Disulfide bonds as switches for protein function

Philip J. Hogg

Centre for Vascular Research, University of New South Wales, and Department of Haematology, Prince of Wales Hospital, NSW 2052, Australia

The prevailing view is that disulfide bonds have been added during evolution to enhance the stability of proteins that function in a fluctuating cellular environment. However, recent evidence indicates that disulfide bonds can be more than inert structural motifs. The function of some secreted soluble proteins and cell-surface receptors is controlled by cleavage of one or more of their disulfide bonds; this cleavage is mediated by catalysts or facilitators that are specific for their substrate.

Most proteins that work in the extracellular milieu contain disulfide bonds, which are covalent links between pairs of cysteine residues. Previously, these bonds have been thought to serve two functions. First, they influence the thermodynamics of protein folding: disulfide bonds stabilize the native conformation of a protein by destabilizing the unfolded form [1,2]; they lower the entropy of the unfolded form, making it less favorable compared with the folded form. However, disulfide bonds can decrease the stability of folded proteins by restricting energetically favorable conformational changes. Second, they maintain protein integrity: oxidants and proteolytic enzymes in the extracellular environment can inactivate proteins. By stabilizing protein structure, disulfide bonds can protect proteins from damage and increase their half-life.

Previously, the disulfide bonds present in mature proteins were thought to be inert, that is, once formed they remain unchanged for the life of the protein. However, it now appears that this is not necessarily the case; disulfide bonds are cleaved in mature proteins and when this happens it has significant consequences for protein function.

Disulfide cleavage in secreted soluble proteins and the extracellular domains of cell-surface receptors will be considered separately in this review, although the same principles apply in both types of protein. How the disulfide cleavage is achieved and how we might be able to predict disulfide cleavage in proteins will be addressed thereafter.

Disulfide cleavage in secreted soluble proteins

One of the first examples of disulfide cleavage described in a secreted protein was in thrombospondin (TSP)-1. TSP-1 is an extracellular glycoprotein that participates in cell–cell and cell–matrix communication, and plays a role in the growth and differentiation of tissues. The TSP family consists of five members in vertebrates [3]. TSP-1 is a homotrimer of disulfide-linked 150-kDa monomers [4] and each subunit contains a free thiol [5]; in Ca^{2+}-depleted TSP-1, this thiol can reside on any one of 12 different cysteine residues in the aspartate-rich Ca^{2+}-binding repeats and C-terminal sequence [5]. Binding of Ca^{2+} to the aspartate-rich repeats influences the position of the free thiol, probably by restricting the conformational flexibility in this region of the molecule [4,6]. A complex intramolecular disulfide interchange, therefore, operates in TSP-1. Different disulfide bonded forms of TSP-1 have different Arg-Gly-Asp-dependent cell adhesive activity [6], have different potencies for inhibition of neutrophil enzymes [7], bind Ca^{2+} with different stoichiometries [8] and bind platelet-derived growth factor with different affinities [9]. The finding that different disulfide-bonded forms of TSP-1 exist *in vivo* [10] supports the idea that disulfide interchange in TSP-1 is important for controlling its function. The thiol–disulfide oxidoreductase, protein disulfide isomerase (PDI), facilitates disulfide interchange in TSP-1 [9,11,12] (Table 1). PDI, like TSP-1, is present on the platelet and mammalian [13–19] cell surfaces.

TSP-1 is also involved in control of the subunit structure of von Willebrand factor (vWF). vWF is a multimeric blood protein that acts by crosslinking platelets, forming plugs to stop hemorrhage from damaged blood vessels; its multimeric size dictates its avidity for platelets. vWF is stored in endothelial cells and platelets as ultra-large multimers, which are reduced in size after secretion. Control of the size of vWF multimers is crucial because the presence of ultra-large multimers in blood is associated with thrombosis. vWF-multimer size can, in fact, be controlled by TSP-1 in plasma [20]. TSP-1 achieves this control by facilitating reversible cleavage of the disulfide bonds that hold vWF multimers together. The

Table 1. Disulfide cleavage in secreted proteins and the catalyst or facilitator

Substrate	Catalyst or Facilitator	Refs
Thrombospondin-1	Protein disulfide isomerase	[5,11,12]
von Willebrand Factor	Thrombospondin-1	[20]
Plasmin	Phosphoglycerate kinase, annexin II	[25,27]
CD4	Thioredoxin	[34]
HIV-1 gp120	Protein disulfide isomerase	[38,39]
$\alpha_{IIb}\beta_3$ integrin	Unknown	[43,46,47]

Corresponding author: Philip J. Hogg (p.hogg@unsw.edu.au).

http://tibs.trends.com 0968-0004/03/$ - see front matter doi:10.1016/S0968-0004(03)00057-4

vWF cleaving activity of TSP-1 centers around Cys974 in the C-terminal sequence [21], which is one of the cysteine residues involved in disulfide interchange in this protein. It is not known whether the disulfide fluidity in TSP-1 is important for control of vWF multimer size.

