Cross-strand disulphides in cell entry proteins: poised to act

Merridee A. Wouters,[1]* Ken K. Lau,[1] and Philip J. Hogg[2]

Summary

Cross-strand disulphides (CSDs) are unusual bonds that link adjacent strands in the same β-sheet. Their peculiarity relates to the high potential energy stored in these bonds, both as torsional energy in the highly strained disulphide linkage and as deformation energy stored in the sheet itself. CSDs are relatively rare in protein structures but are conspicuous by their presence in proteins that are involved in cell entry. The finding that entry of botulinum neurotoxin and HIV into mammalian cells involves cleavage of CSDs suggests that the activity of other cell entry proteins may likewise involve cleavage of these bonds. We examine emerging evidence of the involvement of these unusual disulphides in cell entry events. ***BioEssays*** **26:73–79, 2004.**

Introduction

Cross-strand disulphides (CSDs) are uncommon covalent bonds that link cysteine residues in adjacent strands of antiparallel β-sheet. The position of most disulphide bonds can be rationalized in terms of their stabilizing effect on local protein structure. CSDs are unusual, however, because they occur in a secondary structure that is already non-covalently linked.[1] A clue to the reason for their existence comes from studies of two proteins involved in cell entry events, botulinum neurotoxin[2] and CD4.[3] It was found that cleavage of the CSD in these proteins is required for entry of the neurotoxin or HIV, respectively. This observation led us to explore whether cleavage of CSDs might be a more general mechanism for controlling protein function.

[1]Victor Chang Cardiac Research Institute, Sydney, Australia.
[2]Centre for Vascular Research, University of New South Wales and Department of Haematology, Prince of Wales Hospital, Sydney, Australia.
Funding agency: This work was supported by a Freedman Foundation Fellowship to M.A.W., the National Health and Medical Research Council of Australia, the Australian Research Council, and the New South Wales Cancer Council and Health Department.
*Correspondence to: Merridee A. Wouters, Victor Chang Cardiac Research Institute, Sydney NSW 2010, Australia.
E-mail: m.wouters@victorchang.unsw.edu.au
DOI 10.1002/bies.10413
Published online in Wiley InterScience (www.interscience.wiley.com).

CSDs store potential energy

CSDs are always found in the 'non-H-bonded' site of antiparallel β-sheet,[1] an arrangement where the backbone hydrogen-bonding groups face away from each other (Fig. 1A). This site differs from the alternate 'H-bonded' site by having a smaller separation of the adjacent C_αs (4.5 Å compared to 5.5 Å) and not being directly constrained by two hydrogen bonds that link the backbones of the cross-strand residue pair together. The disulphide linkage forms across the two strands roughly perpendicular to the strand direction and parallel to the hydrogen bonds between the two strands (Fig. 1A). CSDs often join short regions of the polypeptide chain and are commonly found bridging a β-hairpin, a structure where the two antiparallel strands are locally linked to each other by a short turn (Fig. 1B). Both half-cystines in the disulphide assume a highly stressed gauche + χ_1 conformation where the C_α and C_β separations are roughly equal at 4 Å. The deformation of the sheet is mainly apparent as a pucker caused by the strands being drawn closer together and tilting towards each other to accommodate the disulphide linkage (Fig. 1C). Typically, C_α atoms of non-H-bonded residue pairs are separated by a distance of 4.5 Å, whereas CSD C_αs are separated by roughly 4.0 Å. In addition, the strands usually shear in the direction of their C terminus, as well as twisting in the usual direction.

CSDs store potential energy, both as torsional energy in the highly strained disulphide linkage and as deformation energy in the sheet itself (Fig. 1C). Studies of disulphide bonds in model proteins have shown that those bonds with high potential energy are more easily cleaved than disulphide bonds with lower stored energy.[4–7] The strain of a disulphide-bond can be estimated from the five dihedral angles.[4,5] The calculated strain energies only consider the dihedral angles and do not include other factors such as bond lengths, bond angles, and van der Waals contacts in calculating energy. Nevertheless, the findings of Pjura et al.[8] indicate that such calculations can give useful semi-quantitative insights into the amount of strain in a disulphide-bond.

In a survey of release 101 of the PDB (nist.rcsb.org/pdb), 208 CSDs were found in a total of 1620 unique protein structures that contained disulphides. The dihedral strain energy of all disulphides was calculated and compared to the energies of CSDs (Fig. 2). As anticipated, the torsional energy of a CSD is higher than the average for all disulphides. The average

