FEBS Letters 580 (2006) 1897–1902

Mutational analysis of the oxidoreductase ERp57 reveals the importance of the two central residues in the redox motif

Siân M. Beynon-Jones[a], Antony N. Antoniou[a,b], Simon J. Powis[a,c,*]

[a] *Division of Cell Biology and Immunology, University of Dundee, Dundee, DD1 5EH, UK*
[b] *Cancer Sciences Division, University of Southampton School of Medicine, Southampton, S016 6YD, UK*
[c] *Bute Medical School, University of St Andrews, St Andrews, Fife, KY16 9TS, UK*

Received 10 November 2005; revised 31 January 2006; accepted 16 February 2006

Available online 28 February 2006

Edited by Robert Barouki

Abstract **The oxidoreductase ERp57 is involved in the formation and breaking of disulfide bonds in assembling proteins within the environment of the endoplasmic reticulum. Site-directed mutants of the redox-active Cys-Gly-His-Cys motif within an isolated ERp57 sub-domain have been studied. Whereas mutation of either cysteine residue abolished reductase activity, substitution of the central residues resulted in retention of partial activity. Alkylation studies indicated that the central residue mutants retained the normal disulfide bond in the motif, whereas this disulfide bond became more resistant to reduction following addition of a third residue into the redox motif, demonstrating an optimum spacing within the redox-active motif of ERp57.**

Keywords: ERp57; Oxidoreductase

1. Introduction

The folding of many proteins is dependent on correct disulfide bond formation [1]. Members of the thioredoxin superfamily can carry out disulfide bond oxidation, reduction and isomerisation, with important members located in the endoplasmic reticulum (ER), including protein disulfide isomerase (PDI) and its close relative ERp57 [2,3].

Members of the thioredoxin family possess at least one domain containing the redox-active motif CXXC, where XX represents two other amino acids [3]. ERp57 has two such domains, both containing the redox motif Cys-Gly-His-Cys (CGHC). The two domains containing the active sites motifs (denoted **a** and **a′**) are located at either end of ERp57, separated by two non-catalytic domains (**b** and **b′**), which in addition to a tail domain gives the multi-domain order **abb′a′c**. Whereas the **b′** and **a′** domains appear important in the interaction of PDI with its substrates, in the case of ERp57 multiple domains including the **abb′** domains are necessary for its interaction with the P-domain of calreticulin [4]. Mutation of the cysteine residues is known to inhibit the reductase activity of ERp57 [5,6]. The importance of the CXXC motif is further demonstrated in yeast where a PDI homologue, Eug1p, normally contains the motif CXXS, and has low reductase and oxidative activity which can be significantly boosted by mutation of the redox motifs to CXXC [7]. Furthermore in yeast the presence of multiple homologues of PDI have been shown to have essentially non-interchangeable functions [8].

Mutation of the central XX residues of the CXXC motif of this superfamily appears to alter the redox potential and enzyme activity [9]. For example, altering the CGPC of thioredoxin to CGHC of PDI leads to an increase in redox potential and an enzyme which is less reducing and more oxidising compared to wildtype [10,11]. More recently, it has also been shown that the presence of a conserved arginine residue in the locality of the redox motifs is crucial to the catalytic function of PDI, and dramatically influences the pK_a of the active site cysteines [12].

ERp57 also plays an important role in the immune system. It is found in association with folding major histocompatibility complex (MHC) class I molecules and as part of the peptide-loading complex (PLC) in the ER, which comprises MHC class I molecules, calreticulin, ERp57, the class I-specific accessory molecule tapasin, and the cytosol-to-ER peptide transporter TAP [13–15]. In ERp57-deficient cells MHC class I molecules are not stably integrated into the PLC [16], however, the precise role of ERp57 in MHC class I assembly remains somewhat unclear. Disulfide bond intermediates of the MHC class I heavy chain with ERp57 can be detected [6,17], indicating its direct involvement in the folding process. Somewhat unexpectedly it has become evident that in the PLC ERp57 is primarily disulfide linked to an unpaired cysteine residue in tapasin through the redox motif in the ERp57 **a** domain [18,19]. The reason for this linkage is not known, but may suggest that tapasin and ERp57 act in combination in the optimisation of the peptide pool to be loaded into the groove of MHC class I molecules. It has also recently been shown that the disulfide linkage to tapasin inhibits the escape pathway that normally acts to release oxidoreductases from their substrate polypeptides [20].

