REVIEW

3D domain swapping: As domains continue to swap

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Abstract

Three-dimensional (3D) domain swapping creates a bond between two or more protein molecules as they exchange their identical domains. Since the term '3D domain swapping' was first used to describe the dimeric structure of diphtheria toxin, the database of domain-swapped proteins has greatly expanded. Analyses of the now about 40 structurally characterized cases of domain-swapped proteins reveal that most swapped domains are at either the N or C terminus and that the swapped domains are diverse in their primary and secondary structures. In addition to tabulating domain-swapped proteins, we describe in detail several examples of 3D domain swapping which show the swapping of more than one domain in a protein, the structural evidence for 3D domain swapping in amyloid proteins, and the flexibility of hinge loops. We also discuss the physiological relevance of 3D domain swapping and a possible mechanism for 3D domain swapping. The present state of knowledge leads us to suggest that 3D domain swapping can occur under appropriate conditions in any protein with an unconstrained terminus. As domains continue to swap, this review attempts not only a summary of the known domain-swapped proteins, but also a framework for understanding future findings of 3D domain swapping.

Keywords: Functional unit; protein oligomerization; RNase A; IX/X-binding protein; glyoxalase I; T7 gene 4 ring helicase; human prion; human cystatin

Protein oligomers have evolved because of their advantages over their monomers. These advantages include the possibility of allosteric control, higher local concentration of active sites, larger binding surfaces, new active sites at subunit interfaces, and economic ways to produce large protein interaction networks and molecular machines. However, the mechanisms for the evolution of oligomeric interfaces and for the assembly of oligomers during protein synthesis or refolding remain unclear. Different mechanisms have been proposed for the evolution of protein oligomers, among which is three-dimensional (3D) domain swapping (Bennett et al. 1995; Heringa and Taylor 1997). 3D domain swapping holds additional interest because it can also serve as a mechanism for reversible oligomerization, and conceivably for pathological oligomerization, as in amyloids.

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Abbreviations: BS-RNase, bovine seminal ribonuclease; DT, diphtheria toxin; FU, functional unit; Glx I, glyoxalase I; hRNase, human pancreatic ribonuclease; Hsp, heat shock protein; IL, interleukin; IX/X-bp, blood coagulant factors IX/X-binding protein; MBP, mannose binding protein; p13suc1, suppressor of cyclin-dependent kinase 1; PEG, polyethylene glycol; RNase A, bovine pancreatic ribonuclease.

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Historic background of 3D domain swapping

3D domain swapping is a mechanism for two or more protein molecules to form a dimer or higher oligomer by exchanging an identical structural element ("domain"). If both the monomer and the dimer of a molecule exist in stable forms, in which the dimer adopts a domain-swapped conformation and the monomer adopts a closed conformation, then this protein is considered to be a bona fide example of 3D domain swapping (Fig. 1). Some proteins form intertwined, apparently domain-swapped oligomers without a known closed monomer. If these proteins have homologs

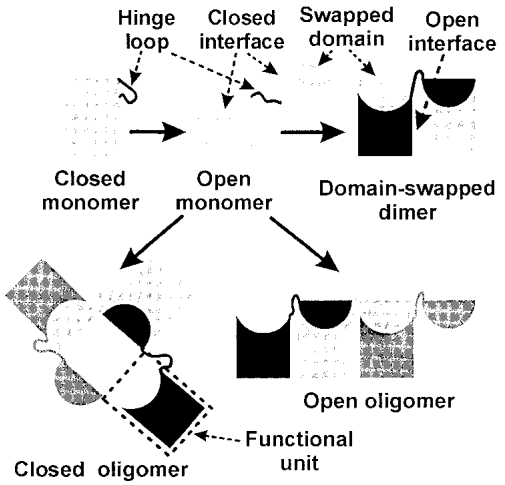

Fig. 1. Schematic diagram illustrating terms related to 3D domain swapping. The swapped domain in an oligomer is a globular domain or a structural element of one subunit that extends into another subunit and interacts with the main domain of this subunit. This interaction is essentially identical to that of the same domain in the monomer. The hinge loop is a segment of polypeptide chain that links the swapped domain and the main domain. This loop adopts different conformations in the monomer and the domain-swapped oligomer. The closed interface is the interface between the swapped domain and the main domain that exists in both the monomer and the domain-swapped oligomer. The open interface exists only in the domain-swapped oligomer, but not in the monomer. The functional unit is shown in the dashed box. Its swapped domain and main domain are from different polypeptide chains. Under certain circumstances, a conformational change in the hinge loop converts a closed monomer to an open monomer with its closed interface exposed to the solvent. Two or more such open monomers form a domain-swapped dimer or oligomer. Domain-swapped oligomers are divided into two types: open oligomers and closed oligomers. The open oligomer is linear and has one closed interface exposed to solvent, whereas the closed oligomer is cyclic and does not expose a closed interface.

known to be closed monomers, these oligomers are considered to be 'quasidomain swapped.' If a protein forms an oligomer by exchanging domains, but there is no monomeric form or homolog for the protein, this protein is considered a candidate for 3D domain swapping (Schlunegger et al. 1997).

The term '3D domain swapping' was first used to describe the structure of a diphtheria toxin dimer (Bennett et al. 1994a). However, the concept of 3D domain swapping can be traced back 40 years. Bovine pancreatic ribonuclease (RNase A) forms dimers during lyophilization in acetic acid. Based on elegant chemical modification experiments, Crestfield et al. (1962) proposed that the dimer forms by exchanging the N-terminal fragments. This mechanism is essentially identical to what is now called 3D domain swapping. Similar ideas emerged from other work (Jackson and Yanofsky 1969; London et al. 1974; Miles 1991). Later, several domain-swapped structures were reported before the concept of 3D domain swapping was generalized. The first structural evidence of 3D domain swapping was shown in the cro repressor from bacteriophage λ, which forms a dimer by swapping its C-terminal strands (Anderson et al. 1981). The structure of the monomeric cro with lengthened hinge loop was reported in 1996 to show cro as an example of 3D domain swapping (Albright et al. 1996). Other possibly domain-swapped structures reported before the term 3D domain swapping include chicken citrate synthase (Remington et al. 1982), beef liver catalase (Fita and Rossmann 1985), βB2-crystallin (Bax et al. 1990), Rec A from *E. coli.* (Story et al. 1992), human CksHs2 (Parge et al. 1993), recombinant human interleukin-5 (IL-5, Milburn et al. 1993), and bovine seminal ribonuclease (BS-RNase, Mazzarella et al. 1993). The structure of γB-crystallin, a homolog of βB2-crystallin, was determined as a monomer in 1981 (Blundell et al. 1981). Thus, βB2-crystallin represents the first structural evidence of quasidomain swapping. Biochemical data showed that the monomeric form of BS-RNase exists (Piccoli et al. 1992), but the structure of the monomer has not been reported. The structure of CksHs1, a homolog of CksHs2, was determined as a monomer in 1995 (Arvai et al. 1995), and the structure of the GM-CSF monomer, a homolog of IL-5, was reported in 1991 (Diederichs et al. 1991). No monomer or monomeric homolog has been reported for chicken citrate synthase, beef liver catalase, or Rec A from *E. coli*. Diphtheria toxin offered the first structural evidence for bona fide 3D domain swapping, the structures of whose monomer and domain-swapped dimer were both reported in 1994 (Bennett et al. 1994a; Bennett and Eisenberg 1994b). These structures also led to the proposal that 3D domain swapping could be a general mechanism for switching between two protein conformers (Bennett et al. 1994c, 1995).