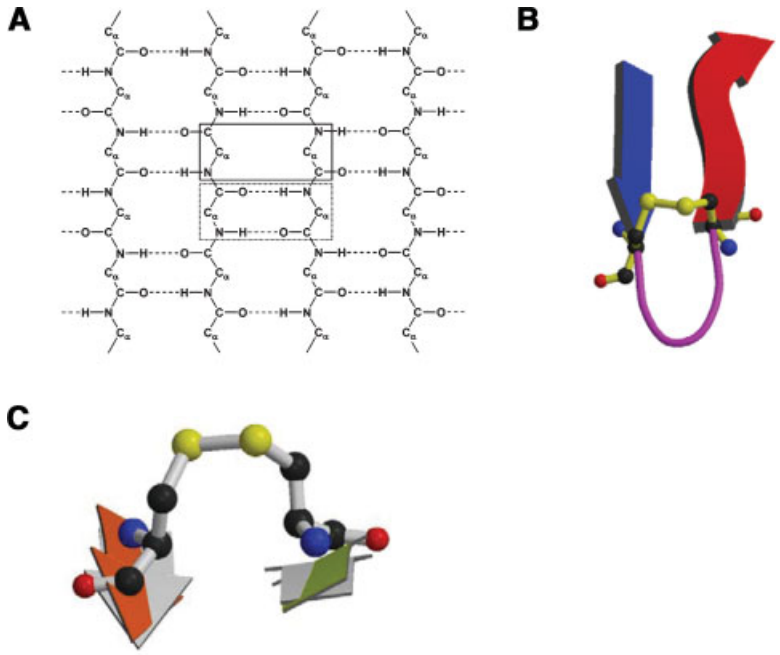

Figure 1. Structural features of CSDs. **A:** CSDs are always located in the non-H-bonded site (solid rectangle) of antiparallel β-sheet. The non-H-bonded site alternates with the H-bonded site (dashed rectangle) both along the strands (y-direction) and parallel to the hydrogen bonds (x-direction), to form regular antiparallel β-sheet. **B:** CSDs are often found bridging a β-hairpin, a structure where the two antiparallel strands are locally linked to each other by a short turn. The β-hairpin that is formed by the Cys^{122}–Cys^{127} CSD in influenza B neuraminidase is shown. The N-terminal strand is blue and the C-terminal strand is red. **C:** CSDs are highly strained linkages that introduce a pucker in the β-sheet in which they reside. The strands are drawn closer together and tilt towards each other to accommodate the disulphide linkage. The CSD in botulinum neurotoxin B is shown. The grey strands are a typical, locally flat β-sheet from CD4 shown for comparison. The green strand (Cys^{436}) is tilted towards the orange strand by ~20° with respect to the CD4 strand, while the orange strand (Cys^{445}) is tilted towards the green by ~30° with respect to the corresponding CD4 strand. The figures were drawn using the coordinates from 1b9v and 1epw and MOLSCRIPT and Raster3D software.

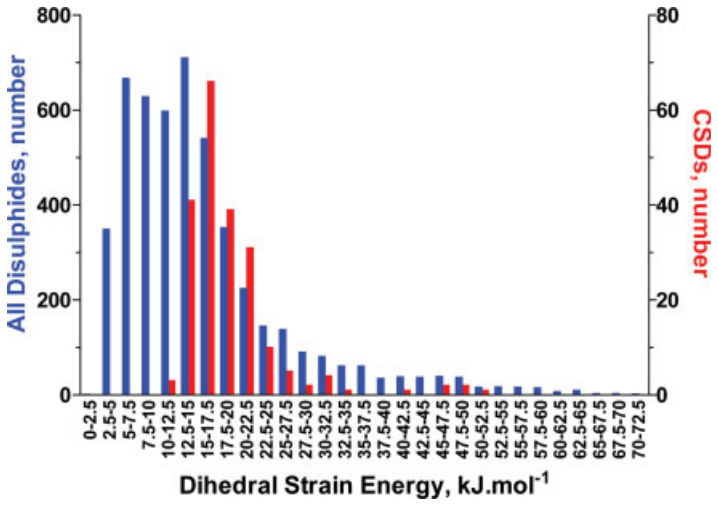

Figure 2. Dihedral strain energies of all disulphides and CSDs in unique protein structures in release 101 of the PDB. The average torsional energy of a CSD (18.8 ± 6.2 kJ · mol^{-1}, n = 208) is higher than the average for all disulphides (15.5 ± 10.9 kJ · mol^{-1}, n = 4,920).

Table 1. Potential involvement of CSDs in the function of bacterial toxins and viral entry proteins

Bacterial toxins

Toxin	Subunit or domain structure and CSDs	Possible involvement of the CSDs in function
Botulinum neurotoxins	Contains a catalytic A subunit and a receptor binding B subunit. The CSD joins the two subunits: Cys^{429}–Cys^{453} in botulinum neurotoxin A and Cys^{436}–Cys^{445} in serotype B.[9]	Cleavage of the CSD is required for the endopeptidase activity of the neurotoxin.[2]
Diphtheria toxin	Contains catalytic (A subunit), transmembrane and receptor-binding (B subunit) domains. The CSD (Cys^{461}–Cys^{471}) straddles a β-hairpin in the receptor-binding domain.[29]	Residues close to the CSD have been implicated as the heparin-binding epidermal growth factor precursor receptor and phospholipid binding sites.[30–32] Cleavage of the CSD may be involved in membrane translocation.[31,33]
Pertussis toxin	A hexamer containing a catalytic subunit (S1) that is tightly associated with a pentameric cell-binding component. The five subunits are pseudo-symmetrically arranged around a central pore. A CSD is present in each of the cell-binding components:[34] Cys^{120}–Cys^{134} in S2 and S3, Cys^{31}–Cys^{51} in both copies of S4 and Cys^{27}–Cys^{41} in S5.	ATP binds in the central pore of the pentamer and destabilizes the interaction with the catalytic subunit.[35,36] Cleavage of one or more of the CSDs surrounding the central pore may be involved in the destabilization.

