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COMMUNICATION

Vicinal disulfide turns

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The formation of a disulfide bond between adjacent cysteine residues is accompanied by the formation of a tight turn of the protein backbone. In nearly 90% of the structures analyzed a type VIII turn was found. The peptide bond between the two cysteines is in a distorted *trans* conformation, the omega torsion angle ranges from 159 to –133°, with an average value of 171°. The constrained nature of the vicinal disulfide turn and the pronounced difference observed between the oxidized and reduced states, suggests that vicinal disulfides may be employed as a 'redox-activated' conformational switch.

Keywords: β-turns/disulfide bridge/protein structure/secondary structure

The disulfide bond formed between the side chains of adjacent cysteines is a rare structural element. In the last few years, vicinal disulfides have been structurally characterized in a variety of proteins including enzymes, receptors and toxins (see Table I). Even though there are no arguments in favor of a common biological function (Blake *et al*., 1994; Czajkowski and Karlin, 1995; Engst and Miller, 1999; Wang *et al*., 2000), it seems plausible that vicinal disulfide bond formation can have both a structural role (e.g. by stabilizing the local backbone conformation) and a redox role (e.g. via facilitating electron exchanges). It has been shown that the acethylcholine cannot bind its receptor when the vicinal disulfide bond of the latter is reduced (Czajkowski and Karlin, 1995). Similarly, the reduction of a vicinal disulfide leads to the inactivation of the methanol dehydrogenase (Blake *et al*., 1994). A Janus-faced atracotoxin keeps its three-dimensional structure upon the reduction of its vicinal disulfide bond, although its neurotoxic activity is completely lost (Wang *et al*., 2000). The disulfide ring is also believed to be necessary in binding and reducing the cation in mercuric ion reductase (Engst and Miller, 1999). A vicinal disulfide bond is supposed to be a significant feature in the function of hepcidin, a polypeptide involved in iron uptake in the human intestine and iron release in macrophages (Hunter *et al*., 2002). Recently, non-native vicinal disulfide bonds were found in transient intermediates observed during the oxidative folding of a small cysteine knot protein, the Amaranth α-amylase inhibitor (Cemazar *et al*., 2003). This points to the fact that this structure may be more frequent than originally thought.

In Figure 1 the structure of the vicinal disulfides is compared with a set of structures in which there are vicinal cysteines in the reduced form. The polypeptide segments containing the oxidized Cys–Cys pair are clearly bent and the two cysteine side chains protrude on the same side of the backbone (Figure 1A). On the contrary, protein segments that contain adjacent cysteines in the reduced form, possess a more-or-less extended backbone and the sulfhydryl side chains tend to be on opposite sides (Figure 1B). The oxidized octapeptide segments also seem to be more constrained than the reduced structures. This is shown by the root-mean-square distance (r.m.s.d.) that is on average smaller for the oxidized octa-peptides (2.51 ± 0.14 Å) than for the reduced octa-peptides (3.31 ± 0.03 Å). These comparisons were calculated only between non-homologous proteins. Figure 1C and D show representative structures of an oxidized and a reduced Cys–Cys motif, respectively.

The backbone conformation of the turn associated with the vicinal disulfide bridge has two characteristic features. (i) The peptide unit formed by the two cysteines frequently deviates from planarity. The omega torsion angle ranges from 159 to –133°, with an average value of 171°. The peptide unit is thus in a distorted *trans* conformation, which is reminiscent of the *cis–trans* isomerization observed in solution of small model peptides (Kim *et al*., 1999). Actually, in the solution structure of hepcidin, the polypeptide moiety around the two cysteines involved in the vicinal disulfide bond is clearly conformationally disordered. (ii) The predominant conformation (Table I) found in 17 out of 20 structures resembles the type VIII β-turn (Wilmot and Thornton, 1990). A different conformation (type II β-turn) is found in the carboxylic esterase [Protein Data Bank (PDB) code 1qlw] and in the complex between a nicotinic acethylcholine receptor fragment with α-bungarotoxin (1idh and 1idg). In this conformation, the φ and ψ torsions of the first cysteine and the φ torsion of the second cysteine have values of –59(7), 161(4) and 58(5)°, respectively. Both conformations compare well with the conformations observed in the solution structure of the model compound Ac–ox–[Cys–Cys]–NH_2 (Creighton *et al*., 2001).

