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**REVIEW**

# Three-Dimensional Domain Swapping in Homooligomeric Proteins and Its Functional Significance

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**Abstract**—In the process of oligomeric structure formation through a mechanism of three-dimensional domain swapping, one domain of a monomeric protein is replaced by the same domain from an identical monomer. The swapped "domain" can represent an entire tertiary globular domain or an element of secondary protein structure, such as an α-helix or a β-strand. Different examples of three-dimensional domain swapping are reviewed; the functional importance of this phenomenon and its role in the development of new properties by some proteins in the process of evolution are considered. The contribution of three-dimensional domain swapping to the formation of linear protein polymers and amyloids is discussed.

*Key words*: three-dimensional domain swapping, oligomeric structure, domains, protein–protein interactions, aggregation

It is known that many proteins in a living cell exist as oligomers composed of identical subunits. A polypeptide chain is often folded in several domains (globular structures formed by different portions of the chain comprising several dozen amino acid residues). In the case of homooligomeric proteins, transition from a monomeric to an oligomeric structure frequently results in altered functional properties. This justifies the considerable interest in the mechanisms by which individual subunits fold into their native conformation and associate into oligomers.

Another problem currently at the focus of much attention concerns the mechanisms responsible for the evolution of oligomeric proteins, which render primitive monomers capable of specifically associating into oligomers with new properties. In recent years a real breakthrough has been made in this area: the discovery of three-dimensional domain swapping as an important mechanism underlying the formation of oligomeric protein structure. The term "domain" in this context can apply, alongside "true" globular structures, also to individual elements of secondary protein structure: α-helices, β-strands, and segments (loops) of the polypeptide chain that connect them. This undeniably elegant mechanism has by now been confirmed in numerous experimental studies; the concept of domain swapping as part of the process of three-dimensional structure formation in oligomers is developing fruitfully.

In this review, three-dimensional domain swapping displayed by different proteins under experimental conditions is considered, and several patterns of native homooligomeric structure formation by this mechanism are described. The role of three-dimensional domain swapping as a mechanism capable of enhancing the functional properties of enzymes is discussed.

## THREE-DIMENSIONAL DOMAIN SWAPPING UPON OLIGOMERIZATION OF MULTIDOMAIN PROTEINS

**Three-dimensional domain swapping observed experimentally.** As noted above, many enzymes (and many other proteins) are composed of several globular domains; the active center of an enzyme is usually located in an interdomain region. In the process of functioning, domains bound to one another via segments of the polypeptide chain ("linkers"), whose function is often performed by loops, undergo ligand-induced movements resulting in the formation of functionally competent active centers.

In 1994, while studying the three-dimensional structure of the diphtheria toxin molecule, Eisenberg and coworkers discovered a new mechanism of oligomer formation that they described as "three-dimensional domain swapping" [1]. Their finding was based on the observation that under certain conditions (low pH), one of the three domains forming a monomer of the diphtheria toxin would break its noncovalent interactions with the two other domains (while still remaining covalently bound

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with the polypeptide chain through a linker), and be replaced by a similar domain from another monomer. In turn, the domain coming from the first monomer would form noncovalent interactions with two domains of the second monomer. A dimer formed in this way appeared to be stabilized by interactions between domains belonging to different subunits.

The discovery of three-dimensional domain swapping gave rise to a series of studies in this field: in the course of the two subsequent years, this mechanism was described for ten other proteins; the number of examples continues to grow [2, 3]. Figure 1 illustrates three-dimensional domain swapping upon association of barnase monomers into a trimer [4]. The native monomer (left) is composed of two domains, an N-terminal domain comprising α-helix 1, loop 1, and α-helix 2 (amino acid residues 1-36), and a C-terminal domain (amino acid residues 42-110). The domains are linked by loop 2. Under conditions which favor a weakening of interdomain interactions, an "open" monomer is formed, with an interdomain interface exposed to the media. Monomers of this sort are capable of domain swapping, which occurs at high protein concentrations and results in the formation of an oligomer whose interdomain interactions are unaltered, yet take place within a trimeric molecule. In this process (see Fig. 1, where identical monomers are shaded differently), the upper monomer transfers its N-terminal domain to the lower monomer, and receives an N-terminal domain from the monomer of the right. In turn, the lower monomer transfers its N-terminal domain to the monomer of the right. The area of contact between the N-terminal and C-terminal domains is similar in a native ("closed") monomer and in a trimer. The active center is formed in the interdomain region; in the case of a trimer at the intersubunit interface.

