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Mechanism and evolution of protein dimerization

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Abstract

We have investigated the mechanism and the evolutionary pathway of protein dimerization through analysis of experimental structures of dimers. We propose that the evolution of dimers may have multiple pathways, including (1) formation of a functional dimer directly without going through an ancestor monomer, (2) formation of a stable monomer as an intermediate followed by mutations of its surface residues, and (3), a domain swapping mechanism, replacing one segment in a monomer by an equivalent segment from an identical chain in the dimer. Some of the dimers which are governed by a domain swapping mechanism may have evolved at an earlier stage of evolution via the second mechanism. Here, we follow the theory that the kinetic pathway reflects the evolutionary pathway. We analyze the structure-kinetics-evolution relationship for a collection of symmetric homodimers classified into three groups: (1) 14 dimers, which were referred to as domain swapping dimers in the literature; (2) nine 2-state dimers, which have no measurable intermediates in equilibrium denaturation; and (3), eight 3-state dimers, which have stable intermediates in equilibrium denaturation. The analysis consists of the following stages: (i) The dimer is divided into two structural units, which have twofold symmetry. Each unit contains a contiguous segment from one polypeptide chain of the dimer, and its complementary contiguous segment from the other chain. (ii) The division is repeated progressively, with different combinations of the two segments in each unit. (iii) The coefficient of compactness is calculated for the units in all divisions. The coefficients obtained for different cuttings of a dimer form a compactness profile. The profile probes the structural organization of the two chains in a dimer and the stability of the monomeric state. We describe the features of the compactness profiles in each of the three dimer groups. The profiles identify the swapping segments in domain swapping dimers, and can usually predict whether a dimer has domain swapping. The kinetics of dimerization indicates that some dimers which have been assigned in the literature as domain swapping cases, dimerize through the 2-state kinetics, rather than through swapping segments of performed monomers. The compactness profiles indicate a wide spectrum in the kinetics of dimerization: dimers having no intermediate stable monomers; dimers having an intermediate with a stable monomer structure; and dimers having an intermediate with a stable structure in part of the monomer. These correspond to the multiple evolutionary pathways for dimer formation. The evolutionary mechanisms proposed here for dimers are applicable to other oligomers as well.

Keywords: compactness; dimerization; domain swapping; intermediate; kinetics; oligomer evolution

Oligomers are often the functional forms of proteins. It has been a challenging problem in protein science to understand the mechanism of oligomerization and the evolution of protein oligomers. The importance of this problem is related not only to the origin of oligomers, but also to the design of functional oligomers. A recent hypothesis proposed by Eisenberg and colleagues provides a new insight to this old topic. The authors found an interesting phenomenon called *three dimensional domain swapping* from a high resolution structure of diphtheria toxin (Bennett et al., 1994). They also extended the mechanism to other oligomers (Bennett et al., 1994; Bennett et al., 1995). In the structure of a domain swapping oligomer, one segment of a monomeric protein is replaced by the same segment from an identical chain. Eisenberg and co-workers further suggest that domain swapping is possibly the mechanism of the evolution of oligomerization, in general. In this hypothesis, a mononer served as the pre-evolved form of an oligomer. The interactions between monomers in an oligomer have been pre-optimized within the monomer at the interface between the swapping segment and the rest of the monomer. Hence, the formation of an oligomer does not rely on chance association or on mutations of surface residues (Bennett et al., 1995).

Despite the inspiring beauty of the hypothesis, it is has been questioned by D'Alessio and colleagues (Piccoli et al., 1992; D'Alessio,

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Abbreviations: BS-RNase, bovine seminal ribonuclease; C, coefficient of compactness; MSA, molecular surface area; PDB, Protein Data Bank.

1995). These authors have argued that domain swapping may not be a general mechanism for oligomer evolution, in particular, not for the case of bovine seminal ribonuclease (BS-RNase). BS-RNase can form two types of dimeric conformations, one with swapped N-terminal segment, and the other without segment swapping. The two structures coexist, and the conformation with swapping occurs only after the non-swapped dimer is formed. Hence, the dimerization occurs independently of the ability of the monomers to swap their N-terminal tails with other monomers. D'Alessio et al. further assume that the kinetic pathway of the dimerization captures the evolutionary pathway of the dimer formation. They suggest that the dimer with segment swapping may not be an early, necessary station in the evolution of the dimerization. Rather, it may be an evolutionary product at a later stage with the versatile biological function of allosteric regulation (D'Alessio, 1995).

Deriving the evolutionary pathway through the kinetic pathway paves the way for studies of the evolution of protein oligomers in general. D'Alessio et al. have discussed only the case of BS-RNase. The kinetic pathways of oligomerization are often complicated. Equilibrium denaturation experiments have shown that there are two types of equilibrium transitions (Neet & Timm, 1994) in dimerization. One type of denaturation is the 2-state, i.e., the native dimer state and the denatured monomer state. There is no stable intermediate in between. The other type of denaturation is the 3-state, i.e., the native dimer state, the stable monomer state, and the denatured monomer state. If the stable monomer state has the same structure as the one it possesses when in the dimer, the dimerization belongs to "rigid body," three-state binding. In other 3-state dimers, the intermediate state deviates its structure from the one in the dimer. For example, aspartate aminotransferase has a molten globule monomer intermediate (Herold & Kirschner, 1990). Such a wide spectrum in the kinetics of oligomerization illustrates the necessity for an analysis of a collection of oligomers.

The evolution-kinetics relationship can be studied from the kinetics-structure relationship. In the case of the monomers, the protein structures can often be divided into several compact units. Due to hydrophobic effects, compact units are generally more stable in the solvent than non-compact ones. In the folding kinetics, large monomers typically form several initial collapsed nuclei as compact units or domains, followed by an assembly of these stable entities (Wu et al., 1994; Tsai & Nussinov, 1997a). Correspondingly, it has been proposed that multi-domain monomers may have evolved from proteins having single-domains via domain insertion (Russell, 1994). Some oligomers can also be divided into several compact units, while others intertwine their chains to form only a single compact unit in one oligomer. The organization of the chains of an oligomer in space reflects the kinetics of the oligomerization, and hence, is likely to show traces of the evolutionary journey of the oligomer.

In this paper, we apply the structure-kinetics-evolution connection to explore the mechanism of oligomerization and the evolution of oligomers. For this purpose, we have carried out a computational analysis on three groups of dimers, i.e, 2-state dimers, 3-state dimers, and domain swapping dimers. All the dimers that we have analyzed are symmetric homodimers, which are the simplest oligomers. A symmetric homodimer consists of two identical peptide chains that are in twofold symmetry in the three dimensional structure. To probe the organization of the two chains, we divide the dimer into two symmetric units. Each unit contains a segment of one chain and the complementary segment of the other chain. Hence, the composition of each unit includes all the amino acids in the sequence of a chain, and the two units are symmetric in their structures. The shape of the units is assessed by their coefficients of compactness (Zehfus & Rose, 1986). We obtain a profile of the compactness for different divisions of the dimer. By describing the landscape of how intimately the two chains of the dimer integrate with each other structurally, the profile is capable of predicting domain swapping. Furthermore, it sheds light on the intermediate states between native dimers and unfolded monomers. Conclusions drawn from the study of dimers can be applied to oligomers in general.

In the following, we present the compactness profiles and the properties of dimer interfaces, illustrating some structural details. We discuss the implications of our computational analysis to the evolution and the mechanism of protein oligomerization. Finally, we describe the data set and the methods which we have employed.

Results

In this section, we present the results of the compactness profiles, together with some structures, for domain swapping, 2-state, and 3-state dimers. We include the results of the calculations of their surface areas and interfacial hydrogen bonds.