Even though the resolution of several of the structures in Table I may not allow the accurate determination of side chain stereochemistry, the overall stereochemical features of the eight-membered heterocycle containing the disulfide bond appear to be close to those observed in Ac–ox–[Cys–Cys]–NH_2 (Creighton *et al*., 2001). χ_1 and χ_2 are ~77 and ~69° for the first cysteine and ~58 and ~–44° for the second cysteine, and χ_3 is ~–100°.

The conserved nature of the vicinal disulfide turn and the pronounced difference that can be expected between the oxidized and reduced states suggests that the vicinal disulfides

Table I. Protein structures with vicinal disulfide bonds, deposited in the PDB

PDB file	Protein (reference)	Source	Resolution (Å)	Type of β-turn conformation
1dl0	J-atracotoxin-HV1C (Wang *et al*., 2000)	*Hadronyche versuta*	NMR	Type VIII
1eh5	Palmitoyl protein thioesterase 1 (Bellizzi *et al*., 2000)	*Bos taurus*	2.50	Type VIII
1ei9	Palmitoyl protein thioesterase 1 (Bellizzi *et al*., 2000)	*B.taurus*	2.25	Type VIII
1exw	Palmitoyl protein thioesterase 1 (Das *et al*., 2000)	*B.taurus*	2.40	Type VIII
1flg	Ethanol dehydrogenase (Keitel *et al*., 2000)	*Pseudomas aeruginosa*	2.60	Type VIII
1g72	Methanol dehydrogenase (Zheng *et al*., 2001)	*Methylophilus methylotrophus*	1.90	Type VIII
1h4i	Methanol dehydrogenase (Ghosh *et al*., 1995)	*Methylobacterium extroquens*	1.94	Type VIII
1h4j	Methanol dehydrogenase (Afolabi *et al*., 2001)	*M.extroquens*	3.00	Type VIII
1hv6	Alginate lyase A1-III (Yoon *et al*., 2001)	*Sphingomonas* spp.	2.00	Type VIII
1i9b	Acetylcholine binding protein (Brejc *et al*., 2001)	*Lymnaea stagnalis*	2.70	Type VIII
1idg[a]	Nicotinic acetylcholine receptor (Zeng *et al*., 2001)	*Torpedo californica*	NMR	Type II
1idh[a]	Nicotinic acetylcholine receptor (Zeng *et al*., 2001)	*T.californica*	NMR	Type II
1kb0	Ethanol dehydrogenase (Oubrie *et al*., 2002)	*Comamonas testosteroni*	1.44	Type VIII
1m4e	20-Hepcidin (Hunter *et al*., 2002)	*Homo sapiens*	NMR	Type VIII
1m4f	25-Hepcidin (Hunter *et al*., 2002)	*H.sapiens*	NMR	Type VIII
1obr	Carboxypeptidase T (Teplyakov *et al*., 1992)	*Thermoactinomyces vulgaris*	2.30	Type VIII
1qaz	Alginate lyase A1-III (Yoon *et al*., 1999)	*Sphingomonas* spp.	1.78	Type VIII
1qlw	Carboxylic esterase (Bourne *et al*., 2000)	*Alcaligenes* spp.	1.10	Type II
1xgc	α Conotoxin GI (Gehrmann *et al*., 1998)	Synthetic non-native	NMR	Type VIII
4aah	Methanol dehydrogenase (Xia *et al*., 1996)	*Methylophilus W3A1*	2.4	Type VIII

[a]The structure of the disulfide turn in acetylcholine receptor in complex with bungarotoxin (1L4W) is virtually identical (Samson *et al*., 2001).

