PROTEIN EXPRESSION AND PURIFICATION 6, 700–706 (1995)

Production of Rat Protein Disulfide Isomerase in *Saccharomyces cerevisiae*

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Received March 17, 1995, and in revised form June 8, 1995

Protein disulfide isomerase (PDI) is an abundant protein of the endoplasmic reticulum that catalyzes the oxidation of protein sulfhydryl groups and the isomerization and reduction of protein disulfide bonds. *Saccharomyces cerevisiae* cells lacking PDI are inviable. PDI is a component of many different protein processing complexes, and the actual activity of PDI that is required for cell viability is unclear. A cDNA that codes for rat PDI fused to the α-factor pre–pro segment was expressed in a protease-deficient strain of *S. cerevisiae* under the control of an ADH2–GAPDH hybrid promoter. The cells processed the resulting protein and secreted it into the medium as a monomer, despite having a KDEL or HDEL sequence at its C-terminus. The typical yield of isolated protein was 2 mg per liter of culture. The catalytic activity of the PDI from *S. cerevisiae* was indistinguishable from that of PDI isolated from bovine liver. This expression system is unique in allowing the same plasmid to be used both to complement *pdi1*Δ *S. cerevisiae* and to produce PDI for detailed *in vitro* analyses. Correlations of the *in vivo* behavior and *in vitro* properties of PDI are likely to reveal structure–function relationships of biological importance. © 1995 Academic Press, Inc.

Protein disulfide isomerase (PDI,[2] EC 5.3.4.1) is present at millimolar levels in the endoplasmic reticulum of eukaryotic cells. This protein is an enzyme that catalyzes the oxidation of protein sulfhydryl groups and the isomerization and reduction of protein disulfide bonds (1–3). The active site of PDI can exist in either a reduced or oxidized state, or as a mixed disulfide with a substrate. The products of reactions catalyzed by PDI depend on the reduction potential of the solution.

PDI also appears to play other roles in cell biology. It has been identified as the β subunit of prolyl hydroxylase (4), as a thyroid hormone binding protein (5–6), and as a component of the microsomal triglyceride transfer protein complex (7). Although PDI is known to catalyze disulfide bond isomerization *in vitro* (8), its role *in vivo* is not known (9). PDI has, however, been shown to increase the heterologous production of other proteins in *Saccharomyces cerevisiae* (10).

The sequence of the cDNA that codes for rat PDI has been determined (11). Within its 527 residues, the protein translated in rat liver contains an N-terminal signal peptide and two pairs of homologous regions: amino acid residues 9–90 (region a) and 353–431 (region a′) and amino acid residues 153–244 (region b) and 256–343 (region b′). Regions a and a′ each contain an active site with the sequence WCGHCK, and these sites have been shown to act independently of one another (12). The C-terminus of PDI ends with the sequence KDEL, which has been implicated as the signal for retention of a protein in the endoplasmic reticulum of mammalian cells (13).

The sequence of the cDNA that codes for PDI from *S. cerevisiae* has also been determined (14–17). The encoded sequence of 522 amino acid residues is 30% identical to that of rat PDI. The amino acid sequence contains an N-terminal signal peptide, two putative active sites, five putative N-glycosylation sites, and a C-terminus ending with HDEL, the *S. cerevisiae* homolog of KDEL (14,18). *S. cerevisiae* mutants in which the PDI gene has been deleted are inviable (14–17).

We are interested in revealing the role of PDI *in vivo* and in correlating that role with properties of PDI determined *in vitro*. Here, we describe the expression in *S. cerevisiae* of the cDNA that codes for rat PDI. In our expression system, PDI is synthesized as a hybrid

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[2] Abbreviations used: DTNB, 5,5′-dithio-bis-(2-nitrobenzoic acid); FPLC, fast protein liquid chromatography; GSH, reduced glutathione; GSSG, oxidized glutathione; MES, 2-(*N*-morpholino)ethanesulfonic acid; M_r, relative molecular mass; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; PDI, protein disulfide isomerase; RNase A, bovine pancreatic ribonuclease A; SDS sodium dodecyl sulfate.

1046-5928/95 $12.00

with the 85-residue yeast α-factor pre–pro segment. The hybrid protein is processed efficiently by endogenous *S. cerevisiae* proteases, and active PDI is secreted into the medium.