Viral entry proteins

Viral protein	Subunit or domain structure and CSDs	Possible involvement of the CSDs in function
HIV gp120	The three CSDs in gp120 straddle the variable loops. Cys^{126}–Cys^{196} straddles V1/V2 on the bridging sheet, while Cys^{296}–Cys^{331} straddles V3 and Cys^{385}–Cys^{418} straddles V4 in the outer domain.[22]	Two of the nine disulphide bonds in gp120 are cleaved during membrane fusion.[14,15]
Influenza A hemagglutinin	The CSD (Cys^{A4}–Cys^{B137}) is on a small 3-stranded β-sheet that flips through 180° during the low pH induced conformational change.[37]	Prior to the pH induced conformational change, the CSD is shielded from solvent. After the sheet has flipped, it is fully exposed to solvent and susceptible to cleavage by reducing agents.[38] Cleavage of this CSD following the conformational change may be important for membrane fusion.
Influenza B neuraminidase	Contains five CSDs that are arranged pseudo-symmetrically around the axis of the β-propeller. Six sheets make up the blades of the β-propeller. A CSD is present in blades 1 (Cys^{122}–Cys^{127}), 3 (Cys^{231}–Cys^{236}) and 6 (Cys^{424}–Cys^{447}), while blade 4 has two CSDs on the same strands that are nested in the primary sequence,[39] Cys^{277}–Cys^{291} and Cys^{279}–Cys^{298}.	All of the CSDs are on the viral membrane side of the molecule. One or more may be involved in mediating the interaction between the β-propeller region and the stem.
Influenza C hemagglutinin-esterase fusion protein	There are three CSDs in the protein. The inter-chain CSD (Cys^{6}–Cys^{137}) anchors the portion of the protein containing the fusion peptide in HEF1 to HEF2. The second CSD (Cys^{106}–Cys^{151}) is in the E′ portion of the esterase domain in HEF1, while the third (Cys^{223}–Cys^{275}) is in the HEF1 receptor-binding domain.[40]	The inter-chain CSD is homologous to the inter-chain CSD in Influenza A hemagglutinin. As suggested for the Influenza A protein, cleavage of this CSD may be important for membrane fusion. The third CSD is very close to residues forming the sialic acid binding site.[40]
Newcastle disease virus fusion protein	The fusion protein is translated as a single chain (F0) that is proteolytically cleaved to form two disulphide-linked chains, F2 (N-terminal) and F1 (C-terminal). The CSD (Cys^{338}–Cys^{347}) in the F1 subunit straddles a β-hairpin in the head region of the complex that nestles against the neck.[41]	Antibodies against the β-hairpin loop neutralize the fusion protein [42] and two loop mutants escape neutralization.[43] Cleavage of the CSD may facilitate opening of the head region and exposure of the fusion peptide sequestered within the head.[44]
Newcastle disease virus hemagglutinin-neuraminidase	The hemagglutinin-neuraminidase is homologous to influenza A and B neuraminidases but contains only one CSD. The CSD (Cys^{531}–Cys^{542}) straddles a β-hairpin in the 6^{th} blade of the β-propeller.[45]	The CSD and β-hairpin loop are close to the active site. Crennell et al.[45] have suggested that the catalytic site is activated by a conformational change that switches between the hemagglutinin and neuraminidase activities. Cleavage of the CSD may play a role in the conformational change.
Ff bacteriophages coat, g3p	G3p consists of three domains, N1, N2, and CT. The N2 domain interacts with the F pilus (primary receptor) whereas the N1 domain forms a complex with TolA (coreceptor) at later stages of the infection process. The CSD (Cys^{46}–Cys^{53}) straddles a β-hairpin in the N1 domain.[46]	The loop of the β-hairpin protrudes into the hinge region between the N1 and N2 domains.[47] It has been predicted that the F pilus binds between N1 and N2 resulting in a conformational change that unmasks the TolA binding site on N1. Cleavage of the CSD may be involved in unmasking of the TolA binding site.
Encephalitis virus glycoprotein	The envelope glycoprotein, which mediates receptor binding and membrane fusion, forms dimers on the surface of mature virions. Exposure to acidic pH induces an irreversible conformational change that includes rearrangement to trimers. The first CSD (Cys^{92}–Cys^{116}) straddles a β-hairpin in the dimerization domain, while the second (Cys^{186}–Cys^{290}) is in the central domain.[48]	The loop region of the β-hairpin is almost fully conserved in all flaviviruses and has been proposed to be important for the fusogenic activity of the virus.[49] Cleavage of one or both CSDs may be involved in membrane fusion.

torsional energy of a disulphide in the dataset of unique proteins is 15.5 ± 10.9 kJ · mol^{-1} (4,920 disulphides in 1620 proteins). CSDs have an average torsional energy of 18.8 ± 6.2 kJ · mol^{-1} (208 disulphides in 171 proteins) and represent 8% of disulphide bonds that have torsional energies higher than 12.5 kJ · mol^{-1}. In contrast, they are basically unrepresented at energies lower than 12.5 kJ · mol^{-1} (3 examples).