The relative importance of the two central residues in the CXXC motif of ERp57 has not been investigated. Because the **a** domain of ERp57 is disulfide linked to tapasin in the PLC we have studied the **a′** domain and its redox motif, reasoning that it might be accessible to possibly influence the disulfide status of MHC class I molecules within the PLC. Our results demonstrate that alteration of these residues in an isolated, functional domain has deleterious effects on the reductase activity, as does the addition of a third residue between the two cysteines.

*Corresponding author.
E-mail address: sjp10@st-andrews.ac.uk (S.J. Powis).

0014-5793/$32.00
doi:10.1016/j.febslet.2006.02.055

2. Materials and methods

2.1. Site-directed mutagenesis

The N-terminally HIS-tagged a′c domain of rat ERp57, contained in the pET15b plasmid vector was subjected to site-directed mutagenesis using Quickchange (Stratagene) methodology, utilising the following primers (and their complementary sequences), with mutations underlined. SGHC (C405S): 5′-ttttatgctccttggtctggccactgtaagaacctggaaccc-3′; CGHS (C408S): 5′-ttttatgctccttggtgtggccactctaagaacctggaaccc-3′; CAHC (G406A): 5′-ttttatgctccttggtgtgcccactgtaagaacctggaaccc-3′; CGAC (H407A): 5′-ttttatgctccttggtgtggcgcctgtaagaacctggaaccc-3′; CAAC (G406A/H407A): 5′-ttttatgctccttggtgtgccgcctgtaagaacctggaaccc-3′; CGDC (H407D): 5′-ttttatgctccttggtgtggcgactgtaagaacctggaaccc-3′; CGHAC: 5′-ttttatgctccttggtgtggccacgcctgtaagaacctggaaccc-3′.

PCR products were transformed into XL10 Gold and DNA from expanded colonies was sequenced to confirm mutagenesis.

2.2. Bacterial expression of mutant A′C domains

Mutant DNA was transformed into BL21 cells and LB broth cultures induced for 1 h with 1 mM IPTG. SDS–PAGE of whole cell lysates was used to confirm protein expression. Solubility of expressed mutants was tested using BPER (Perbio) as per manufacturers instructions. For large-scale purification 100 ml overnight cultures were induced with IPTG as above and cell pellets resuspended in 10 ml BPER. Post-centrifugation supernatants were passed over Ni-agarose, washed, and the histidine-tag removed from the recombinant proteins by cleavage with 5 U thrombin for 2 h at room temperature. Supernatants were dialysed against 25 mM HEPES overnight and samples concentrated and stored at −80 °C.

2.3. Insulin precipitation assay

Freshly dissolved insulin (Sigma) at 1 mg/ml was stored on ice in assay buffer (100 mM KAc, pH 7.5, 2 mM EDTA). 50 μl of insulin was added to a microcuvette at room temperature. ERp57, **a′c** or mutants were added in 10 μl assay buffer to give a final assay concentration of 1 μM. 2 μl of 10 mM DTT was then added, gently mixed and the optical density at 650 nm measured every minute.

2.4. Alkylation of CXXC motifs

One microgram of **a′c** and mutants were made up to 10 μl with 50 mM HEPES (pH 7.5) in triplicate. 0.5 μl of 1 mM DTT was added to the third tube of each set. All samples were incubated at 37 °C for 10 min. Non-reducing sample buffer with or without 0.3 mM 4′-maleimidylstilbene-2,2-disulphonic acid (AMS, Molecular Probes, The Netherlands) was added as required. Samples were incubated at room temperature for 20 min, then analysed by SDS–PAGE. In experiments where the reoxidation of the CXXC motif was studied 0.5 μg of **a′c** and CGHAC polypeptides in 10 μl 50 mM HEPES were supplemented with 5 μl of the reducing agent Tris(2-carboxyethyl) phosphine hydrochloride (TCEP) immobilised on agarose beads (approximately 8 μmol/ml, Perbio) for 45 min at 37 °C. The TCEP-beads were then pelleted by brief centrifugation and the supernatant removed and either immediately subjected to AMS alkylation in non-reducing sample buffer, or incubated at room temperature for a further 45 min before AMS alkylation and SDS–PAGE analysis.