EXPERIMENTAL PROCEDURES

Materials

Escherichia coli strain CJ236 (*dut ung thi relA*; pCJ105[Cm^r]) was from Bio-Rad (Richmond, CA). Protease-deficient *S. cerevisiae* strain BJ2168 (a *prc1-407 prb1-1122 pep4-3 leu2 trp1 ura3-52*) was from the Yeast Genetic Stock Center (Berkeley, CA).

Plasmid prpdi, which contains the cDNA that codes for rat PDI (11) was the generous gift of Dr. P. Tekamp-Olson. Plasmid pMP36 (19), which contains an ADH2–GAPDH hybrid promoter, DNA that codes for the α-factor leader region (pre–pro segment), and the GAPDH terminator, was the generous gift of Dr. M. A. Phillips. Enzymes for the manipulation of DNA were from Promega (Madison, WI) or New England Biolabs (Beverly, MA). Plasmid YEpWL.RNase A was from D. J. Quirk (20).

DNA oligonucleotides were synthesized on an Applied Biosystems Model 392 DNA/RNA synthesizer by using the phosphoramidite method (21) and reagents from Applied Biosystems (Foster City, CA) except for acetonitrile, which was from Baxter Healthcare (McGaw Park, IL). Synthetic oligonucleotides were purified with Oligonucleotide Purification Cartridges from Applied Biosystems. Bacto yeast extract, Bacto peptone, and Bacto yeast nitrogen base without amino acids (YNB) were from Difco (Detroit, MI).

DNA sequencing was performed with a Sequenase 2.0 kit from United States Biochemical (Cleveland, OH). DNA fragments were purified from agarose gels with a GeneClean II kit from BIO-101 (La Jolla, CA). Site-directed mutagenesis was performed by the method of Kunkel (22) on single-stranded DNA isolated from *E. coli* strain CJ236.

Yeast minimal medium (SD) contained (in 1.0 liter) Bacto YNB (6.7 g), dextrose (2%, w/v), and a supplemental nutrient mix (23). Yeast-rich medium (YEPD) contained (in 1.0 liter) Bacto yeast extract (10 g), Bacto peptone (20 g), and dextrose (2%, w/v). Variations of SD and YEPD media were also used. For example, S(8%)D–trp medium contained four times the concentration of dextrose and lacked tryptophan in the supplemental nutrient mix. Filtered medium was prepared by removing high molecular-mass material from YEP(1%)D medium by tangential flow over a 5 kDa-cutoff membrane using a Minitan apparatus (Amicon, Beverly, MA). All media were prepared in distilled, deionized water and autoclaved before use. *S. cerevisiae* cells were transformed as described (24). The cell density of cultures was determined by measuring the absorbance at 600 nm with a Cary Model 3 spectrophotometer, after diluting the culture so that A_{600} = 0.1–0.5 OD. Other manipulations of DNA, *E. coli,* and *S. cerevisiae* were performed as described (23,25).

Bovine PDI was from PanVera (Madison, WI). Reduced glutathione (GSH), oxidized glutathione (GSSG), NADPH, and scrambled RNase A were from Sigma Chemical (St. Louis, MO). 5,5′-Dithio-bis-(2-nitrobenzoic acid) (DTNB) was from Calbiochem (LaJolla, CA). Poly(C) was from Midland Certified Reagents (Midland, TX) and was precipitated from aqueous ethanol (70%, v/v) before use. All other chemicals were of reagent grade or better and were used without further purification.

Methods

Plasmid for production of pre–pro α-factor~PDI~K-DEL. Plasmid pMAL3.1, which directs the production in *S. cerevisiae* of rat PDI with an α-factor pre–pro segment at its N-terminus and a KDEL sequence at its C-terminus, was constructed as follows. Plasmid prpdi was digested with *Nar*I, treated with T4 DNA polymerase to generate blunt ends, and then digested with *Sal*I. The resulting fragment was inserted into plasmid pMP36 that had been digested with *Bal*I (which creates a blunt end) and *Sal*I to yield plasmid pMAL1. An adaptor made from oligonucleotides RR44 and RR45 (Table 1) was inserted into plasmid pMAL1 that had been digested with *Kpn*I and *Asp*I to yield plasmid pMAL2. The *Bam*HI and *Sal*I fragment of pMAL2 was inserted into the appropriate *Bam*HI and *Sal*I sites of plasmid YEpWL.RNase A to yield plasmid pMAL3. The *Nco*I site in the *LEU2*-d gene of plasmid pMAL3 was destroyed by site-directed mutagenesis using oligonucleotide ML1 to yield plasmid pMAL3.1 (which codes for pre–pro α-factor~PDI~KDEL).