The definition of several terms related to 3D domain swapping and the possible mechanism for domain swapping can be found in previous reviews (Bennett et al. 1995; Schlunegger et al. 1997). The possible role of 3D domain swapping in the evolution of protein oligomers has been discussed in other reviews (Bennett et al. 1995; Heringa and Taylor 1997; Schlunegger et al. 1997). In the past few years, the number of structures of domain-swapped proteins has vastly increased. This increase elevates our understanding of 3D domain swapping to a higher level, and forms the foundation of this review.

Definition of functional unit

The definitions of several terms related to 3D domain swapping are summarized in Figure 1. Here we introduce a new term: functional unit (FU).

The FU of a 3D domain-swapped oligomer consists of the portions of two bonded polypeptide chains which form the swapped domain and its associated main domain. It is similar to a closed monomer, except that a closed monomer

consists of one polypeptide chain, whereas an FU is composed of two polypeptide chains (Fig. 1).

Advances in 3D domain swapping

Swapping at the N or C terminus

To date, about 40 domain-swapped proteins have known structures. One common feature of these domain-swapped proteins is that all the swapped domains (except in one protein) are from either the N terminus or the C terminus. In several cases, half of the molecule is swapped, such as in βB2-crystallin (Bax et al. 1990), calbindin D_{9k} (Hakansson et al. 2001), and cyanovirin-N (Yang et al. 1999). In these cases, the proteins are composed of two homologous domains, one of which is swapped. Consequently, it is difficult to define which domain is swapped.

Swapping of more than one domain in a protein

One advance in understanding 3D domain swapping is that more than one domain in a protein can swap. In previous examples of 3D domain swapping, each protein was found with only one domain swapped. It was recently shown that both the N- and C-termini swap in RNase A dimers. RNase A forms a dimer during lyophilization in acetic acid (Crestfield et al. 1962). Further studies showed that there are two types of dimers of RNase A, formed with one dimer more abundant than the other dimer (Libonati et al. 1996; Gotte et al. 1999). Structures of both dimers (Fig. 2) reveal that the N-terminal helix is swapped in the less abundant dimer (the N-terminal swapped dimer, previously named the minor dimer) (Liu et al. 1998), whereas the C-terminal strand is swapped in the other dimer (the C-terminal swapped dimer, previously named the major dimer) (Liu et al. 2001). RNase A also forms trimers (Gotte et al. 1999). Based on the structures of the N- and C-terminal swapped dimers, a trimeric model with both types of swapping (Fig. 2) was proposed (Liu et al. 2001). Further biochemical studies support this model and indicate that the model belongs to the more abundant trimer (linear N- and C-terminal swapped trimer, previously named the major trimer). Thus, structural studies of 3D domain swapping in RNase A show that a protein can have domains swapped at both the N- and C-termini, and that these two types of swapping can occur simultaneously in one oligomer. In the less abundant trimer, only the C-terminal strand is swapped, and the structure is cyclic (cyclic C-terminal swapped trimer, previously named the minor trimer, Fig. 2) (Liu et al. 2002).

RNase A also forms tetramers (Gotte et al. 1999). Based on the structures of the dimers and trimers, models are proposed for the tetramers of RNase A (Fig. 3). Two of these models are linear, with two types of swapping occurring in one molecule (Fig. 3A,B). One model is a combination of cyclic and linear oligomerization, also with both types of swapping occurring in one molecule (Fig. 3C). The last model is a cyclic tetramer, with the swapping only of the C-terminal strand (Fig. 3D).

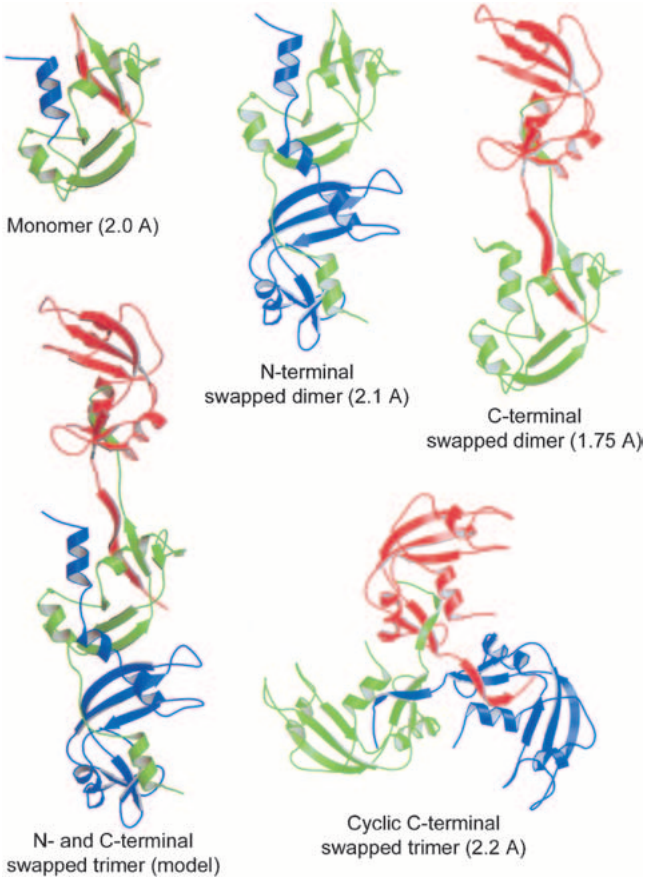

Fig. 2. Ribbon diagrams of the structures of the RNase A monomer (2.0 Å, Wlodawer et al. 1982), the N-terminal swapped dimer (2.1 Å, Liu et al. 1998), the C-terminal swapped dimer (1.75 Å, Liu et al. 2001), the N- and C-terminal swapped trimer model (Liu et al. 2001), and the cyclic C-terminal swapped trimer (2.2 Å, Liu et al. 2002). The N- and C- termini are labeled. The N-terminal helix and the C-terminal strand that are swapped in the oligomers are colored blue and red, respectively, in the monomer. The N-terminal swapped dimer swaps its N-terminal helix, whereas the C-terminal swapped dimer swaps its C-terminal strand. Both types of swapping take place in the N- and C-terminal swapped trimer model: The green subunit swaps its C-terminal strand with the red subunit, and swaps its N-terminal helix with the blue subunit. The cyclic C-terminal swapped trimer is 3D domain-swapped at its three C-terminal strands. The three subunits of the molecule are related by a three-fold axis, giving the molecule the shape of a propeller. The figure was created using Raster 3D (Merritt and Bacon 1997).

Thus, RNase A oligomers display two basic types of 3D domain swapping, and show that with two types of swapping, a protein can form a variety of oligomers.