3. Results

3.1. Reductase activity of isolated *a′c* domain of ERp57

ERp57 contains redox CGHC motifs in both the **a** and **a′** domains (Fig. 1A). In order to analyse the effects of mutation on a single CGHC motif, the **a′c** domain was expressed and examined in isolation. Bacterially expressed and affinity purified full length ERp57 and the **a′c** domain (Fig. 1B) were compared for reductase activity in an insulin precipitation assay [5]. The **a′c** domain retained significant activity in comparison to full length ERp57 (Fig. 1C), validating its use as a target for mutational analyses.

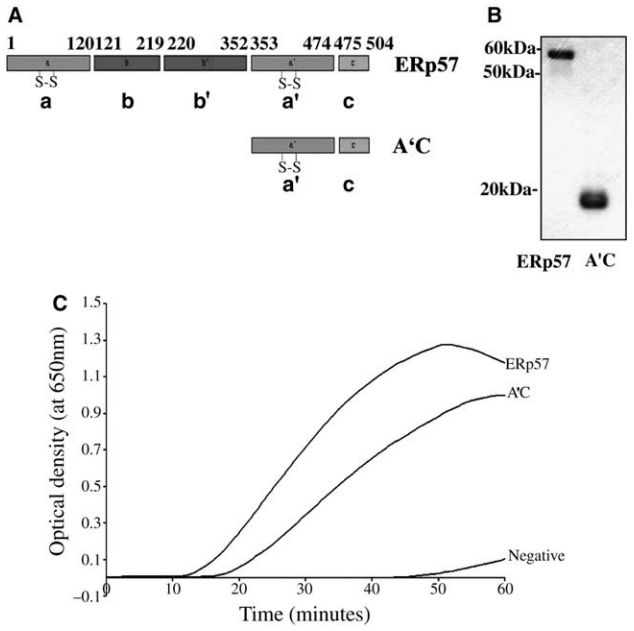

Fig. 1. Purification and reductase activity of the ERp57 **a′c** domain. (A) Domain organisation of ERp57 and the **a′c** fragment. (B) Recombinant ERp57 and **a′c** were affinity purified from bacterial lysates, and analysed by SDS–PAGE and Coomassie Blue staining. (C) Recombinant ERp57 and **a′c** were incubated in the presence of insulin and a final DTT concentration of 0.32 mM. The control sample (Negative) included insulin and DTT alone.

3.2. Reductase activity of *a′c* redox motif mutant molecules

Site-directed mutagenesis was performed on the **a′c** domain to generate mutants of the four residues in the CGHC motif. Additionally, a mutant was generated such as to add a third residue in between the two cysteine residues, resulting in the motif CGHAC. The full list of the 7 mutants generated is given in Table 1. All the mutants were successfully affinity purified from the soluble fraction of bacterial lysate preparations, and their purity assessed by non-reducing SDS–PAGE analysis (Fig. 2A). In the case of mutant CGHS, and less so in the case of mutant SGHC, a band of approximately 40 000 molecular mass was detected in several purification preparations. Upon analysis in reducing conditions this band disappeared, suggesting that it was a dimer of the **a′c** domain induced by the unpaired cysteine in these mutants.

Insulin precipitation assays were performed to determine the reductase activity of the **a′c** mutants. The assays were repeated four times overall, from two separate purifications, with a reproducible pattern of results. Mutants from which the cysteine residues were removed (SGHC and CGHS) were both

Table 1
Summary and nomenclature of the mutations in the **a′c** domain fragment of ERp57 used in this study

Mutant number	Mutation	Mutant name
1	C405S	SGHC
2	C408S	CGHS
3	G406A	CAHC
4	H407A	CGAC
5	G406A/H407A	CAAC
6	H407D	CGDC
7	Insertion of A between H407/C408	CGHAC

