Expression and secretion of human protein disulphide isomerase in Saccharomyces cerevisiae


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Protein disulphide isomerase (PDI), an enzyme which catalyses thiol-disulphide interchange, is a major protein of the endoplasmic reticulum (ER) of plants, animals and yeast [1, 2, 3]. This enzyme seems to be critical for attaining the correctly folded tertiary structure of disulphide-bonded proteins both in vivo and in vitro. PDI is ubiquitous, but its activity is highest in cells actively producing secretory proteins [4]. Most ER luminal proteins have a C-terminal tetra-peptide which functions as an ER retention signal [5]. This tetra-peptide (HDEL in yeast and KDEL in mammalian cells, [6]) does not prevent ER luminal proteins from escaping the ER lumen by means of its KDEL signal [10, 11]. A receptor for ER proteins, that cycles between the ER and the Golgi apparatus, has been found in yeast [8].

The human PDI gene codes for a protein that acts simultaneously as PDI and the β-subunit of polyol-4-hydroxylation (P4H, [9]). Recent reports suggest that the main function of the P4H β-subunit is to retain the protein in the ER lumen by means of its KDEL signal [10, 11]. A variety of other functions have been described for PDI (see [12] for a review).

PDI isolated in solution is a homodimer [5], and the human monomer is 55-60kDa [11, 13, 14]. The yeast enzyme is ~70kDa, with a shift to 60kDa after endoglycosidase H treatment, indicating that the protein is N-glycosylated [15].

It is known that the expression of functional hPDI in the yeast S. cerevisiae and demonstrate that the tetra-peptide C-terminal retention signal can be overridden in yeast by either overexpression or by use of the mating factor α (MFα) pre-pro sequence at the N-terminus of the polypeptide. A hPDI expression cassette was integrated at the LYS2 locus of Saccharomyces cerevisiae. The expression cassette consisted of the GAL1.10 promoter, the leader sequence of the MFα fused in frame with the sequence for mature hPDI, with either the native C-terminal KDEL sequence or a mutated sequence coding for HDEL signal. Yeast strains were grown in 3YEPD (4.8% glucose, 6% Oxoid yeast extract, 3% Bacto peptone), for 24 hours at 30°C, and then transferred to 3YGAL (3YEPE with glucose substituted by galactose) for a further 24 hours. Protein extraction was achieved by glass bead lysis, and for analysis of secreted proteins, the culture medium was precipitated by addition of TCA to a final concentration of 6%. After SDS-PAGE analysis, proteins were transferred to a nitrocellulose membrane and incubated with antibodies raised against hPDI [16], yPDI [17] or BiP [18].

We found that the transformed strains expressing either the -KDEL or the -HDEL hPDI, secreted the protein into the culture medium, with little if any protein being detectable intracellularly. Furthermore, ultrafiltration of the culture medium through a 100 000 kDa cut-off membrane (Amicon), followed by a step of gel filtration in Superose 12 (Pharmacia), indicated that hPDI was found in the column fractions containing polypeptides in the molecular weight range 92-150kDa, suggesting that the protein exists as a dimer in the culture medium. When the Superose 12 peak fractions were analysed for PDI activity, the activity of recombinant hPDI (10-2 units) was found to be comparable to PDI purified from bovine liver. The first eight N-terminal amino-acids of hPDI secreted by yeast were sequenced and found to be Asp-Ala-Pro-(Glu)₃-Asp-His, i.e., identical to the sequence derived from human placenta PDI [9]. Expressing hPDI in the presence of the N-glycosylase inhibitor tunicamycin failed to promote a shift in the migration of the protein in SDS-PAGE. In contrast, endogenous yeast PDI is N-glycosylated.

We have therefore demonstrated that S. cerevisiae can express hPDI as an active secreted dimer. Secretion of hPDI-HDEL may represent saturation of the ER retention system, an assumption confirmed by the secretion , in the hPDI recombinant strains, of an endogenous luminal ER protein, BiP. The absence of yPDI in the culture medium from the hPDI recombinant strains indicates the absence of a mixed population of hPDI-yPDI dimers.

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