Compactness profile

The principle of the calculation of the compactness profile of a dimer is illustrated in Figure 1. A dimer consists of two identical protein chains whose structures are in twofold symmetry. Assume that each chain has residues 1, 2, 3, ..., $N-1$, N. The dimer is cut into two identical units, with each unit containing two non-contiguous segments, as divided by the dashed lines in the figure. The left figure describes the cutting at position $i = k$. The newly formed unit is composed of k contiguous residues from one chain from its N-terminus (black balls in the figure), i.e, residues 1, 2, 3, ..., $k-1$, k, and $N-i$ contiguous residues from the other chain until its C-terminus (shown in white balls), i.e., residues $k+1$, $k+2$, ..., $N-1$, N. Such a cutting method reorganizes a dimer into two structurally symmetric units, each having the same composition as the original chain. We define the cutting ratio t as:

$$t = i/N. \tag{1}$$

Next, we proceed to cut the dimer at $i = k+1$ (shown in the right figure), and so on. The dimer is cut progressively at $i = 0, 1, 2, \ldots k, k+1, \ldots, N-1, N$. The compactness profile is the coefficient of compactness (C) of the units as a function of the cutting ratio t, where $0 \le t \le 1$.

As t increases, the cutting position shifts from the N-terminus to the C-terminus. At $t = 0$ or $t = 1$, the value of C gives the coefficient of compactness for the whole chain. For a symmetric homodimer, C at $t = 0$ should be identical to C at $t = 1$. However, there is a minor difference in some actual calculations due to the crystal packing of the dimer and the errors in the structure. Nevertheless, the difference is insignificant. In the following, we will classify each group of dimers (domain swapping, 2-state, or 3-state dimers) into several types according to the behavior of their compactness profiles, as summarized in Table 1. The numbering of (a), (b), ... for different types in Table 1 is consistent with the numbering of (a), (b), etc., marked in the figures of the compactness profiles and of the structures (Figs. 2–7).

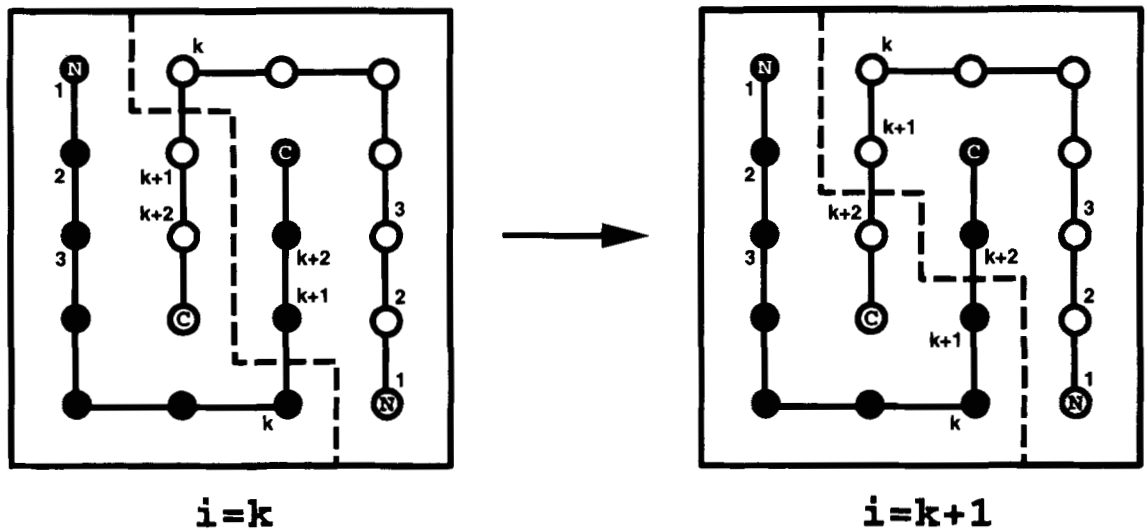

Fig. 1. Schematic diagram showing the cutting method of a dimer. The chain with black balls and the one with white balls represent two identical peptide chains of the dimer, whose boundary is shown in the square boxes. A cutting divides the dimer into two units, each including a segment from one chain and the complementary segment from the other chain. The two units also have 2-fold spatial symmetry. The dashed lines show the cutting at position k (left) and at position $k + 1$ (right).

Figure 2 shows the compactness profiles of domain swapping dimers. Assume $t = t_m$ is the cutting position obtaining the global minimum of C, i.e., t_m reflects the most compact unit among all possible units derived from the progressive cutting of the dimer. Hence, t_m identifies the exact residue range of the swapping segment. The results are listed in Table 2. The landscape of the profile can be roughly classified into four types. Type (a) shown in Figure 2A describes the case where the global minimum is around the middle and the two ends ($t = 0$, 1) are around local minima. Diphtheria toxin (1ddt), depicted in the left graph in Figure 3A, is

Table 1. *Summary of dimer groups and types*[a]

Group	Type	Compactness profile	Structural feature	PDB
Domain swapping dimers	(a)	Global min. around middle	A compact domain swaps	2bb2
(Table 3) (Figs. 2, 3)		Local min. around both ends	to another compact domain	1ddt
	(b)	Global min. close to side	Compact domain with short	1fia
		Local min./max. around ends	uncompact swapping segment	1obp
	(c)	Global min. close to center	Compact domain with long	1bsr, 1hum
		Local min./max. around ends	uncompact swapping segment	1puc
	(d)	Global min. around middle	No compact domain in monomer;	1cdc,1hul,1ilk
		Global max. around both ends	two chains intertwined	1rfb,2spc
2-state dimers	(a)	Global max. around middle	Flat large interface;	1bet, 1lfb
(Table 4) (Figs. 4, 5)		Global min. around both ends	no swapping segment	1rpo
	(b)	No deep min. or max.	Chains are short;	1cta, 1myk
		The profile is flat.	two chains intertwined	2zta
	(c)	Global min. around middle	Uncompact segment swaps to	1hhp
		Local min./max. around ends	a relatively compact domain	3wrp
	(d)	Local min. around middle	Uncompact swapping segment	2gvb
		Global min. close to an end	in the middle of a sequence	
3-state dimers	(a)	Global max. around middle	Flat small interface;	1lyn, 1tim
(Table 5) (Figs. 6, 7)		Global min. around both ends	no swapping segment	1xso, 3ssi
	(b)	Global min. close to an end	Compact domain with short	1lmb
		Local min./max. around ends	uncompact swapping segment	1tar
	(c)	Local min. around middle	Inter-chain domain packing;	1glq
		Global min. around both ends	no swapping segment	1ttq

[a]The features of the compactness profiles and related structures in the different groups and types of dimers. "min": minimum; "max": maximum.