Further inspection of the data set indicated that CSDs were present in proteins having some common functions. Conspicuously, CSDs are over-represented in molecules involved in cell entry. 29 CSDs are found in 14 proteins involved in cell entry (Fig. 3), which equates to an average of 2.1 CSDs per protein. In contrast, the frequency of a CSD in any disulphide-containing protein is 0.13. The demonstrated involvement of CSDs in botulinum neurotoxin and HIV entry will be reviewed first and then the implied or potential role of CSDs in other cell entry proteins will be considered.

Involvement of the CSD in clostridial neurotoxin action

The clostridial neurotoxins, comprising tetanus neurotoxin and the seven serotypes of botulinum neurotoxin (A–G), bind specifically to neuronal cells and disrupt neurotransmitter release by cleaving proteins involved in synaptic vesicle membrane fusion.[9] Translated as a single chain, the neurotoxins are subsequently cleaved to form the heavy and light chains. The two portions of the toxin remain covalently associated through a disulphide-bond. Structures of botulinum neurotoxins A and B have been solved, while only the heavy chain of tetanus neurotoxin has been determined.

In both botulinum structures, the CSD is the disulphide bond that joins the two chains; Cys^{429}–Cys^{453} in botulinum neurotoxin A and Cys^{436}–Cys^{445} in serotype B. Reference to sequence alignments show that this disulphide is conserved in all the clostridial neurotoxins.[9] Cleavage of this disulphide-bond is required for the endopeptidase activity of botulinum neurotoxins, a step that is rate limiting during the toxification process.[2] Upon cleavage of the CSD, neurotoxin A assumes an enzymatically active molten globule conformation, characterized by the existence of a native-like secondary structure and fold, but a loss of rigidity in tertiary structure.[10] This conformational rearrangement is apparent in altered UV spectra and lower thermal stability constants with respect to the native tertiary structure. These changes make the protein less likely to crystallize, however, and to date no protein has been structurally characterized in this state. The CSD of tetanus toxin is cleaved by reduced thioredoxin and by rat brain homogenate.[11]

Involvement of CSDs in human immunodeficiency virus (HIV) entry

The HIV envelope glycoprotein (Env) is translated as a single polypeptide chain (gp160) that is proteolytically cleaved by host cell subtilisins into two non-covalently associated fragments, the surface glycoprotein subunit (gp120) and the transmembrane (gp41) subunit that is anchored in the viral membrane.[12] Env is activated by binding to CD4 and chemokine receptor CXCR4 or CCR5 on susceptible cells. gp120 dissociates from gp41, which allows the fusion peptide to be inserted into the target membrane. The end result is formation of a six-helix bundled gp41 ectodomain that drives the membrane merger and eventual fusion.[13] Disulphide cleavage appears to be an important part of this process, both in CD4 and in gp120. The CSD in CD4[3] is cleaved on the cell surface and two of the nine disulphide bonds in gp120[14,15] are cleaved during HIV/cell membrane fusion.

The extracellular portion of CD4 consists of four immunoglobulin-like domains, D1 to D4.[16–18] The D1, D2 and D4 domains of CD4 each contain a disulphide-bond. The D1 and D4 disulphides are conventional cross-sheet immunoglobulin domain disulphides, while the atypical D2 disulphide is a CSD. The D2 bond is most likely cleaved by thioredoxin,[3] which is a thiol-disulphide oxidoreductase secreted by CD4$^+$ T cells.[19] It has been suggested that cleavage of the D2 bond is important for conformational changes in CD4 required for fusion of the viral and cell membranes.[20]

Comparison of primate immunodeficiency viruses identified five variable regions (V1–V5) in gp120 sequences.[21] The gp120 structure contains seven disulphides, three of which are CSDs in at least one of the solved structures.[22] Binding of gp120 to CD4$^+$ cells results in protein disulphide isomerase (PDI)-mediated cleavage of, on average, two of the nine disulphide bonds in gp120.[14,15] PDI, like thioredoxin, is a thiol-disulphide oxidoreductase[23] that interacts with CD4 both in solution and on the cell surface.[14] PDI cleavage of gp120 occurs after chemokine receptor binding.[15] The conformational change in gp120 that accompanies cleavage is believed to facilitate the unmasking of the gp41 fusion peptide and its insertion into the target cell membrane.[15]

The three CSDs in gp120 straddle the variable loops. Cys^{126}–Cys^{196} straddles V1/V2, Cys^{296}–Cys^{331} straddles V3, while Cys^{385}–Cys^{418} straddles V4 (Fig. 3). The gp120 monomer forms a bilobal structure with the two halves referred to as the 'inner' and 'outer' domains. The V3 and V4 loops and their resident CSDs are located in the outer domain. The two lobes are joined by a 'bridging sheet' composed of two β-hairpins donated from distal regions of the polypeptide chain. The N-terminal β-hairpin of the bridging sheet is straddled by the Cys^{126}–Cys^{196} CSD on strands 2 and 3 and long range interactions within the primary structure of the molecule are mediated by four hydrogen bonds between strands 2 and 21. CD4 binding increases exposure of epitopes involving residues in the bridging sheet.[24] Considering that V3 is the principal determinant of chemokine receptor specificity and that cleavage of gp120 disulphide bonds ablates chemokine receptor binding,[15] the Cys^{296}–Cys^{331} CSD is most likely one of the

two disulphide bonds reduced by PDI. We think it likely that either the Cys^{126}–Cys^{196} or Cys^{385}–Cys^{418} CSD is the other.