Plasmid for production of pre–pro α-factor~PDI~H-DEL. A plasmid, pMAL5.1, that directs the production in *S. cerevisiae* of rat PDI with an α-factor pre–pro segment at its N-terminus and an HDEL sequence at its C-terminus was constructed as follows. An adaptor made from oligonucleotides ML9 and ML10 was inserted into plasmid M13mp18 that had been digested with *Eco*RI and *Hind*III to yield plasmid pMAL10. The *Nhe*I/*Sal*I fragment of pMAL3.1 was inserted into pMAL10 that had been digested with *Nhe*I and *Sal*I to yield plasmid pMAL13. The cDNA in pMAL3.1 that codes for the KDEL sequence at the C-terminus of PDI was changed to code for an HDEL sequence by site-directed mutagenesis using oligonucleotide ML19. The region in the pMAL13-derived vector containing the desired mutation was amplified by using the PCR. The amplified region was digested with *Nhe*I and *Sal*I and ligated to the *Nhe*I/*Sal*I fragment of pMAL3.1 to yield plasmid pMAL5.1 (pre–pro α-factor~PDI~HDEL).

TABLE 1

DNA Oligonucleotides Used in This Work

Name	Sequence
ML1	GCTTGGTACGGTGCCAAGACGAGG
ML9	AATTCGGATCCCCATGGGTCGACATATATATACGGCCGATATATATAGCATGCGCTAGCTCTAGAA
ML10	AGCTTTCTAGAGCTAGCGCATGCTATATATATCGGCCGTATATATATGTCGACCCATGGGGATCCG
ML19	CAGAAAGCCGTGCACGATGAACTGTAG
ML25	AGTGGCCACACCATGGGGCATAGAACTCCA
ML26	CATCCTCCGCATTAGCTGACGCTCTGGAGGAGGA
ML27	CCTCCTCCAGAGCGTCAGCTAATGCGGAGGATG
ML28	TTCGTCTTCAGAGCTCATTGTTTGTTTGAA
RR44	CTTTGGATAAAAGAGACGCTCTGGAGGAGGAGGACA
RR45	TTGTCCTCCTCCTCCAGAGCGTCTCTTTTATCCAAAGGTAC

Plasmid for production of pre α-factor~PDI~KDEL and pre α-factor~PDI~HDEL. The overlap extension method of Pease and coworkers (26) was used to excise from pMAL3.1 the DNA that codes for the pro region of the pre–pro α-factor segment. Plasmid pMAL3.1 was digested with *Bam*HI and *Nhe*I. The resulting 2577-bp fragment was used as a template in two PCRs—first with oligonucleotides ML25 and ML26 and then with oligonucleotides ML27 and ML28. The resulting fragments were purified and again subjected to PCR conditions using oligonucleotides ML25 and ML28. This PCR product was digested with *Sac*I and *Nco*I and ligated to plasmid pGEM5f(−) (Promega) that had been digested with *Sac*I and *Nco*I, to yield plasmid pMAL14. The *Sac*I/*Nco*I fragment of pMAL14 was inserted into pMAL3.1 and pMAL5.1 that had been digested with *Sac*I and *Nco*I, to yield plasmids pMAL6.1 (pre α-factor~PDI~KDEL) and pMAL7.1 (pre α-factor~PDI~HDEL).

Production and purification of recombinant rat PDI. *S. cerevisiae* cells that had been transformed with pWL (20) served as a control for all experiments. *S. cerevisiae* BJ2168 cells were transformed with plasmids pMAL3.1 (which codes for pre–pro α-factor~PDI~KDEL) and transformants were selected by plating on SD–trp medium. Stock cultures were prepared in S(8%)D–trp medium, which contained a high concentration of dextrose to repress expression from the ADH2–GAPDH hybrid promoter. These cultures were allowed to grow for 1 day by shaking at 30°C. The stock culture was diluted to A_{600} = 0.05 OD in filtered YEP(1%)D medium, and the resulting culture (1.0 liter) was grown by vigorous shaking at 30°C until A_{600} = 4.0 OD. The cells were removed by centrifugation, and the culture medium was concentrated to 10 ml by tangential flow over a 5-kDa-cutoff membrane. The concentrate was loaded on a Hi Load 26/60 G-75 gel filtration column (Pharmacia, Uppsala, Sweden) that had been equilibrated with 20 mM imidazolium chloride buffer, pH 7.2. The loaded column was eluted with the same buffer at a flow rate of 0.75 ml/min. Peak fractions were analyzed by SDS–PAGE, and fractions containing PDI were pooled and concentrated to 10 ml by ultrafiltration.