Diversity of the swapped domains

The swapped domains have diverse sizes and sequences. A swapped "domain" can be one structural element made of several residues. It can also be an entire tertiary domain consisting of hundreds of residues (Tables 1–3). Sequence

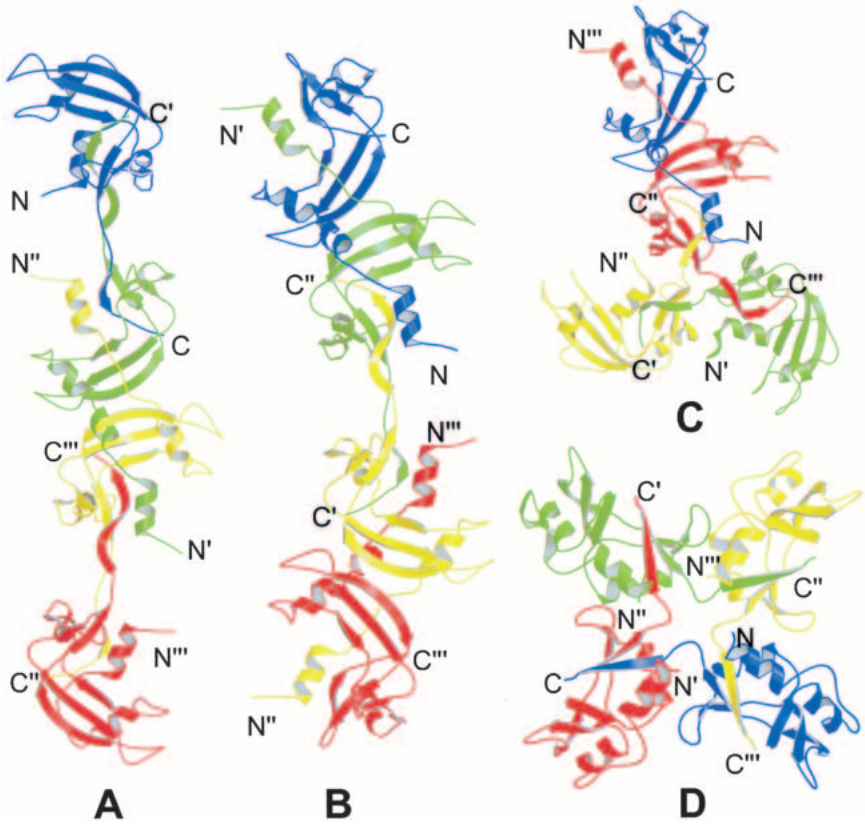

Fig. 3. Ribbon representations of hypothetical models of RNase A tetramers. RNase A is known to form tetramers, but their structures are unknown. These models are based on the structures of the RNase A minor dimer, major dimer, and minor trimer. (*A*) A tetramer model with two C-terminal strands swapped and one N-terminal helix swapped. (*B*) A tetramer model with one C-terminal strand swapped and two N-terminal helices swapped. (*C*) A tetramer model with a combination of cyclic and linear oligomerization. In this model, three subunits form a cyclic trimer by swapping the C-terminal strand. One of these three subunits swaps its N-terminal helix with the fourth subunit. This mode of domain swapping can lead to branching chains. (*D*) A cyclic tetramer model with the swapping of four C-terminal strands. The figure was created with Raster3D (Merritt and Bacon 1997).

comparison shows the lack of sequence similarity among these domains. No specific sequence motif can be found among these domains. Therefore, based on its sequence, a protein cannot be predicted to be domain-swapped or not. The diverse size and sequence of the swapped domains indicates that the closed interfaces of these domain-swapped proteins are different from each other. In addition, various types of interactions are formed at different closed interfaces, including hydrophobic interactions, hydrogen-bonding, electrostatic interactions, and even disulfide bridge interactions (Diederichs et al. 1991; Milburn et al. 1993; Knaus et al. 2001). The interactions at the closed interface contribute to the energy required for the disruption of the closed interface during domain swapping. This energy is called the activation energy for 3D domain swapping (Bennett et al. 1995). Therefore, the diverse sizes and sequences of the swapped domains suggest that the activation energy for 3D domain swapping varies among domain-swapped proteins.

The swapped domains also have diverse secondary structures. Among the domain-swapped proteins with reported structures, a swapped domain can be one α-helix (BS-RNase, RNase A N-terminal swapped dimer, staphylococcal nuclease dimer, Spo0A, etc.), one β-strand (CksHs2 dimer, cro dimer, RNase A C-terminal swapped dimer, BTB domain of PLZF, etc.), several α-helices (calbindin D_{9k}, barnase, phosphoenolpyruvate mutase, human IL-10, etc.), several β-strands (β-B2 crystallin, diphtheria toxin dimer, SH3 domain of Eps8, N-terminal domain of CD2, etc.), or a mixture of α-helix and β-strand (T7 gp 4 ring helicase, human cystatin C, human IL-5, T4 endonuclease VII, etc.). This diversity shows that 3D domain swapping does not require or prefer certain types of secondary structure.

In summary, the diversity of the swapped domains indicates that 3D domain swapping does not depend on the protein sequence or secondary structure.

Flexibility and diversity of the hinge loops

According to the definition in Figure 1, a hinge loop has the intrinsic flexibility to adopt different conformations in

Table 1. *Examples of bona fide 3D domain-swapped proteins*[a]