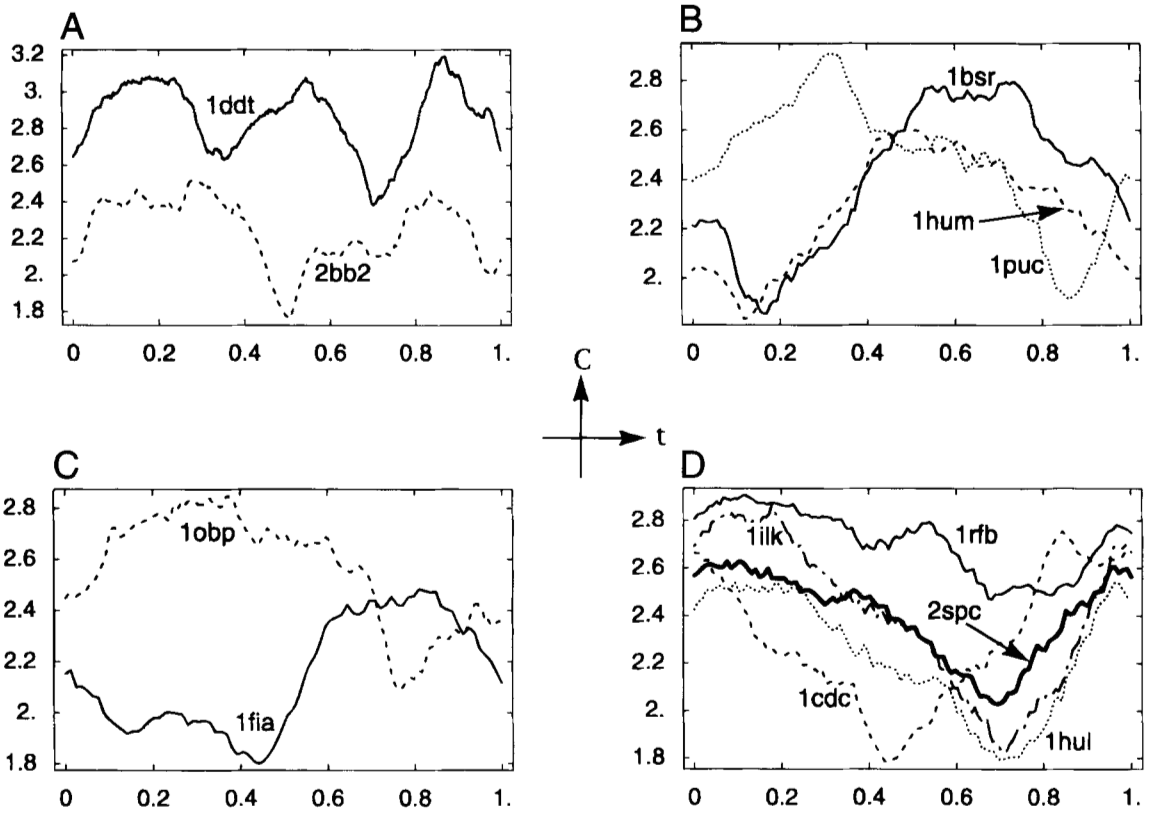

Fig. 2. Compactness profile, i.e., coefficient of compactness (C) as a function of cutting ratio t, for the domain swapping dimers. **A:** The global minimum is close to the middle and the two ends ($t = 0, 1$) are around local minima. **B:** The global minimum is close to the one end ($N < 0.2$ or $N > 0.8$). **C:** The global minimum is close to the middle and the one end is around a local minimum and the other end is around a local maximum. **D:** The global minimum is close to the middle and both ends are around global maxima. The protein names of marked PDB codes are listed in Table 2.

Table 2. *Domain swapping dimers*[a]

Interface	Molecule	Resol. (Å)	Size (residues)	Swapped segment (residues)	Stability
1ddt (sym)	Diphtheria toxin	2.0	535	380–535	Metastable
1bsrAB	BS-RNase	1.9	124	1–21	Stable
1cdcAB	N-terminal domain CD2	2.0	99	46–99	Metastable
2spcAB	Spectrin	1.8	107	74–106	Unknown
2bb2 (sym)	βB2 crystallin	2.1	181	−2–86	Stable
1hulAB	Interleukin-5	2.4	108	89–112	Stable
1ilk (sym)	Interleukin-10	1.8	151	117–160	Stable
1rfbAB	Interferon γ	3.0	119	82–119	Stable
1obpAB	Odorant-binding protein	2.0	159	122–159	Stable
1puc (sym)	Protein sucl	1.95	105	89–106	Stable
1humAB	HMIP-1B	(NMR)	69	1–8	Stable
1fiaAB	FIS protein	2.0	98	56–98	Stable

[a]Domain swapping dimers employed in the analysis. The table describes the PDB code with the chain names, name of the molecule, the resolution of the structure, number of amino acids in a chain, the swapping segment predicted by the compactness profile, and the dimer stability. "(sym)" in the "Interface" column indicates that the dimer was generated from a single chain in the PDB by using the symmetry operation (same in Tables 3 and 5). "NMR" in the "Resolution" column indicates the dimer is from an NMR structure. The residue numbers in the swapping segment are according to the ones in the dimer's PDB code. The stability indicates whether a dimer can maintain its structure under normal physiological conditions. The metastable structures only exist under some special conditions. For example, diphtheria toxin (1ddt) is in a dimeric form in the crystal structure, but it is a monomer in solvent (Bennett et al., 1994). The first seven cases were taken from the reference (Bennett et al., 1995). The next three cases are from other experimental papers: 1rfb (Bennett et al., 1994) 1obp (Tegoni et al., 1996) 1puc (Bourne et al., 1995). The last two cases, 1hum and 1fia, were predicted from the cutting of hydrophobic folding units (Tsai & Nussinov, 1997b).

an example. The thick line indicates the cutting segment corresponding to t_m. In this cutting, a segment (residues 380–535 shown in thick red line) of one chain, swaps to integrate with its complementary portion (residues 1–379 in the thick blue line) of the other chain. There is a clear barrier between either of the ends and the global minimum in the compactness profile. The compactness is reduced as the cutting units start to deviate from the single chain, until t approaches t_m. Hence, the conformation of each chain alone is relatively compact. Since a compact structure in the solvent is energetically more favorable than a non-compact segment, the monomeric conformation observed in the dimer is in a meta-stable state prior to dimerization. Given the compactness both at $t = t_m$ and at $t = 0$, 1, each chain can be divided into two compact blocks, one with residues 1–379 and one with residues 380–535, as shown by the thick and thin lines, respectively, within a chain. There is a local minimum at $t = 0.35$, with the conformation shown in the right graph of Figure 3A. The cutting unit is formed by residues 1–187 (thick blue line) of one chain, and residues 200–535 (thick red line) of the second chain. This local minimum demonstrates that the segment 1–379 can be further divided into two compact domains: 1–187 and 200–397 (there are no coordinates available for residues 188–199 in the PDB).

Types (b) and (c) of domain swapping dimers shown in Figures 2B and 2C are similar. In both cases, there is a local minimum at one end of the profile ($t = 0$ or $t = 1$) and a local maximum at the other end. In (b) the global minimum is close to one end ($t < 0.2$ or $t > 0.8$), while in (c), the global minimum is around the middle. However, the difference between (b) and (c) is not clear cut. Figure 3B shows the conformation of BS-RNase (1bsr), an example of the cutting in Figure 2B. The unit at $t = t_m$ contains residues 22–124 (thick blue line) of one chain and residues 1–21 (thick red line) of the other. Figure 3C depicts an example taken from Figure 2C, i.e., the cutting of odorant-binding protein (1obp) at $t = t_m$. It represents the conformation with residues 2–121 (thick blue line) from one chain, and residues 122–159 (thick red line) from the other chain. Both type (b) and (c) dimers have compact domains starting from a sequence terminus, i.e., residues 22–124 in 1bsr and 2–121 in 1obp. The other portion of the chain (residues 1–21 of 1bsr or residues 122–159 of 1obp) is swapping to the compact domain. This portion is not compact therefore, its structure in the dimer is unstable before dimerization. The swapping segments of (b) and (c) differ in their contribution to the stability of the dimer. The stability of the dimer is mostly contributed by the segment swapping in (c). The swapping segments in (b) contribute much less and may only help further strengthen the dimers, in particular in the case of BS-RNase (Piccoli et al., 1992; D'Alessio, 1995).