The concentrated sample was loaded on a Mono-Q column (Pharmacia) that had been equilibrated with the same buffer. PDI was eluted with a linear gradient (30 ml + 30 ml) of NaCl (0.15–0.75 M) in 20 mM imidazolium chloride buffer, pH 7.2. Fractions containing PDI eluted at 0.25–0.28 M NaCl, and were analyzed individually by SDS–PAGE. Those fractions containing homogeneous PDI were pooled and concentrated with a 10 kDa-cutoff Centriprep concentrator (Amicon).

Assays for enzymatic activity. The ability of PDI to catalyze the reduction of insulin by GSH was assayed as described (27). The ability of PDI to catalyze the isomerization of the disulfide bonds of scrambled RNase A to those of native RNase A was assayed as described (28), with minor modifications. Assays were performed at 30°C in 50 mM Tris–HCl buffer, pH 7.6, containing PDI (0.7 μM), GSH (1 mM), and GSSG (0.2 mM). Reactions were initiated by the addition of scrambled RNase A (1.0 μl of a 0.50 mg/ml solution in 10 mM acetic acid). At 10-min intervals, aliquots (10 μl) were removed and added to 500 μl of 100 mM MES–HCl buffer, pH 6.0, containing poly(C) (10 mM). Ribonucleolytic activity was monitored by following the change in absorbance at 238 nm.

Values of specific activity were precise to ±10%.

S. cerevisiae cell extracts. Intracellular protein was isolated from *S. cerevisiae* cells as described (29).

Polyclonal antibodies against bovine PDI. A chicken was injected with bovine PDI (200 μg) and 7 days later boosted with additional bovine PDI (100 μg). Eggs were collected 21 days after the initial injection and IgY isolated as described (30).

Immunoblots. Yeast cultures were grown to late log phase in liquid medium. Cells were removed by centrifugation, and the supernatant was concentrated with a 10-kDa-cutoff Centriprep concentrator. Denaturing

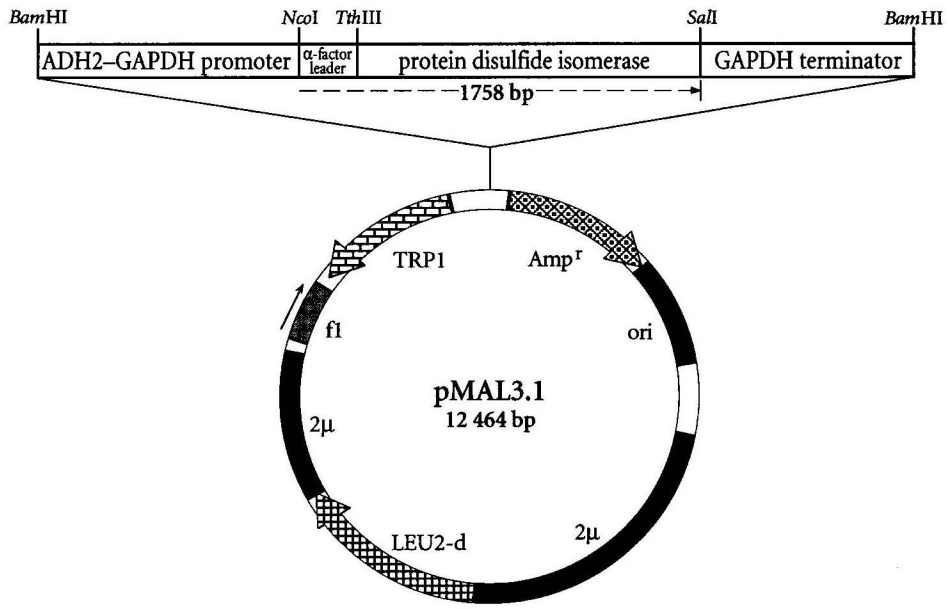

FIG. 1. Map of plasmid pMAL3.1, which directs the expression of rat PDI in *S. cerevisiae*. The plasmid is designed to facilitate the genetic manipulations required in protein engineering (20).

protein gel electrophoresis was performed as described (23). Immunoblotting was performed using the Renaissance kit (DuPont NEN, Boston, MA) as directed by the manufacturer. Antibodies to bovine PDI were used at a 1:500 dilution for probing the blots. Peroxidase-conjugated rabbit affinity purified antibody to chicken IgG was from Cappel (Durham, NC) and was used as directed by the manufacturer.