Certain disulfide bonds in plasmin – a serine proteinase that functions in thrombolysis and cell migration – are also manipulated in the extracellular space. Plasmin contains five consecutive triple-disulfide-linked domains called kringle domains, followed by a serine proteinase module. Cleavage of two disulfide bonds in the fifth kringle domain (kringle 5) triggers proteolysis of plasmin and release of an N-terminal fragment called angiostatin [22,23]. Angiostatin is an inhibitor of blood-vessel formation (angiogenesis) [24]. This disulfide-bond cleavage can be facilitated by both large and small molecules. The best-characterized large molecule is phosphoglycerate kinase (PGK), the sixth enzyme of the glycolytic pathway. PGK is secreted by tumor cells in a regulated manner, and facilitates extracellular cleavage of plasmin disulfide bonds and angiostatin formation both *in vitro* and *in vivo* [25,26]. Binding of plasmin to annexin II that is expressed on the surface of tumor cells can also facilitate cleavage of plasmin disulfide bonds [27], although the *in vivo* contribution of this mechanism is unknown. The small molecule facilitators are low-molecular-weight thiols such as *N*-acetyl–*L*-cysteine or reduced glutathione [28] and hydroxide ions [29]. There is emerging evidence that PGK and hydroxide ions work together to cleave disulfide bonds in kringle 5 [22,23,26].

Disulfide cleavage in cell-surface receptors

Disulfide bonds in the extracellular domains of some cell-surface receptors are also cleaved. The best-characterized examples to date are the immune-cell receptor CD4, the HIV-1 envelope glycoprotein gp120 and the integrin receptor $\alpha_{IIb}\beta_3$.

CD4 is a member of the immunoglobulin (Ig) superfamily of receptors that mediates cell–cell interactions in the immune system and is the primary receptor for HIV-1. HIV-1 binds to CD4 via its gp120 envelope protein. This binding leads to interaction of the complex with a chemokine receptor, triggering fusion of the viral and cell membranes, leading to HIV-1 entry and infection [30].

The extracellular region of CD4 consists of four Ig-like domains, D1 to D4 [31–33]. The D1, D2 and D4 domains each contain one disulfide bond. Cleavage of the D2 disulfide bond can occur on the T-cell surface [34], and appears to be mediated by thioredoxin, which is secreted by CD4 + T cells [35,36]. The two thiols in the cleaved domain react with a trivalent arsenical, which implies that they are close enough to reform the disulfide bond. Cleavage of the D2 disulfide bond might be important for conformational changes in CD4 that are required for fusion of the viral and cell membranes [37].

PDI interacts with CD4 both in solution and on the cell surface [38]. Binding of gp120 to CD4 + cells results in PDI-mediated cleavage of probably two of the nine disulfide-bonds in gp120 [38,39]. Cleavage of disulfide bonds in gp120 appears to occur after chemokine receptor binding [39] – the idea is that the conformational change in gp120 that accompanies disulfide-bond cleavage drives envelope-mediated cell–cell fusion.

The importance of these disulfide cleavage events in HIV-1 entry is highlighted by the finding that mono- and di-thiol alkylating agents – which inactivate thioredoxin and PDI, and react with reduced CD4 and gp120 – inhibit HIV-1 entry and envelope-mediated cell–cell fusion [34,38,40,41]. Anti-PDI monoclonal antibodies also inhibit HIV-1 entry and cell–cell fusion [40,41]. Notably, thioredoxin and PDI do not cross-catalyze: thioredoxin cleaves disulfide bonds in CD4 but not in gp120 [34], whereas PDI cleaves disulfide bonds in gp120 but not in CD4 [38].