Type (d) of domain swapping dimers shown in Figure 2D has the global minimum of C around the middle and the degenerated

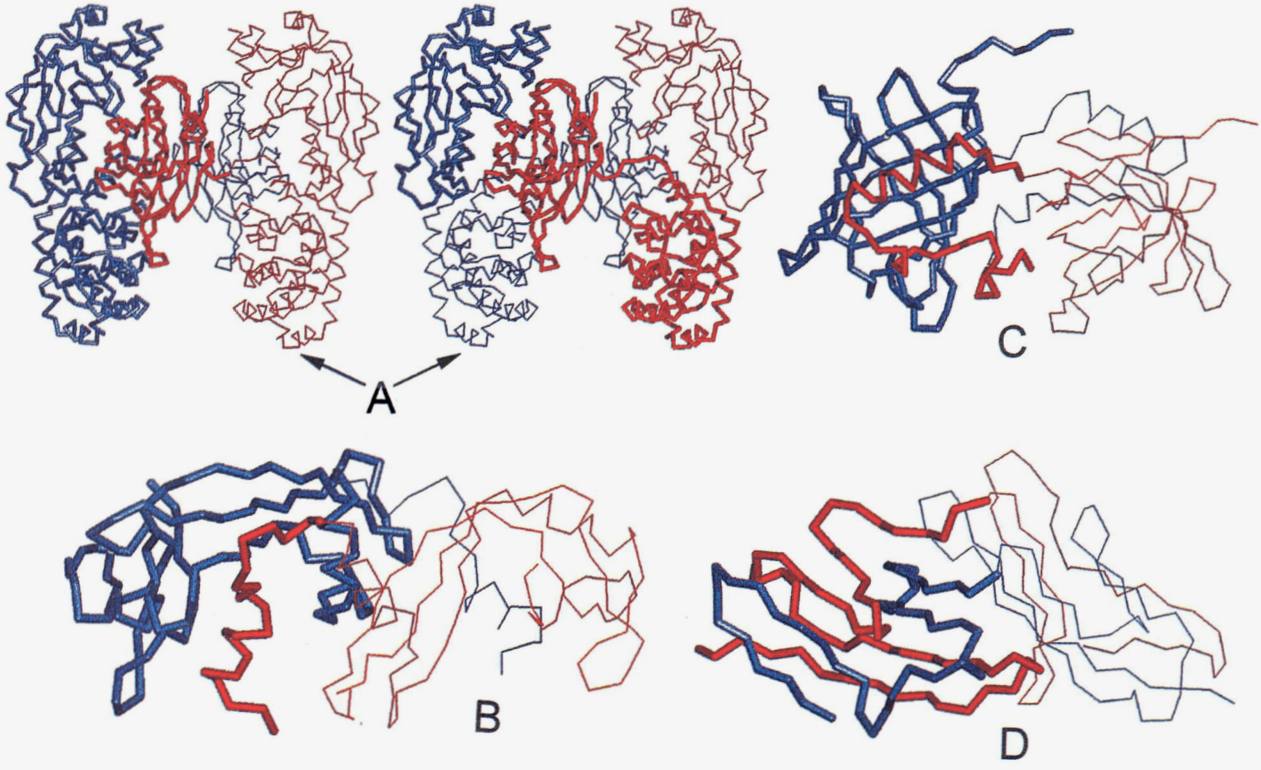

Fig. 3. Structural examples of domain swapping dimers for Figure 2. The two chains are differentiated utilizing blue and red colors. **A:** Diphtheria toxin (1ddt). Left graph: residues 1–379 in the thick blue line and residues 380–535 in the thick red line; right graph: residues 1–187 in the thick blue line and residues 200–535 in the thick red line. Residues 188–199 are not shown, since they are unavailable in the crystal structure. **B:** BS-RNase (1bsr) with residues 22–124 in the thick blue line and residues 1–21 in the thick red line. **C:** Odorant-binding protein (1obp) with residues 2–121 in the thick blue line and residues 122–159 in the thick red line. **D:** N-terminal domain CD2 (1cdc) with residues 4–45 in the thick blue line and residues 46–99 in the thick red line. The thick lines and thin lines show the two selected symmetric units which have the lowest C value in the compactness profile, except for the right graph of (A), which shows a local minimum of C. This picture and Figures 5 and 7 were generated by the program QUANTA (Molecular Simulations, 1994).

global maxima of C at both ends. As an example in Figure 3D, the cutting at $t = t_m$ in the N-terminal domain CD2 (1cdc) composes residues 4–45 (thick blue line) of one chain, and residues 46–99 (thick red line) of the other. The two chains in the dimer intertwine with each other. The conformation of the whole chain or any segment within the chain is not compact, and hence, the monomer is unstable by itself prior to dimerization.

Figure 4 gives the compactness profiles of dimers shown by experiments to have 2-state kinetics (listed in Table 3). The landscape of the profile can be roughly divided into four types. In type (a), both ends of the compactness profile are around the degenerated global minima of C. The repressor of primer (1rpo), whose structure is shown in Figure 5A, provides an example. The dimer interface is flat, and there is no swapping segment between the two chains. In type (b), C is around 2.0 and the fluctuation is relatively small along t (less than 10%). As demonstrated in Figure 5B for troponin C site III (1cta), the dimers are very small. Each chain is relatively compact and a large portion of either chain's surface area is buried in the interface. The compactness profile of type (c) is similar to that of domain swapping dimers. The compactness profile of HIV-1 protease (1hhp) matches type (b) of domain swapping, which involves only a short segment exchange across the two chains. Trp aporepressor (3wrp) matches type (c) of the domain swapping dimers. As shown in Figure 5C, residues 44–108 (thick blue line) and residues 8–43 (thick red line) intertwine with each other. However, residues 44–108 form a relatively compact segment. In type (d), both ends are around the degenerated global minimum, while there is a deep local minimum around the middle. The left graph of Figure 5D shows the conformation of gene V protein (2gvb) at $t = t_m$. The structure consists of residues 1–61 from one chain (thick blue line) and residues 62–87 from the other chain (thick red line). The value of C at $t = t_m$ is 2.196. Since there is no global minimum in the middle, it is not predicted as a domain swapping dimer by the compactness profile.

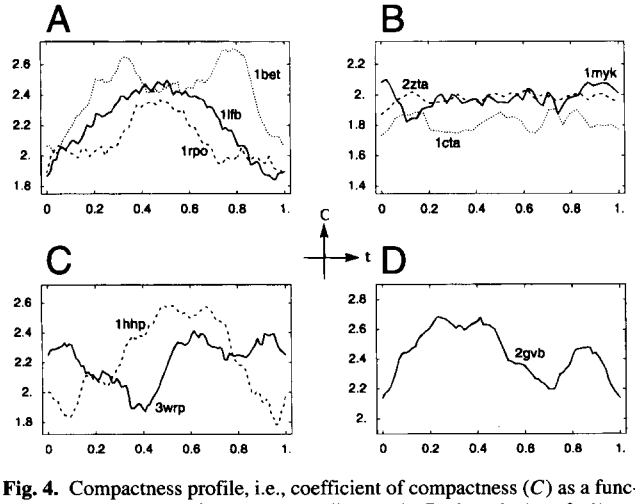

Fig. 4. Compactness profile, i.e., coefficient of compactness (C) as a function of cutting ratio t for the 2-state dimers. **A:** Both ends ($t = 0, 1$) are around the degenerated global minima. **B:** C does not vary significantly along t. **C:** Domain swapping dimers. **D:** Both ends ($t = 0, 1$) are around the degenerated global minima, while there is a deep local minimum in the middle. The protein names of marked PDB codes are listed in Table 3.