Protein sequence analysis. Protein sequencing was performed by Dr. G. Grant (Department of Molecular Biology, Washington University, St. Louis, MO).

RESULTS

Production of Rat PDI in S. cerevisiae

The cDNA sequence that codes for rat PDI was inserted in a plasmid between ADH2–GAPDH hybrid promoter and GAPDH terminator sequences. The resulting plasmid, pMAL3.1 (Fig. 1), was used to transform the protease-deficient *S. cerevisiae* strain BJ2168. The expression of the PDI cDNA became derepressed as the concentration of fermentable carbon sources in the medium decreased. The PDI produced was secreted into the culture medium.

Purification of Rat PDI from S. cerevisiae Medium

PDI had been purified from bovine liver and other animal tissues by using heat treatment, ammonium sulfate precipitation, and cation exchange chromatography steps (31). These steps were not effective in purifying PDI from *S. cerevisiae* medium (data not shown). Accordingly, we developed a new purification scheme. A key method in this scheme was tangential flow concentration of the growth medium. We grew our cells in medium that had been filtered to remove components of $M_r > 5000$. After cell growth, we concentrated the medium to remove components of $M_r < 5000$ (Fig. 2, lane 1). We then used gel filtration chromatography to remove components of $M_r < 30{,}000$. Concentrated medium was loaded directly onto a Pharmacia HiLoad 26/60 G-75 gel filtration column. The fractions containing PDI were approximately 15% pure as assessed by SDS–PAGE (Fig. 2, lane 2) and specific activity (Ta-

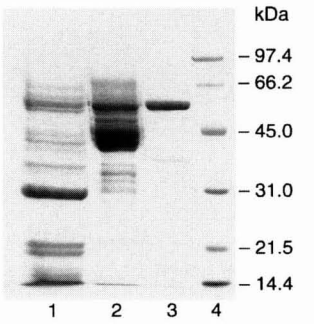

FIG. 2. SDS–polyacrylamide gel showing the purification of PDI from *S. cerevisiae*. Recombinant PDI was produced in protease-deficient *S. cerevisiae* strain BJ2168. Concentrated proteins were separated by electrophoresis under reducing conditions after the following purification steps. Lane 1, crude extract from culture medium; lane 2, gel filtration chromatography; lane 3, anion exchange chromatography; lane 4, unstained molecular mass markers (in kDa).

TABLE 2

Summary of Purification of Rat Protein Disulfide Isomerase from *S. cerevisiae*[a]

Purification step	Protein (mg)	Total activity (U)	Specific activity (U/mg)[b]	Fold purification	Yield (%)
Concentrated crude extract	177	0.87	0.0049	—	100
Gel filtration chromatography	12.8	0.60	0.047	9.6	69
Anion exchange chromatography	2.13	0.70	0.33	67	80

[a] From a 1.0-liter culture.

[b] One unit of PDI will catalyze the reduction of 1 μmol of insulin per min at pH 7.2 in the presence of 0.5 mM GSH. PDI purified from bovine liver has a specific activity of 0.41 U/mg.

ble 2). Since PDI is an acidic protein [p*I* 4.2 (31)], we used anion exchange chromatography as the final purification step. The solution of PDI obtained after gel filtration chromatography was concentrated and loaded onto a Mono-Q anion exchange column, and PDI was eluted with a NaCl gradient. The fractions containing PDI were >95% pure as assessed by SDS–PAGE (Fig. 2, lane 3). The yield of isolated PDI was typically 2 mg per liter of culture medium.

During gel filtration chromatography, PDI eluted at 120–125 ml–beyond the void volume of the column. Experiments using M_r standards showed that PDI is produced as a monomer (data not shown). This finding is in contradiction to reports that PDI is a dimer (32), but agrees with results from Gilbert (33) and Kim (8) who have also characterized the M_r of PDI by using gel filtration chromatography.

