REVIEW ARTICLE

The protein disulphide-isomerase family: unravelling a string of folds

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The mammalian protein disulphide-isomerase (PDI) family encompasses several highly divergent proteins that are involved in the processing and maturation of secretory proteins in the endoplasmic reticulum. These proteins are characterized by the presence of one or more domains of roughly 95–110 amino acids related to the cytoplasmic protein thioredoxin. All but the PDI-D subfamily are composed entirely of repeats of such domains, with at least one domain containing and one domain lacking a redox-active -Cys-Xaa-Xaa-Cys- tetrapeptide. In addition to their known roles as redox catalysts and isomerases, the last few years have revealed additional functions of the PDI proteins, including peptide binding, cell adhesion and perhaps chaperone activities. Attention is now turning to the non-redox-active domains of the PDIs, which may play an important role in all of the known activities of these proteins. Thus the presence of both redox-active and -inactive domains within these proteins portends a complexity of functions differentially accommodated by the various family members.

Key words: calsequestrin, ERp28, ERp57, ERp72, P5, PDI-D, PDIp, PDIR, thioredoxin.

INTRODUCTION

Even as the translocation of a protein into the endoplasmic reticulum (ER) proceeds, the translocating polypeptide is engaged by a host of ER-resident, luminal proteins that subject it to various modes of post-translational processing. These proteins form part of the elaborate mechanisms of retention, retrieval, regulation of protein expression, assisted folding and degradation that have evolved so as to ensure that only the desired, properly folded and oligomerized species of secretory proteins exit the ER. Although significant progress has been made in recent years regarding the nature of the molecular effectors partaking in these processes, much still remains obscure. This haziness in detail has, until recently, been an unfortunate characteristic of our knowledge of the crucial oxidative/non-oxidative folding processes that occur in this cellular compartment. Recent insight into the cell's response to the accumulation of misfolded protein in the ER [1], the role played by the lectins calreticulin and calnexin [2,3], as well as the mechanism of reverse transport of malfolded proteins into the cytoplasm for degradation by the proteasome [4–6], is shedding invaluable light on the matter. Part of the puzzle is the role played in protein folding by protein disulphide-isomerase (PDI) and members of the PDI family. This review is devoted to these latter proteins, summarizing a growing, though yet incomplete, wealth of data on their structure/function relationships.

PDI

PDI (EC 5.3.4.1) is a member of the thioredoxin superfamily. Its structure encompasses two double-cysteine, redox-active sites, each within domains with high sequence similarity to thioredoxin [7,8], separated by a further two thioredoxin-related domains (hereafter referred to as thioredoxin fold domains) lacking reactive cysteines [9,10]. It is a highly abundant ER luminal protein in mammalian cells and in yeast, constituting approx. 0.8% of total cellular protein [8] and reaching near-millimolar concentrations in the ER lumen of some tissues [11]. First isolated from liver in 1963 by Anfinsen's group [12], PDI has since been discovered in a variety of tissues and organs [13], and is highly conserved between species. The protein, about 510 amino acids in length, carries a typical C-terminal KDEL (HDEL in yeast) ER retrieval sequence, and is usually isolated as a homodimer [8], although monomers and homotetramers are also known to occur [14]. PDI is a catalyst of the rate-limiting reactions of disulphide-bond formation, isomerization and reduction within the ER [8], and it displays chaperone activity (described below) *in vitro* and *in vivo*. In *Saccharomyces cerevisiae* PDI is an indispensable protein [15], and its essential role in this organism has been shown to be the isomerization of disulphide bonds [16], although this issue has been disputed for some time. Association between PDI and malfolded proteins (such as overexpressed lysozyme mutants) or endogenous, nascent proteins (e.g. immunoglobulins, thyroglobulin and procollagen) in the ER has been observed [17–20], where the complexes may range up to 1.5×10^6 Da or more [20]. PDI can complement DsbA-deficient *Escherichia coli*, and increases the yield of heterologously expressed disulphide-containing protein in *E. coli* [21] and cultured insect cells [22] (DsbA is the *E. coli* periplasmic PDI homologue). PDI is also an essential structural subunit of the enzyme prolyl 4-hydroxylase (P4H) [23] and the microsomal triacylglycerol transfer protein (MTP) [24] in mammalian cells.

Several other ER luminal proteins with high sequence identity to PDI have been identified (see below), and their putative functions have only recently started becoming apparent.

THIOREDOXIN AND THE THIOREDOXIN FOLD

Thioredoxin is a small, ubiquitous protein of approx. 12 kDa that is involved in several cellular activities [25,26]. For instance, it functions as a protein disulphide reductase (together with thioredoxin reductase and NADPH) and a hydrogen donor for

Abbreviations used: BiP, immunoglobulin heavy chain binding protein; BPTI, bovine pancreatic trypsin inhibitor; ER, endoplasmic reticulum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MTP, microsomal triacylglycerol transfer protein; PDI, protein disulphide-isomerase; P4H, prolyl 4-hydroxylase.

