on how the glucocorticoid receptor works; in eyedrops in treatment of glaucoma; possibly in the local treatment of skin wounds; and as a potential birth-control method. Baulieu, RU 486's developer, sees three different ways in which the drug could be used as a birth-control method. First is as what Baulieu calls a 'menses inducer': if a woman takes RU 486 in the second half of her cycle, there is an 80% chance that she will begin to bleed. A second approach is to use very small amounts of RU 486 during the second, luteal phase of the cycle. The final and perhaps most promising potential use of RU 486 is as a contraceptive in the conventional sense.

WHO says Roussel has promised to deliver the drug to any WHO member country that requests it for the purpose of further study. The People's Republic of China, a participant in WHO-sponsored trials of RU 486, is the only country besides France to approve the drug for use as an abortifacient. In India, an ICMR-funded study has been conducted at the Central Drug Research Institute in Lucknow on the embryotoxicity of RU 486 in rabbits (see *Current Science*, 1990, **59**, 56).

Joseph Palca says in *Science*: 'However it may ultimately be used, RU 486 has forced participants in the debate over the moral issues of human reproduction to reconsider their points of view. But it seems likely that legal prohibitions will not be able to stop a drug with the promise of RU 486.'

Protein disulphides: More than just bonds

Utpal Tatu

The compactly folded three-dimensional structure of globular proteins is stabilized by a complex interplay of different intramolecular interactions. Unique among these are disulphide bonds, which are the only commonly observed covalent cross-links. A major attribute of disulphide bonds appears to be their ability to impart conformational stability to proteins [1]. At first sight these cross-links would seem to stabilize proteins in a mechanical fashion by imposing physical constraints to chain unfolding. However, it is now widely accepted that disulphide bonds stabilize the conformation of proteins by their effect on the unfolded state and not on the folded state; by decreasing the degrees of freedom available to the unfolded state, disulphides shift the unfolding equilibrium towards the native state of the protein. Thus their contribution is subtle, influencing the entropy of the unfolded chain. X-ray crystallography and NMR studies indicate that in some globular proteins disulphide bridges may allow a large amount of internal motion, without disturbing the correct folded state of the protein. According to Williams [2]: 'Within a framework, disulphide bonds give an elastic quality to the protein, which allows it to deform somewhat with change in condition but ensures that it returns to its original state on removal of the stress. The protein behaves like a small piece of rubber.' Such molecular fluctuations are known to be linked to biological functions in many proteins.

Disulphide bonds are not merely a kind of structural glue but also influence function. In some cases disulphide bonds participate directly in catalytic mechanisms involving redox reactions, e.g. the small disulphide loops in thioredoxin and glutathione reductase located at the active site. In other cases they play an indirect role, e.g. the disulphide bridge located adjacent to the active site of serine proteases is essential for maintaining the proper orientation of catalytically important amino-acid side-chains. A recent finding [3] points to new ways by which nature harnesses disulphide bonds for regulatory purposes. A search of the Protein Identification Resource (PIR) data base for sequences homologous to the active site of thioredoxin (–Trp–Cys–Gly–Pro–Cys–Lys–) identified sequences from the β-subunits of the gonadotropic hormones luteinizing hormone (–His–Cys–Gly–Pro–Cys–Arg–) and follicle-stimulating hormone (–His–Cys–Gly–Lys–Cys–Asp–). Encouraged by the sequence homology and also a close similarity in the hydropathy profiles and predicted secondary structure in this region, the authors [3] examined these hormones for 'thioredoxin-like' functional activity also. Indeed, LH and FSH exhibited thioredoxin-like activity, catalysing disulphide isomerization in other proteins. The authors invoke a role for disulphide isomerization or reshuffling in the mechanism of signal transduction resulting from hormone–receptor interaction. The binding of the hormone to the receptor might then induce disulphide isomerization in the receptor, resulting in structural alteration required for signal transduction.