Both monomeric and trimeric forms are catalytically active and are readily interconverted, and this means that a composite active center, formed in the trimer at subunit junctions and comprising amino acid residues which belong to different monomers, can be converted into a simple active center formed by a single polypeptide chain. Similarly, glyoxalase can exist either as a monomer comprising two domains or as a dimer that arises as a result of three-dimensional domain swapping between monomers [5]. A dimer has composite active centers located at subunit interfaces. The amino acid residues that constitute these active centers belong to neighboring subunits; two residues from each subunit are involved in the coordination of catalytically important zinc ions. In the presence of reduced glutathione (one of the substrates), a dimer slowly dissociates into monomers possessing catalytic activity and containing one zinc ion in the active center. The removal of glutathione results in a re-association of monomers into a dimer. Since both forms are catalytically active, these data suggest, just as in the case of barnase described above, that a composite active center formed through domain swapping may undergo a rearrangement turning it into an active center located at a domain interface within a single subunit. It is interesting that the reversible transition from a monomer to a dimer depends on the presence of reduced glutathione; this is the first case ever described of three-

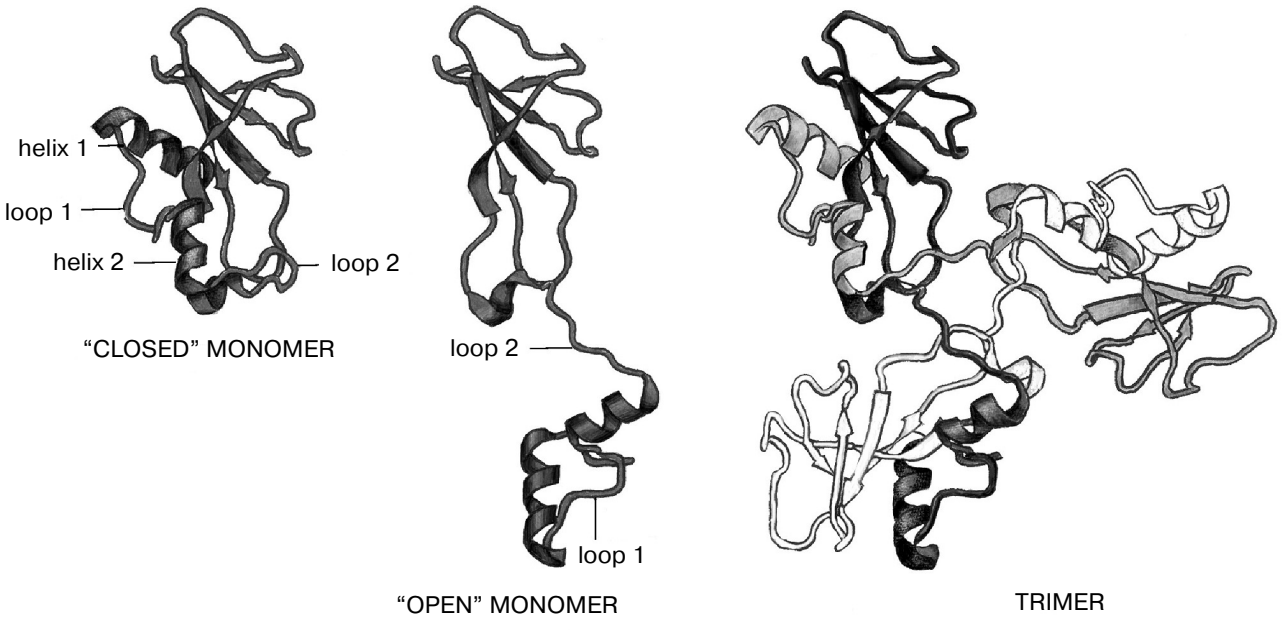


**Fig. 1.** Three-dimensional domain swapping upon association of barnase monomers into a trimer. Identical monomers within a trimer are shaded differently. Taken from [4] with alterations.

dimensional domain swapping being regulated by a low molecular weight effector.