Integrins comprise a large family of cell-adhesion molecules that mediate interactions between the extracellular environment and the cytoplasm [42]. Most integrins contain an on/off switch that regulates ligand binding. Several reports have shown that reducing agents, such as dithiothreitol, can turn integrins on. The redox switch in the extracellular domain of the platelet $\alpha_{IIb}\beta_3$ integrin is the best characterized; dithiothreitol cleaves two disulfide bonds in the cysteine-rich domain of $\alpha_{IIb}\beta_3$, which causes conformational changes in both its subunits leading to exposure of ligand-binding sites [43]. A role for this mechanism of activation is supported by the observations that $\alpha_{IIb}\beta_3$ has unpaired thiols [44] and endogenous thiol-isomerase activity [45], and that thiol-alkylating agents inhibit $\alpha_{IIb}\beta_3$-mediated platelet aggregation [46,47]. A physiological $\alpha_{IIb}\beta_3$ reductant has not yet been identified and does not appear to be platelet PDI [47]. Interestingly, the disulfide bonding in the integrin epidermal growth factor 3 (I-EGF-3) domain of the $\alpha_v\beta_3$ integrin is different in its X-ray structure [48] compared with its NMR [49] structures – this might reflect disulfide interchange in this integrin.

How disulfide bonds are cleaved outside the cell

There is more than one way that disulfide bonds can be cleaved in the extracellular environment: three possible mechanisms of disulfide-bond cleavage have been identified or suggested in secreted proteins (Fig. 1).

Dithiol–disulfide exchange

The first mechanism is conventional dithiol–disulfide redox exchange. The disulfide cleavage is typically facilitated by oxidoreductases of the PDI superfamily. The active-site(s) of these enzymes contain a reactive dithiol in the common sequence Cys-Gly-Xaa-Cys [50]. One of the thiol groups of the dithiol attacks the substrate disulfide bond, forming a mixed disufide that then undergoes intramolecular thiol–disulfide exchange releasing the (now oxidized) enzyme and the cleaved substrate. This appears to be the operative mechanism in CD4 and gp120 where secreted thioredoxin and PDI are the oxidoreductases, respectively. PDI can also facilitate disulfide interchange in TSP-1.

How thioredoxin and PDI are exported from the cell is not known. Thioredoxin is found throughout the cell, including the endoplasmic reticulum (ER), but does not contain a recognizable secretory signal sequence. Nevertheless, it is constitutively secreted by plasma cells and is secreted from other cells in response to stress [35,36]. By