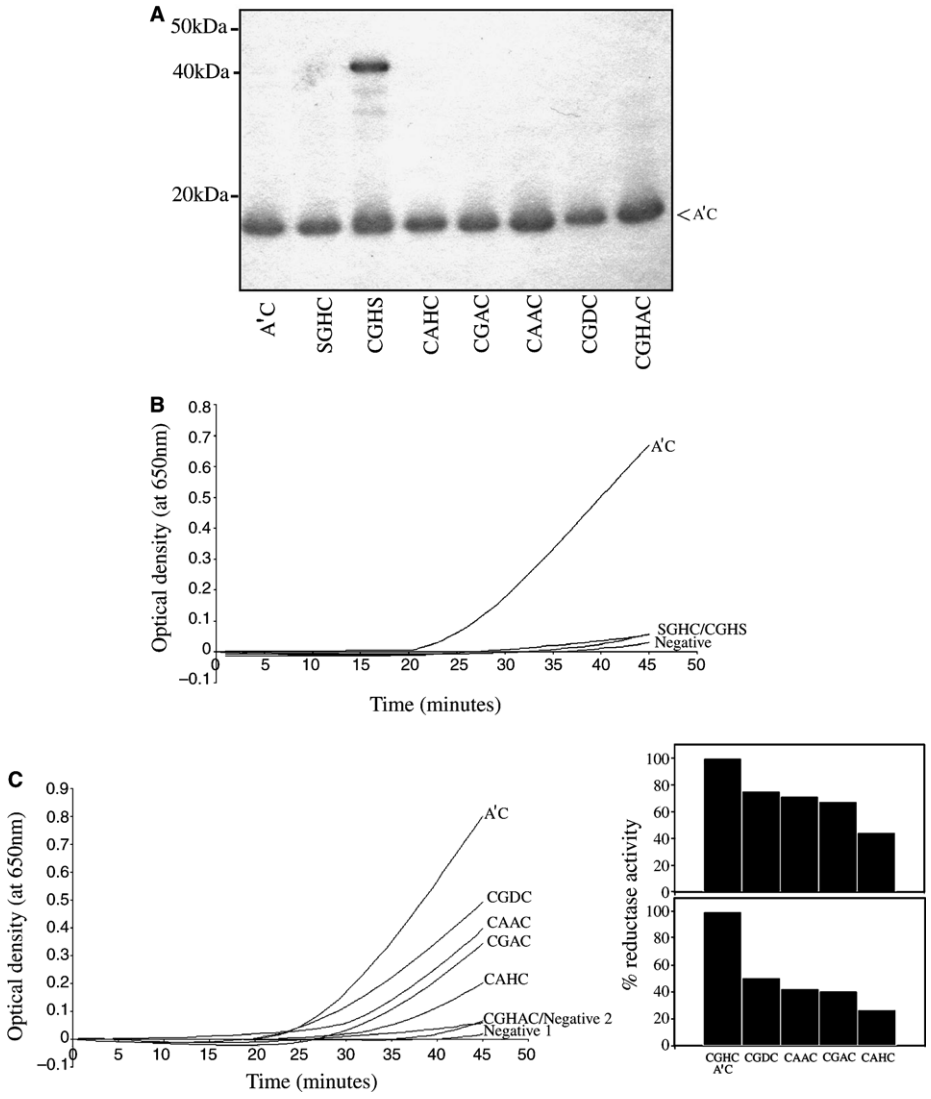

Fig. 2. Purification and reductase activity of A′C domain redox motif mutants. (A) Bacterial expressed and affinity purified recombinant **a′c** domain mutants were analysed under non-reducing conditions by SDS–PAGE. (B) **a′c** domain and cysteine-mutants were incubated with insulin and DTT as in Fig. 1. Negative control was insulin and DTT alone. (C) **a′c** redox motif central residue mutants were tested for reductase activity against insulin. Negative samples were run at the start (1) and the end (2) of the assay, comprising insulin and DTT alone. The percent final reductase activity of two further experiments using the mutants is shown in the two panels.

inactive (Fig. 2B). In contrast, mutants in which the central two residues were altered all retained partial activity (Fig. 2C). Notably, mutant CGDC, where the positive charge of the histidine was replaced with the negatively charged aspartic acid, was the most active of the alterations, whereas in mutant CAHC, replacement of the glycine residue resulted in considerable impairment of activity. In all the assays mutant CGHAC, where an additional third residue was present between the cysteines, produced no activity.