The importance of these disulphide cleavage events in HIV entry is supported by the finding that mono- and di-thiol alkylating agents, which inactivate thioredoxin and PDI and react with cleaved CD4 and gp120, inhibit HIV entry and envelope-mediated cell–cell fusion.[3,14,25,26] Anti-PDI monoclonal antibodies also inhibit HIV entry and cell–cell fusion.[25,26]

Involvement of CSDs in other bacterial toxins and viral entry proteins

There are two types of bacterial toxins: those that kill cells by pore formation and those that kill cells by translocation to the cytosol of a catalytic subunit that abrogates normal cellular function. CSDs are commonly found in the translocation type also known as AB toxins. While all these toxins are not homologous, they share the common feature of having a receptor binding B subunit that binds to the target cell to enable translocation of the catalytic A subunit into the cytosol. In most AB toxins, these two subunits are initially linked by both a peptide-bond and a disulphide-bond. Separation of the two subunits is required for the toxin's enzymic function. To date, AB toxins of four types have been structurally characterized: clostridial neurotoxins, diphtheria toxins, AB5 toxins such as pertussis toxin and anthrax protective antigen. A CSD is involved in the action of the clostridial neurotoxins (see above) and may be involved in the action of diphtheria toxins and pertussis toxin. Unlike the CSD of the clostridial neurotoxins, which links the A and B subunits, the CSDs of diphtheria and pertussis toxins are found in their receptor-binding B subunits.

Viral envelope glycoproteins penetrate host cells by binding to endogeneous receptors and fusing with the host cell membrane. For some well-characterized viruses, such as influenza A, HIV and tick-borne encephalitis, both of these functions are performed by a single viral envelope glycoprotein. Other types of viruses including most paramyxoviruses (e.g., Newcastle disease virus) and filamentous Ff bacteriophages achieve this feat with two interacting envelope glycoproteins, one of which binds the receptor and another that fuses with the membrane. CSDs are present in the envelope proteins of both types of virus. In characterized structures where the functions are performed by separate glycoproteins, the CSDs are in the binding glycoprotein.

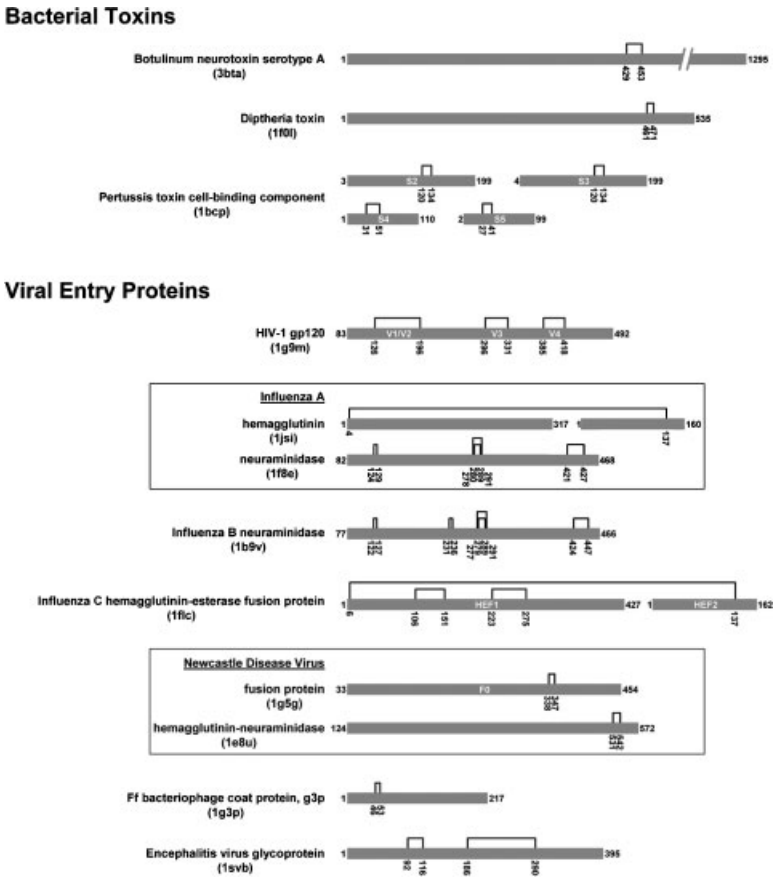

Figure 3. Relative positions of the CSDs in bacterial and viral entry proteins. The grey bars represent the portion of the polypeptide for which there is structure information. The PDB number is indicated in brackets for each protein. The CSDs are indicated by bridges, which link the indicated cysteine residues. Other disulphide bonds are not shown.