Protein (Reference)	PDB code	Number of residues per subunit	Number of residues in swapped domain	Residues in hinge loop	Function	Structure of swapped domain
Barnase monomer (Buckle and Fersht, 1994)	1BRN	110			Ribonuclease	
Barnase trimer (Zegers et al. 1999)	1YVS	110	36	37–41	Ribonuclease	N-terminal helices
Calbindin D9k wild-type monomer (Svensson et al. 1992)	4ICB	76			Transcellular calcium transport and magnesium uptake in the intestine	
Calbindin D9k mutant dimer (Hakansson et al. 2001)	1HT9	76	27	38–47	Unknown	C-terminal helices
Soluble domain CD2 monomer (Jones et al. 1992)[b]	1HNG	177			Binding domain of lymphocyte adhesion protein	
N-terminal domain of CD2 dimer (Murray et al. 1995)[b]	1CDC	99	43	44–50	Unknown	N-terminal β-strands
Insertion mutant cro monomer (Albright et al. 1996)	1ORC	71		55–56d	Unknown	
Wild-type cro dimer (Anderson et al. 1981)[b]	1CRO	66	11	55	DNA repressor	C-terminal β-strand
Cyanovirin-N monomer (Bewley et al. 1998)	2EZM	101			HIV inactivation	
Cyanovirin-N dimer (Yang et al. 1999)	3EZM	101	48	50–53	Unknown	N- or C-terminal globular domain
DT monomer (Bennett and Eisenberg 1994b)[b]	1MDT	535			ADP-ribosylating toxin	
DT dimer (Bennett et al. 1994a)[b]	1DDT	535	148	379–387	Speculative receptor binding	C-terminal globular domain
Human prion monomer (Zahn et al. 2000)	1QLX	108			Unknown	
Human prion dimer (Knaus et al. 2001)	1I4M	108	28	188–198	Unknown	C-terminal helices
Protein L B1 domain monomer (O'Neill et al. 2001)	1HZ5	62			Binding of Ig G	
Protein L B1 domain dimer (Kuhlman et al. 2001)	1JML	61	7	52–55	Unknown	C-terminal β-strand
RNase A monomer (Wlodawer et al. 1982)	5RSA	124			Ribonuclease	
RNase A N-terminal swapped dimer (Liu et al. 1998)	1A2W	124	14	15–22	Ribonuclease	N-terminal α-helix
RNase A C-terminal swapped dimer (Liu et al. 2001)	1F0V	124	9	112–115	Ribonuclease	C-terminal β-strand
RNase A cyclic C-terminal swapped trimer (Liu et al. 2002)	1JS0	124	9	112–115	Ribonuclease	C-terminal β-strand
BS-RNase dimer (Mazzarella et al. 1993)[b]	1BSR	124	14	15–22	Ribonuclease	N-terminal α-helix
Single-chain Fv monomer of antibody NC10 (Malby et al. 1998)	1NMC	246		113–127	Antigen binding	
Diabody (Perisic et al. 1994)	1LMK	243	122 or 116	123–127	Antigen binding	N- or C-terminal globular domain
Triabody (Pei et al. 1997)	1NQB	236	120 or 116	0	Antigen binding	N- or C-terminal globular domain
Phosphorylated N-Spo0A (Lewis et al. 1999)	1QMP	129			Response regulator related to sporulation	
N-domain of Spo0A dimer (Lewis et al. 2000)	1DZ3	129	21	103–109	Response regulator related to sporulation	C-terminal α-helix
Wild-type staphylococcal nuclease monomer (Loll and Lattman 1989)[b]	1SNC	149		112–120	Nuclease	
Deletion mutant of staphylococcal nuclease dimer (Green et al. 1995)	1SND	143	29	112–114	Unknown	C-terminal α-helix
suc1 monomer (Endicott et al. 1995)	1SCE	113			Cell cyle regulation	
suc1 dimer (Khazanovich et al. 1996)[b]	1PUC	113	22	85–91	Cell cycle regulation	C-terminal β-stand
TrkA-d5 and NGF complex (Wiesmann et al. 1999)	1WWW	120			Binding of nerve growth factor	
TrkA-d4 dimer	1WWA	109	13	297–299	Unknown	N-terminal β-strand
TrkC-d5 dimer	1WWB	103	14	299–301		
TrkC-d5 dimer (Ultsch et al. 1999)	1WWC	118	14	317–319		

[a] The oligomers of these proteins are domain-swapped and their monomers adopt a closed conformation.
[b] These entries are taken from the previous review (Schlunegger et al. 1997).

Table 2. *Examples of proteins that exhibit quasi-domain swapping*[a]

Protein (Reference)	PDB code	Number of residues per subunit	Number of residues in swapped domain	Residues in hinge loop	Function	Structure of swapped domain
CksHs1 monomer (Arvai et al. 1995)[b]	1DKS	79			Cell cycle regulation	
CksHs2 dimer (Parge et al. 1993)[b]	1CKS	79	14	60–65	Cell cycle regulation	C-terminal β-strand
γ-crystallin II monomer (Blundell et al. 1981)	4GCR	174			Eye lens protein	
βB2-crystallin dimer (Bax et al. 1990)	1BLB	204	97	79–87	Eye lens protein	N- or C-terminal globular domain
Chicken cystatin monomer (Bode et al. 1988)	1CEW	108			Cysteine protease inhibitor	
Human cystatin C dimer (Janowski et al. 2001)	1G96	120	54	55–59	Unknown	N-terminal helix and strand
E. coli glyoxalase I dimer (He et al. 2000)	1FA5	135			Interconversion of glutathione thiohemiacetal of methylglyoxal and S-D-lactoyl-glutathione	
Human glyoxalase I dimer (Cameron et al. 1997)	1BH5	183	19	20–32	Interconversion of glutathione thiohemiacetal of methylglyoxal and S-D-lactoyl-glutathione	N-terminal α-helix
GM-CSF monomer (Diederichs et al. 1991)[b]	1GMF	127		87–99	Granulocyte macrophage growth factor	
IL-5 dimer (Milburn et al. 1993)[b]	1HUL	113	26	82–89	B and T cell growth factor	C-terminal strand and helix
IFN-β monomer (Senda et al. 1992)[b]	1RM1	160		97–114	Fibroblast interferon	
IL-10 dimer (Zdanov et al. 1995)[b]	1ILK	160	46	108–118	Cytokine inhibitory synthesis factor	C-terminal helices
Mannose binding protein (Weis et al. 1991)	1MSB	115			Mannose binding	
IX/X-binding protein (Mizuno et al. 1997)	1IXX	Heterodimer 129 and 123	17	Loop1: 72–75 Loop2: 93–98	Anticoagulation	Middle loop
Major urinary protein monomer (Bocskei et al. 1992)[b]	1MUP	166		126–130	Rodent pheromone transporter	
Odorant binding protein dimer (Tegoni et al. 1996)[b]	1OBP	159	35	121–124	Odorant binding and transport	C-terminal stand and helix
Human pancreatic ribonuclease chimera (Canals et al. 2001)	1H8X	128	15	16–23	RNA digestion (homolog of RNase A and BS-RNase)	N-terminal α-helix
Grb2 adaptor (SH2 + SH3) (Maignan et al. 1995)	1GR1	217			Signal transduction	
Grb2-SH2 domain dimer (Schiering et al. 2000)	1FYR	93	27	121–123	Binding phosphorylated peptide	C-terminal α-helix
Fyn-SH3 monomer (Musacchio et al. 1994)[b]	1FYN	62		112–118	Signal transduction	
SH3 domain of Eps8 (Kishan et al. 1997)	1AOJ	65	26	34–39	Recognition of proline-rich sequences	C-terminal β-strands
SigE dimer (Luo et al. 2001)	1K3S	113			Molecular chaperone in type III secretion system	
CesT dimer (Luo et al. 2001)	1K3E	156	32	33–36	Molecular chaperone in type III secretion system	N-terminal α-helix and β-strand
Monomer of two repeats of α-spectrin (Grum et al. 1999)	1CUN	211			Cytoskeletal protein	
Dimer of one repeat of α-spectrin (Yan et al. 1993)[b]	2SPC	107	32	72–75	Cytoskeletal protein	C-terminal α-helix

[a] These proteins show oligomeric structures with 3D domain swapping, and their homologs adopt the closed monomeric conformation.
[b] These entries are taken from the previous review (Schlunegger et al. 1997).