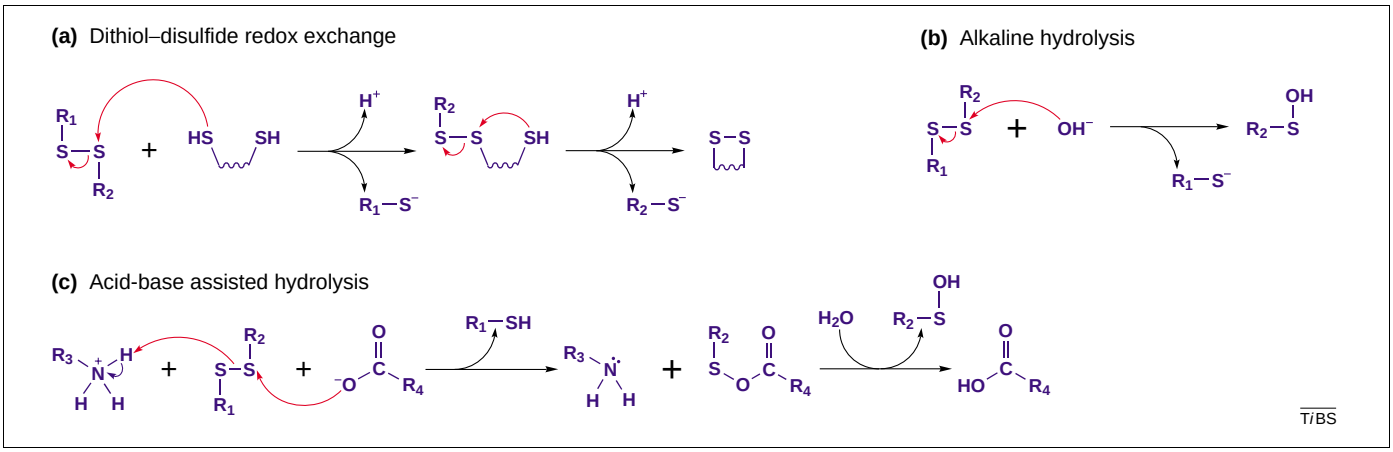

Fig. 1. Three possible mechanisms of disulfide-bond cleavage in secreted proteins. (a) Dithiol–disulfide redox exchange: one of the thiol groups of the oxidoreductase dithiol attacks the substrate disulfide bond, cleaving off the thiolate $R_1–S^-$. The mixed disulfide can then spontaneously decompose via an intramolecular thiol–disulfide exchange giving the more stable disulfide, in the process cleaving off the thiolate $R_2–S^-$. (b) Alkaline hydrolysis: a hydroxide ion attacks the disulfide bond, giving the sulfenic acid $R_2–S–OH$ by cleaving off the thiolate $R_1–S^-$. (c) Acid-base assisted hydrolysis: the carboxylate anion $R_4–CO_2^-$ attacks one of the sulfur atoms of the disulfide bond, cleaving off the thiolate $R_1–S^-$ (as the thiol $R_1–SH$) with the assistance of the nearby ammonium cation $R_3–NH_3^+$ (and giving the amine $R_3–NH_2$). The intermediate sulfenic ester $R_4–CO_2–S–R_2$ is spontaneously hydrolyzed by H_2O, giving the sulfenic acid $R_2–S–OH$ and the carboxylic acid $R4–CO_2H$.

contrast, PDI assists the folding of nascent proteins in the ER and is recycled back to the ER from the Golgi and intermediate compartment through interaction with the KDEL (Lys-Asp-Glu-Leu) receptor via its C-terminal KDEL motif. However, secreted PDI retains the KDEL anchor [15,16], which means that it can escape this retrieval mechanism. A route to the cell surface for these oxidoreductases is suggested by the finding that the ER can fuse with the plasma membrane in macrophages. Gagnon *et al.* [51] showed that the ER contributes to the macrophage phagosome membrane. It might be that thioredoxin and/or PDI are carried directly to the plasma membrane by the ER in some cells.

It is not known whether thioredoxin and PDI function as single turnover reductants outside the cell, or if they act catalytically to reduce several substrate molecules. A mechanism is required to reduce the oxidized form of the protein for these enzymes to act catalytically; for example, thioredoxin reductase and its cofactor NADPH reduce oxidized thioredoxin inside the cell. It is possible that systems similar to the NADH–oxidoreductase complex [52], which has been implicated in reduction of extracellular protein disulfide bonds, can reduce the oxidized enzymes.

Alkaline hydrolysis

The second mechanism is simple alkaline hydrolysis in which a hydroxide ion cleaves a disulfide bond generating a cysteine thiol and a cysteine sulfenic acid. This mechanism is favored at alkaline pH, but might occur at neutral pH when facilitated. For example, the cleavage events in plasmin at alkaline pH are indistinguishable from those that are facilitated by PGK at neutral pH [23]. This observation has led Lay *et al.* [26] to propose that binding of PGK to plasmin triggers alkaline hydrolysis of plasmin disulfide bonds.

Acid-based assisted hydrolysis

The third possibility is acid-based assisted hydrolysis. Brandt *et al.* [53] used quantum chemical simulations to show that the interaction of a carboxylic acid and a primary amine with a disulfide bond can polarize and cleave the bond. Although this mechanism is theoretically feasible, it has not been demonstrated experimentally. It is possible that this might occur in plasmin; in the crystal structure of plasmin kringle 5 [54], the primary amine of Arg474 is within ~5 Å of the Cys536 sulfur atom. The Cys512–Cys536 disulfide bond is cleaved in plasmin upon incubation with PGK. It is possible that PGK positions a glutamic or aspartic carboxylic acid near the Cys512 sulfur, thus leading to polarization and cleavage of the disulfide bond.

Prediction of disulfide cleavage in proteins?

As more examples of disulfide cleavage in proteins are uncovered and the mechanism of cleavage is elucidated, it will be possible to begin making predictions about similar events in other proteins. With this in mind, my colleagues and I wondered whether the unusual features of the disulfide bond in CD4 D2 are mirrored in other proteins.