3.3. Accessibility of cysteines to alkylation in redox motif mutants

In purified recombinant form it was anticipated that the cysteines of the **a′c** domain exist as an oxidised disulfide pair. To determine if the alterations in reductase activity observed in the redox motif mutants in Fig. 2 were due to changes in the status of the disulfide pair, we undertook experiments to evaluate if the cysteines were paired or unpaired in the mutants. We made use of 4′-maleimidylstilbene-2,2-disulphonic acid (AMS), which alkylates free cysteines, and with a molecular mass of 536.44 allows the detection of modified cysteines by an increase in size of the target polypeptide on SDS–PAGE. Wildtype and mutant **a′c** preparations were incubated in the presence or absence of DTT at 48 μM (the latter to fully reduce any disulfide pair), and modified with AMS. As shown in Fig. 3A, both cysteine mutants SGHC and CGHS undergo minor increases in size indicating the modification of the single available cysteine. Of note, DTT-sensitive dimers of SGHC and CGHS were detected in this experiment. All the mutants with modifications to their central two residues required the presence of DTT to achieve AMS alkylation (indicated by an asterisk). Thus under the conditions in which these polypeptide have been purified from bacterial cell lysates the cysteines in these mutants appear to be disulfide paired.

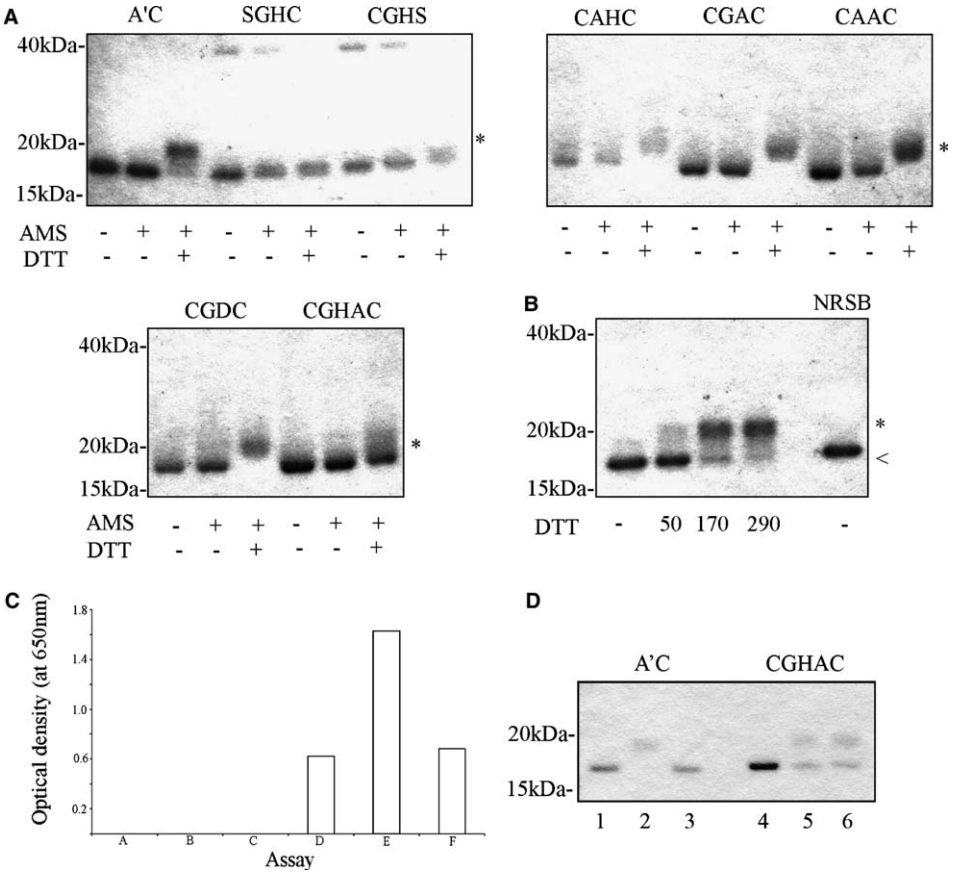

Fig. 3. Alkylation of **a′c** domain redox motif mutants. (A) Recombinant **a′c** samples were incubated for 10 min at 37 °C with or without DTT (48 μM final concentration), before addition of sample buffer containing the alkylating agent AMS, and analysis by SDS–PAGE. An asterisk indicates the AMS-modified product. (B) Mutant CGHAC was incubated with the indicated levels of DTT prior to AMS alkylation. (C) Mutant CGHAC was tested at 37 °C in the insulin precipitation assay, and the optical density read after 60 min. Samples were: A, insulin at room temperature; B, insulin and DTT at room temperature; C, insulin at 37 °C; D, insulin and DTT at 37 °C; E, **a′c** domain and insulin and DTT at 37 °C; F, mutant CGHAC and insulin and DTT at 37 °C. (D) Disulfide bond formation after removal of reducing agent. **a′c** and CGHAC were alkylated with AMS in the absence of reducing agent (lanes 1 and 4), or incubated with TCEP-agarose beads at 37 °C and immediately treated with AMS (lanes 2 and 5) or reincubated at room temperature before addition of AMS (lanes 3 and 6).

