrate cysteines into disulfides with concomitant reduction of disulfides of the PDI active site (Figure 3). The oxidase activity requires only a or *a'* domain. This oxidase activity occurs because of high reactivity of the active site disulfides, which are approximately 500-fold more reactive than the disulfide of glutathione^[52]. The N-terminal of the two cysteines in both redox-active domains possess a low pKa (4.5) compared to free cysteine (8.7), and both N-terminal cysteines are solvent exposed, giving rise to two highly reactive thiol groups. On the contrary, the C-terminal cysteines are buried in the interior core and have high pK value around 9.0; therefore they are unreactive. In the oxidized state, the active-site cysteines of PDI are in the disulfide form, which can act as an electron acceptor to form disulfide bonds in sulfhydryl-containing proteins. Disulfide formation in a substrate protein probably involves direct transfer of a disulfide bond from the catalyst to the protein via a mixed disulfide intermediate between the two^[3,8].

Disulfide bond formation that occurs during folding of protein is often error prone, resulting in an erroneous pairing of two cysteines or pairing of correct cysteines but in a temporal order that impedes further folding^[53]. When protein contains wrong pairs of disulfide bonds, PDI catalyzes disulfide isomerization of these bonds into correct pair of disulfides. Thus, the disulfide bonds are broken and reformed with different cysteines. The active site cysteine near the N-terminus of PDI (CGHC), initiates isomerization by attacking on a non-native disulfide bond and consequently forms a mixed disulfide intermediate. Once the mixed disulfide between PDI and the substrate is formed, the resulting substrate thiol is free to attack another protein disulfide bond. The mixed disulfide formed between substrate and PDI can also be mediated by the second active-site cysteine of the PDI, but under this condition it is not involved in the formation of mixed disulfide^[54]. The isomerization reaction is driven by energy minimization where the native disulfide bond is energetically favored and thus forms more quickly than the reoxidation of the same non-native disulfide bond. Once the catalysis reaction is over, PDI is

regenerated in its original reduced state^[3].

Another mechanism by which PDI catalyzes disulfide isomerization is the reduction of incorrect disulfide bonds into thiol group by one PDI molecule or redox-active domain, in the next step, PDI molecule or redox-active domain catalyzes oxidation of disulfides bonds which becomes correctly paired^[3]. Interestingly, both a and *a'* domains can reduce and oxidize protein disulfide bonds, but they are incapable of shuffling disulfides^[55]. Therefore, combination of domain *b'* with domains *a'* and c is required for the isomerase activity, but this combination of domains is not sufficient for catalyzing isomerase reactions that involve large conformational changes^[3].

PDI IS A CHAPERONE

PDI has the ability to distinguish between partially folded, unfolded, and properly folded protein substrates. It shows high affinity for misfolded proteins rather than native proteins^[56]. It binds misfolded protein through hydrophobic interaction. These properties, together with its conformational flexibility, make PDI a highly effective chaperone^[57]. In the chaperone activity PDI binds misfolded proteins within the ER and activates the unfolded protein response (UPR) where the main function of UPR is to reduce the load of unfolded proteins by increasing the curvature of ER, thereby reducing protein biosynthesis. Other function of UPR is the induction of PDI and other chaperones that further increase protein folding capacity of protein substrate^[58,59]. Thus, UPR shows protective role, but it has been found that prolong activation of UPR leads to apoptosis of the cell^[60]. PDI facilitates the degradation of misfolded proteins via ER association degradation (ERAD) pathway. This occurs by translocation of proteins from the ER to the cytoplasm, where it is degraded by the proteasomal machinery: ubiquitin proteasome system^[61,62]. PDI also helps in protein quality control by retaining unassembled procollagen until the correct native structure is formed^[63]. PDI also possesses chaperone activity. For instance, it stabilizes the native conformation of the β subunit of collagen prolyl 4- hydroxylase^[64]. PDI also exhibits both chaperone and anti-chaperone activity depending upon its initial concentration. When PDI's chaperone concentration is high, virtually all of the substrate protein molecules are correctly folded. At low concentrations, PDI possesses antichaperone activity which promotes intermolecular disulphide crosslinking among protein substrates leading to the formation of large amorphous aggregates^[65].

ROLE OF PDI IN DISEASES STATES

Recent studies have convincingly shown that PDI plays important roles in the physiology as well as pathophysiology of the disease states including diabetes^[28], cardiovascular diseases^[66], cancer^[67] neurodegenerative conditions^[68] and the entry of pathogens in infectious diseases^[69]. However, the precise roles for PDI in each of these diseases have not yet been elucidated. PDI is upregulated in various tissues during disease. Interestingly, both protective and harmful effects have been assigned for PDI. The diseases states results from either a loss of normal protective function in some situations, or gain of toxic function in others. Hence, association between the PDI family and human diseases requires further validation. Currently, it has been thought that PDI plays an important physiological role *in vivo*.