Table 3. *Candidates for 3D domain swapping*[a]

Protein (Reference)	PDB code	Number of residues per subunit	Number of residues in swapped domain	Residues in hinge loop	Function	Structure of swapped domain
Bleomycin resistance protein dimer (Dumas et al. 1994)	1BYL	122	8	9–11 (?)	Bleomycin resistance	N-terminal β-strand
BTB domain of PLZF (Ahmad et al. 1998)	1BUO	120	12	13–23 (?)	Evolutionarily conserved protein-protein interaction motif	N-terminal β-strand
Cab-type β class carbonic anhydrase (Strop et al. 2001)	1G5C	170	12	13–23 (?)	Catalyzing reversible hydration of CO_2	N-terminal α-helix
Catalase dimer (beef liver) (Fita and Rossmann 1985)	7CAT	500	65	66–70 (?)	H_2O_2 hydrolysis	N-terminal helices
Citrate synthase dimer from chicken heart (Remington et al. 1982)	1CTS	433	10	417–423 (?)	Citrate synthesis	C-terminal helix
Designed coiled coil dimer (Ogihara et al. 2001)	1G6U	48	14	33–34 (?)	Unknown	C-terminal α-helix
dUTPase trimer (Larsson et al. 1996)	1DUD	136	10	125–126 (?)	dUTP hydrolysis	C-terminal strand
Heat shock protein 33 dimer (Vijayalakshmi et al. 2001)	1HW7	255	75	178–184 (?)	Molecular chaperone	C-terminal helices and strands
Phosphoenolpyruvate mutase dimer (Huang et al. 1999)	1PGM	295	35	240–260 (?)	Converting phosphoenolpyruvate to phosphonopyruvate	C-terminal helices
T4 endonuclease VII dimer (Raaijmakers et al. 1999)	1EN7	157	60	63–72 (?)	Mismatch repair, Resolving branchpoint before phage package	N-terminal helix and strands
T7 gene 4-ring helicase hexamer fragment (Singleton et al. 2000)	1E0J	326	40	283–305 (?)	Separation of nucleic acid duplexes into strands	N-terminal α-helix and β-strand
T7 gene 4-ring helicase fragment (Sawaya et al. 1999)	1CR0	296	12	283–305 (?)	Unknown	N-terminal α-helix
RecA hexamer from *E. coli* (Story et al. 1992)	2REB	352	26	27–39 (?)	DNA recombination and repair	N-terminal α-helix
Simian virus 40 oligomer (Stehle et al. 1996)	1SVA	361	61	296–300 (?)	Virus coat protein	C-terminal helix and strands

[a] These proteins show oligomeric structures with exchanging domains, but have not been shown to have monomers or monomeric homologs with a similar structure. Therefore, the residues forming hinge loops in these proteins are speculative, and are indicated here with a question mark.

the monomer and in the domain-swapped oligomer. Several recent structural studies of 3D domain swapping further support the flexibility of the hinge loops. The flexibility is evident in RNase A, BS-RNase, and human pancreatic ribonuclease (hRNase) chimera. RNase A and BS-RNase show 80% sequence identity, and BS-RNase and hRNase chimera share the common hinge loop. All three of these proteins swap the N-terminal helix; however, the relative orientations of the subunits in their dimers are different, resulting in different conformations for the three hinge loops (Mazzarella et al. 1993; Liu et al. 1998; Canals et al. 2001). Flexibility is also displayed in the C-terminal hinge loop of RNase A: the C-terminal strand of RNase A is swapped in both the C-terminal swapped dimer and the cyclic C-terminal swapped trimer of RNase A; however, the subunits are related by a two-fold axis in the C-terminal swapped dimer and by a three-fold axis in the cyclic C-terminal swapped trimer (Fig. 2). Therefore, the same hinge loop adopts different conformations in the monomer, the C-terminal swapped dimer, and the cyclic C-terminal swapped trimer of RNase A, showing the great flexibility of this hinge loop.

Hinge loops display a variety of secondary structures in domain-swapped proteins. Some hinge loops are coils, some form β-strands, and others form α-helices. In the RNase A N-terminal swapped dimer, one hinge loop forms a coil, and the other forms a helix (Liu et al. 1998). A common feature is that when the hinge loop forms a β-strand or an α-helix, the oligomeric form is favored over the monomer. These proteins usually exist as dimers in vivo or have dimeric forms more stable than the monomeric forms. Other cases of domain-swapped oligomers that are more stable than their monomers are those for which the hinge loop is not long enough for the swapped domain to fold back to the same peptide chain (Bennett et al. 1995).

New examples of 3D domain swapping in proteins

To date, about 40 domain-swapped proteins have been reported. These proteins are involved in different biological

functions. The reported domain-swapped proteins are listed in Tables 1–3. Here, we discuss six domain-swapped proteins presenting aspects of 3D domain swapping that were not known at the time of previous reviews.

IX/X-binding protein

As mentioned above, most swapped domains are at either the N or C terminus. The only exception found to date is blood coagulant factors IX/X-binding protein (IX/X-bp, Mizuno et al. 1997). IX/X-bp is an anticoagulant isolated from the venom of the habu snake. It consists of two homologous subunits linked by an intermolecular disulfide bond. The two subunits form a heterodimer by exchanging a loop in the central part of the molecules (Fig. 4). Structural comparison of the two subunits with mannose binding protein (MBP) shows that they adopt the same fold, except that the exchanged loop in the IX/X-bp folds back to the same polypeptide chain in MBP. Thus, IX/X-bp is quasidomain-swapped. IX/X-bp is the only known example of 3D domain swapping taking place in the middle of the molecule, in contrast to other domain-swapped proteins, in which domain swapping takes place at either the N or C terminus.

Fig. 4. Ribbon diagram of the structure of blood coagulant factor IX/X-bp (Mizuno et al. 1997). IX/X-bp is a heterodimer, with subunit A in red and subunit B in green. The N- and C-termini are indicated. The middle loops of the two subunits are swapped. IX/X-bp is the first example of 3D domain swapping of a heterodimer and with the central part of the molecule swapped. The figure was created with Raster3D (Merritt and Bacon 1997).

Because domain swapping takes place in the middle of IX/X-bp, there are two hinge loops in each subunit. In addition, IX/X-bp is the only known example of a domain-swapped heterodimer; all other domain-swapped proteins are homooligomers.

RNase A linear N- and C-terminal swapped trimer and cyclic C-terminal swapped trimer

The structures of the RNase A trimers support the earlier proposal that a protein can form a linear or cyclic oligomer by 3D domain swapping. RNase A forms two types of trimers during lyophilization in acetic acid (Gotte et al. 1999). One trimer is slightly more abundant than the other trimer. A linear model with the swapping of both the N- and C-termini was proposed for the more abundant trimer (Fig. 2; Liu et al. 2001). Further biochemical studies support this model (Liu et al. 2002). With both types of swapping taking place, the linear model does not have an exposed closed interface, and therefore has no dangling domains. This model is thermodynamically more favorable than the previous linear model (Fig. 1; Bennett et al. 1995), which had an exposed closed interface with one type of swap. In addition, the crystal structure of the RNase A N-terminal swapped trimer shows that it is a cyclic molecule, with swapping of the C-terminal strands (Fig. 2; Liu et al. 2002). Thus, RNase A represents the first protein found to form both linear and cyclic domain-swapped oligomers.

The two different modes of domain swapping in the RNase A linear N- and C-terminal swapped trimer result in two hinge loops in the molecule. However, this is different from the dimer of IX/X-bp, which also has two hinge loops. In the RNase A major trimer, there is a swapped domain at both termini, whereas in IX/X-bp, there is only one domain swapped in the middle of the molecule.