Of significant note, mutant CGHAC did not follow a similar pattern to the other mutants. Its ability to be alkylated was investigated further using increased concentrations of DTT. CGHAC required more DTT to observe reduction, with AMS modification occurring at DTT concentrations of 170 μM and above (Fig. 3B). To further study the ability of mutant CGHAC to act as a reductase we also determined its activity at 37 °C in the insulin precipitation assay (Fig. 3C). However, although the increased temperature led to an increase in wildtype **a′c** activity (column E), as shown by comparing the optical density readings in Figs. 2 and 3, mutant CGHAC activity (column F) did not increase markedly above that of the relevant control sample (column D), indicating its inability to act as an efficient reductase. A possibility to account for the failure of CGHAC to act as a reductase is the inability to complete the escape pathway due to spatial restraints imposed by the additional residue. This was examined by inducing reduction with an immobilised agent (TCEP), and analysing AMS alkylation following reincubating of the protein after removal of the reduction agent. As shown in Fig. 3D **a′c** was able to reform the disulfide in the CXXC motif, although in some experiments only approximately 50% became refractory to AMS alkylation (data not shown). In contrast mutant CGHAC displayed no loss of AMS alkylation after removal of the reducing agent.

4. Discussion

Previously we have shown that an ERp57 mutant containing only the more N-terminal cysteines of the two CXXC motifs, i.e., mutant $(CGHS)_2$, was partially active as a reductase, while the corresponding mutant, $(SGHC)_2$ with only the more C-terminal cysteines, was inactive [6]. The NMR structure of the PDI **a** domain indicates that it is the N-terminal cysteine which is more solvent exposed and therefore able to interact with free cysteines [21]. This may account for the inactivity of ERp57 $(SGHC)_2$, and also that of the **a′c** mutant SGHC observed in this study (Fig. 2B). For PDI, $(CGHS)_2$ mutants accumulate as enzyme–substrate intermediates because the C-terminal residues are usually required to perform the escape pathway to release the intermediate form [22], which may account for the partial reductase activity of ERp57 $(CGHS)_2$ [6]. It was therefore unexpected in this study that mutant CGHS was inactive as a reductase (Fig. 2B). A possible explanation may be that the **a** and **a′** domains are not functionally equivalent, comparable to the differences in function of the corresponding domains of PDI [23,24], although recent data indicates that the two redox motifs in ERp57 have very similar redox potentials and that the isolated domains can act similarly in RNAase refolding [4,25]. Alternatively, it is possible that the absence of a cysteine in the CXXC

motif may lead to an instability of the **a′c** domain, as seen for the **a′** domain of PDI [26], and that the presence of the non-catalytic **b′** domain may be additionally required.

In PDI [27], and in bacterial DsbA [28], histidine is the second central residue, providing a positive charge to stabilise ionisation of the N-terminal cysteine. Interestingly, replacing histidine with the negative charge of aspartate produced a mutant (CGDC) with more reductase activity than a simple alanine replacement (CGAC). This indicates that the charge status of this residue is not crucial for maintaining reductase activity, and that in fact the size of the residue in this second position may be more important. Alternatively the aspartate may also contribute to the stabilisation of the reduced motif, which could be tested in the future with further mutants such as CGEC or CGNC. The absence of a glycine residue next to the histidine (CAHC) also proved detrimental to reductase activity, possibly by restricting conformations that could be adopted in the active site. However, changing both central residues to alanine (CAAC) may relax constraints induced by the alanine–histidine pair, leading to the increase in reductase activity we observed. Overall, small conformational changes that may have been induced by the central residue mutations may have contributed to a stabilisation of the reduced form of **a′c**, similar to observations made in the thioredoxin active site [29]. Nevertheless, in purified recombinant form, the cysteines appeared to be in a disulfide pair in the absence of a reducing agent (Fig. 3). It should be noted that this situation differs from the status of ERp57 in vivo where it appears to exist mostly, excluding its interaction with tapasin, in reduced form [18,30].