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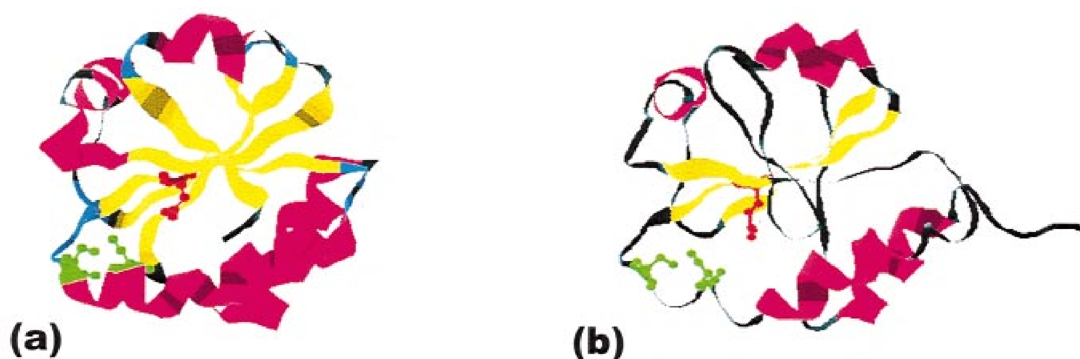

Figure 1 Comparison of the tertiary structure of thioredoxin with the thioredoxin fold of the a domain of PDI

(**a**) Ribbon model of the crystal structure of *E. coli* thioredoxin, as determined by Katti et al. [34]. (**b**) The **a** domain of human PDI, based on a model suggested by NMR data [9]. The Cys residues of the reactive site (green), Asp-26 of thioredoxin (red) and Glu-30 of PDI (red) are shown as ball-and-stick representations. α-Helical elements are shown in red, and β-elements in yellow.

several enzymes, including ribonucleotide reductase [26], as a subunit of T7 DNA polymerase [27,28], and as a subunit of the heterodimeric protein LMA1, required for vacuole inheritance in yeast [29,30]. The last two proteins do not require the redox activity of thioredoxin.

Thioredoxins from different species may show less than 25% identity, but all contain the -Cys-Gly-Pro-Cys- thioredoxin box [31,32]. The crystal structure of the *E. coli* protein has been determined [33,34], and is depicted in Figure 1.

Cys-32 occurs near the start of an α-helix [35], is surface-exposed and has a pK_a of 7.1 [36], much lower than that of the free cysteine (pK_a of 8.7 at neutral pH), rendering it highly reactive. At neutral pH the reactive sulphur atom of Cys-32 may share a hydrogen bond to the -SH hydrogen of Cys-35 [37]. The pK_a of Cys-32 is thought to be decreased by a nearby buried partial charge on Asp-26 [36,38], which may serve as a general acid/base in thioredoxin-catalysed redox reactions [39]. The Cys-32 thiolate can make a nucleophilic attack on disulphides, generating a mixed disulphide that is then disrupted by Cys-35 to produce a reduced substrate protein. The reactivity of Cys-35 follows upon its loss of a proton to Asp-26. The thiolate species generated is well poised to attack the intermolecular disulphide-bonded Cys-32. Thioredoxin is then recycled by thioredoxin reductase and NADPH. In close proximity to the reactive-site pocket are the hydrophobic residues Gly-33, Pro-34, Ile-75, Pro-76, Gly-92 and Ala-93, which may be important for protein–protein interactions [32,40].

Most, but perhaps not all [41], proteins catalysing the redox reactions involving reactive dithiols *in vivo* belong to the thioredoxin superfamily. This large family accommodates thioredoxin-like, glutaredoxin-like and PDI-like proteins, as well as members of the bacterial Dsb family [14,26,42,43], all of which contain one or more copies of the highly conserved thioredoxin fold, i.e. a β–α–β–α–β–α–β–β–α structure (as shown in Figures 1 and 2) encompassing a reactive -Cys-Xaa-Xaa-Cys- tetrapeptide [9,34,44–46].

It is interesting to note that, despite the lack of sequence similarity between thioredoxin and a bacterial homologue of PDI, i.e. the periplasmic protein DsbA, a marked similarity exists between their deduced three-dimensional structures [35]. The crystallized DsbA protein displays an extra four-helix insertion within the thioredoxin fold.

PDI MODEL

The groups of Creighton [9] and Söling [10] have recently shown that PDI is composed of four thioredoxin domains, and not two as was previously believed. The model of the domain structure of PDI, as shown in Figure 2, is based on our previous work [10] and has been adjusted for and combined with the structural data supplied for the **b** domain [9] and extended to accommodate all domains and all members of the family (see Figure 3). This model differs significantly from the earlier model suggested by Freedman's group [8].

The current model includes two domains, **a** and **a′**, with high sequence similarity to thioredoxin, each of which contains one copy of the -Cys-Gly-His-Cys- active-site sequence. The **b′** domain may have lost the -Cys-Gly-His-Cys- active-site sequence via point mutations [10], and sequence similarity upstream of the reactive cysteines (A boxes in Figures 2 and 4) can only be implied with the support of experimental data. The role of the **b** and **b′** domains, which share only a low degree of similarity with one another, has only recently started to be unravelled. PDI has a putative low-affinity, high-capacity Ca^{2+}-binding site at its C-terminus [47], within the **c** domain. Each **a** and **b** domain is composed of A, B and C boxes. The A boxes encompass β–α–β elements and indicate regions that are the least conserved between **a** and **b** domains and members of the PDI family, while the C box (β–β–α) indicates the most highly conserved region.

The redox/isomerase activities of PDI, as in thioredoxin, are due to the reactivity of the N-terminal Cys residue in two thioredoxin-like boxes (-Cys-Gly-His-Cys-) within the **a** and **a′** domains of the protein [38,48–51], which can function independently of each other. The N-terminal Cys residue of the first active site of PDI has a pK_a of 4.5, and is stabilized partly by the nearby histidine imidazole group (within the -Cys-Gly-His-Cys- sequence) and partly by partial positive charges at the N-terminus of an α-helix occurring just after this cysteine. Hence the disulphide form of the protein is much less stable than the corresponding disulphide of thioredoxin. This is reflected in the oxidative function of PDI, in contrast with the reductive role of thioredoxin [51,52].

