CHAPTER 9

IMPLICATIONS OF 3D DOMAIN SWAPPING FOR PROTEIN FOLDING, MISFOLDING AND FUNCTION

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Abstract: Three-dimensional domain swapping is the process by which two identical protein chains exchange a part of their structure to form an intertwined dimer or higher-order oligomer. The phenomenon has been observed in the crystal structures of a range of different proteins. In this chapter we review the experiments that have been performed in order to understand the sequence and structural determinants of domain-swapping and these show how the general principles obtained can be used to engineer proteins to domain swap. We discuss the role of domain swapping in regulating protein function and as one possible mechanism of protein misfolding that can lead to aggregation and disease. We also review a number of interesting pathways of macromolecular assembly involving β-strand insertion or complementation that are related to the domain-swapping phenomenon.

INTRODUCTION

Three-dimensional domain swapping (referred to subsequently as “domain swapping”) is the process by which two identical protein chains exchange a part of their structure to form an intertwined dimer or higher-order oligomer. The phenomenon was first proposed in the 1960s to explain the behaviour of RNase A1 dimer and somewhat later also tryptophan synthetase2 and tryptophanase3 but the first crystal structures of domain-swapped proteins only emerged in the 1980s.4–8 The terminology that is currently used was introduced in 1994 by Eisenberg and colleagues who also put forward a mechanistic framework within which to understand how and why domain swapping occurs.9–11 In this chapter we will:
(1) highlight recently determined structures that suggest roles for domain swapping in regulating protein function, (2) discuss quantitative studies of the energetics and kinetics of the domain-swapping process and (3) review the evidence for domain swapping as a mechanism of protein misfolding leading to aggregation and disease.

**DOMAIN SWAPPING TERMINOLOGY**

The structure of the subunits within the domain-swapped oligomer is identical to that of the monomer with the exception of the region that connects the exchanging domain with the rest of the protein (Figs. 1, 2A). In most cases, this so-called “hinge loop” region folds back on itself to form the monomer and adopts an extended conformation in the domain-swapped dimer. Although the process is known as “domain” swapping, proteins are often found to swap only a single secondary structure element such as a β-strand or α-helix rather than a whole domain of structure. The swapped structure can be located in any part of the polypeptide sequence although it is generally at the N- or C-terminus. In some cases, approximately half of the molecule is swapped and it is therefore difficult to define which half constitutes the swapped domain. The interactions made between the swapped domain and the rest of the protein are the same in the oligomer as in the monomer but they are formed in an inter- rather than an intramolecular fashion. These intermolecular interactions comprise the “primary” interface. Since the subunits are often close to each other in the domain-swapped oligomer, an intermolecular new interface may be created that is not present in the monomeric form and this is known as the “secondary” interface. Domain swapping can potentially occur in a reciprocal manner to form a dimer, in a cyclical manner to form a trimer, tetramer etc., or in an open-ended manner to form an oligomer leaving uncomplemented ends available to assemble further. In order to be classed as a domain-swapping protein, both monomer and domain-swapped forms need to have been observed. In some cases, however, there is a structure of the domain-swapped form of a protein but no structure of the closed monomer and the protein is therefore considered to be a ‘candidate’ for domain swapping. In other cases, the protein has a homolog that is a closed monomer; these oligomers are classed as “quasi domain swapped”.

**DOMAIN-SWAPPED STRUCTURES AND REGULATION OF PROTEIN FUNCTION**

Many of the proteins and protein domains that are commonly used as model systems for studying protein folding and molecular recognition have been crystallized as domain-swapped forms in addition to the monomeric forms, most notably SH2 and SH3 domains, staphylococcal nuclease, chymotrypsin inhibitor 2 (all domain-swapped dimers) and barnase (a domain-swapped trimer). These examples suggest that, although domain swapping is relatively rare (there are less than 60 structures of domain-swapped proteins to date), many proteins have regions with features suggesting they could act as hinge loops and it may therefore be possible to induce many proteins to domain swap simply by making a few amino acid substitutions. The potential ease with which a domain-swapped species may become stabilized suggests that domain swapping could be a mechanism for the evolution of larger, complex folds from smaller, simpler ones via a domain-swapped intermediate followed by a gene duplication or fusion process. There is a subset of proteins for which evidence suggests