Chirality of the Disulfide in the Prion Proteins

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In the Transmissible Spongiform Encephalopathies (TSEs) it has been generally assumed that the normal prion proteins (PPr) occurring on neural cells have the same composition of amino acids and the same sequence as the pathological forms (PPr^{Sc}) but differ in the manner of folding. The mechanism(s) by which the conversion of PPr into PPr^{Sc} takes place remain unknown. This paper calls attention to some aspects of chirality inherent in the disulfide function and suggests the possibility that handedness in the disulfide bond of prions may transmit stereochemical information that can influence the manner of folding or refolding into pathogenic forms.

The central problem of the fatal **prion diseases**[1] (also known as Transmissible Spongiform Encephalopathies, or TSEs) is the elucidation of the mechanism(s) by which a naturally occurring protein, PPr, is influenced by a misfolded isomer, PPr^{Sc}, to refold itself into a duplicate of the latter infective form. PPr is the general expression for the normal prion proteins occurring on the surfaces of neural cells in mammals, including humans, which are capable of being transformed into pathologic forms. They are similar but differ characteristically in each species, varying in certain amino acid replacements. In addition, there may be genetic differences and adventitious changes. The functions of PPr proteins are not well defined, and it is not certain whether they are essential.

In an animal infected with the PPr^{Sc} protein, the conversion of good PPr into PPr^{Sc} slowly builds up to a critical level of the unnatural molecules. Then the symptoms of one of the prion diseases manifests itself and runs its inexorable course to ultimate death of the infected animal.

The PPr proteins are composed mainly of α-helices. The disease-associated PPr^{Sc} forms are mostly in β-sheets. The nature of the resulting disease depends on the species of mammal and the structures of PPr and of PPr^{Sc}. In the spontaneous occurrences of a prion disease, the PPr and PPr^{Sc} are generally assumed to consist of the same chain of amino acids, in the same sequence, differing only in the manner of the folding of the chains.

The composition and three-dimensional structures of PPr have been determined by both X-ray crystallographic[2] and NMR[3] methods and are in agreement. The structure of PPr^{Sc} is not well defined. It remains an open question whether the active agent in the transformation is monomeric or polymeric. Polymeric forms do occur and have been found to have predominantly β-sheet folding.

PPr proteins contain one disulfide bond, as first reported by Turk, Teplow, Hood, and Prusiner.[4] In the typical human PPr, the disulfide joins the Cys units at positions 179 and 214, closing a large ring of 108 atoms. Maiti and Surewicz[5] discussed the role of the disulfide in the stability and folding in native prion protein. Analogs were studied in which the −S−S− group linking Cys units was reduced or was replaced with sulfur-free amino acids. These forms did not behave in ways typical of native prion protein. Welker, Raymond, Scheraga, and Caughey[6] demonstrated that the monomers of PPr^{Sc} are not linked by intermolecular disulfide bonds. They also provided evidence that "PPr^{Sc} can induce the conversion of the oxidized, disulfide-intact form without the temporary breakage and subsequent reformation of the disulfide bonds in cell free reactions".

The main purpose of this paper is to call attention to potential chirality, an inherent general property of the disulfide function, and **the possibility that the sense of helix (right-handed or left-handed) in the disulfide may transmit essential information affecting the manner in which the PPr protein molecules subsequently fold**—either to propagate duplicate copies or to misfold to generate the pathological forms PPr^{Sc}. Two isomeric forms of PPr differing only in the sense of helix of the disulfide would be **diastereoisomers** and would be expected to have different physical properties, including preferences for folding. In normal animals the unfolded, or partially folded, precursors of natural PPr must undeviatingly acquire the correct sense of helix and accurately fold into its correct form. We suggest that an intermediate complex of PPr^{Sc}, or some portion of it, with a partially folded PPr may reverse the sense of helix of the disulfide, with the consequence that the now unnatural diastereoisomer folds into a duplicate of PPr^{Sc}.