It is unknown whether the side chain of the active-site proximal Glu-30 residue has a role in catalysis similar to that of Asp-26 of thioredoxin (see Figure 1).

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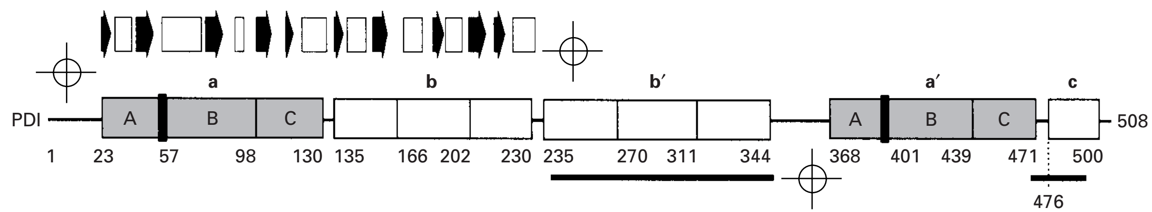
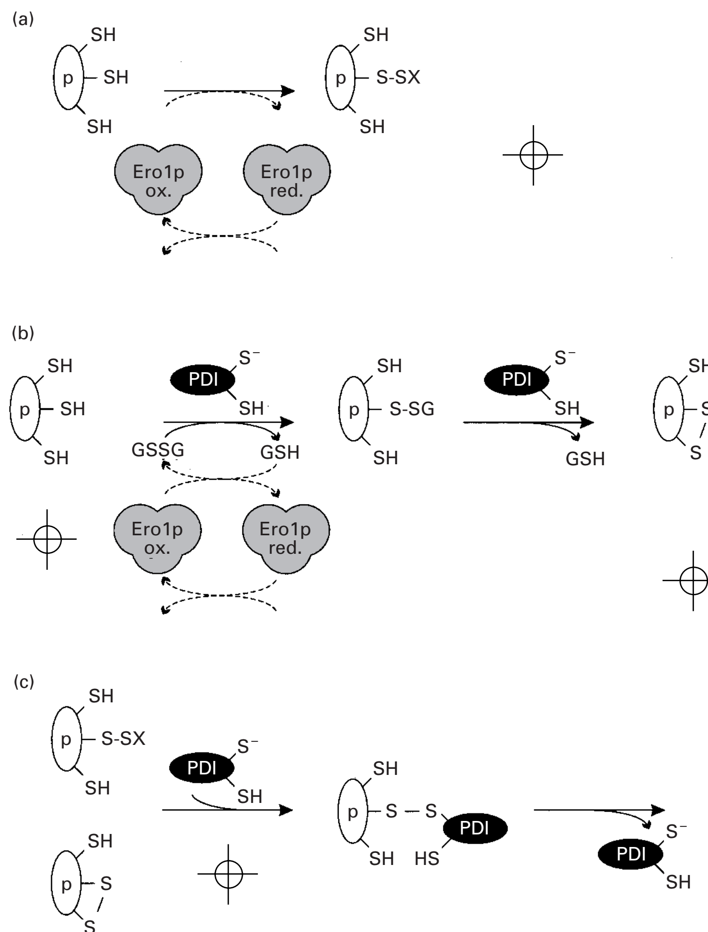


Figure 2 Domain structure of PDI

Domains are depicted as follows: **a** and **a′** (grey boxes) are redox-active thioredoxin fold domains, **b** and **b′** (red boxes) are redox-inactive thioredoxin fold domains, and **c** (white box) is a putative calcium-binding domain. The positions of secondary-structure elements, as determined by NMR [9], are shown above the domain structure (α-helices and β-strand elements are represented by red boxes and black arrows respectively). Horizontal bars below the domain structure indicate possible peptide-binding sites. The numbers below the boxes refer to the corresponding amino acid residues of the human protein. A, B and C designations are as in Figure 3.

REDOX/ISOMERASE FUNCTION

The redox activity of PDI is dependent on the redox potential of the medium, reflected in the ratio of reduced to oxidized glutathione in the ER (GSH/GSSG $\approx 2:1$) [53]. Evidence exists for the presence of a glutathione transporter in the ER membrane [53]. In addition, recent work suggests that oxidizing equivalents may enter the ER via electron transport machinery involving the novel Ero1p protein [54,55], resulting, perhaps, in the direct oxidation of GSH or of nascent proteins. In the latter case, the main role of PDI would be as an isomerase [54–56], and it would therefore be independent of re-oxidation by GSSG (see Scheme 1). Indeed, PDI does preferentially catalyse the net oxidation of proteins through glutathione–protein mixed-disulphide species [49,57].



Scheme 1 Possible mechanisms of disulphide-bond formation, reduction and isomerization

Disulphide-bond formation in a substrate protein (p; depicted in red) may be catalysed directly (**a**) or indirectly (**b**) by Ero1p. PDI may also catalyse this step (**b**). Disulphide-bond isomerization is catalysed by PDI using either the mixed protein/GSH disulphide (**b**) or some other inter- or intra-molecular disulphide (**c**) as substrate. S^- indicates the more reactive, N-terminal cysteine residue (as thiolate). Observe that the direct oxidation pathway in (**a**) followed by an isomerization pathway (**c**) obviates the requirement for glutathione (proposed by Kaiser and co-workers [55]). In this scheme, the source of oxidizing equivalents (Ox.) for the regeneration of oxidized Ero1p (Ero1p ox.) from the reduced form (Ero1p red.) is unknown.