PDI IN CANCER

PDI is highly expressed and up-regulated in various cell types; including kidney, lungs, brain, ovarian, melanoma, prostate, and

male germ cell thereby it induces cancers or tumors in these organs and cells^[67]. The mechanism involved in these diseases states include suppression of apoptosis by PDI which results in the progression of tumor growth and metastasis. Cell surface PDI is also implicated in cancer progression and administering anti-PDI monoclonal antibodies inhibits the invasion of glioma cells^[70]. This further validates that PDI promotes survival and progression of various forms of cancer cells. Hence, inhibitors of PDI may possess therapeutic actions against cancer progression^[67]. Recently a series of PACMA (propynoic acid carbamoyl methyl amides) compounds were synthesized that showed potent anticancer activity for human ovarian cancer by inhibiting overexpressed PDI^[71]. In a similar vein, bacitracin, a pharmacological inhibitor of PDI, reduced the *in vitro* migration and invasion of human brain glial cells^[70]. However, the specificity of bacitracin as an inhibitor of PDI is in dilemma^[72]. Small-molecule inhibitors of PDI which bind to the CGHC active site may also inhibit PDI function in melanoma cells^[73]. However, the role of PDI in supporting tumor survival is dependent on the specific type of cancer cell. Hence, it is important to recognize the specific type of cancer cell for future applications in cancer therapy.

PDI IN NEURODEGENERATIVE DISEASES

Neurodegenerative diseases are also known as protein misfolding diseases occur due to misfolding of protein molecule which associates with complementary molecule to form oligomers and consequently amyloid fibrils. The amyloid fibrils accumulate as amyloid deposit within the affected tissues of the brain and central nervous system. Protein misfolding within the ER triggers ER stress, hence up-regulation of PDI^[58]. Most studies suggest that the induction of PDI during ER stress in neurodegenerative diseases reduces the load of misfolded proteins or increase the propensity of protein to fold correctly, thus restoring proteostasis and increase the neuronal cells viability. PDI is upregulated in dopaminergic neurons and Lewy bodies of patients with Parkinson's disease. Here, it reduces aggregation of synphilin-1 protein in neuroblastoma cells of Parkinson diseases patient^[74]. Similarly, PDI also prevents aggregation of another Parkinson's associated protein, α -synuclein, in cell-free *in vitro* systems^[75]. Furthermore, PDI is upregulated in response to hypoxia in the brain and it prevents neuronal and cardiomyocyte apoptosis by assisting proper folding reaction of misfolded proteins^[76]. In prion diseases, PDI and ERp57 have protective function against prion induced toxicity *in vitro*^[77] because inhibition of PDI raises the levels of misfolded prion proteins^[78]. Furthermore, over-expression of PDI showed protective role against the formation of mutant SOD1 inclusions and ER stress, whereas knockdown of PDI using siRNA increases mutant SOD1 aggregation and inclusion formation^[79]. Small molecule mimics of PDI reduce mutant SOD1 aggregation *in vitro*^[79].

ROLES OF PDI IN CARDIOVASCULAR DISEASE

PDI plays both protective and harmful roles in cardiovascular disease. PDI prevents misfolding of proteins in the myocardium during ischemic myocardial injury^[80]. PDI is also up-regulated in hypoxia induced in myocardial capillary endothelial cells^[81] hence is associated with significant decrease in the rate of cardiomyocyte apoptosis in murine models^[82]. PDI plays an important role in the maintenance of endothelial cell structure^[82]. It is also involved in platelet derived growth factor (PDGF)-induced vascular smooth muscle cell migration^[83] which is an important therapeutic target in atherosclerosis^[84]. Conversely, increased expression of PDI is

protective against endothelial cellular migration, adhesion, and tubular formation in mice suggesting an important role for PDI in angiogenesis^[85]. The diabetes is associated with, coronary artery disease and an increased risk of heart failure, and consequently PDI function is impaired in mouse models of diabetes. This may be due to alterations in the oxidoreductive state^[80]. This is supported by the findings that reduced PDI has been detected in the diabetic heart after ischemia, therefore it is virtually incapable of protecting diabetes^[80]. PDI has been implicated in detrimental activities in cardiovascular diseases. Since over-expression of PDI in myocytes decreases the levels of misfolded pro-insulin molecule as well as insulin secretion, thereby it induces ER stress leading to apoptosis^[86].

PDI MEDIATES PATHOGEN ENTRY LEADING TO INFECTIOUS DISEASES

PDI is also implicated in mediating the entry of pathogens during infectious disease because over-expression of PDI increases, the fusion of viral membrane to host cell, leading to internalization of HIV-1^[87] resulting in HIV diseases. Similarly, cell surface PDI facilitates infection of HeLa cells by fusing mouse polyoma virus with host cell membrane^[88]. Recent study has shown that toxoplasma protein disulfide isomerase (PDI) gondii participates in human infection^[89].

CONCLUSIONS

PDI consists of four tandem thioredoxin-like domains *a*, *b*, *b'*, and *a'* plus a C-terminal extension^[3-5,22], which are arranged into U-shape structure^[16-18]. It is a major ER protein that functions as a molecular chaperone and a folding enzyme by catalyzing the formation, cleavage, and rearrangement of the disulfide bonds in unfolded or misfolded proteins^[3-5]. Recent studies have convincingly demonstrated that PDI indeed plays important roles in both the physiology and pathophysiology of disease states including diabetes^[29], cardiovascular diseases^[30], cancer^[32], neurodegenerative conditions^[33] and the entry of pathogens in infectious diseases^[34]. However, the precise roles for PDI in these disease states have not yet been established. Thus, in future precise role of PDI in each of the disease state should be clearly dissected for the therapeutic interventions.

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CONFLICTS OF INTEREST

The authors have not conflicts of interest to declare.

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