Cleavage of the CD4 D2 disulfide bond was suggested by the unusual geometry and strain of the bond [34]. The backbone of Ig domains are defined by two β sheets, the single disulfide bond in these domains nearly always straddle the β sheets [55]. The disulfide bond in D2, however, links strands in the same β sheet. This geometry introduces a high strain on the disulfide bond – the strands tilt towards each other, which distorts the β sheet and results in a high torsional energy across the bond (Fig. 2). Indeed, the dihedral strain energy of the D2 bond is approximately twice that of the D1 and D4 disulfide bonds [34]. The more strain there is on a disulfide bond the more readily it is cleaved [2,56–58], so it was not that surprising to find that the D2 bond could be cleaved on the cell surface.

Disulfide bonds that straddle strands in the same β sheet are relatively rare in proteins; a search of release 101 of the Protein Data Bank (http://nist.rcsb.org/pdb) identified 118 functionally distinct proteins with one or more cross-stranded bonds (M. Wouters and P. Hogg, unpublished observations). These bonds are conspicuous,

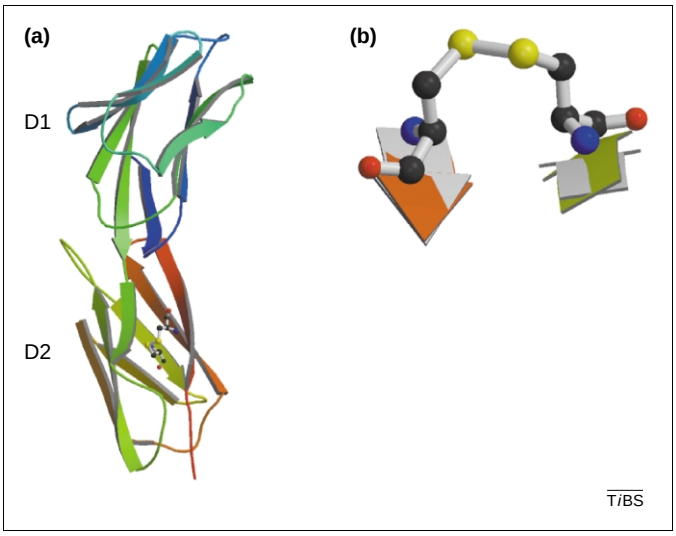

Fig. 2. Figure of domains 1 (D1) and 2 (D2) of human CD4 and the cross-strand disulfide bond in D2. (a) The D2 disulfide bond (ball and stick) is shown straddling the C (green) and F (red) β strands. (b) Linkage of the disulfide between the strands is accomplished by distorting the β sheet so that the strands are tilted towards each other. The distortion imparts a high torsional energy on the bond. When the disulfide straddles the sheets the strands involved are much less distorted. The grey strands are the homologous residues from rat CD4 D4, which has a conventional cross-sheet disulfide bond and, like D2, is a C2-set immunoglobulin domain. The green strand from human CD4 is tilted towards the orange strand by ~30° with respect to the rat CD4 strand, whereas the orange strand is tilted towards the green with respect to rat CD4 by ~15°. The figures were drawn using the coordinates from Ryu *et al.* [60] and Brady *et al.* [33], and MOLSCRIPT and Raster3D software.

however, by their presence in proteins that have a common function. Cross-strand bonds are found in mammalian cell-surface receptors (e.g. tissue factor, thrombomodulin, growth hormone receptor, erythropoietin receptor, interferon γ receptor and interleukin receptors), bacterial and viral proteins involved in membrane fusion (e.g. bacterial toxins and viral coat proteins, including HIV-1 gp120), trypsin-like serine proteinases and amyloid-forming proteins (e.g. amyloid precursor protein, cystatin, gelsolin and serum amyloid P). The function of some of these proteins might be controlled by cleavage of their cross-strand disulfide bond. For example, the three cross-strand disulfide bonds in gp120 straddle the variable loops: Cys126–Cys196 straddles V1 and V2; Cys296–Cys331 straddles V3; and Cys385–Cys418 straddles V4. Considering that V3 is the principal determinant of chemokine-receptor specificity and that cleavage of gp120 disulfide bonds reduces chemokine receptor binding [39], the Cys296–Cys331 cross-strand bond is probably one of the two disulfide bonds cleaved by PDI.

Concluding remarks

The evidence indicates that disulfide bonds have been added to proteins not only to help hold them together, but also as a way of controlling how they work. Identification of the common molecular events that lead to cleavage of disulfide bonds in extracellular proteins, whether the cleavage is reversible and how prevalent this form of protein regulation is in biology, are all pressing issues.