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The standard redox potential of PDI is about −180 mV, much more oxidizing than that of thioredoxin (−260 mV) but less than that of bacterial DsbA (−100 mV) [58–60]. The difference in redox potentials is due mainly to the nature of the two intervening residues of the reactive -Cys-Xaa-Xaa-Cys- sequence [61]. PDI is about 50-fold more active than thioredoxin at catalysing the isomerization of disulphide bonds in scrambled RNase [58]. Studies on bacterial thioredoxin in which the thioredoxin-box -Cys-Gly-Pro-Cys- was mutated to -Cys-Gly-His-Cys- resulted in a protein with 10-fold higher oxidizing and disulphide-isomerase activity [8,62] and which had gained the ability to complement *S. cerevisiae* PDI1 null mutants (see below) [38], highlighting the importance of these residues.

Catalysis of disulphide-bond formation can occur co-translationally [63–65] and, in the case of isomerization, the reaction may proceed at several thousand times the uncatalysed rate [66]. A possible mechanism of PDI-catalysed disulphide-bond formation and isomerization is outlined in Scheme 1.

The second Cys residue is required for net oxidation of substrate proteins *in vitro*, but its chief role *in vivo* may be to facilitate rapid disruption of intermolecular disulphides, allowing efficient scanning of several disulphide isomers and preventing trapping of PDI in disulphide-linked complexes [16,38,67,68].

Darby and Creighton [69] have shown that the **a** and **a′** thioredoxin domains of PDI, when expressed alone, though soluble and retaining near-full redox activity, show greatly reduced isomerase activity, comparable with that of the bacterial protein DsbA [70]. In the *E. coli* periplasm, isomerase activity is mainly attributed to the related DsbC protein [71]. Furthermore, it has recently been shown [72] that high redox/isomerase activity of PDI on bovine pancreatic trypsin inhibitor (BPTI), a model substrate, requires even the redox-inactive domains. The **b′** domain seems to be especially important for isomerization reactions, and this together with the **a′** and **c** domains constitutes the minimum PDI fragment observed to catalyse disulphide-bond rearrangement in BPTI folding intermediates at a significant rate. Addition of the **b** domain to generate a **b**–**b′**–**a′**–**c** fragment further increased this rate to about 50% that of wild-type PDI [72]. Interestingly, the **b′** domain has also been indicated to be a major peptide-binding site (see below).

The fact that PDI is an essential protein in *S. cerevisiae* [15] (see below), where its critical role is the isomerization of disulphide bonds [16] rather than its redox properties, further highlights the importance of investigating the role of the **b**/**b′** domains in PDI function. Finally, it may be of importance that the **b**/**b′** domains of PDI are a target for phosphorylation and may even catalyse ATP hydrolysis, which increases upon binding of peptides but not during redox reactions [73,74].

PDI-DEFICIENT YEAST STRAINS AND THE MATURATION OF CARBOXYPEPTIDASE Y

PDI is essential for the viability of *S. cerevisiae* [15,75–78], and its essential role is in the isomerization of disulphide bonds [16,38], although its redox activity is clearly important for substrates such as carboxypeptidase Y [79]. Mammalian PDI can rescue PDI-deficient yeast, despite only low sequence identity (30%) between the two proteins [81]. In fact, even thioredoxin can complement Δ*Pdi1* yeast, providing that the -Cys-Gly-Pro-Cys- thioredoxin box sequence is mutated to either a PDI-type thioredoxin box, which results in a 10-fold increase in disulphide isomerase activity [38,62], or to a -Cys-Gly-Pro-Ser- box [38]. These mutations raise the redox potential of thioredoxin to a value closer to those of PDI (−180 mV) and the GSH/GSSG redox couple in the ER lumen.

In light of the preceding discussion on the role of the N-terminal Cys residue within the thioredoxin box in isomerase activity, it is not surprising to learn that Eug1p, a PDI-related protein with active-site sequences -Cys-Leu-His-Ser- and Cys-Ile-His-Ser- [80], can complement the isomerase deficiency of PDI-deficient yeast, and yet cannot effect maturation of carboxypeptidase Y [79] to wild-type levels. Integrity of the reactive site of the first **a** domain of PDI is clearly more important for maturation of carboxypeptidase Y than is the **a′** domain. For the mammalian proteins ERp72 and P5, both of which are known to complement PDI-deficient yeast, no similar preference for any particular **a** domain is observed [81] (B. Kramer and H.-D. Söling, unpublished work). The finding that ERp57 could not complement this yeast strain [81] may simply be due to the facts that (1) ERp57 did not translocate well (some complementation occurred when the TRG1 leader was used) and (2) ERp57 perhaps did not remain in, or was not retrieved effectively by, the ER. The retrieval signal of ERp57, QEDL, is unusual, and does not occur in *S. cerevisiae*. Finally, it is interesting to note that the recently described PDI-D subfamily of PDI-related proteins (D. M. Ferrari and H.-D. Söling, unpublished work; see Figure 3) contains both redox-active (PDI-Dα) and redox-inactive (PDI-Dβ) homologues. Only the PDI-Dα proteins can compensate for yeast PDI deficiency (D. M.Ferrari, J. Monnat, B. Kramer, T. Soldati and H.-D. Söling, unpublished work).

It is thus probable, and very plausible, that all members of the PDI family containing reactive thioredoxin boxes can complement PDI-deficient yeast strains with their isomerase activity. The redundancy of the system could be due to varying efficiencies of catalysis with different substrates.

CHAPERONE FUNCTION

A chaperone is a protein that can assist unfolded or incorrectly folded proteins to attain the native state by providing a micro-environment in which losses due to competing aggregation reactions are reduced, and which mediates the reversibility of pathways leading to incorrectly folded structures. For PDI, the extent to which the chaperone and redox/isomerase activities are apparent in the folding of proteins is substrate-dependent, as is the degree of involvement of individual **a** or **a′** domains in redox/isomerase activity [69,79,82–84]. Whereas chaperone activity has been shown for disulphide-containing proteins, such as lysozyme [85,86], and independence of the isomerase and chaperone activities has been shown *in vitro* in the refolding of acidic phospholipase A_2, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and rhodanese [87–89], chaperone activity is lacking and does not seem to be required for folding of substrates such as antibody Fab fragments with intact disulphide bonds [90].