Figure 2A schematically shows the process of three-dimensional globular domain swapping. The interconversion of monomeric and oligomeric forms observed experimentally and described above can be explained by the existence of reversible steps *1* and *2* that lead to the formation of open monomers and their association into oligomers. It should be emphasized that oligomerization of proteins through the mechanism of three-dimensional domain swapping requires only minor structural rearrangements to create the intersubunit contact regions, which actually preexist, since they are identical to the interdomain contact regions within initial monomers; monomeric and oligomeric forms have comparable free energies. At the same time, since the oligomerization process is coupled with the formation of open monomers, which requires the rupture of interdomain interactions, it needs energy to overcome a substantial barrier. This explains the slow rate of this process and its dependence on factors lowering the energy barrier (e.g., low pH values).

Progress in the application of genetic engineering methods, aimed in particular at structural modification of the loops connecting different elements of the protein secondary structure, combined with crystallographic analysis of the three-dimensional structure of the mutants obtained, led in the middle of the 1990s to the discovery

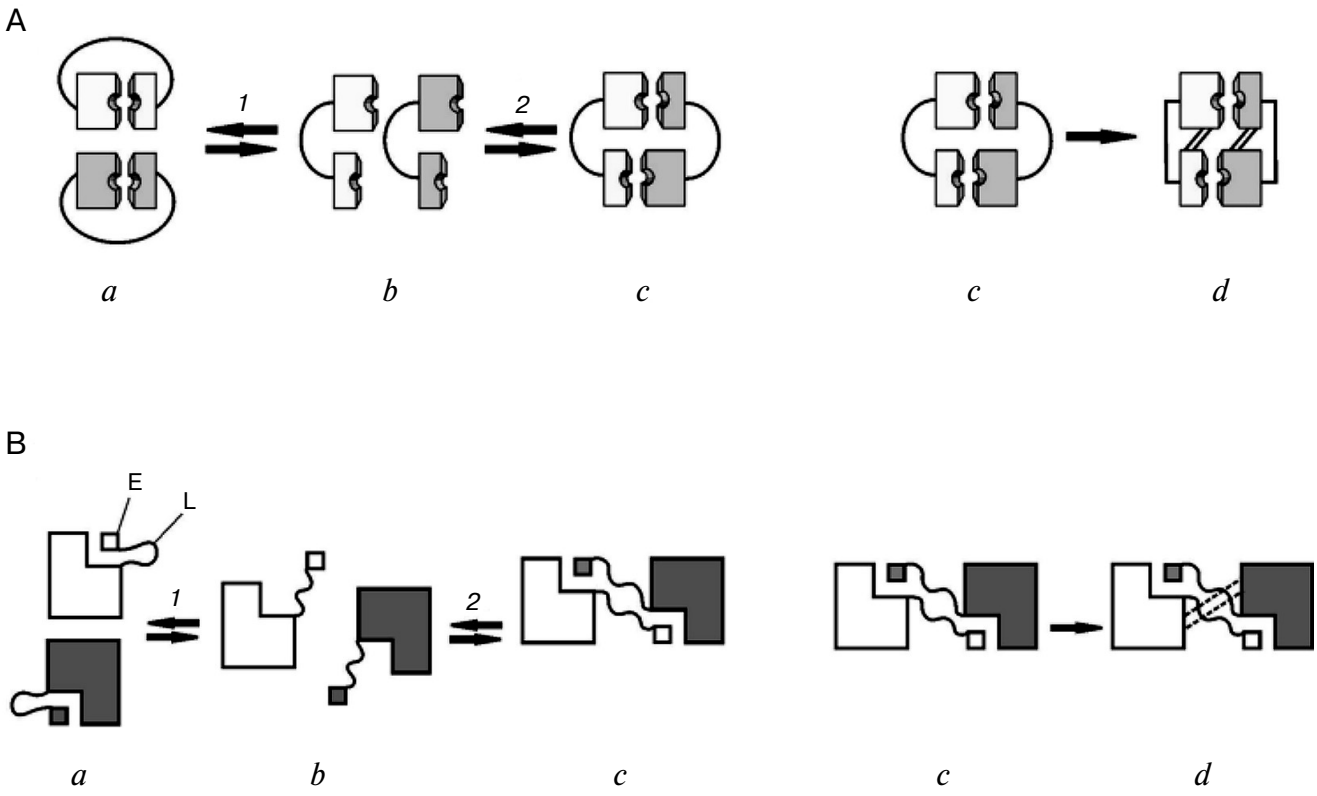


**Fig. 2.** Schematic representation of three-dimensional domain swapping. A) Association of monomers into a dimer upon swapping of globular domains: *a*) closed identical monomers shaded differently. The domains are connected by a linker (loop). The active center (shown by two semicircles) of each monomer is formed at the interdomain interface. Protein–protein interactions between monomers are absent; *b*) open monomers. Under conditions weakening interdomain interactions (lower pH, temperature change) the interdomain regions become exposed to the medium. This is a prerequisite for the formation of interdomain contacts between different monomers, i.e., for three-dimensional domain swapping. As a result, dimer *c* is formed, in which intersubunit contacts are simultaneously interdomain ones. In dimer *c*, the subunits are held together by noncovalent interactions between domains, which are not different in any way from those existing within the initial monomers *a*; this is why steps *1* and *2* are reversible. The transition from the metastable *c* form to the stable *d* form is suggested hypothetically as the way present-day homooligomers with active centers located at the subunit interface are created. The resulting oligomers may be stabilized by different mechanisms (see text). B) Association of monomers into a dimer by three-dimensional swapping of protein secondary structure elements: *a*) closed monomers; E, a secondary structure element (α-helix, β-strand, or their combination) occupying its position in the three-dimensional structure of a monomer; L, a loop connecting the above secondary structure element with the rest of the polypeptide chain; *b*) open monomers; *c*) a dimer stabilized by the interaction of secondary structure elements belonging to one monomer, with the second monomer. As in the case of globular domains (A), steps *1* and *2* are reversible, and dimer *c* is metastable. In the right-hand portion of the figure, the transformation of a metastable dimer into a stable present-day oligomer *d* is shown, which may have occurred over time as a result of mutations favorable to the strengthening of intersubunit interactions.