The dithiol–disulfide redox exchange mechanism for cleaving disulfide bonds in the extracellular milieu might prove to be the exception rather than the rule. PDI family enzymes have broad substrate specificity and they require at least two additional factors to act catalytically, so it is difficult to see how specificity and efficiency of action will be achieved. By contrast, cleavage of disulfide bonds in the extracellular environment by the thiol-independent mechanisms, facilitated alkaline hydrolysis or acid–based assisted hydrolysis, have the advantage of exquisite substrate specificity and can act catalytically.

Cleavage of disulfide bonds might, in many instances, be reversible. Perturbation of the balance between the cleaved and disulfide-bonded forms could be the key to the regulation of proteins in some cases. For example, the disulfide cleavage in vWF [20], and perhaps also in CD4 [34], is reversible. How the disulfide bond reforms is an important question: does the bond reform spontaneously or does it require facilitation or catalysis? Notably, nitric oxide is emerging as an important facilitator of disulfide-bond formation in some proteins by reacting with and activating cysteine thiols [59].

Whether disulfide-bond cleavage is a mechanism frequently used for controlling protein function or whether it is only a property of a few select proteins, is not known at this stage. The technical challenges of detecting and measuring disulfide cleavage in trace amounts of protein has made progress on this topic slow. Bioinformatics approaches such as the identification of cross-strand disulfide bonds, will be useful for understanding how important this protein chemistry is in biology.

Acknowledgements

I thank Merridee Wouters for many helpful discussions about cross-strand disulfides and Neil Donoghue for his help with describing the mechanisms of disulfide-bond cleavage. This work was supported by the National Health and Medical Research Council of Australia, the Australian Research Council, and the New South Wales Cancer Council and Health Department.

References

1 Thornton, J.M. (1981) Disulphide bridges in globular proteins. *J. Mol. Biol.* 151, 261–287
2 Wetzel, R. (1987) Harnessing disulfide-bonds using protein engineering. *Trends Biochem. Sci.* 12, 478–482
3 Adams, J.C. (2001) Thrombospondins: multifunctional regulators of cell interactions. *Annu. Rev. Cell Dev. Biol.* 17, 25–51
4 Lawler, J. and Hynes, R.O. (1986) The structure of human thrombospondin, an adhesive glycoprotein with multiple calcium-binding sites and homologies with several different proteins. *J. Cell Biol.* 103, 1635–1648
5 Speziale, M.V. and Detwiler, T.C. (1990) Free thiols of platelet thrombospondin. *J. Biol. Chem.* 265, 17859–17867
6 Sun, X. *et al.* (1992) Disulfides modulate RGD-inhibitable cell adhesive activity of thrombospondin. *J. Cell Biol.* 118, 693–701
7 Hogg, P.J. (1994) Thrombospondin 1 as an enzyme inhibitor. *Thromb. Haemost.* 72, 787–792
8 Misenheimer, T.M. and Mosher, D.F. (1995) Calcium ion binding to thrombospondin 1. *J. Biol. Chem.* 270, 1729–1733
9 Hogg, P.J. *et al.* (1997) Interaction of platelet-derived growth factor with thrombospondin 1: dependence on the disulfide-bond arrangement in thrombospondin 1. *Biochem. J.* 326, 709–716
10 Matthias, L.J. *et al.* (1996) Identification of monoclonal antibodies that recognize different disulfide-bonded forms of thrombospondin 1. *Biochim. Biophys. Acta* 1296, 138–144
11 Hotchkiss, K.A. *et al.* (1996) Catalysis of disulfide isomerization in thrombospondin 1 by protein disulfide isomerase. *Biochemistry* 35, 9761–9767
12 Milev, Y. and Essex, D.W. (1999) Protein disulfide isomerase catalyzes