Glyoxalase I

Glyoxalase I (Glx I) catalyzes the interconversion of the glutathione thiohemiacetal of methylglyoxal and S-D-lactoyl-glutathione. Human Glx I is a dimer with 183 amino acid residues per monomer. Each monomer is composed of an N-terminal helix and two homologous domains. The crystal structure of this dimer is 3D domain-swapped at the N-terminal helix (Fig. 4; Cameron et al. 1997). Sequence comparison with *E. coli* Glx I shows that this N-terminal helix does not exist in *E. coli* Glx I. The function of this N-terminal helix may be to stabilize the human Glx I dimer, although it is not required for dimerization of Glx I (He et al. 2000). The active sites exist at the dimer interface, and are therefore composite. According to Cameron et al. (1997), the dimer also swaps its C-terminal globular domain, in addition to swapping the N-terminal helix. The swapping of the C-terminal domain is ambiguous, however, because there are two ways to define the monomer. As shown in Figure 5, in one way, the monomer can be divided

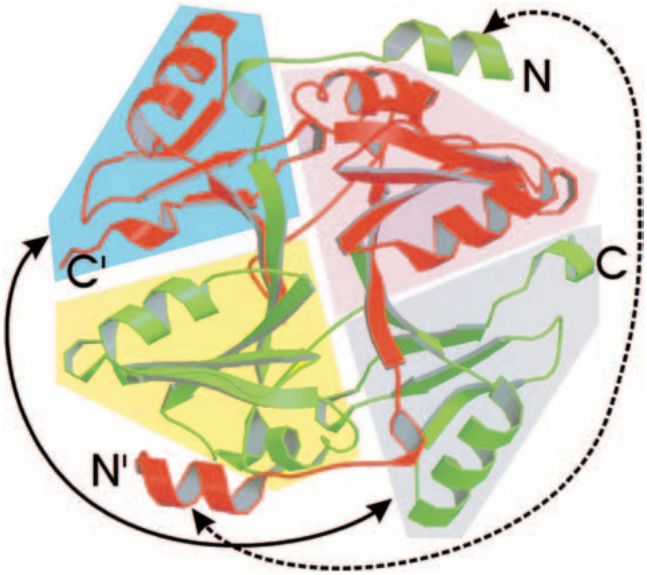

Fig. 5. Ribbon diagram of the structure of human glyoxalase I (Cameron et al. 1997). Glyoxalase I is a homodimer, shown with one subunit colored red and one green. The N-and C-termini are labeled. The N-terminal helix is swapped (shown by the arrowheads on the dashed line). There are two ways to define the monomer. In one way, the monomer is composed of the domains in the pink- and blue-shaded areas. These two globular domains of the monomer are from the same polypeptide chain (red or green), and therefore, the C-terminal domain is not swapped. In the second way, the monomer is composed of the domains in the blue- and gray-shaded areas. To obtain such a monomer, the domain in the gray-shaded area must be replaced by the domain in the blue-shaded area in the crystal structure, as shown by the arrowheads on the solid line. Then the C-terminal domain is considered to be swapped, and therefore there are two swapped domains in this case. The figure was created with Raster3D (Merritt and Bacon 1997).

into two domains in the pink- and blue-shaded areas, as seen in the crystal structure. These two domains are from the same polypeptide chain, and therefore, the C-terminal domain is not swapped in the crystal structure. In such a monomer, the two domains have extensive interactions and would be stable in solution. But in this conformation, the active site is incomplete and the monomer would be inactive. The second way to define a monomer is to divide the monomer into two domains in the pink- and gray-shaded areas (Fig. 5), as proposed by Cameron et al. (1997). According to this definition, one has to move the C-terminal domain in the blue-shaded area in the crystal structure to replace the same domain in the gray-shaded area from the other subunit to obtain a monomer (shown by the arrowheads connected by a solid line in Fig. 5). In such a case, the C-terminal domain is swapped and the active site is complete, but the interactions between the two domains are limited, which may result in an unstable monomer. Biochemical data show an equilibrium of monomer and dimer in Glx I from *Pseudomonas putida* (55% sequence identity to human Glx I), and both the monomer and the dimer are active (Saint-Jean et al. 1998). This indicates that the active site in the monomer of *P. putida* Glx I should be complete, as in the second definition of monomer mentioned above. Based on these biochemical data, we regard human Glx I as a protein with two domains swapped, as proposed by Cameron et al. (1997). The crystal structure of the monomer will give a definitive answer to this question.

T7 gene 4 ring helicase

T7 gene 4 ring helicase (T7 helicase) is a 5′-3′ helicase from bacteriophage T7 whose crystal structure displays a screw-axis form of open oligomeric 3D domain swapping. It contains 566 amino acid residues, and comprises separate helicase and primase domains (Bird et al. 1997; Frick et al. 1998). The active form of T7 helicase is a hexamer. The helicase loses the ability to form a hexamer, and thus loses its activity, after the truncation of its N-terminal residues 1–271. However, in crystals, T7 helicase 4E fragment (residues 272–566) forms a helical filament along a six-fold screw axis by swapping its N-terminal helix (Fig. 6; Sawaya et al. 1999). The active fragment, T7 helicase 4D (residues 241–566), forms a stable hexamer by swapping its N-terminal helix and strand (Singleton et al. 2000). Therefore, 3D domain swapping is required for the hexamerization and the activity of T7 helicase. T7 helicase provides the structural evidence for 3D domain swapping in a hexamer, supporting the proposal that 3D domain swapping is a mechanism for extended protein oligomerization (Bennett et al. 1995).

In T7 helicase 4E, each subunit involved in domain swapping is related by a six-fold screw axis. Therefore, these molecules do not form a closed ring and 3D domain swapping is extended in the helical filaments throughout the whole crystal (Sawaya et al. 1999). This type of swapping is what was proposed in the linear open oligomer (Fig.1; Bennett et al. 1995). This phenomenon is also seen in the crystal structure of the "cab"-type β class carbonic anhydrase from Archaeon *Methanobacterium thermoautotrophicum*, and RecA from *E. coli*. In RecA, domain swapping is also displayed along a six-fold screw axis (Story et al. 1992), whereas in carbonic anhydrase, domain swapping is displayed along a two-fold screw axis throughout the crystal (Strop et al. 2001).

In summary, the "cab"-type β class carbonic anhydrase, RecA, and the T7 helicase display open-oligomeric 3D domain swapping, which can be seen in crystals because the oligomer screw-axis symmetry is also a symmetry of the crystal.

Human prion and human cystatin C

3D domain swapping has been proposed as a mechanism for amyloid formation (Klafki et al. 1993; Schlunegger et al. 1997; Cohen and Prusiner 1998; Liu et al. 1998, 2001). However, there was no structural evidence for amyloidogenic proteins to be domain-swapped until human prion (Knaus et al. 2001) and human cystatin C (Janowski et al.