of a mechanism of protein oligomerization based on the swapping of small elements of the secondary structure between separate monomers. Such a mechanism, shown schematically in Fig. 2B, was first explicitly recognized in the study of staphylococcal nuclease [6]. The deletion of a small segment in the long loop connecting the C-terminal α-helix with the rest of the monomeric enzyme molecule resulted in a weakening of the noncovalent interactions which held this helix in its position in the native structure, and to its stripping, accompanied by the formation of an open monomer (Fig. 2B, *b*).

The shortening of the loop, which precluded a correct orientation of the C-terminal α-helix within a monomer, did not however prevent interactions between this helix and complementary structural elements of the neighboring open monomer, which remained free after the stripping of its own α-helix. These interactions turned out to be very strong and caused the formation of a dimer (Fig. 2B, *c*), whose structure, when determined by X-ray crystallography analysis, confirmed that it had indeed been formed by the swapping of C-terminal α-helices between monomers. Further studies in this direction carried out on native proteins have shown that the swapping of secondary structure elements (in all cases described to date, these are N-terminal or C-terminal portions of the molecule) may be considered, along with globular domain swapping, as a probable mechanism of oligomeric protein formation. The term presently accepted for both these mechanisms is "three-dimensional domain swapping" [2].

**Three-dimensional domain swapping as a mechanism for the enhancement of the functional properties of enzymes.** The experimental evidence of three-dimensional domain swapping has shed light on the possible mechanisms of oligomeric structure formation in a number of present-day proteins, including enzymes whose active sites are located at subunit junctions. It seems plausible that such homooligomers may have evolved from initial monomers through the formation, at the first stage, of metastable oligomers similar to those observed experimentally (form *c* in Fig. 2A). If the production of such primitive oligomers were accompanied by the acquisition of new properties conferring an advantage on the organism, mutations that stabilize the oligomeric structure would be favored in natural selection. A hypothetical mechanism describing the emergence of stabilized forms (*d*) is shown in Fig. 2A, right; it presumes that amino acid replacements arise in the subunit interface region and in the loop connecting the domains, but allows for other possibilities as well.

Such a mechanism could be at the origin of many present-day oligomeric proteins whose intersubunit contacts serve as interdomain contacts at the same time; enzymes built in this manner have composite active centers which include amino acid residues belonging to neighboring subunits (aspartate transcarbamoylase, glutamine synthetase, catalase, and many other enzymes). The fact that the interdomain interface, where active center is usually located, also becomes an intersubunit interface in an oligomer creates additional potential for regulation. Let us consider such a possibility, taking aspartate transcarbamoylase as an example.