Mutant CGHAC was virtually inactive as a reductase. It was anticipated that this was due to inhibition of disulfide bond formation caused by the extra residue. However, the cysteines proved inaccessible to AMS modification unless exposed to increased DTT concentrations. This unexpected result suggested that CGHAC formed a more stable disulfide bond in comparison to **a′c**. Incubation at 37 °C did not restore CGHAC reductase activity. Furthermore removal of reducing agent failed to allow reformation of the disulfide, which in contrast did occur for **a′c**. Our data suggests that once the more stable disulfide bond in CGHAC has been broken it is unable to reform, making this mutant inactive.

The ability to modify the behaviour of individual redox motifs within ERp57, as demonstrated here, may allow further analysis of the MHC class I peptide-loading complex. We have studied the **a′c** domain here because it is most likely to be available for substrate interactions when part of the PLC. Thus, it is the **a′** domain that may potentially be relevant to controlling the disulfide status of MHC class I molecules. We have demonstrated here the ability to modify the activity of ERp57. By introducing such mutants into ERp57 deficient cells [16] we may be able to discern the role of ERp57 within the peptide-loading complex.

Acknowledgements: This work was supported by a Medical Research Council (UK) Senior Fellowship to S.J.P. A.N.A is funded by an Arthritis Research Campaign (UK) Career Development Fellowship (Grant No. 15293). We thank Susana Santos and Sarah Lynch for comments on the manuscript.

References

[1] Rietsch, A. and Beckwith, J. (1998) The genetics of disulfide bond metabolism. Annu. Rev. Genet. 32, 163–184.

[2] Koivunen, P., Helaakoski, T., Annunen, P., Veijola, J., Raisanen, S., Pihlajaniemi, T. and Kivirikko, K.I. (1996) ERp60 does not substitute for protein disulphide isomerase as the beta-subunit of prolyl 4-hydroxylase. Biochem. J. 316 (Pt 2), 599–605.

[3] Ferrari, D.M. and Soling, H.D. (1999) The protein disulphide-isomerase family: unravelling a string of folds. Biochem. J. 339, 1–10.

[4] Silvennoinen, L., Myllyharju, J., Ruoppolo, M., Orru, S., Caterino, M., Kivirikko, K.I. and Koivunen, P. (2004) Identification and characterization of structural domains of human ERp57: association with calreticulin requires several domains. J. Biol. Chem. 279, 13607–13615.

[5] Hirano, N., Shibasaki, F., Sakai, R., Tanaka, T., Nishida, J., Yazaki, Y., Takenawa, T. and Hirai, H. (1995) Molecular cloning of the human glucose-regulated protein ERp57/GRP58, a thiol-dependent reductase. Identification of its secretory form and inducible expression by the oncogenic transformation. Eur. J. Biochem. 234, 336–342.

[6] Antoniou, A.N., Ford, S., Alphey, M., Osborne, A., Elliott, T. and Powis, S.J. (2002) The oxidoreductase ERp57 efficiently reduces partially folded in preference to fully folded MHC class I molecules. EMBO J. 21, 2655–2663.

[7] Norgaard, P. and Winther, J.R. (2001) Mutation of yeast Eug1p CXXS active sites to CXXC results in a dramatic increase in protein disulphide isomerase activity. Biochem. J. 358, 269–274.

[8] Norgaard, P., Westphal, V., Tachibana, C., Alsoe, L., Holst, B. and Winther, J.R. (2001) Functional differences in yeast protein disulfide isomerases. J. Cell. Biol. 152, 553–562.

[9] Huber-Wunderlich, M. and Glockshuber, R. (1998) A single dipeptide sequence modulates the redox properties of a whole enzyme family. Fold Des. 3, 161–171.

[10] Krause, G., Lundstrom, J., Barea, J.L., Pueyo de la Cuesta, C. and Holmgren, A. (1991) Mimicking the active site of protein disulfide-isomerase by substitution of proline 34 in *Escherichia coli* thioredoxin. J. Biol. Chem. 266, 9494–9500.

[11] Lundstrom, J., Krause, G. and Holmgren, A. (1992) A Pro to His mutation in active site of thioredoxin increases its disulfide-isomerase activity 10-fold. New refolding systems for reduced or randomly oxidized ribonuclease. J. Biol. Chem. 267, 9047–9052.