The understanding of protein folding

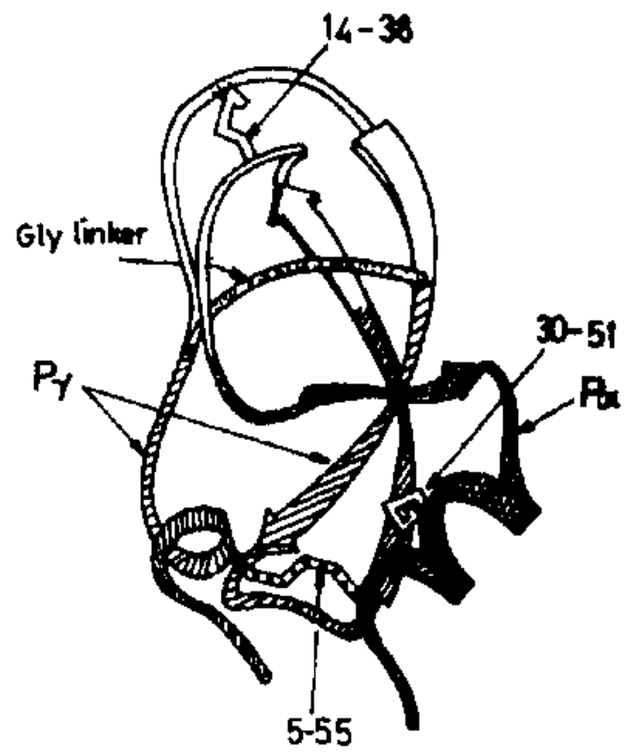

Figure 1. Ribbon diagram of native BPTI (from ref. 6). The hatched, crossed and dotted regions together form the $P_{\alpha\text{-}\gamma}$ subdomain linked by the 5–55 disulphide bond (closed circles). The dotted region indicates the glycine linker which is used to covalently join the N-terminal segment (residues 1–9) to the central β-sheet (residues 20–33). A peptide model lacking the central β-sheet and the glycine linker sequences fails to adopt a 'native-like' conformation.

Figure 2. C^{α}-carbon tracing of chicken skeletal troponin C plotted using co-ordinates available in the Brookhaven Protein Data Bank. The engineered disulphide has been modelled (arrow) using the program MODIP[10]. (Figure prepared by R. Sowdhamini, Molecular Biophysics Unit, Indian Institute of Science, Bangalore.)

mechanisms is a major concern of contemporary protein chemistry. Success has thus far eluded molecular biologists attempting to crack the 'folding code' which relates one-dimensional sequence to three-dimensional structure of proteins. Where do disulphide bonds stand in the hierarchy of interactions determining folding? Creighton and Goldenberg[4] have elegantly described the kinetically most accessible pathway for the folding of reduced bovine pancreatic trypsin inhibitor (BPTI) based on the sequence in which the three native disulphide bonds (30–51, 14–38, 5–55) are formed during the refolding process. An unanswered question in this connection has been whether development of secondary and tertiary structure precede disulphide-bond formation or vice versa. Two recent reports[5,6] suggest that disulphide bonds are important for development of structure in proteins. These authors[5,6] have synthesized disulphide-bonded peptide fragments of BPTI ($P_{\alpha-\beta}$ 30–51 and $P_{\alpha-\gamma}$ 5–55) and shown that these subdomains contain secondary and tertiary structure similar to that found in native BPTI (Figure 1). Interestingly, these peptide fragments do not show any substantial folded structure once the disulphide is reduced. This finding strengthens the view that a strong co-operativity exists between disulphide-bond formation and development of 3D structure in proteins

Introduction of disulphide cross-links into proteins lacking them would appear to be an attractive approach to stabilizing the native structures of enzymes, particularly those with useful commercial applications. It is therefore not surprising that 'protein engineers' have enthusiastically inserted disulphide bonds into several proteins. Most recently, Matthews and others[7] have unequivocally demonstrated that introduction of three disulphide bonds into native T_4 lysozyme (which is otherwise devoid of disulphides) results in an increase in denaturation temperature by 23° C. Further, each of the three disulphides was shown to have an additive stabilization effect on the reversible thermal denaturation. The same group of workers has succeeded in introducing a novel regulatory mechanism to control the catalytic function of T_4 lysozyme[8]. They introduced a redox-dependent on/off switch into T_4 lysozyme by engineering a disulphide bond across its active-site cleft. This disulphide completely abolishes the enzyme activity of T_4 lysozyme by interfering with substrate binding. However, reduction of the disulphide bond results in near-total recovery of enzyme activity. Thus the enzyme can be turned on/off by changing the redox potential of the medium. A dramatic recent example of protein engineering are studies on the mechanism of regulation of muscle contraction[9]. The triggering event in muscle contraction is thought to be Ca^{2+}–induced changes in the conformation of troponin C (TnC), which are then transmitted to other components of thin filaments. Crystal-structure analysis of TnC indicates that Ca^{2+} induces changes in the disposition of two N-terminal helices, C and D, and this relative movement of helices may be important in transmitting signals to the other components of thin filament. This possibility was addressed[9] by asking: Will TnC retain its normal regulatory properties if the movement of its two helices is constrained by introducing a disulphide between them? When a disulphide was introduced between helices C and D (Figure 2) TnC lost its regulatory properties, re-emphasizing the importance of the movement of helices C and D in signal transmission. Engineered disulphides may thus be useful in modulating relative motions of distant segments of polypeptide chains in proteins.

It is clear that disulphide bonds can no longer be considered as mere rigid, stabilizing structures used solely for the purpose of holding segments of proteins together. Undoubtedly, nature has used this simple covalent link to tackle complex issues of structure, function and regulation in biology.

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