*Aspartate transcarbamoylase* from *E. coli* is a well-known allosteric enzyme [7-9] composed of two catalytic trimers and six regulatory subunits. Each active center in a catalytic trimer is formed by amino acid residues belonging to the aspartate-binding domain of one monomer and the carbamoylphosphate-binding domain of the neighboring monomer. Each regulatory subunit forms contacts with the catalytic subunits composing each of the trimers. Upon binding of substrates, the oligomeric enzyme molecule undergoes a concerted conformational transition from the T state to the R state. As this takes place, the domains participating in the formation of active centers draw closer to each other, while the distance between catalytic trimers increases.

Recently, it was shown that for conformational transition to occur it appears sufficient that just one of the six active centers be filled with specific ligands [9], which suggests a very high degree of subunit cooperativity. The location of the active centers at the interfaces between subunits constituting the trimers can be regarded as a structural feature important for a concerted change of their conformation. However, this alone does not seem to suffice since isolated trimers, out of contact with regulatory subunits, lose allosteric properties and can exist only in the R state. Thus, the regulatory subunits play an important structural role, ensuring the integrity of the complex system of interdomain interactions necessary for the enzyme to function.

Under the hypothetical scheme shown in Fig. 2A, the advent of regulatory subunits in the process of evolution can be seen as the factor responsible for the formation of a stable enzyme from "primitive" trimers which, due to three-dimensional domain swapping, had the potential ability to undergo concerted conformational transitions.

*Glucose-fructose oxidoreductase*. Another example illustrating the functional advantages acquired by an enzyme whose oligomeric structure is formed by three-dimensional domain swapping is glucose-fructose oxidoreductase. This enzyme, isolated from the bacterium *Zymomonas mobilis*, catalyzes NADP-dependent oxidation of glucose to gluconolactone with subsequent reduction of fructose to sorbitol. According to X-ray crystallography data [10], each of the monomers in the tetrameric enzyme molecule is folded in two independent domains tightly bound to one another. NADP(H) is located between the domains, its adenine portion covered by an N-terminal loop belonging to a neighboring subunit.

Thus, tetramer formation occurs owing to three-dimensional swapping of N-terminal loops between adja-

cent monomers [10-12]. These loops, or "arms", extend from each subunit and wrap around a portion of the nucleotide-binding domain of a neighboring subunit, reducing the solvent exposure of NADP. The protein's area of contact with the N-terminal loops is quite extensive; it amounts to 78% of the entire surface that is buried on tetramerization. A deletion of the N-terminal loops ("arms") leads to the dissociation of the tetramer into dimers, and also greatly increases the solvent exposure of the adenine portion of the coenzyme molecule as a result of the disruption of three hydrogen bonds with the protein. The weakening of the protein–cofactor interaction causes considerable changes in the functional properties of the enzyme: it loses the ability to catalyze the fructose-reductase reaction and converts into a glucose dehydrogenase [11].

These results lead to the conclusion that three-dimensional swapping of the N-terminal loops, which ensures a sufficiently tight fixation of NADP and its shielding from the solvent, provides the means to hold the cofactor in complex with the enzyme throughout the catalytic cycle, including its reduction in the glucose oxidation reaction and subsequent oxidation in the fructose reductase reaction. According to the scheme in Fig. 2B, glucose-fructose oxidoreductase can be counted among the proteins whose properties correspond to case *d*, since a tetramer is the only stable present-day form of this enzyme.

*Nitric oxide synthase.* The dimeric nitric oxide synthase molecule shown schematically in Fig. 3 was probably formed as a result of three-dimensional swapping of globular domains between initial monomers. The enzyme consists of two domains: the N-terminal oxygenase domain containing heme (Fe), tetrahydrobiopterin ($H_4B$), and arginine (ARG) binding sites, and the C-terminal reductase domain that binds FAD, FMN, calmodulin (CAM), and NADPH. To synthesize NO, NADPH-derived electrons must transfer from the reductase domain flavins to the oxygenase domain heme irons [13]. Despite the fact that a monomer of the enzyme contains both domains (the reducing and the oxidizing ones), nitric oxide synthase is only active as a dimer.