Interestingly, sub-stochiometric concentrations of PDI to non-native proteins may result in the facilitated aggregation of the species, a peculiarity also shared by BiP (immunoglobulin heavy chain binding protein) and termed anti-chaperone activity [85,91,92]. Although indicated to be independent of isomerase or even chaperone functions [91], Wang and colleagues [93] have recently shown that this effect may in fact be an alternative expression of the chaperone activity produced by the experimental conditions.

PEPTIDE BINDING

At the heart of the chaperone activity of PDI, and perhaps also important for its redox/isomerase activities, is its ability to

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interact with (incorrectly folded) polypeptides. Although two peptide-binding sites have been suggested in the literature, one located to the **b′** domain and another to the C-terminal 57 residues following the **a′** domain [84,97], to date very little is known of the identity of the residues involved and the nature of the interactions.

PDI interacts weakly ($K_D > 100$ μM) with polypeptides. The binding affinity increases with increasing length of the substrate backbone, but for peptides of similar length, those containing Cys residues may bind 4–8-fold more strongly [94,95]. In contrast with earlier data [94], Klappa and co-workers [95] observed that hydrophobic interactions may determine the efficiency of binding.

Peptide-binding studies using either somatostatin or a 28-residue peptide from the N-terminus of staphylococcal nuclease indicated that the interaction of peptides with PDI competes with the refolding of reduced, denatured RNase and scrambled RNase, reduces the efficiency of catalysis of insulin reduction, and inhibits the chaperone function of PDI in the refolding of denatured GAPDH [94–96]. This indicates not only that both peptide- and polypeptide-binding sites are the same or spatially juxtaposed, but also that they are close enough to the active site to inhibit redox activity when occupied by a polypeptide chain.

One peptide-binding site has been mapped to amino acids 451–475 of the mature PDI sequence (corresponding to the last three residues of the **a′** domain and most of the **c** domain; see Figure 2) using photoaffinity labelling techniques and tripeptides as substrates [97]. Although deletion of this sequence does not disrupt the redox activity of the protein [98], elimination of the terminal 51 amino acids from PDI results in an inability to refold denatured lysozyme and a reduced rate of refolding of certain disulphide-containing substrates (lysozyme, acidic phospholipase A_2), indicating the presence of a distinct peptide-binding site and the co-operation of peptide-binding and disulphide redox activities in the folding process [99].

The second, perhaps more general, binding site (mentioned above) localizes to the **b′** domain of PDI [84] (Figure 2). This domain has been shown to be sufficient for peptide binding, as well as being essential, but not sufficient, for the binding of larger polypeptides such as BPTI and scrambled RNase [84]. In the latter case, a **b′**–**a′**–**c** fragment was the smallest fragment capable of binding peptides and proteins efficiently, followed by an **a**–**b**–**b′** construct. Thus the **b′** domain seems to be of central importance, not only for the isomerase activity of PDI, but also for its substrate-binding ability.

Interestingly, Michalak and co-workers [100] have published findings on the possibly Zn^{2+}-dependent interaction of PDI and calreticulin. These authors found that not only did the interaction abolish the binding of Ca^{2+} to the high-affinity P site of calreticulin, but it also inhibited the ability of PDI to refold scrambled RNase A. The latter interaction could be localized to the N domain of calreticulin, and could be disrupted by the addition of Zn^{2+} or Ca^{2+}. Finally, recent data suggest that PDI may be a major peptide-binding protein of the ER, accepting TAP (transporter associated with antigen processing)-translocated polypeptides [101].

What about possible peptide-binding sites in other members of the PDI family? The **b′** domain of PDI shares 39 % identity with the corresponding domain of the pancreas-specific PDIp protein (49 % identity overall), but less than 20 % identity with the **b** domains of the other family members. The highly acidic C-terminal putative peptide-binding site shows no significant similarity with other family members, except for the inescapable similarity to the acidic residues of the **c** domains of ERp72 and P5. Furthermore, none of P5, ERp72 or ERp28 can replace PDI in assisting the refolding of denatured GAPDH *in vitro* (D. M. Ferrari and H.-D. Söling, unpublished work), and although ERp72, ERp57, PDIp and ERp28 have been reported to interact with peptides and/or malfolded proteins [10,102–108], only for PDIp has the interaction been indicated to be direct [108].

In *S. cerevisiae* PDI (accession no. X54535), it has been noted that a sequence with similarity to the C-terminal peptide-binding site of PDI locates to residues 252–277 [98]. Whether this sequence constitutes a *de facto* peptide-binding site is unknown.

SUBUNIT ASSOCIATION

P4H, an ER luminal soluble enzyme that catalyses procollagen pro-α-chain prolyl hydroxylation, is a heterotetrameric ($\alpha_2\beta_2$) protein containing two PDI molecules as β-subunits [23]. Here, the function of PDI may be the retention or stabilization of the complex in the ER [109,110], although Wells et al. [111] have shown that the β-subunit, as glutaredoxin, has dehydroascorbate reductase activity, and may therefore function in generating one of the cofactors of P4H, namely ascorbate. Although the active-site cysteines of the N-terminal thioredoxin domain are largely inaccessible (or inactivated) in the complex, and PDI activity as measured by a scrambled RNase assay is reduced by about 50 % [110], the interaction between α- and β-subunits is independent of the active-site cysteines.