[12] Lappi, A.K., Lensink, M.F., Alanen, H.I., Salo, K.E., Lobell, M., Juffer, A.H. and Ruddock, L.W. (2004) A conserved arginine plays a role in the catalytic cycle of the protein disulphide isomerases. J. Mol. Biol. 335, 283–295.

[13] Morrice, N.A. and Powis, S.J. (1998) A role for the thiol-dependent reductase ERp57 in the assembly of MHC class I molecules. Curr. Biol. 8, 713–716.

[14] Lindquist, J.A., Jensen, O.N., Mann, M. and Hammerling, G.J. (1998) ER-60, a chaperone with thiol-dependent reductase activity involved in MHC class I assembly. EMBO J. 17, 2186–2195.

[15] Hughes, E.A. and Cresswell, P. (1998) The thiol oxidoreductase ERp57 is a component of the MHC class I peptide-loading complex. Curr. Biol. 8, 709–712.

[16] Garbi, N., Tanaka, S., Momburg, F. and Hammerling, G.J. (2006) Impaired assembly of the major histocompatibility complex class I peptide-loading complex in mice deficient in the oxidoreductase ERp57. Nat. Immunol. 7, 93–102.

[17] Antoniou, A.N., Ford, S., Taurog, J.D., Butcher, G.W. and Powis, S.J. (2004) Formation of HLA-B27 homodimers and their relationship to assembly kinetics. J. Biol. Chem. 279, 8895–8902.

[18] Antoniou, A.N. and Powis, S.J. (2003) Characterization of the ERp57-Tapasin complex by rapid cellular acidification and thiol modification. Antioxid. Redox Signal. 5, 375–379.

[19] Dick, T.P., Bangia, N., Peaper, D.R. and Cresswell, P. (2002) Disulfide bond isomerization and the assembly of MHC class I-peptide complexes. Immunity 16, 87–98.

[20] Peaper, D.R., Wearsch, P.A. and Cresswell, P. (2005) Tapasin and ERp57 form a stable disulfide-linked dimer within the MHC class I peptide-loading complex. EMBO J. 24, 3613–3623.

[21] Kemmink, J., Darby, N.J., Dijkstra, K., Scheek, R.M. and Creighton, T.E. (1995) Nuclear magnetic resonance characterization of the N-terminal thioredoxin-like domain of protein disulfide isomerase. Protein Sci. 4, 2587–2593.

[22] Walker, K.W., Lyles, M.M. and Gilbert, H.F. (1996) Catalysis of oxidative protein folding by mutants of protein disulfide isomerase with a single active-site cysteine. Biochemistry 35, 1972–1980.
[23] Holst, B., Tachibana, C. and Winther, J.R. (1997) Active site mutations in yeast protein disulfide isomerase cause dithiothreitol sensitivity and a reduced rate of protein folding in the endoplasmic reticulum. J. Cell. Biol. 138, 1229–1238.
[24] Lyles, M.M. and Gilbert, H.F. (1994) Mutations in the thioredoxin sites of protein disulfide isomerase reveal functional nonequivalence of the N- and C-terminal domains. J. Biol. Chem. 269, 30946–30952.
[25] Frickel, E.M., Frei, P., Bouvier, M., Stafford, W.F., Helenius, A., Glockshuber, R. and Ellgaard, L. (2004) ERp57 is a multifunctional thiol-disulfide oxidoreductase. J. Biol. Chem. 279, 18277–18287.
[26] Darby, N.J. and Creighton, T.E. (1995) Characterization of the active site cysteine residues of the thioredoxin-like domains of protein disulfide isomerase. Biochemistry 34, 16770–16780.
[27] Kortemme, T., Darby, N.J. and Creighton, T.E. (1996) Electrostatic interactions in the active site of the N-terminal thioredoxin-like domain of protein disulfide isomerase. Biochemistry 35, 14503–14511.
[28] Grauschopf, U., Winther, J.R., Korber, P., Zander, T., Dallinger, P. and Bardwell, J.C. (1995) Why is DsbA such an oxidizing disulfide catalyst? Cell 83, 947–955.
[29] Chivers, P.T., Laboissiere, M.C. and Raines, R.T. (1996) The CXXC motif: imperatives for the formation of native disulfide bonds in the cell. EMBO J. 15, 2659–2667.
[30] Jessop, C.E. and Bulleid, N.J. (2004) Glutathione directly reduces an oxidoreductase in the endoplasmic reticulum of mammalian cells. J. Biol. Chem. 279, 55341–55347.