The spike glycoproteins of orthomyxoviruses (e.g., influenza) and paramyxoviruses (e.g., Newcastle disease virus) have three functions: to bind to a receptor on the cell surface, to mediate viral fusion with the cell membrane and to destroy the receptor. For influenza A and B, the binding and fusion functions are performed by haemagglutinin, while a separate protein, neuraminidase, destroys the receptor. In influenza C virus, all three cell entry functions are mediated by a single glycoprotein, the hemagglutinin–esterase fusion protein. The hemagglutinin or fusion proteins of orthomyxoviruses and paramyxoviruses are translated as a single chain that is proteolytically cleaved to form two chains linked by a single disulphide-bond, a situation reminiscent of the AB translocation toxins (see above). The CSD in the orthomyxovirus fusion proteins is the inter-chain disulphide, similar to the clostridial neurotoxins.

A summary of the possible involvement of CSDs in the function of bacterial toxins and viral entry proteins is given in Table 1. The relative position of the CSDs in the primary structure and the cysteine residues that they link are indicated in Fig. 3.

Concluding remarks

A recurring feature of the CSDs in entry proteins is their presence in stressed regions of β-sheet, often bridging β-hairpins. Notably, residues in the loop region of the β-hairpin have been implicated in the mechanism of action of several of the proteins discussed herein. CSDs are also often associated with potentially stressed regions of the sheet known as β-bulges, where the sheet departs from the standard hydrogen-bonding pattern shown in Fig. 1A. These structural features imply that CSDs form locks on regions of protein structure of high potential energy. We suggest that cleavage of CSDs in perhaps a number of the entry proteins releases this stored energy, which is used for conformational changes that trigger the action of the protein. The nature of such a conformational change has been described for a few entry proteins.

The two-chain influenza A hemagglutinin is in a metastable state until it is exposed to low pH, whereupon the smaller C-terminal chain spontaneously adopts a new conformation.[27] It appears, therefore, that the larger N-terminal chain kinetically traps the C-terminal chain in a non-native fold. Exposure to low pH tips the balance in favour of the native fold that is adopted spontaneously. This scenario is supported by the observation that the smaller chain adopts the low pH conformation when it is expressed in the absence of the larger chain. Although the low pH structure is often thought of as the ground state, further conformational changes are implicated during fusion of the viral and host membranes. The protein appears to transit through a series of metastable states before reaching the ground state.

The CSD in influenza A hemagglutinin may be considered as a lock on a metastable region of structure and its cleavage facilitates transition to lower energy states. This notion is supported by how the CSD is initially formed. Six of the eight disulphide bonds in the protein appear to form spontaneously, while the CSD Cys^{4}–Cys^{466} (Cys^{A4}–Cys^{B137} in the two-chain form) and the bond linking Cys^{52} and Cys^{277} receive special attention.[28] Binding of nearby glycans to calnexin protects Cys^{4} from oxidation until Cys^{466} is translocated into the endoplasmic reticulum, while soluble calreticulin protects Cys^{52} until Cys^{277} is translocated. These two disulphides are then formed by the oxidoreductase ERp57, which is recruited by calnexin and calreticulin.

Probing the involvement of CSDs in the function of cell entry proteins is possible by manipulating the Cys residues that comprise the CSD. Thiol alkylating agents such as 5′-dithiobis(2-nitrobenzoic acid) or 3-(N-maleimidylpropionyl) biocytin, which are largely membrane impermeable, could be used to block the Cys thiols of a cleaved CSD, while thiol-oxidising agents such as $HgCl_2$ may be used to catalyse formation of a CSD. Cleavage of a CSD may be achieved using the protein reductants, thioredoxin or PDI. Should one or more of these reagents perturb membrane fusion of the protein being investigated, it would suggest that a CSD might be involved in the process. A particular CSD could be investigated by eliminating the bond by mutating both Cys to Ser or Ala, or a novel disulphide-bond could be engineered into the protein to restrict conformational changes that are predicted to occur upon cleavage of the CSD.

Certain proteins, such as hemaglutinnin and the serpins, undergo dramatic conformational changes during function. Such conformational changes may be preceded by cleavage of CSDs in some cases. The preponderance of CSDs in bacterial and viral entry proteins and the demonstration of their cleavage in two of the proteins implies that manipulation of CSDs may be a key event during breach of the cell membrane.

References

1. Wouters MA, Curmi PM. An analysis of side chain interactions and pair correlations within antiparallel β-sheets: the differences between backbone hydrogen-bonded and non-hydrogen-bonded residue pairs. Proteins 1995;22:119–131.
2. Erdal E, Bartels F, Binscheck T, Erdmann G, Frevert J, Kistner A, Weller U, Wever J, Bigalke H. Processing of tetanus and botulinum A neurotoxins in isolated chromaffin cells. Naunyn Schmiedebergs Arch-Pharmacol 1995;351:67–78.
3. Matthias LJ, Yam PTW, Jiang X-M, Vandegraaff N, Li P, Poumbourios P, Donoghue N, Hogg PJ. Disulphide exchange in domain 2 of CD4 is required for entry of the Human Immunodeficiency Virus Type 1. Nature Immunol 2002;3:727–732.
4. Weiner SJ, Kollman PA, Case DA, Singh UC, Ghio C, Alagona G, Profeta S Jr, Weiner P. A new force field for molecular mechanical simulation of nucleic acids and proteins. J Am Chem Soc 1984;106:765–784.
5. Katz BA, Kossiakoff A. The crystallographically determined structures of atypical strained disulphides engineered into subtilisin. J Biol Chem 1986;261:15480–15485.
6. Wells JA, Power DB. In vivo formation and stability of engineered disulphide bonds in subtilisin. J Biol Chem 1986;261:6564–6570.
7. Wetzel R. Harnessing disulphide bonds using protein engineering. Trends Biochem Sci 1987;12:478–482.