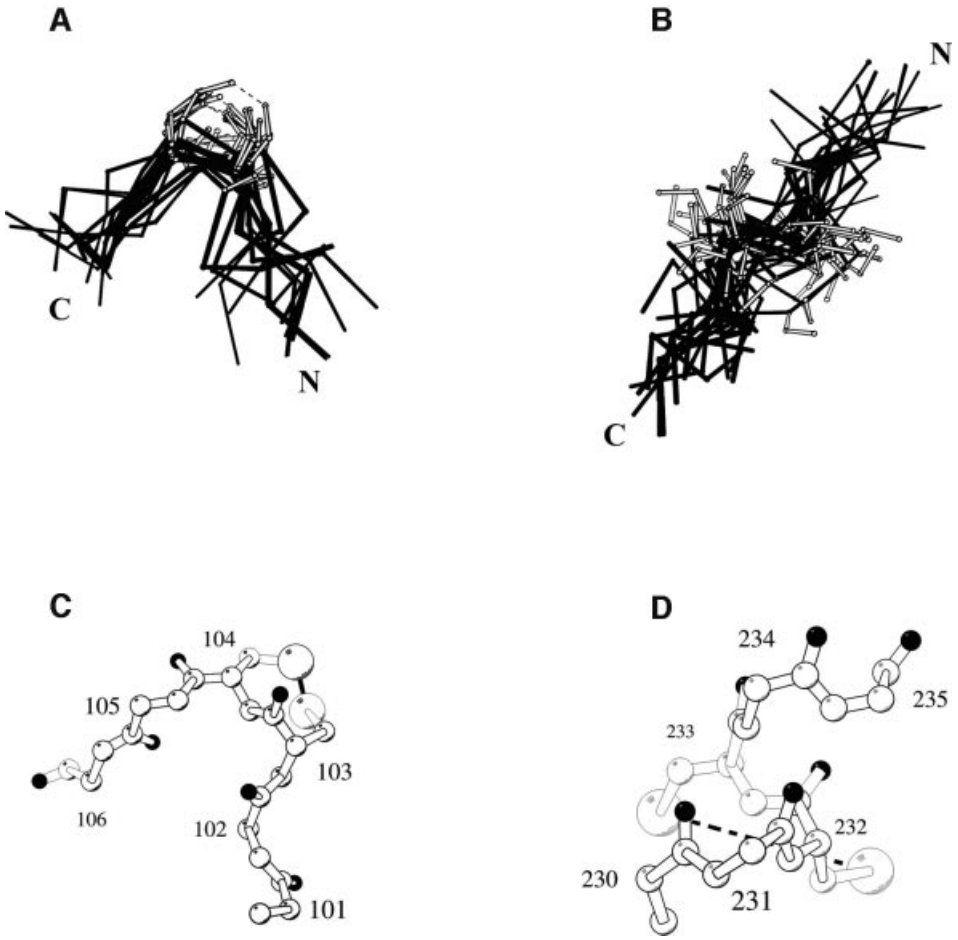

Fig. 1. (**A**) Superposition of octa-peptides (Cys–Cys motif and three residues one each side of it), containing the vicinal disulfide to J-atracotoxin-HV1C (1dl0). A few disulfide bonds are indicated with dashed lines. (**B**) Superposition of the octa-peptides, where the two adjacent cysteines are reduced. The data set contained 62 octa-peptides taken from a non-homologous set of protein structures (maximal sequence identity 25%, worst crystallographic resolution 2.5 Å) (Hobohm and Sander, 1994). The r.m.s.d. calculated between the Cα atoms of non-homologous octa-peptides is 2.51 ± 0.14 and 3.31 ± 0.03 Å in (A) and (B), respectively. (**C**) Molscript (Kraulis, 1991) view of the hexa-peptide centered on the oxidized cysteine pair in methanol dehydrogenase (4aah). (**D**) Molscript view of the hexa-peptide centered on the reduced cysteine pair in herpes virus cycline (1f5q). Nitrogen and carbon atoms in (C) and (D) are shown as white spheres, oxygen atoms as smaller black spheres, and sulfur atoms as larger white spheres. A black line connects the two sulfur atoms that are 2.0 Å away in (C) and 6.6 Å away in (D).

may be used in protein engineering to construct 'redox activated' conformational switches. While this hypothesis can be tested with peptides end-labeled with suitable spectroscopic probes, it is worth mentioning that the activity of ribonuclease A has been reversibly modulated via the reduction and oxidation of two vicinal cysteines 'engineered' into the molecule (Park and Raines, 2001).

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