Another similar function of PDI is as the β-subunit of the heterodimeric MTP [24], which facilitates the incorporation of triacylglycerols into lipoproteins. Here again, PDI seems necessary to keep the complex in solution, as irreversible inactivation and aggregation ensues upon removal of the β-subunit [112].

Neither of the more active (i.e. N-terminal) Cys residues of the two thioredoxin-like boxes in PDI are required for P4H [110] or MTP [113], although it has been shown that P4H probably contains disulphide bonds [114] and undergoes aggregation under reducing conditions even in the presence of PDI [115]. Furthermore, although the C-terminal 27 residues of PDI (amino acids 452–478 in human PDI) are essential for interaction with P4H, replacement of the corresponding residues of ERp60 with these PDI residues does not confer P4H-binding ability upon ERp60 [116]. Therefore, although the C-terminal peptide-binding site of PDI is essential for P4H binding, it is not sufficient. The nature of the remaining site(s) remains to be elucidated. In MTP, the C-terminal 30 amino acid residues are essential for interaction with PDI [117]. It is interesting that, in the MTP dimer, only very little PDI activity (in refolding of reduced, denatured RNase) can be measured [24], indicating that access to or reactivity of both active sites is blocked.

DIFFERENT LOCALIZATIONS OF PDI

There have been several reports on the possible extracellular localization of PDI in some cell types, despite the integrity of the KDEL signal [118–120]. Of these, perhaps the most noteworthy is the cell-adhesion protein retina cognin [121], a 50 kDa retina-specific protein which is probably a truncated version of PDI lacking the **a** domain and the C-terminal KDEL tetrapeptide [122,123]. This protein is involved in cell recognition and neuronal differentiation of the embryonic retina, and the exported protein has been observed to enhance specific aggregation of retina cells. Both active and inactive domains seem to be involved in the process [124].

Within the cell, PDI family members may also be found in different compartments. Two outstanding examples have been reported: (1) a chloroplast-specific PDI in plants (RB60) [125]

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that retains a KDEL ER-retrieval tag but is directed to the chloroplast on the basis of an N-terminal chloroplast pre-sequence, and which regulates the light-induced translation of chloroplast mRNAs through its redox activity, and (2) the sarcoplasmic reticulum protein calsequestrin, which is composed entirely of **b**-type domains (see Figure 3). The crystal structure of this protein has recently been solved [126].

THE HAPPY FAMILY

It is becoming increasingly clear that most, if not all, members of the PDI family have both enzymic and chaperone functions to fulfil in the ER. All but the PDI-Dβ protein ERp28 are believed to be redox-active, although experimental evidence to confirm this has not yet been procured for all proteins. Most have been shown to catalyse the reduction of disulphide bonds *in vitro* and/or *in vivo*, and to be able to complement the lethal isomerase deficiency of a PDI-deficient yeast strain (see above). Differences between these proteins may be reflected in their slightly different tissue distributions [127], expression levels, substrate ranges and modes of regulation [108,128–130]. Known members of the PDI family are listed in Table 1, and domain structures of the various family members are depicted in Figure 3.

ERp57

ERp57 has the same modular structure of active and inactive domains as PDI, but lacks the C-terminal acidic region. The protein has the retrieval sequence QEDL [145,146]. On the amino acid level, overall identity with PDI is 33%, and identity with the **a**–**b**–**b**′–**a**′ domains of ERp72 is 40%. The protein responds to stress stimuli in a manner similar to that of the glucose-regulated proteins GRp78 and GRp94 [146,147], and may be up-regulated additionally by oestrogens in brain tissue and by luteinizing-hormone-releasing hormone in the pituitary [148].

ERp57 interacts with nascent monoglycosylated glycoproteins, but not with non-glycosylated proteins, in a disulphide-independent manner [104,105], but probably only indirectly via interactions with calnexin or calsequestrin [106,107], as ERp57 itself lacks lectin-like properties [106]. This is in contrast with PDI, which interacts with proteins independently of their glycosylation status [65,104,107]. Furthermore, it is interesting that this calnexin- or calreticulin-mediated interaction greatly increases the disulphide-isomerase activity of ERp57 on a monoglucosylated protein, whereas the same type of interaction has been indicated to reduce PDI activity and peptide binding [100,106,149].

ERp57 exhibits a lower redox activity than PDI, as seen in a glutathione–insulin transhydrogenase assay [150,151], and cannot substitute for PDI in P4H [116]. Although ERp57 has been claimed to possess carnitine medium/long-chain acyltransferase (CPT) activity [152,153] and proteolytic activity [137,154,155] dependent on its active-site cysteines [156], both of these claims have been challenged [157].

PDIp

PDIp, the pancreas-specific PDI-family member [139], is the only known family member to date to show restricted tissue expression, being detectable only in the acinar cells of the pancreas [158]. It has the same domain organization as human PDI (apart from lacking the acidic region), with which it shares 45% identity.

Since PDIp has been shown, by chemical cross-linking techniques, to bind misfolded proteins (scrambled RNase A) and peptides (including zymogen-derived peptides) *in vitro* [108], and since it may be in transient contact with secretory proteins as they translocate into the ER [159], it is tempting to speculate that PDIp may be required for the folding of pancreas-specific proteins, e.g. zymogens. Interestingly, this peptide-binding interaction could be inhibited by competition experiments using stochiometric concentrations of oestrogens, such as 17β-oestradiol. Binding of proteins/peptides *in vitro* is also observed for PDI, but not for P5 [108]. PDI binding, however, is much

Table 1 Domain composition of members of the PDI family and related proteins

All mammalian PDI proteins known to date have at least one redox-inactive thioredoxin fold, as suggested by sequence analysis (D. M. Ferrari and H.-D. Söling, unpublished work). For PDI, experimental evidence exists for the presence of a thioredoxin fold in the **b** domain [9].