Chirality in the disulfide grouping is a consequence of the repulsive forces among the four unshared pairs of electrons on the two adjacent sulfur atoms and the interactions of the attached carbon-containing groups. In a sequence of atoms, $-C^1-S^2-S^3-C^4-$, the plane determined by atoms 1−2−3 ordinarily prefers to assume a configuration that is inclined at an approximately 90° angle to the plane determined by atoms 2−3−4. Boyd[7] has made a theoretical study of the electron distribution in disulfides generally, and of the special case of disulfides under torsion,[8] i.e., disulfides in which the dihedral angle is substantially less than 90° and may approach *cis*-coplanarity.

In simple disulfide compounds, e.g., dialkyl disulfides, the barrier to free rotation about the sulfur−sulfur bond is too low to allow optically active forms to exist at ambient

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temperatures, but when one or more fixed chiral centers coexist in the same molecule with the disulfide function, the fixed chiral center(s) induce a preference for one helical sense in the disulfide over its opposite. The presence of many asymmetric centers in a protein generally results in the existence of only one of the two alternative screw senses in the disulfide. The absolute configuration of that disulfide can, in favorable circumstances, be determined by measurements of the circular dichroism spectrum (CD) in the near-ultraviolet region, as will be explained later.

There is further subtlety inherent in the disulfide function. Neubert and Carmack[9ab] observed that a nearly linear relationship exists between the frequency of the peak nearest to the visible region of the spectrum and the dihedral angle in the disulfide. Thus, under favorable conditions, it is possible to define the absolute configuration of a sequence of atoms in the molecule containing not only the two sulfur atoms but also the attached carbon atoms.

The stabilizing role of disulfide bridges in proteins is well illustrated in the structural studies of decorsin,[10] a 39-residue protein isolated from the blood-sucking leech, *Macobdella decora*, which has the powerful ability to prevent blood clot formation. Decorsin has been intensively studied as a potential therapeutic agent or a model for other inhibitors of platelet aggregation. Research on decorsin also illlustrates the utility of circular dichroism measurements to define the geometry of protein disulfides. For example, decorsin contains three disulfides, all shown by circular dichroism studies to be right-handed. The dihedral angle of each disulfide was unique and was determined by measurements of the frequencies of the three bands in the near-UV circular dichroism spectrum. The sulfur-reduced peptide could be synthesized and purified in its reduced form and allowed to refold in the presence of glutathione. The disulfide bridges were found to reform exactly as in the original and were important in maintaining the correct three-dimensional structure of biologically active decorsin.

The disulfide group can preserve discrete helical configurations even in small rings in which the angle is close to 0°. In naturally occurring optically active thioctic acid, the fixed asymmetry of the carbon bearing the carboxyl (C*-COOH) induces optical activity in the disulfide. **Both possible diastereoisomers** are observed, with slightly different dihedral angles, resulting in two CD bands of opposite sign but slightly displaced in frequency. The two CD bands partially cancel, and the observed CD has the uncharacteristic appearance of a small sine wave.[11]

Two interesting stereochemical effects have been observed in a compound having a small but definite, dihedral angle. (a) When the disulfide bond is forced into near *cis*-coplanarity by structural influences (such as being incorporated in rings, or by other fixed asymmetric centers), the sulfur−sulfur bond is elongated, and the geometry of nearby carbon−carbon bonds is altered. It may be predicted that such "torsional strain" (as defined by Boyd[8]) would have an effect on the chemical reactivity of the disulfide bond and might become the basis for stereoselective chemical modification. (b) In one example cited below, a hydrogen bond was observed between a hydoxyl group and a torsionally strained disulfide in the same ring.

The torsional strain effects that were observed in a pair of steroidal derivatives, **I** and **II**. X-ray crystallographic structure determinations[12] of these compounds [1α,5α-epidithioandrostane-3α,17β -diol ($C_{19}O_2S_2H_{30}$) (**I**), and a similar sterol (**II**) lacking the disulfide], showed several unusual phenomena. Compound **I** had a sulfur−sulfur bridge across the 1,5-positions of the A ring. The nearly (but not quite) *cis*-coplanar disulfide retains a small dihedral angle. The compression of the dihedral angle from its preferred orthogonal configuration results in a lengthening of the sulfur−sulfur bond from its usual value, and nearby carbon−carbon bonds are also affected. Within the crystal lattice of **I** are "two crystallographically independent molecules," with slightly different coordinates.