8. Pjura PE, Matsumura M, Wozniak JA, Matthews BW. Structure of a thermostable disulphide-bridge mutant of phage T4 lysozyme shows that an engineered cross-link in a flexible region does not increase the rigidity of the folded protein. Biochemistry 1990;29:2592–2598.
9. Lacy DB, Stevens RC. Sequence homology and structural analysis of the clostridial neurotoxins. J Mol Biol 1999;291:1091–1104.
10. Cai S, Singh BR. Role of the disulphide cleavage induced molten globule state of type a botulinum neurotoxin in its endopeptidase activity. Biochemistry 2001;40:15327–15333.
11. Kistner A, Habermann E. Reductive cleavage of tetanus toxin and botulinum neurotoxin A by the thioredoxin system from brain. Evidence for two redox isomers of tetanus toxin. Naunyn Schmiedebergs Arch Pharmacol 1992;345:227–234.
12. Einfeld D. Maturation and assembly of retroviral glycoproteins. Curr Top Microbiol 1996;214:133–176.
13. Gallo SA, Finnegan CM, Viard M, Raviv Y, Dimitrov A, Rawat SS, Puri A, Durell S, Blumenthal R. The HIV Env-mediated fusion reaction. Biochim Biophys Acta 2003;1614:36–50.
14. Gallina A, Mandel R, Trahey M, Broder CC, Ryser HJ. Inhibitors of protein disulphide isomerase (PDI) prevent cleavage of disulphide-bonds in receptor-bound gp120 and prevent HIV-1 entry. J Biol Chem 2002;277: 50579–50588.
15. Barbouche R, Miquelis R, Jones IM, Fenouillet E. Protein disulphide isomerase-mediated reduction of two disulphide-bonds of HIV Env gp120 occurs post CXCR4 binding and is required for fusion. J Biol Chem 2003;278:3131–3136.
16. Wang JH, Yan YW, Garrett TP, Liu JH, Rodgers DW, Garlick RL, Tarr GE, Husain Y, Reinherz EL, Harrison SC. Atomic structure of a fragment of human CD4 containing two immunoglobulin-like domains. Nature 1990; 348:411–418.
17. Ryu SE, Kwong PD, Truneh A, Porter TG, Arthos J, Rosenberg M, Dai XP, Xuong NH, Axel R, Sweet RW et al. Crystal structure of an HIV-binding recombinant fragment of human CD4. Nature 1990;348:419–426.
18. Brady RL, Dodson EJ, Dodson GG, Lange G, Davis SJ, Williams AF, Barclay AN. Crystal structure of domains 3 and 4 of rat CD4: relation to the NH2-terminal domains. Science 1993;260:979–983.
19. Tagaya Y, Maeda Y, Mitsui A, Kondo N, Matsui H, Hamuro J, Brown N, Arai K, Yokota T, Wakasugi H et al. ATL-derived factor (ADF), an IL-2 receptor/Tac inducer homologous to thioredoxin: possible involvement of a dithiol-reduction in the IL-2 receptor induction. EMBO J 1989;8: 757–764.
20. Matthias LJ, Hogg PJ. Redox control on the cell surface: Implications for HIV-1 entry. Antioxid Redox Signal 2003;5:133–138.
21. Starcich BR, Hahn BH, Shaw GM, McNeely PD, Modrow S, Wolf H, Parks ES, Parks WP, Josephs SF, Gallo RC et al. Identification and characterization of conserved and variable regions in the envelope gene of HTLV-III/LAV, the retrovirus of AIDS. Cell 1986;45:637–648.
22. Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J, Hendrickson WA. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature 1998;393: 648–659.
23. Freedman RB, Klappa P, Ruddock LW. Protein disulphide isomerases exploit synergy between catalytic and specific binding domains. EMBO Rep 2002;3:136–140.
24. Wyatt R, Kwong PD, Desjardins E, Sweet RW, Robinson J, Hendrickson WA, Sodroski JG. The antigenic structure of the HIV gp120 envelope glycoprotein. Nature 1998;393:705–711.
25. Ryser HJ-P, Levy EM, Mandel R, DiSciullo GJ. Inhibition of human immunodeficiency virus infection by agents that interfere with thiol-disulphide interchange upon virus-receptor interaction. Proc Natl Acad Sci USA 1994;91:45459–45463.
26. Fenouillet E, Barbouche R, Courageot J, Miquelis R. The catalytic activity of protein disulphide isomerase is involved in human immunodeficiency virus envelope-mediated membrane fusion after CD4 cell binding. J Infect Dis 2001;183:744–752.
27. Bullough PA, Hughson FM, Skehel JJ, Wiley DC. Structure of influenza haemagglutinin at the pH of membrane fusion. Nature 1994;371:37–43.
28. Daniels R, Kurowski B, Johnson AE, Hebert DN. N-linked glycans direct the cotranslational folding pathway of influenza hemagglutinin. Mol Cell 2003;11:79–90.