Investigation of the properties of different heterodimeric mutant forms of the enzyme comprising full-length subunits and isolated domains has shown that flavin-to-heme electron transfer proceeds exclusively

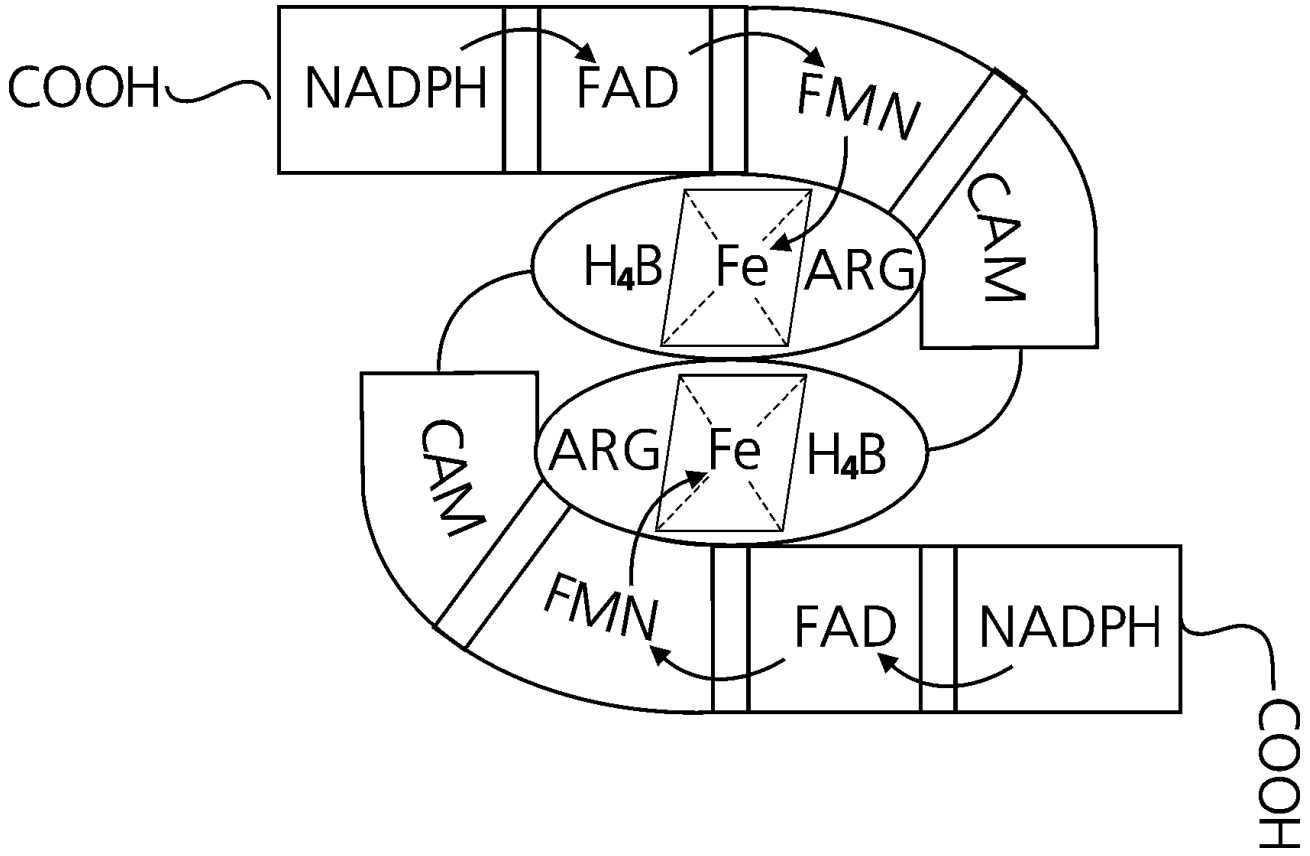


**Fig. 3.** Proposed arrangement of the reductase (C-terminal) and oxygenase (N-terminal) domains of dimeric nitric oxide synthase. As a consequence of the swapping of oxidase domains between monomers, a functionally active dimer is formed, capable of transferring electrons from NADPH to the heme irons. See text for details. CAM, calmodulin. Taken from [13] with alterations.

between adjacent subunits in the dimeric species, indicating that the functionally important domain–domain interaction takes place between the reductase domain of one monomer and the oxygenase domain of the other [13]. In this way, electron transfer is directed from NADPH, bound in the reductase domain of one monomer, to the heme iron located in the oxygenase domain of the adjacent monomer (see Fig. 3). The architecture of the nitric oxide synthase dimer formed by domain swapping probably allows for an alignment of the reductase and oxygenase domains which is best for NO synthesis and which cannot be achieved while these domains are in direct contact within a single monomer.