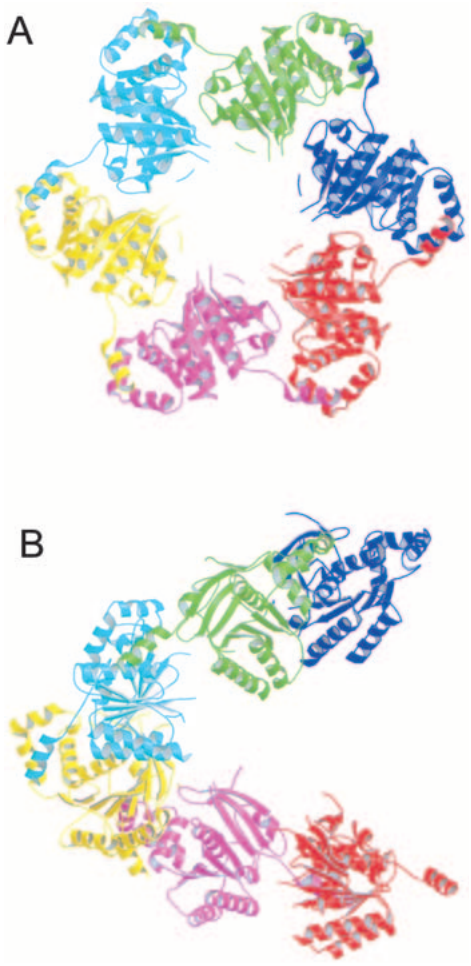

Fig. 6. An open oligomeric domain-swapped structure, illustrated by a ribbon diagram of the structure of T7 helicase 4E fragment (Sawaya et al. 1999). The hexamer of T7 helicase 4E has a six-fold screw axis in the molecule, which is coincident with the crystallographic symmetry. 3D domain swapping is displayed along the screw axis throughout the crystal. (*A*) A view of the hexamer along the screw axis. (*B*) A view of the hexamer perpendicular to the screw axis. The figure was created with Raster3D (Merritt and Bacon 1997).

2001; Staniforth et al. 2001) were reported to be domain-swapped. Both human prion and cystatin C form fibers and are related to amyloid diseases. Crystal and NMR structures of these proteins show that they form domain-swapped dimers. Based on these structures, models with 3D domain swapping were proposed for amyloid formation (Janowski et al. 2001; Knaus et al. 2001; Staniforth et al. 2001). In addition, Rousseau et al. (2001) observed a qualitative correlation between domain swapping and aggregation propensity of p13suc1 mutants. Although it is unclear whether the domain-swapped dimer is the building block for these fibers, these studies show that domain swapping and amyloid formation may share common intermediates, and thus suggest that 3D domain swapping is a possible mechanism for amyloid formation.

Physiological relevance or crystallographic artifact?

As the number of domain-swapped proteins continues to increase, the question of the physiological relevance of 3D domain swapping grows in importance. If domain swapping is biologically relevant, how does domain swapping regulate biological functions of the swapped molecules? By examining domain-swapped proteins, we conclude that domain swapping is physiologically relevant in some proteins, but not in others. Here, we list several examples for both situations.

Physiological relevance

As mentioned above, RNase A forms dimers and trimers during lyophilization in acetic acid (Crestfield et al. 1962; Gotte et al. 1999). Can these oligomers form under physiological conditions? RNase A was reported to dimerize at pH 6.5 and 37°C, similar to the physiological conditions. The dissociation constant for the dimer at this condition is ~2 mM, which is about 20-fold greater than the concentration of RNase A in the bovine pancreas, suggesting that a small amount of RNase A dimer does exist in vivo (Park and Raines 2000). In addition, RNase A oligomers display higher enzyme activity on double-strand RNA than does the monomer (Gotte et al. 1999). These results suggest that 3D domain swapping in RNase A exists in vivo and may play some physiological role.

In addition, 3D domain swapping in bovine seminal ribonuclease (BS-RNase, sharing 80% sequence identity with RNase A, Mazzarella et al. 1993) was reported to be necessary for its immunosuppression activity and allostery activity (Piccoli et al. 1988; Cafaro et al. 1995), indicating the physiological role of 3D domain swapping.

Important progress has recently been made in understanding the role of 3D domain swapping in biological functions. Single point mutations at the closed interface of the suppressor of cyclin dependent kinase 1 (p13suc1) shifted the equilibrium between monomer and dimer (Schymkowitz et al. 2001). Since the closed interface exists in both the monomer and the dimer, the mutation at the closed interface should affect both forms, and therefore should not affect the equilibrium. It was reported that there is a strain at the hinge loop of suc1 which controls the equilibrium of the monomer and the dimer through 3D domain swapping (Rousseau et al. 2001). The mutations that shift the equilibrium are distant from the hinge loop, suggesting that the strain at the hinge loop can be "sensed" by the remote mutation sites. Ligand binding to suc1, which is distant from the hinge loop, also shifted the equilibrium, further supporting the suggestion of sensing remote strain (Schymkowitz et al. 2001). These studies provide evidence for 3D domain swapping as a mechanism for allostery and signal sensing in a macromolecule, and therefore for regulating biological functions of proteins.

Macromolecular crowding supports the possible physiological relevance of 3D domain swapping. Cells are crowded with macromolecules (Goodsell 1993). The effect of other macromolecules on a specific macromolecule in cells has been studied and termed "macromolecular crowding" (Minton 2001). Macromolecular crowding increases protein local concentration and facilitates protein oligomerization (Cole and Ralston 1994; Lindner and Ralston 1995; Rivas et al. 1999). Since high concentration of a protein favors 3D domain swapping, macromolecular crowding may facilitate 3D domain swapping in vivo. Macromolecular crowding also stabilizes protein oligomers (Eggers and Valentine 2001). This effect was seen during the crystallization of the RNase A minor trimer, which is stabilized by polyethylene glycol (PEG) 10,000 even at pH 3.5 (Liu et al. 2002). Therefore, even though the dissociation constant for the dimer of RNase A is greater than the concentration of RNase A in bovine pancreas, the amount of dimers formed in vivo may be higher than the amount calculated from the dissociation constant, due to macromolecular crowding. That is, thermodynamic activity exceeds concentration. Thus, macromolecular crowding in cells increases the population of domain-swapped oligomers and thus in a general way adds support to the possible physiological relevance to 3D domain swapping.

3D domain swapping induced by receptor/ligand binding provides more evidence for the physiological relevance of domain swapping. Diphtheria toxin (DT), which enters cells by endocytosis, was first found to form a domain-swapped dimer upon lowering pH (Carroll et al. 1986; Bennett et al. 1994a). Although this low pH may mimic the environment of an endosome, more direct evidence of the physiological relevance of 3D domain swapping in DT comes from the crystal structure of the complex of DT and a domain of its receptor showing that DT forms domain-swapped dimer upon binding to its receptor at neutral pH (Louie et al. 1997). 3D domain swapping regulated by ligands was reported in glyoxalase I (Saint-Jean et al. 1998) and p13suc1 (Schymkowitz et al. 2001), where the equilibrium between the monomer and the dimer is regulated by glutathione and phosphopeptide, respectively, suggesting that ligand binding may regulate the functions of its receptor through 3D domain swapping. Similarly, 3D domain swapping was proposed as a mechanism for the oligomerization of membrane-associated guanylate kinases regulated by their ligand binding (McGee et al. 2001).

In addition, support for physiological relevance of 3D domain swapping can be found in the proteins that exist as domain-swapped oligomers in vivo. These proteins include BS-RNase (Mazzarella et al. 1993), T7 helicase (Singleton et al. 2000), cro repressor (Anderson et al. 1981), phosphoenolpyruvate mutase (Huang et al. 1999), T4 endonuclease VII (Raaijmakers et al. 1999), IX/X-binding protein (Mizuno et al. 1997), and bleomycin resistance protein (Dumas et al. 1994). The specific role of 3D domain swapping in these proteins is still unclear. However, the active forms of these proteins are domain-swapped, suggesting that their 3D domain swapping is related to their biological functions in vivo.