Protein	Molecular mass (kDa)	Domain structure	Redox-active-site sequence	No. of domains			Refs.
				a	**b**	**a + b**	
Thioredoxin (*E. coli*)							
Thioredoxin	12	**a**	-Cys-Gly-Pro-Cys-	1	0	1	[25,31,131]
PDI-like (mammalian)							
PDI	55	**a–b–b′–a′–c**	-Cys-Gly-His-Cys-	2	2	4	[7,9,10,59,60]
P5	46	**a⁰–a–b–c**	-Cys-Gly-His-Cys-	2	1	3	[132–134]
ERp72	71	**c–a⁰–a–b–b′–a′**	-Cys-Gly-His-Cys-	3	2	5	[134–136]
ERp57	54	**a–b–b′–a′**	-Cys-Gly-His-Cys-	2	2	4	[137,138]
PDIp	~ 55	**a–b–b′–a′**	-Cys-Gly-His-Cys- -Cys-Thr-His-Cys-	2	2	4	[139]
PDIR	57	**b–a⁰–a–a′**	-Cys-Ser-Met-Cys- -Cys-Gly-His-Cys- -Cys-Pro-His-Cys-	3	1	4	[140]
PDI-Dβ (mammalian)							
ERp28	26	**b–D**	None	0	1	1	[10,141,142]
PDI-Dα (*Dictyostelium discoideum*)							
Dd-PDI	38	**a⁰–a–D**	-Cys-Gly-His-Cys-	2	0	2	[143]
DsbA (*E. coli*)							
DsbA	21	**a**	-Cys-Pro-His-Cys-	1	0	1	[60,144]

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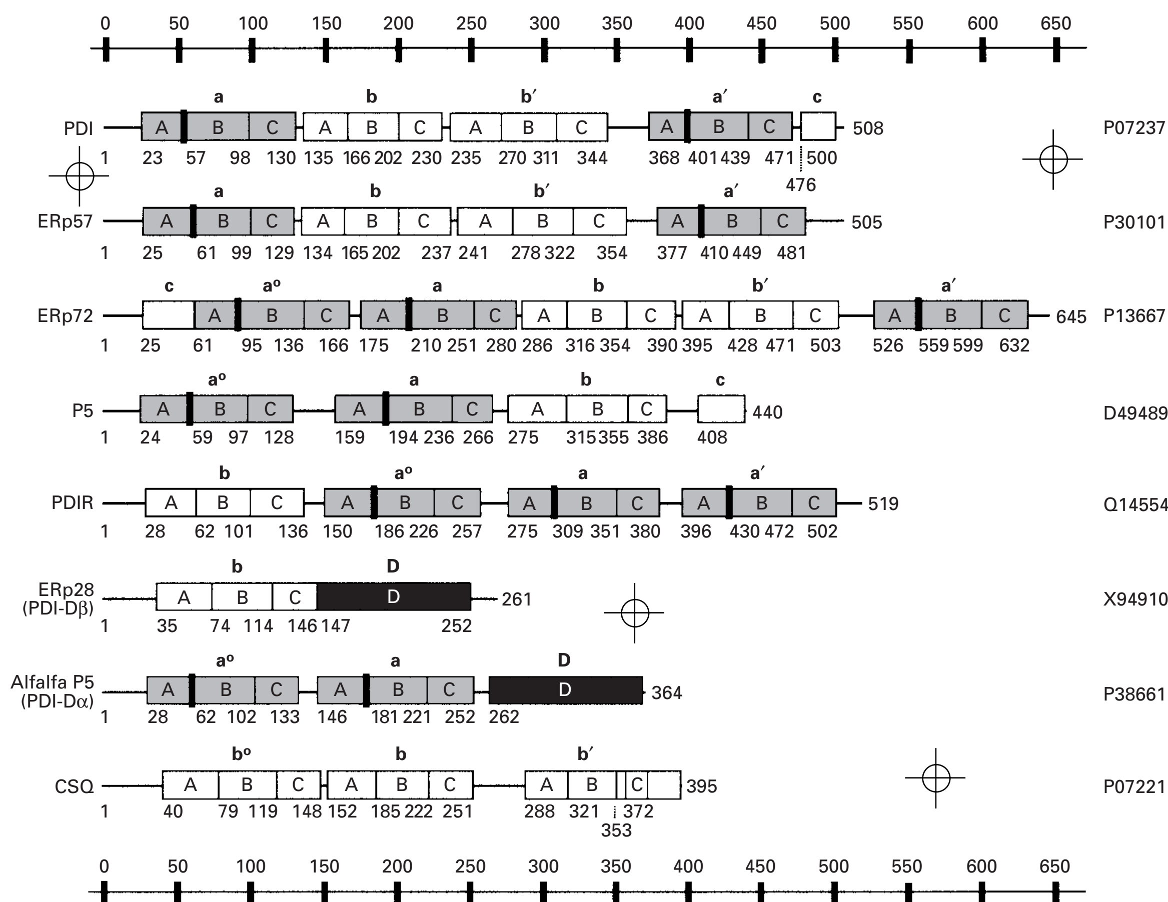

Figure 3 Domain structures of PDI-related proteins

All proteins are the human homologues, apart from PDI-Dα and calsequestrin (CSQ), which are from alfalfa and rabbit respectively. Domains **a°**, **a** and **a′** (grey boxes) are redox-active thioredoxin domains, whereas domains **b°**, **b** and **b′** (red boxes) are redox-inactive thioredoxin domains. Black bars indicate the positions of the active-site -Cys-Xaa-Xaa-Cys- sequence. **D** indicates the α-helical domain unique to PDI-D proteins (black box), and domain **c** (white box) is a highly acidic region. The letters A, B and C refer to regions with sequence similarity to the β–α–β, α–β–α and β–β–α secondary-structure elements respectively of the thioredoxin fold. The numbers below the boxes indicate the amino acid residues in the protein concerned. Accession numbers for the corresponding sequences are shown on the right.

less sensitive to oestrogens, although it was the finding that 17β-oestradiol could inhibit PDI-catalysed insulin reduction [160] that led to the suggestion of an oestrogen-receptor-like domain of PDI (domain **e**) [8].