the formation of disulfide-linked complexes of thrombospondin-1 with thrombin-antithrombin III. *Arch. Biochem. Biophys.* 361, 120–126
13 Chen, K. *et al.* (1995) Characterization of protein disulfide isomerase released from activated platelets. *Br. J. Haematol.* 90, 425–431
14 Essex, D.W. *et al.* (1995) Localization of protein disulfide isomerase to the external surface of the platelet plasma membrane. *Blood* 86, 2168–2173
15 Yoshimori, T. *et al.* (1990) Protein disulfide-isomerase in rat exocrine pancreatic cells is exported from the endoplasmic reticulum despite possessing the retention signal. *J. Biol. Chem.* 265, 15984–15990
16 Terada, K. *et al.* (1995) Secretion, surface localization, turnover, and steady state expression of protein disulfide isomerase in rat hepatocytes. *J. Biol. Chem.* 270, 20410–20416
17 Lawrence, D.A. *et al.* (1996) Surface thiols of human lymphocytes and their changes after *in vitro* and *in vivo* activation. *J. Leukoc. Biol.* 60, 611–618
18 Tager, M. *et al.* (1997) Membrane-bound proteindisulfide isomerase (PDI) is involved in regulation of surface expression of thiols and drug sensitivity of B-CLL cells. *Exp. Hematol.* 25, 601–607
19 Jiang, X-M. *et al.* (1999) Redox control of exofacial protein thiols/disulfides by protein disulfide isomerase. *J. Biol. Chem.* 274, 2416–2423
20 Xie, L. *et al.* (2001) Control of von Willebrand factor multimer size by thrombospondin-1. *J. Exp. Med.* 193, 1341–1349
21 Pimanda, J.E. *et al.* (2002) The von Willebrand Factor reducing activity of thrombospondin-1 is located in the calcium-binding/C-terminal sequence and requires a free thiol at position 974. *Blood* 100, 2832–2838
22 Stathakis, P. *et al.* (1997) Generation of angiostatin by reduction and proteolysis of plasmin. Catalysis by a plasmin reductase secreted by cultured cells. *J. Biol. Chem.* 272, 20641–20645
23 Stathakis, P. *et al.* (1999) Angiostatin formation involves disulfide-bond reduction and proteolysis in kringle 5 of plasmin. *J. Biol. Chem.* 274, 8910–8916
24 O'Reilly, M.S. *et al.* (1994) Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. *Cell* 79, 315–328
25 Lay, A.J. *et al.* (2000) Phosphoglycerate kinase acts in tumour angiogenesis as a disulphide reductase. *Nature* 408, 869–873
26 Lay, A.J. *et al.* (2002) Plasmin reduction by phosphoglycerate kinase is a thiol-independent process. *J. Biol. Chem.* 277, 9062–9068
27 Kwon, M. *et al.* (2002) Identification of annexin II heterotetramer as a plasmin reductase. *J. Biol. Chem.* 277, 10903–10911
28 Gately, S. *et al.* (1997) The mechanism of cancer-mediated conversion of plasminogen to the angiogenesis inhibitor angiostatin. *Proc. Natl. Acad. Sci. U. S. A.* 94, 10868–10872
29 Kassam, G. *et al.* (2001) Purification and characterization of A61. An angiostatin-like plasminogen fragment produced by plasmin autodigestion in the absence of sulfhydryl donors. *J. Biol. Chem.* 276, 8924–8933
30 Eckert, D.M. and Kim, P.S. (2001) Mechanisms of viral membrane fusion and its inhibition. *Annu. Rev. Biochem.* 70, 777–810
31 Wang, J.H. *et al.* (1990) Atomic structure of a fragment of human CD4 containing two immunoglobulin-like domains. *Nature* 348, 411–418
32 Ryu, S.E. *et al.* (1990) Crystal structure of an HIV-binding recombinant fragment of human CD4. *Nature* 348, 419–426
33 Brady, R.L. *et al.* (1993) Crystal structure of domains 3 and 4 of rat CD4: relation to the NH_2-terminal domains. *Science* 260, 979–983
34 Matthias, L.J. *et al.* (2002) Disulfide exchange in domain 2 of CD4 is required for entry of the human immunodeficiency virus type 1. *Nat. Immun.* 3, 727–732
35 Martin, H. and Dean, M. (1991) Identification of a thioredoxin-related protein associated with plasma membranes. *Biochem. Biophys. Res. Commun.* 175, 123–128
36 Rubartelli, A. *et al.* (1992) Secretion of thioredoxin by normal and neoplastic cells through a leaderless secretory pathway. *J. Biol. Chem.* 267, 24161–24164