Artifact

Several domain-swapped oligomers are obtained under nonphysiological low pH, and the biological functions of the oligomers are unknown. Barnase is active as a monomer. At pH 4.5, it forms a domain-swapped trimer (Zegers et al. 1999). The N-terminal domain of sporulation response regulator Spo0A forms a domain-swapped dimer at pH 4.0. However, when this domain is phosphorylated, it exists as a monomer. The contradictory fact is that the phosphorylated whole Spo0A is a dimer in solution (Lewis et al. 2000). It is unclear whether domain swapping takes place in this dimer, since the structure of the whole Spo0A is not available. Cyanovirin-N (Yang et al. 1999) was also crystallized under low pH and was shown to be domain-swapped. Although low pH environments exist in some compartments of cells, there is no indication of a relationship of these domain-swapped proteins to those compartments.

In addition, several domain-swapped proteins are fragments of their complete molecules, whereas the intact molecule is a monomer. The most obvious example is Domain 5 of TrkA, TrkB, and TrkC. Domain 5 alone is domain-swapped at its N-terminal strand (Ultsch et al. 1999). However, the domain-swapped dimer is incapable of binding to the natural ligand (Urfer et al. 1995). In addition, there are four domains N-terminal to Domain 5 in the intact molecule. These four domains may block the swapping of the N-terminal strand of Domain 5 and result in a monomer of the intact molecule. Therefore, domain swapping in Domain 5 is regarded as a consequence of the truncation of the whole protein (Ultsch et al. 1999) and is not of physiological significance.

Other examples include the N-terminal domain of Spo0A (Lewis et al. 2000), the BTB domain from PLZF (Ahmad et al. 1998), the SH3 domain of Eps8 (Kishan et al. 1997), and the SH2 domain of Grb2 (Schiering et al. 2000), which are also part of their whole molecules, and show domain swapping. However, whether their entire polypeptide chains are domain-swapped remains to be seen. Although the physiological relevance of domain swapping in these domains remains controversial, these examples suggest that smaller domains form domain-swapped oligomers more easily than larger domains, and may do so under nonphysiological conditions.

The mechanism of 3D domain swapping

Although about 40 proteins have been reported to be domain-swapped, studies on the mechanism of 3D domain

swapping are few (Hayes et al. 1999; Kuhlman et al. 2001; Rousseau et al. 2001; Schymkowitz et al. 2001), and to date, the mechanism of 3D domain swapping remains elusive. Based on the monomeric and dimeric structures of DT and the conditions to form its dimer, a free energy diagram was proposed for the pathways of 3D domain swapping (Bennett et al. 1995). According to this proposal, the closed interface in a closed monomer is disrupted under certain conditions to form an open monomer. There is a high energy difference between the closed and the open monomers, which is the activation energy. Two or more open monomers aggregate to form a domain-swapped dimer or oligomer. The free energy difference between the closed monomer and domain-swapped oligomer is small, because they share the same structures except at the hinge loop. Therefore, there is a high energy barrier between the closed monomer and the domain-swapped oligomer. This energy barrier can be reduced under certain circumstances, such as change of pH, change of temperature, mutation in the protein, presence of denaturants, and binding of a ligand. In short, the current energetic model for the formation of 3D domain swapping is that of a high energy barrier that can be reduced by a change in solution conditions.

Recent studies on 3D domain swapping show that there are three factors that affect the free energy difference between the monomer and the domain-swapped oligomer. First, the greater entropy of the monomer makes it more favored thermodynamically. Second, hinge loops may form new interactions in the domain-swapped dimer, which favor dimerization. Also, there may be strains introduced or relieved when a protein forms a domain-swapped dimer. Therefore, the conformational changes at the hinge loop also contribute to this free energy difference. Third, new interactions at the open interface make the domain-swapped oligomer more favorable thermodynamically (Kuhlman et al. 2001; Liu et al. 2001; Rousseau et al. 2001; Schymkowitz et al. 2001). Therefore, by changing the hinge loop or engineering the open interface, one can change the equilibrium between the monomer and the domain-swapped oligomer.

Engineering the hinge loop has been shown to affect 3D domain swapping. After the hinge loop is shortened, Domain 1 of CD2 (Murray et al. 1995), staphylococcal nuclease (Green et al. 1995), and single chain Fv (Kortt et al. 1994; Perisic et al. 1994) form domain-swapped dimers. On the other hand, lengthening the hinge loop of the domain-swapped dimer of cro repressor leads to the monomer formation (Albright et al. 1996).

Other examples of the effect of the hinge loop on domain swapping include p13suc1, in which there are two prolines at the hinge loop. These two prolines control the balance of the monomeric and the dimeric forms by the strains at the hinge loop. In the monomer, there is a strain on residue Pro90 but not Pro92, whereas in the dimer there is a strain on residue Pro92 but not Pro90. Therefore, there is an equilibrium between the monomer and the dimer in the wild-type p13suc1. By mutating the hinge loop to change the strains on the hinge loop, the authors shifted the equilibrium and obtained all monomer or all dimer (Rousseau et al. 2001). Similar examples include cystatin (Staniforth et al. 2001) and Protein L (Kuhlman et al. 2001; O'Neill et al. 2001), in whose monomers there is a strain on residues at their hinge loops. By dimerization, this strain is removed and thus the dimer is thermodynamically favored.

Structural studies of DT and RNase A suggest that 3D domain swapping occurs in these proteins through partial unfolding of the monomer, to a core whose structure remains intact and to intact terminal domains free to move and to swap (Bennett et al. 1995; Liu et al. 2001). However, studies on p13suc1, CD2, and Protein L suggest that these proteins are completely unfolded on the pathway to 3D domain swapping (Hayes et al. 1999; Kuhlman et al. 2001; Rousseau et al. 2001). Apparently different proteins have different pathways for 3D domain swapping. Nevertheless, they all require the disruption of the closed interface, which contributes to the high activation energy.

Summary

In this review, we have summarized structures showing that 3D domain swapping takes place in proteins involved in diverse biological functions. Although biochemical data indicate that 3D domain swapping may affect the regulation of protein functions, further studies are required to understand the role of domain swapping in these biological functions. In several proteins, 3D domain swapping is found in the active form of these proteins, whereas in other proteins, domain swapping seems an artifact of truncation of the whole molecules.

We also discussed several domain-swapped proteins with unique features. These examples show that: (1) one protein can swap more than one domain; (2) a protein can also swap its middle domain, in addition to the domains at the termini; (3) the swapped domains have diverse primary and secondary structures; (4) the hinge loops have high flexibility and display diverse primary and secondary structures; (5) domain-swapped open oligomers can form using a screw-axis symmetry element; and (6) two amyloid proteins have been reported to be domain-swapped, strengthening the link of 3D domain swapping to amyloid formation. These structures broaden our view of 3D domain swapping.

Studies of the mechanisms of 3D domain swapping have been reported, but much remains to be learned. The independence of 3D domain swapping from protein sequence, secondary structure and hinge loop suggests that any protein can be domain-swapped under appropriate conditions where the terminal domain of the protein is unconstrained. As domains continue to swap, new examples will raise our

understanding of 3D domain swapping to a higher level, and new functions and mechanisms of 3D domain swapping will be revealed.

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