However, one can see that there is a swapping segment in the middle of the sequence. We carried out a search for the minimum C around residues 62–87 in all possible symmetric cuttings, i.e., without the limitation that a chain can only be divided into two contiguous segments. The search yields a compactness of 2.053, which is lower than C at $t = t_m$ in the previous cutting. The conformation of the new symmetric unit is composed of residues 1–64 and 80–87 (both in the thick blue lines) from one chain, and residues 62–79 (thick red line) from the other chain, as shown in the right graph of Figure 5D. In this particular case, the compactness profile failed to predict 2gvb as a domain swapping dimer, since the swapping segment does not start from a terminus of the chain.

Figure 6 presents the compactness profiles of dimers showing 3-state kinetics in experiments (see Table 5). There are roughly three types. In type (a), both ends are around the degenerated global minima, as shown in the example of superoxide dismutase (1xso) in Figure 7A. The dimer interface is flat, with no swapping segment exchanges between the two chains. It is similar to type (a) of the 2-state. However, the interface of the 3-state dimer occupies a much smaller portion of the monomer's surface area than the one of the 2-state. In type (b), there is a small segment swapping in the dimer. This belongs to type (b) of the domain swapping dimers. As shown in Figure 7B, aspartate aminotransferase (1tar) swaps residues 3–13 (thick blue line) to the major portion of the monomer, i.e., residues 14–410 (thick red line). In type (c), both ends are around the degenerated global minima with a deep local minimum in the middle. The profile is similar to type (d) of the 2-state. However, structurally they do not share a common feature. Figure 7C shows the conformation of glutathione S-transferase (1glq) at $t = t_m$. Residues 79–209 (thick blue line) and residues 1–78 (thick red line) are two compact domains of the monomer. They do not intertwine. The local minimum originates from the packing of the two domains between the two chains rather than within a monomer.

Properties of dimer interfaces

We evaluated the hydrogen bonds across dimer interfaces for the 2-state, 3-state, and the domain swapping dimers, as shown in Table 4. There is no significant signature in this aspect for the three groups of dimers. We calculated the total buried molecular surface area (MSA) across the dimer interface, i.e., ΔMSA. It is obtained from the difference between the surface area of the dimer and the sum of the surface areas of the two separate chains, i.e., MSA_1 and MSA_2 (MSA_1 and MSA_2 should be identical in a symmetric homodimer, but they may be slightly different in the actual PDB due to crystal packing and errors in the structure). The ratio p between the interfacial MSA of a monomer and its total MSA is derived as

$$p = \frac{\Delta MSA}{MSA_1 + MSA_2}. \quad (2)$$

ΔMSA and p are listed in Table 5. Figure 8 draws the relationship between ΔMSA and p. 3-state dimers (dashed line) have a small p value around 0.1, and p does not increase significantly with increasing ΔMSA. The p of the 2-state dimers (solid line) increases dramatically with increasing ΔMSA. This indicates that the interface of a 3-state dimer only involves a small portion of the surface

Table 3. *2-state protein dimers*[a]

Interface	Molecule	Resol. (Å)	Size (residues)	Reference
1bet (sym)	β nerve growth factor	2.3	107	Timm et al., 1994
3wrp (sym)	Trp aporepressor	1.8	108	Mann et al., 1993
1rpo (sym)	Repressor of primer	1.4	65	Steif et al., 1993
2gvbAB	Gene V protein	NMR	87	Liang & Terwilliger, 1991
1hhp (sym)	HIV-1 protease	2.7	99	Grant et al., 1992
1lfb (sym)	LFB1 transcription factor	2.8	99	De Francesco et al., 1991
1ctaAB	Troponin C site III	NMR	36	Monera et al., 1992
1mykAB	Arc repressor	2.4	53	Milla & Sauer, 1994
2ztaAB	Leucine zipper	1.8	34	Neet & Timm, 1994

[a]The 2-state dimers. The table describes the PDB code with the chain names, name of the molecule, the resolution of the structure, number of amino acids in a single chain, and the reference which shows the dimerization is 2-state.

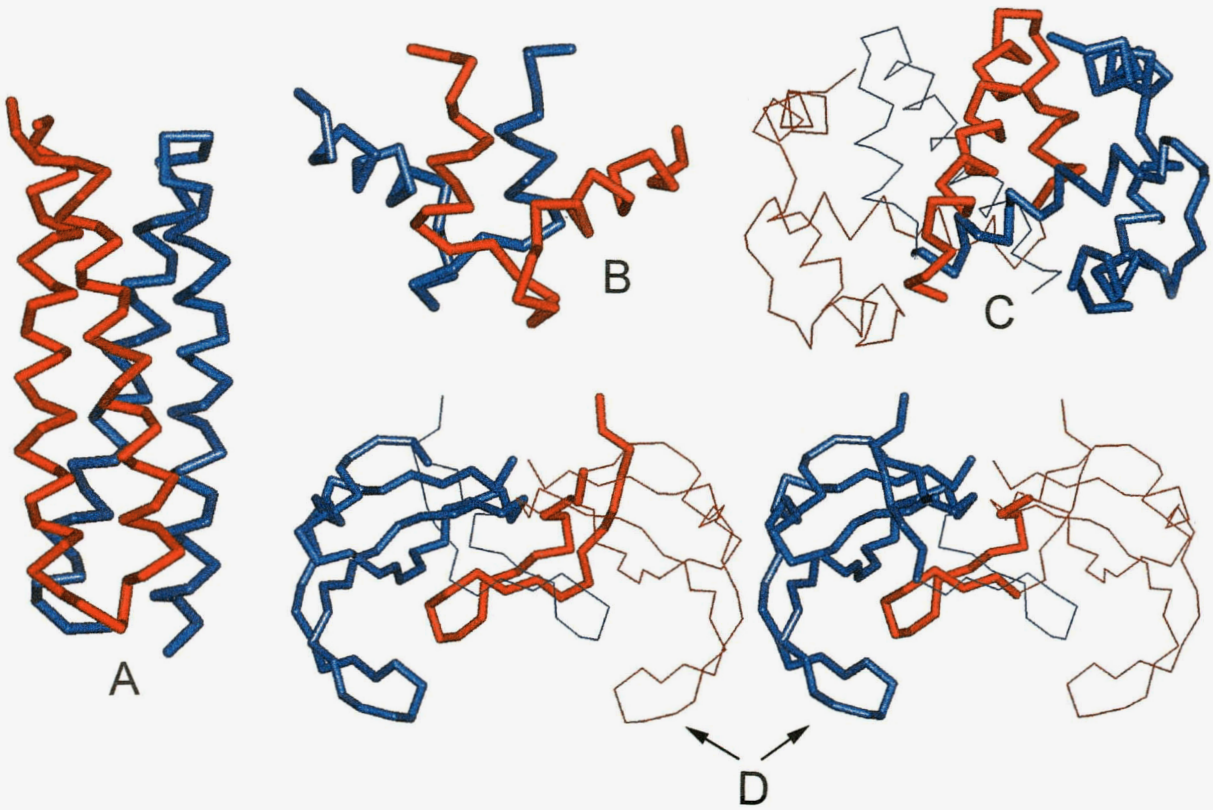

Fig. 5. Structural examples of 2-state dimers for Figure 4. The two chains are differentiated by blue and red. **A:** Two monomers of repressor of primer (1rpo), shown in blue and red, respectively. **B:** Two monomers of troponin *C* site III (1cta), shown in blue and red respectively. **C:** Trp aporepressor (3wrp) with residues 44–108 in the thick blue line and residues 8–43 in the thick red line. The two units shown in the thick line and in the thin line corresponds to the cutting with the lowest C value in the compactness profile. **D:** Gene V protein (2gvb). Left graph: residues 1–61 in the thick blue line and residues 62–87 in the thick red line. The correspondent *C* value of the cutting is 2.196, which is the lowest along the compactness profile. Right graph: residues 1–64 and 80–87 in the thick blue line and residues 62–79 in the thick red line. The cutting yields a *C* value of 2.053, which is the lowest in all possible cuttings which divide the dimer into two symmetric blocks.

area of the monomer as shown in Figure 7, while the interface of a 2-state dimer often composes a large portion of the surface area in each chain as shown in Figure 5. The p values of domain swapping dimers (shown by dots and crosses) scatter in between the 2-state and the 3-state. Type (d) of domain swapping dimers, marked by crosses, shows the 2-state behavior, except for interferon (1rfb), whose compactness profile is shallow compared to the other four cases of this type (see Figure 2D). This again shows that most type (d) dimers of domain swapping follow a 2-state profile.