It is interesting that in both units in the crystal of **I**, the 3α-hydroxyls form **intramolecular hydrogen bonds with the 1,5α-dithio function**. The 17β-hydroxyl groups are involved in **intermolecular hydrogen bonds**. The two crystallographically independent molecules of **I** have what appear to be slightly different dihedral angles of −2.5° and −4.5°. This potential for torsionally strained dithio bridges to form hydrogen bonds may be of general significance in some protein structures.

In the examples of the prion proteins, the sense of helix of the disulfide in typical PPr proteins does not seem to have been spelled out but should be available in the various structural determinations by X-ray crystallography[2] or NMR.[3]

That the disulfide can be a significant participant in stereochemistry changes in PPr is shown in the equilibrium between monomeric and dimeric human PPr. Knaus et al.[2] demonstrated that reconstituted human PPr can be oxidatively refolded correctly into the normal human form, containing presumably the normal helical sense of the disulfide. They found that the recrystallization of their pure monomeric form slowly deposited a dimeric form in which two original PPr molecules form a tight complex. The authors report that the crystal structure of the dimer "requires that a dramatic conformational transition must have occurred: the intrachain disulfide bonds in the monomers must be reduced, the two helices 3 must swing out across the dimer interface to swap and pack against the other half of the dimer, and the two disulfide bridges must re-form between polypeptide chains".

In the case of the pathogenic changes in PPr, the suggestion is that complexe(s) form between PPr, or an incompletely folded precursor, and PPr^{Sc}, or some portion of PPr^{Sc}, in which the sense of helix in the disulfide has been inverted.

In his recent book, Yam[14] summarizes two leading concepts as to how the transformation of native to misfolded forms might occur. In the **template-directed model**, PPr^{C} "can exist in a stable intermediate state PrP* somewhere between its normal and pathogenically folded state. Called PrP*, the intermediate form then interacts with a different form, which Prusiner dubbed Protein X. As a result, PPr* is able to bind with PPr^{Sc}, forming a dimer. PPr* then spontaneously adopts the β-sheet dominated shape of PPr^{Sc}. The two split apart and go on to recruit other PPr^{C} molecules."

"In this template-directed model, the Protein X might well be PPr^{Sc} (or a portion of it), the binding of which with PPr* enforces a reversal of the chirality of the disulfide helix."

The other working hypothesis, as described by Yam is "the **nucleated polymerization model**—basically a chunk of PPr^{C} serves as a seed. Charles Weissman is quoted as

saying 'The seeding hypothesis says that the infectious agent is really an assembly of molecules—simply a crystal. So the idea is that, depending upon the structure of the crystal, the molecules that add to it will adapt to whatever the conformation is.'"

It would seem that either of these two schemes could be adapted to incorporate the idea that the fundamental change is the transformation of one diastereoisomeric form of an incompletely folded PPr into its diastereoisomer, which has a preference for β-folding. The spontaneous crystal seeding phenomenon has long been observed in organic chemistry (called "*impfen*" in early German publications of organic chemistry).

The sequence of intermediate steps that occur during the transformation of PPr to PPr^{Sc} remains unknown despite much investigation. Although this general picture is now widely accepted, some skepticism still exists, and other alternative explanations are sought. The current views have been summarized in papers by Priola et al.[15] and by LeBlanc and Roucou[16] describing the discussions at a major conference held in Breckinridge, CO, in April 2003.

Some experimental attention to the chirality of the disulfide in PPr and PPr^{sc} and also in intermediate forms may be worthwhile.

REFERENCES AND NOTES

(1) The term Transmissible Spongiform Encephalopathies, or TSEs, also known as the prion diseases, includes scrapie in sheep; bovine spongiform encephalopathy (BSE) in cows, often referred to as "mad cow disease"; variant Creutzfeldt−Jacob disease in humans; kuru, in the Gore mountain people of New Guinea; chronic wasting disease in elk, deer, and various other animals; Gerstmann−Sträussler−Scheinker Disease (GSS) disease in humans; fatal familial insomnia (FFI) in humans. These are all diseases of the neural system involving gradual disintegration of the brain, loss of bodily functions, dementia, and ultimate death. They are characterized by long incubation periods between infection and the appearance of obvious symptoms. The infective agents appear to be misfolded forms of naturally occurring proteins. The mechanism(s) of the infective process are unknown and the subject of extensive investigation.

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