29. Louie GV, Yang W, Bowman ME, Choe S. Crystal structure of the complex of diphtheria toxin with an extracellular fragment of its receptor. Mol Cell 1997;1:67–78.
30. Proia RL, Wray SK, Hart DA, Eidels L. Characterization and affinity labeling of the cationic phosphate-binding (nucleotide-binding) peptide located in the receptor-binding region of the B-fragment of diphtheria toxin. J Biol Chem 1980;255:12025–12033.
31. Papini E, Schiavo G, Tomasi M, Colombatti M, Rappuoli R, Montecucco C. Lipid interaction of diphtheria toxin and mutants with altered fragment B. 2. Hydrophobic photolabelling and cell intoxication. Eur J Biochem 1987;169:637–644.
32. Bennett MJ, Choe S, Eisenberg D. Refined structure of dimeric diphtheria toxin at 2.0 Å resolution. Protein Sci 1994;3:1444–1463.
33. Stenmark H, Olsnes S, Madshus IH. Elimination of the disulphide bridge in fragment B of diphtheria toxin: effect on membrane insertion, channel formation, and ATP binding. Mol Microbiol 1991;5:595–606.
34. Hazes B, Boodhoo A, Cockle SA, Read RJ. Crystal structure of the pertussis toxin-ATP complex: a molecular sensor. J Mol Biol 1996;258: 661–671.
35. Moss J, Stanley SJ, Watkins PA, Burns DL, Manclark CR, Kaslow HR, Hewlett EL. Stimulation of the thiol-dependent ADP-ribosyltransferase and NAD glycohydrolase activities of Bordetella pertussis toxin by adenine nucleotides, phospholipids, and detergents. Biochemistry 1986; 25:2720–2725.
36. Hausman SZ, Manclark CR, Burns DL. Binding of ATP by pertussis toxin and isolated toxin subunits. Biochemistry 1990;29:6128–6131.
37. Ha Y, Stevens DJ, Skehel JJ, Wiley DC. X-ray structures of H5 avian and H9 swine influenza virus hemagglutinins bound to avian and human receptor analogs. Proc Natl Acad Sci USA 2001;98:11181–11186.
38. Wiley DC, Skehel JJ. The structure and function of the hemagglutinin membrane glycoprotein of influenza virus. Annu Rev Biochem 1987;56: 365–394.
39. Finley JB, Atigadda VR, Duarte F, Zhao JJ, Brouillette WJ, Air GM, Luo M. Novel aromatic inhibitors of influenza virus neuraminidase make selective interactions with conserved residues and water molecules in the active site. J Mol Biol 1999;293:1107–1119.
40. Rosenthal PB, Zhang X, Formanowski F, Fitz W, Wong CH, Meier-Ewert H, Skehel JJ. Wiley DC. Structure of the haemagglutinin-esterase-fusion glycoprotein of influenza C virus. Nature 1998;396:92–96.
41. Chen L, Gorman JJ, McKimm-Breschkin J, Lawrence LJ, Tulloch PA, Smith BJ, Colman PM, Lawrence MC. The structure of the fusion glycoprotein of Newcastle disease virus suggests a novel paradigm for the molecular mechanism of membrane fusion. Structure (Camb) 2001;9: 255–266.
42. Yusoff K, Nesbit M, McCartney H, Meulemans G, Alexander DJ, Collins MS, Emmerson PT, Samson AC. Location of neutralizing epitopes on the fusion protein of Newcastle disease virus strain Beaudette C. J Gen Virol 1989;70:3105–3109.
43. Toyoda T, Gotoh B, Sakaguchi T, Kida H, Nagai Y. Identification of amino acids relevant to three antigenic determinants on the fusion protein of Newcastle disease virus that are involved in fusion inhibition and neutralization. J Virol 1988;62:4427–4430.
44. Peisajovich SG, Shai Y. New insights into the mechanism of virus-induced membrane fusion. Trends Biochem Sci 2002;27:183–190.
45. Crennell S, Takimoto T, Portner A, Taylor G. Crystal structure of the multifunctional paramyxovirus hemagglutinin-neuraminidase. Nature Struct Biol 2000;7:1068–1074.
46. Lubkowski J, Hennecke F, Pluckthun A, Wlodawer A. The structural basis of phage display elucidated by the crystal structure of the N-terminal domains of g3p. Nat Struct Biol 1998;5:140–147.
47. Holliger P, Riechmann L, Williams RL. Crystal structure of the two N-terminal domains of g3p from filamentous phage fd at 1.9 Å: evidence for conformational lability. J Mol Biol 1999;288:649–657.
48. Rey FA, Heinz FX, Mandl C, Kunz C, Harrison SC. The envelope glycoprotein from tick-borne encephalitis virus at 2 Å resolution. Nature 1995;375:291–298.
49. Roehrig JT, Hunt AR, Johnson AJ, Hawkes RA. Synthetic peptides derived from the deduced amino acid sequence of the E-glycoprotein of Murray Valley encephalitis virus elicit antiviral antibody. Virology 1989; 171:49–60.