P5

P5 is the smallest redox-active [134] PDI protein to have an inactive domain of the **b** type, with an **a°**–**a**–**b** domain distribution (D. M. Ferrari and H. D. Söling, unpublished work). P5 is not stress-inducible, although it is highly over-amplified in a hydroxyurea-resistant cell line [161]. The relevance of this amplification is not known.

Only mammalian homologues of the protein are known; previously defined P5 proteins from plants (such as alfalfa P5) have recently been re-designated to the novel PDI-D family (D. M. Ferrari and H.-D. Söling, unpublished work; see below).

ERp72

ERp72 is an abundant, ubiquitous, stress-inducible protein with calcium-binding capacity [132,135,136,162–165] that differs from other PDI-like proteins in having three active thioredoxin domains rather than two. The domain distribution is **c**–**a°**–**a**–**b**–**b′**–**a′** (see Figure 3). The protein possesses significant redox and disulphide-isomerase activity [136,166], and can complement PDI-deficient yeast [81]. The level of expression of the protein is up-regulated in antibody-producing cells [127] and differentiated F9 culture cells [129].

Although no peptide-binding capacity has been found for ERp72 [108], the protein and BiP have been shown to co-precipitate with an overexpressed, mutated substrate protein lacking glycosylation sites (human chorionic gonadotropin β-subunit) in a process that probably involves disulphide-bond formation [102] and which is independent of calnexin. ERp72 interacts *in vitro* with denatured proteins in association with molecular chaperones, including PDI, BiP and GRp94 [167,168]. Similar findings have been made *in vivo* for thyroglobulin and another secretory protein, thrombospondin [103,169].

ERp72, like ERp57, has been implicated in ER proteolytic activity [137,154,170]. However, as the role of ERp57 as a protease has been questioned, and since ERp72 in our hands fails to show protease activity (D. M. Ferrari and P. Van Nguyen, unpublished work), this issue remains unresolved.

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PDIR

Although little is known about the function of PDIR [140], its domain structure (**b**–**a°**–**a**–**a′**) is interesting. Each **a**-type domain has a different active-site sequence: -Cys-Ser-Met-Cys-, -Cys-Gly-His-Cys- and -Cys-Pro-His-Cys-. From the previous discussion of redox potentials, it could be speculated that each **a** domain has a different rate of catalysis, which may be optimal for the particular target substrate(s). There is no correlation between the sequential occurrence of each domain and its degree of sequence similarity with similarly positioned domains in other PDI proteins.

PDI-D proteins

Members of the recently described PDI-D subfamily ([10,141,142]; D. M. Ferrari, and H.-D. Söling, unpublished work) of PDI proteins may be either redox-active (PDI-Dα) or redox-inactive (PDI-Dβ), but all are characterized by an N-terminal α-helical domain of about 110 amino acids (termed the D domain), the function of which is unknown. These proteins are the only known PDI members to display a domain not related to thioredoxin. PDI-Dα, but not PDI-Dβ, proteins can complement PDI-deficient yeast. Furthermore, PDI-Dβ proteins cannot chaperone the folding of denatured GAPDH (D. M. Ferrari and H.-D. Söling, unpublished work). These proteins may be involved in the maturation of secretory proteins. ERp28, the human PDI-Dβ homologue, was found to co-precipitate with overexpressed hepatitis B surface antigen in COS cells [10]. In addition, Windbeutel, the *Drosophila* PDI-Dβ homologue, which may be essential for development of the embryo, has been suggested to be involved in folding/maturation of an essential factor that participates in a pathway leading to dorsoventral patterning [171,172].

CONCLUDING REMARKS

The data of Kemmink and co-workers [9] on the **b** domain of PDI and the recently solved crystal structure of calsequestrin, a PDI-derived protein [126], suggest that each inactive domain has retained its structural integrity. Indeed, the thioredoxin fold seems to be a robust scaffold that is not particular to much of its overall composition. Highly diverged, yet structurally intact, thioredoxin domains have been found in other, non-ER, non-redox-active proteins. This includes the C-terminal domain of the transducin regulator phosducin, and the N-terminal domain of HORF6, a human peroxidase enzyme of the novel peroxiredoxin (Prx) class. [173,174].

It is interesting that, for all PDI proteins known to date, probably all but the PDI-D subfamily contain at least one redox-inactive thioredoxin domain in addition to the active domain(s). Why is an inactive domain on the same polypeptide so advantageous? With recent work suggesting an important role for the **b** domains of PDI in peptide binding and stimulation of catalytic activity, it may be speculated that the substrate range and specificity of each PDI homologue is determined to a similar extent by the number, position and sequence of its inactive domains.

It could be that, within the ER, simple, uncontrolled disulphide-bond formation/isomerization of protein substrates at random is less advantageous than spatially or temporally controlled reactions, evolutionarily determined by the optimal chaperone/redox folding requirements of the substrate and its specific interaction with one or more co-evolving chaperone(s)/foldase(s).

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