Processing of Rat PDI in S. cerevisiae

The location of the PDI produced by various plasmids was analyzed by immunoblotting. Polyclonal antibodies raised against bovine PDI recognize rat PDI but not endogenous *S. cerevisiae* PDI (data not shown). The medium from *S. cerevisiae* cultures was concentrated and probed with polyclonal antibodies against bovine PDI. Only those cells carrying plasmids that code for PDI fused to the α-factor pre–pro segment directed the secretion of PDI into the medium (Fig. 3A), and the presence of KDEL (lane 1) or HDEL (lane 2) did not alter dramatically the amount of protein secreted. This secreted protein had M_r 55,000, which is that expected for mature rat PDI. Amino acid sequence analysis of the rat PDI produced from pMAL3.1 showed that the nine N-terminal residues of the protein were identical to those predicted from the cDNA sequence. Thus, the fusion protein was processed correctly by the secretory system of *S. cerevisiae*.

To evaluate how much protein was being retained inside the cells, total protein was isolated from *S. cerevisiae* cells and probed by immunoblotting with polyclonal antibodies raised against bovine PDI. Extract from cells carrying plasmids that code for PDI fused to the α-factor pre segment contained one cross-reacting protein of M_r 60,000 (Fig. 3B, lanes 3 and 4), which is equivalent to that of rat PDI plus the pre segment. Extract from cells carrying plasmids that code for PDI fused to the α-factor pre–pro segment contained two cross-reacting proteins of M_r 74,000 and 77,000 (Fig. 3B, lanes 1 and 2). These proteins had an M_r greater than that of rat PDI plus the α-factor pre–pro segment. Control samples transformed with the parental vector pWL, showed no PDI bands (Fig. 3B, lane 6).

The extra molecular mass in the PDI fused to the α-factor pre–pro segment suggests that this protein was glycosylated. Rat PDI does not contain an Asn–X–Ser/Thr sequence, which is the consensus site for N-glycosylation. In contrast, endogenous *S. cerevisiae* PDI contains five such sites, and it is glycosylated extensively (14). The α-factor pro segment also contains two N-glycosylation sites. Immunoblotting after endoglycosidase H treatment was used to determine if the fusion protein was indeed glycosylated in the pro segment.

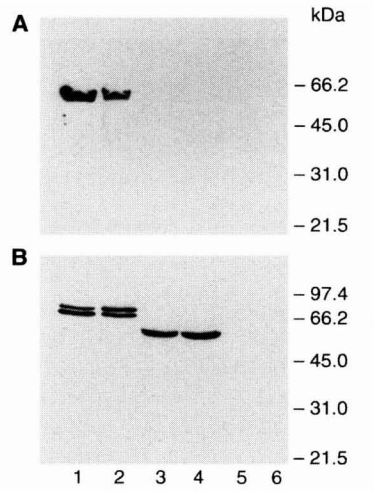

FIG. 3. Immunoblot of secreted (A) and intracellular (B) proteins from *S. cerevisiae* strain BJ2168 probed with antibodies against bovine PDI. Lane 1, proteins from cells carrying plasmid pMAL3.1 (pre–pro α-factor~PDI~KDEL); lane 2, pMAL5.1 (pre–pro α-factor~PDI~HDEL); lane 3, pMAL 6.1 (pre α-factor~PDI~KDEL); lane 4, pMAL 7.1 (pre α-factor~PDI~HDEL); lane 5, empty; lane 6, pWL.

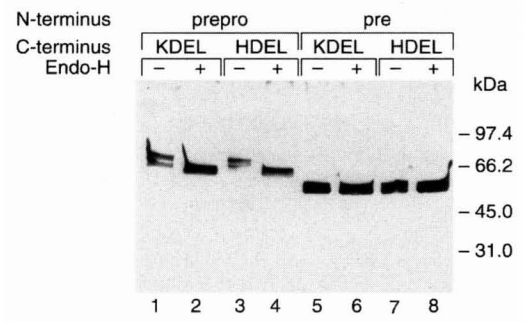

FIG. 4. Immunoblot of intracellular proteins from *S. cerevisiae* strain BJ2168 before and after treatment with endoglycosidase H for 12 h. Lanes 1 and 2, extract from cells carrying plasmid pMAL3.1 (pre–pro α-factor~PDI~KDEL); lanes 3 and 4, pMAL5.1 (pre–pro α-factor~PDI~HDEL); lanes 5 and 6, pMAL6.1 (pre α-factor~PDI~KDEL); lanes 7 and 8, pMAL7.1 (pre α-factor~PDI~HDEL).