37 Matthias, L.J. and Hogg, P.J. (2003) Redox control on the cell surface: implications for HIV-1 entry. *Antioxid. Redox Signal.* 5, 133–138
38 Gallina, A. *et al.* (2002) Inhibitors of protein disulfide isomerase (PDI) prevent cleavage of disulfide-bonds in receptor-bound gp120 and prevent HIV-1 entry. *J. Biol. Chem.* 277, 50579–50588
39 Barbouche, R. *et al.* (2003) Protein disulfide isomerase-mediated reduction of two disulfide-bonds of HIV Env gp120 occurs post CXCR4 binding and is required for fusion. *J. Biol. Chem.* 278, 3131–3136
40 Ryser, H.J. *et al.* (1994) Inhibition of human immunodeficiency virus infection by agents that interfere with thiol-disulfide interchange upon virus-receptor interaction. *Proc. Natl. Acad. Sci. U. S. A.* 91, 4559–4563
41 Fenouillet, E. *et al.* (2001) The catalytic activity of protein disulfide isomerase is involved in human immunodeficiency virus envelope-mediated membrane fusion after CD4 cell binding. *J. Infect. Dis.* 183, 744–752
42 Hynes, R. (2002) Integrins: bidirectional, allosteric signaling machines. *Cell* 110, 673–687
43 Yan, B. and Smith, J.W. (2001) Mechanism of integrin activation by disulfide-bond reduction. *Biochemistry* 40, 8861–8867
44 Yan, B. and Smith, J.W. (2000) A redox site involved in integrin activation. *J. Biol. Chem.* 275, 39964–39972
45 O'Neill, S. *et al.* (2000) The platelet integrin $\alpha_{IIb}\beta_3$ has an endogenous thiol isomerase activity. *J. Biol. Chem.* 275, 36984–36990
46 Lahav, J. *et al.* (2002) Sustained integrin ligation involves extracellular free sulfhydryls and enzymatically catalyzed disulfide exchange. *Blood* 100, 2472–2478
47 Essex, D.W. *et al.* (2001) Protein disulfide isomerase and sulfhydryl-dependent pathways in platelet activation. *Biochemistry* 40, 6070–6075
48 Xiong, J.P. *et al.* (2001) Crystal structure of the extracellular segment of integrin $\alpha_v\beta_3$. *Science* 294, 339–345
49 Beglova, N. *et al.* (2002) Cysteine-rich module structure reveals a fulcrum for integrin rearrangement upon activation. *Nat. Struct. Biol.* 9, 282–287
50 Freedman, R.B. *et al.* (2002) Protein disulfide isomerases exploit synergy between catalytic and specific binding domains. *EMBO Rep.* 3, 136–140
51 Gagnon, E. *et al.* (2002) Endoplasmic reticulum-mediated phagocytosis is a mechanism of entry into macrophages. *Cell* 110, 119–131
52 Berridge, M.V. and Tan, A.S. (2000) High-capacity redox control at the plasma membrane of mammalian cells: trans-membrane, cell surface, and serum NADH-oxidases. *Antioxid. Redox Signal.* 2, 231–242
53 Brandt, W. *et al.* (1999) A molecular mechanism for the cleavage of a disulfide-bond as the primary function of agonist binding to G-protein-coupled receptors based on theoretical calculations supported by experiments. *Eur. J. Biochem.* 261, 89–97
54 Chang, Y. *et al.* (1998) Structure and ligand binding determinants of the recombinant kringle 5 domain of human plasminogen. *Biochemistry* 37, 3258–3271
55 Richardson, J.A. (1981) Protein anatomy. *Adv. Protein Chem.* 34, 167–339
56 Weiner, S.J. *et al.* (1984) A new force field for molecular mechanical simulation of nucleic acids and proteins. *J. Am. Chem. Soc.* 106, 765–784
57 Katz, B.A. and Kossiakoff, A. (1986) The crystallographically determined structures of atypical strained disulfides engineered into subtilisin. *J. Biol. Chem.* 261, 15480–15485
58 Wells, J.A. and Power, D.B. (1986) In vivo formation and stability of engineered disulfide-bonds in subtilisin. *J. Biol. Chem.* 261, 6564–6570
59 Lipton, S.A. *et al.* (2002) Cysteine regulation of protein function – as exemplified by NMDA-receptor modulation. *Trends Neurosci.* 25, 474–480
60 Ryu, S.E. *et al.* (1994) Structures of an HIV and MHC binding fragment from human CD4 as refined in two crystal lattices. *Structure* 2, 59–74