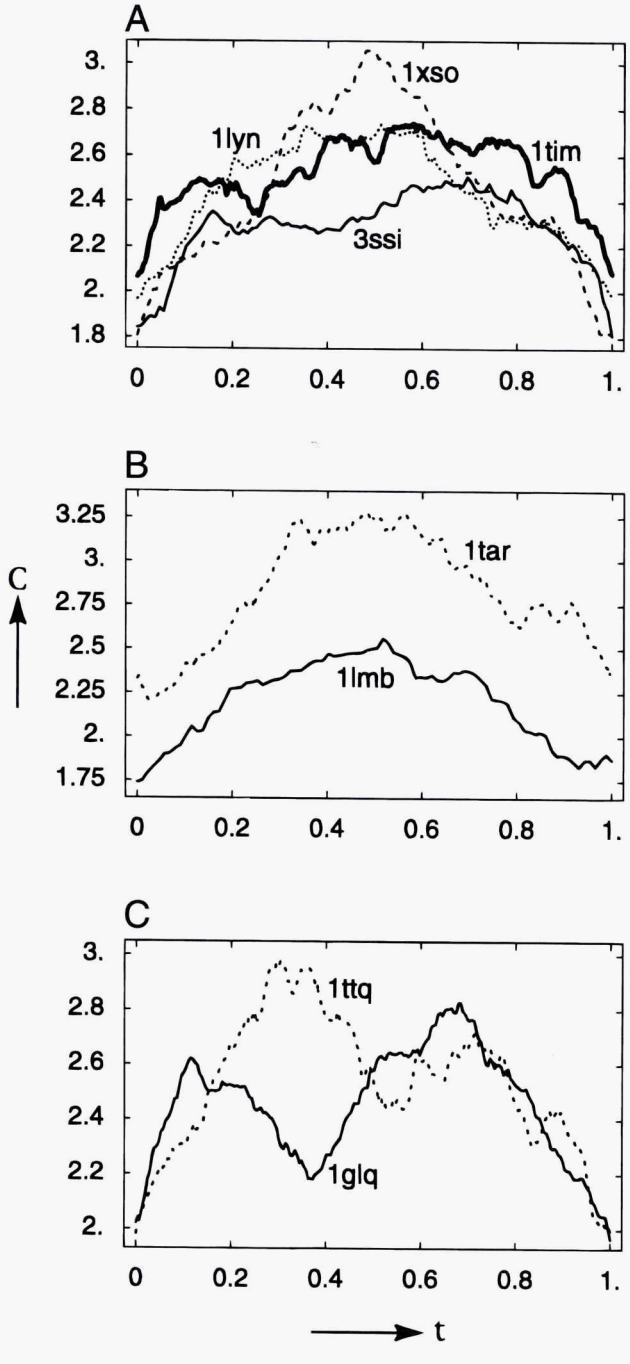

Fig. 6. Compactness profile, i.e., coefficient of compactness (C) as a function of cutting ratio t for the 3-state dimers. **A:** Both ends (t = 0, 1) are around the degenerated global minima. **B:** Dimers with small segment swapping. **C:** Both ends (t = 0, 1) are around the degenerated global minima with a deep local minimum around the middle. The protein names of marked PDB codes are listed in Table 5.

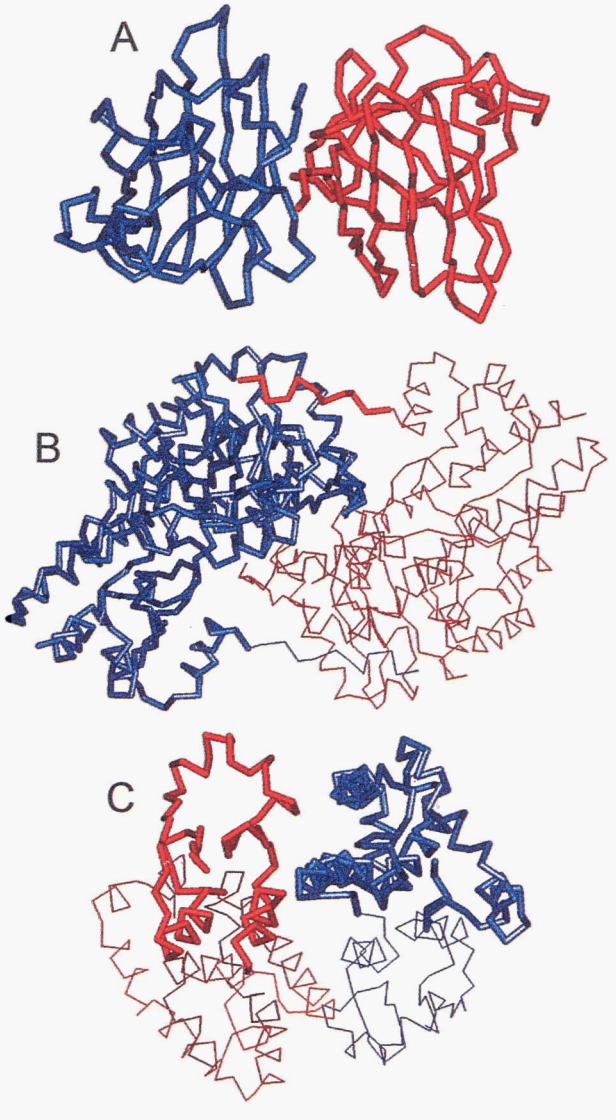

Fig. 7. Structural examples of 3-state dimers for Figure 6. The two chains are differentiated by blue and red. **A:** The two monomers of superoxide dismutase (1xso), shown in blue and red, respectively. **B:** Aspartate aminotransferase (1tar) with residues 3–13 in the thick blue line and residues 14–410 in the thick red line. **C:** Glutathione S-transferase (1glq) with residues 79–209 in the thick blue line and residues 1–78 in the thick red line. In (B) and (C), the thick lines and thin lines show the two selected symmetric units which have the lowest C value in the compactness profile.

Discussion

In this section, we discuss the attributes of the compactness profile method and the characteristics of domain swapping. We further address the kinetics of dimerization and the evolution of dimers based on our computational analysis.