Treatment with endoglycosidase H produced a single product of M_r 65,000 (Fig. 4, lanes 3 and 5), which is equivalent to that predicted for rat PDI fused to the α-factor pre–pro segment.

Enzymatic Activity of Rat PDI from S. cerevisiae. The specific activity of recombinant PDI in the GSH-dependent reduction of insulin was 0.34 μmol/min/mg at pH 7.2 in the presence of GSH (0.5 mM). The specific activity of the rat PDI from *S. cerevisiae* was indistinguishable from that of the enzyme isolated from bovine liver (Table 2).

The specific activity of recombinant PDI in the isomerization of scrambled RNase A was 0.64 nmol RNase A reactivated/min/mg at pH 7.6 in the presence of GSH (1 mM) and GSSG (0.2 mM). Under these conditions, the enzyme isolated from bovine liver reactivated 0.68 nmol RNase A/min/mg.

DISCUSSION

Protein disulfide isomerase is an endoplasmic reticulum enzyme that has been implicated in the catalysis of protein disulfide bond formation *in vivo* (34). Null PDI mutants of *S. cerevisiae* cell are inviable. Nonetheless, since PDI is a component of many different protein processing complexes *in vivo,* the actual activity of PDI that is required for cell viability is unclear (9). We anticipate that *in vivo* and *in vitro* comparisons of wild-type and mutant PDIs will resolve this dilemma.

Several systems have been developed for the heterologous production of PDI. Four groups have reported the production of human, rat, or murine PDI in *E. coli* (12,35–37), with yields as high as 30 mg/l. Of these groups, two reported that the expression of a PDI cDNA in *E. coli* resulted in PDI molecules that were truncated because of the initiation of protein synthesis from internal start codons (36,37). PDI has also been produced in *Spodoptera frugiperda* (38) and *Bacillus brevis* (39), with yields of 10 and 5 mg/l, respectively. In the former system, the aim was the study of the formation of the tetramer of prolyl-4-hydroxylase in which PDI is the β subunit. Finally, a functional expression cassette for human PDI has been integrated into the *LYS2* locus of an *S. cerevisiae* chromosome (32).

We have constructed a vector that directs the expression of rat PDI under the control of an ADH2–GAPDH hybrid promoter. In our vector, the endogenous signal sequence for the rat PDI has been replaced with the α-factor pre–pro segment. This sequence is effective in directing the secretion of PDI into the medium (Fig. 3, lane 1). We have also constructed a vector in which the rat PDI KDEL retention sequence was mutated to the *S. cerevisiae* HDEL retention sequence. Still, protein was secreted into the medium (Fig. 3, lane 2). Apparently, the α-factor pre–pro segment overrides the retention provided by the HDEL sequence. Alternatively, the retention system may become saturated so that excess protein is secreted into the medium (18).

To determine the basis for the targeting of PDI *in vivo,* we deleted the 66 residues of the pro region α-factor pre–pro segment, leaving only the 19 residues of the pre region. This deletion should direct the protein into the endoplasmic reticulum, but not signal it to be secreted. Our results show that PDI is indeed retained within the cell.

Other heterologous expression systems can produce PDI at levels higher than does ours. Nevertheless, our system results in the synthesis of nonglycosylated, properly processed, full-length protein, which is easily purified from the culture medium in a enzymatically active, monomeric form. In addition, we have recently demonstrated that our plasmid pMAL3.1 complements *pdi1Δ S. cerevisiae* cells (M. C. A. Laboissière, S. L. Sturley, and R. T. Raines, unpublished data), which are otherwise inviable. Thus, our system allows for the meaningful study of wild-type and mutant PDIs both *in vivo* and *in vitro.* We believe that this system will enable us to reveal biologically relevant structure–function relationships in PDI.

ACKNOWLEDGMENTS

M.C.A.L. is a CNPq (Brazil) predoctoral fellow. P.T.C. is a Wisconsin Alumni Research Foundation predoctoral fellow. R.T.R. is a Presidential Young Investigator (NSF), Searle Scholar (Chicago Community Trust), and Shaw Scientist (Milwaukee Foundation).

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