Implication of the compactness profile

The compactness profile is a useful tool to probe the geometric organization of the two chains in dimers. It gives not only a single number of the coefficient of compactness for the whole chain, but also how the two chains of a dimer pack together. A special advantage of the method is that the calculation of the compactness coefficients versus different cuttings in a dimer are carried out for units with identical amino acid composition. Hence, such a comparison between compactness coefficients of a dimer is related solely to the geometry (shape) of the units, rather than to their physical or chemical properties. If a dimer is known to have domain swapping, one can find the global minimum of the compact-

Table 4. *Dimer properties*[a]

Group	Interface	ΔMSA (Å^2)	p	hbond
2-state dimers	1bet (sym)	1,435	0.12	8
	3wrp (sym)	2,947	0.23	18
	1rpo (sym)	1,995	0.26	10
	2gvbAB	1,118	0.11	2
	1hhp (sym)	2,271	0.21	20
	1lfb (sym)	717	0.08	4
	1ctaAB	843	0.18	2
	1mykAB	2,315	0.34	17
	2ztaAB	1,128	0.22	2
3-state dimers	1ttqB (sym)	1,960	0.07	12
	1lmb34	931	0.10	6
	1tarAB	4,100	0.12	36
	1xsoAB	842	0.07	4
	1glqAB	1,677	0.09	9
	3ssi (sym)	1,140	0.11	4
	1lynAB	1,142	0.08	4
	1timAB	2,183	0.10	18
Domain swapping dimers	1ddt (sym)	4,940	0.11	32
	1bsrAB	2,443	0.17	34
	1cdcAB	5,493	0.38	61
	2spcAB	3,458	0.23	16
	2bb2 (sym)	2,590	0.15	26
	1hulAB	4,802	0.33	15
	1ilk (sym)	6,567	0.33	32
	1rfbAB	2,382	0.13	4
	1obpAB	3,360	0.18	13
	1puc (sym)	2,934	0.21	26
	1humAB	1,118	0.13	4
	1fiaAB	2,958	0.29	9

[a] ΔMSA: total buried surface areas of the dimer interface; p: the ratio between ΔMSA and the total surface area of the two unbound monomers in the dimer; hbond: number of hydrogen bonds across the dimer interface.

ness profile to pinpoint accurately the domain swapping segment. In most cases, the compactness profile can be used to predict whether a dimer is domain swapped, and what type of swapping it is, as summarized in Table 1. It may fail to identify the case where the swapping domain takes place in the middle of a chain (see Fig. 5D). However, this is very rare in dimers. The compactness profile can also identify which segment forms a compact domain in a chain. The compactness profile can be similarly applied to other oligomers.

Domain swapping

The information obtained from the compactness profile allows us to revisit the definition of domain swapping. Many dimers are referred to as domain swapping dimers in the literature based on the structures, as shown in Table 3. However, among the four types of domain swapping dimers shown in Figures 2 and 3, only type (a) fits the "classical" meaning of domain swapping dimers. There, the parts swapping between the two chains are stable domains. In types (b) and (c), there is a stable domain in each chain. However, the swapping portions between the two chains are not compact, and do not fit the definition of a protein domain in the conventional sense. This type of dimers may be dubbed "segment swapping dimer," rather than domain swapping dimers. Type (d) of domain swapping is similar to the types (a) and (b) in the 2-state. It cannot form a compact unit along any portion of the chain. Hence, we suggest that this type of dimers be classified as 2-state, rather than domain swapping. The compactness profile provides a computational tool determining whether a dimer has domain/segment swapping. On the other hand, if the compactness profile has a global minimum in the middle, and there are local minima at both ends, the dimer is predicted to be a domain swapping dimer; if the compactness profile has a global minimum in the middle and only one end has a local minimum, the dimer is predicted to be a segment swapping dimer. Lastly, if there is a local minimum in the middle and two global minima at both ends, as shown in Figure 4D, it is possible that a segment in the middle of one chain swaps to form a compact unit with two complementary segments of its sister chain.

Dimer kinetics

The difference between 2-state and 3-state dimers can be revealed in several ways. First, it can be detected directly by kinetics, i.e., whether a monomer has a stable structure on its own in the solvent prior to dimerization. Second, as our early work shows, the differences between the two groups of dimers are also found in energetics, in thermodynamics, and in structures. Hydrophobic effects are generally more important to 2-state complexes than to 3-state ones (Tsai et al., 1997; Xu et al., 1997). The interfacial motif of a 2-state dimer is far more likely to find a monomer in the PDB with a good structural alignment than a 3-state dimer does (Tsai et al., 1997). A 2-state complex is more likely to reach its global minimum state in free energy than a 3-state one (Xu et al., 1997). 2-state binding is similar to folding of a single-domain monomer, a process which typically fits the 2-state kinetics as well (Zwanzig, 1997; Dill & Chan, 1997). In both cases, the formation of the final state from the initial state does not depend on a unique reaction pathway or a stable intermediate. The lack of linkage between two chains in a 2-state dimer is not critical. This is well illustrated by barnase, where an assembly of the polypeptide fragments of the monomer forms a native-like conformation (Kippen et al., 1994). Consistently cutting a chain in dimeric trp repressor still keeps the native dimer structure basically intact (Tasayco & Carey, 1992). On the other hand, a 3-state dimer depends substantially on the kinetic pathway from the denatured monomers to the complex. In particular, it has to go through the intermediate, i.e., the stable monomer state.

Our study sheds some light on the classification of dimers. As seen in Figure 8, many domain swapping dimers are in between 2-state and 3-state. This suggests that the kinetics of their dimerizations is not 2-state. On the other hand, their intermediates are not as stable as the intermediates in the 3-state dimerizations. As shown by the compactness profiles, in the domain swapping dimers of types (b) and (c), only a portion of the chain is folded into a compact unit. The remainder of the chain is not compact in the dimer structure. This suggests that the intermediates of these dimers are most likely monomers having some, relatively stable, structures for the compact domains but no unique structures for the other portions. That is, in the intermediate state, the compact domain of the chain has substantially less structural fluctuations than the non-compact portion. From this standpoint, the definition of 2-state versus 3-state is not clear cut for dimers. There is a broad

Table 5. *3-state protein dimers*[a]

Interface	Molecule	Resol. (Å)	Size (residues)	Reference
1ttqB (sym)	β_2 subunit of Trp synthase	2.0	268	Zetina & Goldberg, 1980
1lmb34	λ repressor	1.8	92	Banik et al., 1992
1tarAB	Aspartate aminotransferase	2.2	401	Herold & Kirschner, 1990
1xsoAB	Superoxide dismutase	1.49	150	Mei et al., 1992
1glqAB	Glutathione S-transferase	1.8	209	Aceto et al., 1992
3ssi (sym)	Serine protease inhibitor	2.3	113	Akasaka et al., 1982
1lynAB	Sperm lysin	2.75	136	Shaw et al., 1995
1timAB	Triose phosphate isomerase	2.5	247	Borchert et al., 1995

[a]The 3-state dimers. The table describes the PDB code with the chain names, name of the molecule, the resolution of the structure, number of amino acids in a single chain, and the reference which shows the dimerization is 3-state.

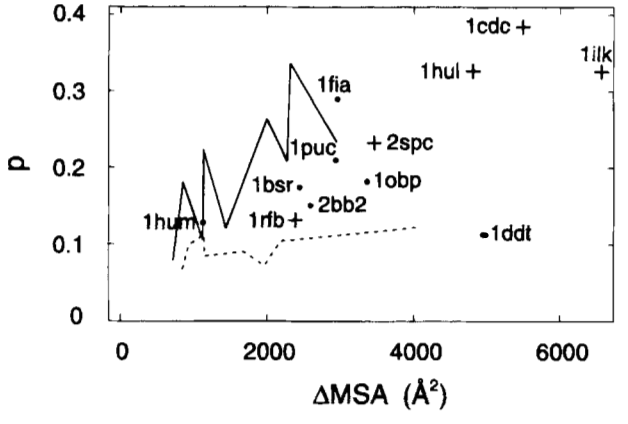

Fig. 8. The relationship between ΔMSA and p for the 2-state dimers (solid line), the 3-state dimers (broken line), and the domain swapping dimers marked by PDB codes (dots and crosses). ΔMSA is the total interfacial buried surface area of the dimer; p is the ratio between ΔMSA and the total surface area of the two unbound monomers in the dimer. Type (d) of the domain swapping dimers are indicated by crosses, and other types are shown in dots. The protein names of marked PDB codes can be found in Table 2.

spectrum in the dimerization: from no intermediate at one end to rigid binding at the other.

Evolution of dimers

The kinetics of dimerization can be revealed in the structures of the dimers. Based on the assumption that the kinetic pathway reflects the evolutionary path (D'Alessio, 1995), we may propose evolutionary pathways from monomer to dimer. Although the kinetics of dimerization takes place on a time scale of seconds while the evolution of dimer may have taken millions of years, both processes share a similarity in terms of energetic stability. The kinetics of dimerization is along the pathway which is optimal among all possible paths governed by the reaction free energy function. The evolution of the dimer has been along the pathway which optimized the biological function of the protein, typically accompanied by the optimization of its energetic stability. Hence, the kinetic pathway of dimerization is likely to follow the same course trodden by evolution. As discussed above, dimerizations have a spectrum of kinetics, from the 2-state with no intermediate, to the rigid binding where the intermediate monomer has the same conformation as that in a dimer. In between, the intermediate may have some structure but different from the one observed in the dimeric configuration. For example, some intermediates have molten globular structures or have stable domains connected by unstable loops. The diversity of the kinetics indicates that evolution may have multiple pathways. According to the types of the kinetics, we suggest three possible pathways.

The first evolutionary pathway of dimerization follows the mechanism which governs the evolution of single-domain monomers, without going through an ancestor monomer. Monomeric proteins have evolved through gene mutation, deletion, and fusion to become foldable and functional proteins. Although a dimer contains two chains, the linkage between the chains is not important in many cases (Tasayco & Carey, 1992; Kippen et al., 1994). The motifs of some dimer interfaces align perfectly with the motifs of some protein monomers. For example, the repressor of primer, a 2-state type (a) dimer shown in Figure 5A, matches well a four-helix bundle motif in the 1rpr monomer (Tsai et al., 1997). The tethered dimer, which consists of two 99-amino acid HIV-PR subunits linked together by a pentapeptide, maintains basically the same structure as the natural HIV-PR dimer (Cheng et al., 1990). We suggest that during evolution, some dimers have evolved through gene mutation, deletion, and fusion, similarly to the monomer evolution. Here, the protein chain was optimized directly for the functional dimer, rather than for any intermediate. The dimers did not have stable monomers in their evolutionary pathways. Types (a) and (b) of the 2-state dimers, and type (d) of the domain swapping dimers, may have evolved through such an evolutionary mechanism, since their dimerization kinetics resembles the folding kinetics of monomers.

The second evolutionary pathway to dimerization is through a stable monomer as an intermediate followed by mutations of surface residues in the monomer. Types (a) and (c) of the 3-state fit into this category. The ancestors of these dimers were structured monomers. Since the monomers were stable in the solvent, mutations of surface residues could effectively make the surface and energetics complementary between two monomers to form a dimer. The interfaces of such dimers are likely to be small, and key residues for the binding are few (Novotny et al., 1989; Cunningham & Wells, 1993). Hence, not many mutations are needed to evolve a dimer from a monomer in this case.

The third evolutionary pathway is through a domain swapping mechanism, as suggested by Eisenberg and colleagues (Bennett et al., 1994; Bennett et al., 1995). This includes types (a) and (c) of domain swapping dimers, and types (c) and (d) of the 2-state dimers. These dimers contain at least one compact domain in a chain. This domain is stable and forms a unique structure in the solvent in the monomeric state. Although no stable intermediate is detected in kinetics for types (c) and (d) of the 2-state dimers, it is likely that under certain solvent conditions or through mutations of a few residues, the compact units in these dimers may be stable in the monomeric form. From the energetics point of view, the swapping between the compact domain and the remainder of the chain, whether stable or unstable, contributes to stabilize the recognition between the two monomers. From the evolutionary standpoint, mutations and deletions of residues may have facilitated the conversion of the protein to be more stable in the domain swapping dimer than in the monomeric form (Bennett et al., 1995). This type of evolutionary process has been nicely demonstrated by deleting six residues in staphylococcal nuclease converting a native monomer to an engineered dimer (Green et al., 1995).

It is also likely that in some cases, two evolutionary pathways took place on the same dimer at different stages. This was probably the case for type (b) of domain-swapping dimers and type (b) of the 3-state dimers. However, unlike type (c) of domain-swapping dimers, which are mostly stabilized by the exchange of the swapping segments, here the exchanged segments in the dimers are small. The small swapping segments help strengthen the dimer, but may not have been its initial evolutionary pathway. This has been illustrated in the case of BS-RNase (Piccoli et al., 1992; D'Alessio, 1995). These dimers may have initially evolved through the second evolutionary pathway. They were further optimized through the domain swapping mechanism at a later stage of their evolution. This two-step evolution is consistent with the kinetics of BS-RNase. There, the dimer conformation with the swapped segments occurs only after the dimer without swapping is formed (Piccoli et al., 1992).

In summary, the domain swapping mechanism is one, but not the only possible evolutionary pathway of protein dimerization. Some domain swapping dimers, which are referred to as such in the literature, actually follow the evolutionary mechanism observed for most 2-state dimers. Such evolution does not go through a stable monomeric ancestor. At the other end of the spectrum, most 3-state dimers have evolved through stable monomers followed by mutations of their surface residues. Some such 3-state dimers may have also experienced domain swapping at a later stage of evolution. We further note that the evolutionary mechanisms proposed and discussed here for dimers, can be applied to other oligomers as well.

Materials and methods

Data set selection

We collected three groups of dimers from the literature. The structures of the dimers are known from crystallography or NMR. The first group includes the ones which were referred to as domain swapping dimers in the literature (see Table 2). The second and third groups include the 2-state and 3-state dimers, as determined in kinetic experiments. These are listed in Tables 3 and 5.

Calculation of the compactness profile

The coefficient of compactness of a structure is defined as (Zehfus & Rose, 1986)

$$C = \frac{MSA \text{ of the structure}}{MSA \text{ of a sphere of equal volume}}, \tag{3}$$

C can be simplified to

$$C = \frac{MSA}{(36\pi V^2)^{1/3}}. \tag{4}$$

MSA and V are the molecular surface area and the volume of the structure, respectively. We employed the molecular surface area (Richards, 1977) rather than the solvent accessible surface area (Lee & Richards, 1971), which was used by Zehfus and Rose (1986), since the former is a more sensitive measurement of the molecular shape.

We have employed the united atom model, which excludes hydrogen atoms, for MSA calculation. The areas and volumes were calculated by the molecular surface (MS) program (Connolly, 1983; Connolly, 1993). The coordinates were obtained from the Brookhaven Protein Database (PDB) (Bernstein et al., 1977). The atomic radii were taken from the CHARMM parameters (Brooks et al., 1983). The solvent probe has a radius of 1.4 Å

Calculation of hydrogen bonds

Hydrogen bonds across the dimer interfaces are analyzed by HBPLUS (McDonald et al., 1993; McDonald & Thornton, 1994). The program determines the positions of missing hydrogens in the PDB and checks each donor-acceptor pair to ascertain whether it fits the geometric criteria as follows: The maximum distances are 3.9 Å between donor and acceptor and 2.5 Å between acceptor and hydrogen; the minimum angles are 90.0 degrees for the angle of donor-hydrogen-acceptor, for the angle of donor-acceptor-acceptor antecedent, and for the angle of hydrogen-acceptor-acceptor antecedent (Baker & Hubbard, 1984). Amino-aromatic hydrogen bonds are not taken into account in our analysis.

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