*Ribonuclease.* As early as 1962, pancreatic ribonuclease (RNase A) was found to be capable of forming dimers and more complex oligomers upon lyophilization in 50% acetate; the results of chemical modification experiments suggested that dimerization might have occurred through swapping of N-terminal chain segments between monomers [14]. After 36 years, this suggestion was confirmed by X-ray crystallography data, which showed a three-dimensional swapping of N-terminal α-helices (amino acid residues 1-15) [15]. Later it was found that along with dimers of this kind, another type of dimers could also be formed, considerably different in their physicochemical properties [16]. Subsequent analysis revealed an interesting feature of these dimers: they appeared to be formed by three-dimensional swapping of C-terminal segments of the polypeptide chain (the β-strands including amino acid residues 116-124) [17]. In both cases, domain swapping led to the formation of composite active centers, since the functionally important amino acid residues His12 and His119 are located in the N-terminal and the C-terminal portions of the chain, respectively; the dimers retained full activity.

Figure 4A is a schematic representation of two possible ways in which three-dimensional domain swapping can occur between monomers of pancreatic RNase under conditions which favor a weakening of the contacts formed by the terminal segment of the chain with the rest of the molecule (low pH values). A different case is that of bovine seminal ribonuclease (BS RNase), whose special characteristic is the existence of a dimeric form resulting from an exchange of N-terminal α-helices under physiological conditions [18]. As shown in Fig. 4B, this form (M × M) exists in equilibrium with another dimeric form of the enzyme, created without domain swapping (M = M).

The ability of BS RNase to form stable dimers has to do with the peculiarities of its primary structure. A comparison with bovine pancreatic RNase reveals 23 amino acid substitutions in each subunit, including two cysteine residues at positions 31 and 32. These form two intersubunit disulfide bridges with cysteine residues (32′ and 31′, respectively) of the other subunit. The subunits constituting the M = M form have independent active centers and are stabilized within the dimer by disulfide bridges. The somewhat greater stability of the M × M form compared to the M = M form is reflected in the fact that an equilibrium ratio of 2 : 1 is observed between these forms in solution.

The existence of equilibrium between two different forms of dimeric BS RNase suggests that a continuous unfolding/refolding of structural elements into two alternative conformations takes place in the native protein. The unusual characteristics of BS RNase raise several questions. What is the biological role of the dimeric forms of the enzyme? Are there any functional differences between the M = M and M × M forms? How can the coexistence of these forms be explained? It is believed that an explanation can be given in evolutionary terms, assuming that present-day seminal RNase could be a product of an evolutionary experiment yet to be completed [19]. Transition from the M = M form to the M × M form is accompanied by the formation of a composite active center comprising amino acid residues from adjacent subunits, which opens up new possibilities for interaction between active centers, and hence for allosteric regulation. Actually, the M × M form has been shown to be allosterically regulated [20, 21] and to exhibit antitumor, immunosuppressive, and antiviral activities [22-24]. One may think therefore that "evolution is still experimenting with this protein" [19], endowing it with new properties and a more complex structure.

**Three-dimensional domain swapping as an element of the mechanisms of cell cycle regulation.** *Cell cycle regulatory proteins.* The progression of the cell cycle is regulated by the activity of cyclin-dependent kinases, which is controlled by the binding of cell cycle regulatory proteins. Among these is CksHs2 of human tissue. The polypeptide chain of CksHs2, containing 79 amino acid residues, is folded in a 4-stranded antiparallel β-sheet with two short α-helices [25]. In the dimer, the fourth β-strand (i.e., the C-terminal segment comprising amino acid residues 66-79) is extended and exchanged for an identical strand from the other subunit. The domain-swapped dimer structure is stabilized by the binding of metal ions ($Zn^{2+}$). Glu63 residues located in a hinge loop connecting the third and fourth β-strands in each subunit contribute to a metal-binding site in the dimer.

It is significant that the monomeric form of CksHs2 is incapable of binding $Zn^{2+}$. In the absence of metal ions, the dimeric structure is destabilized by electrostatic repulsion between Glu63 residues of the adjacent subunits. At neutral pH, metal ion binding compensates this repulsion, stabilizing the dimer. It is suggested that ion-regulated dimerization produces structures whose interaction promotes the assembly of a hexamer that is probably the functional unit of CksHs2 [25]. Therefore, oligomerization through three-dimensional domain swapping in CksHs2 and some other cell cycle regulatory proteins can be regarded as an important element of cell cycle control mechanisms.

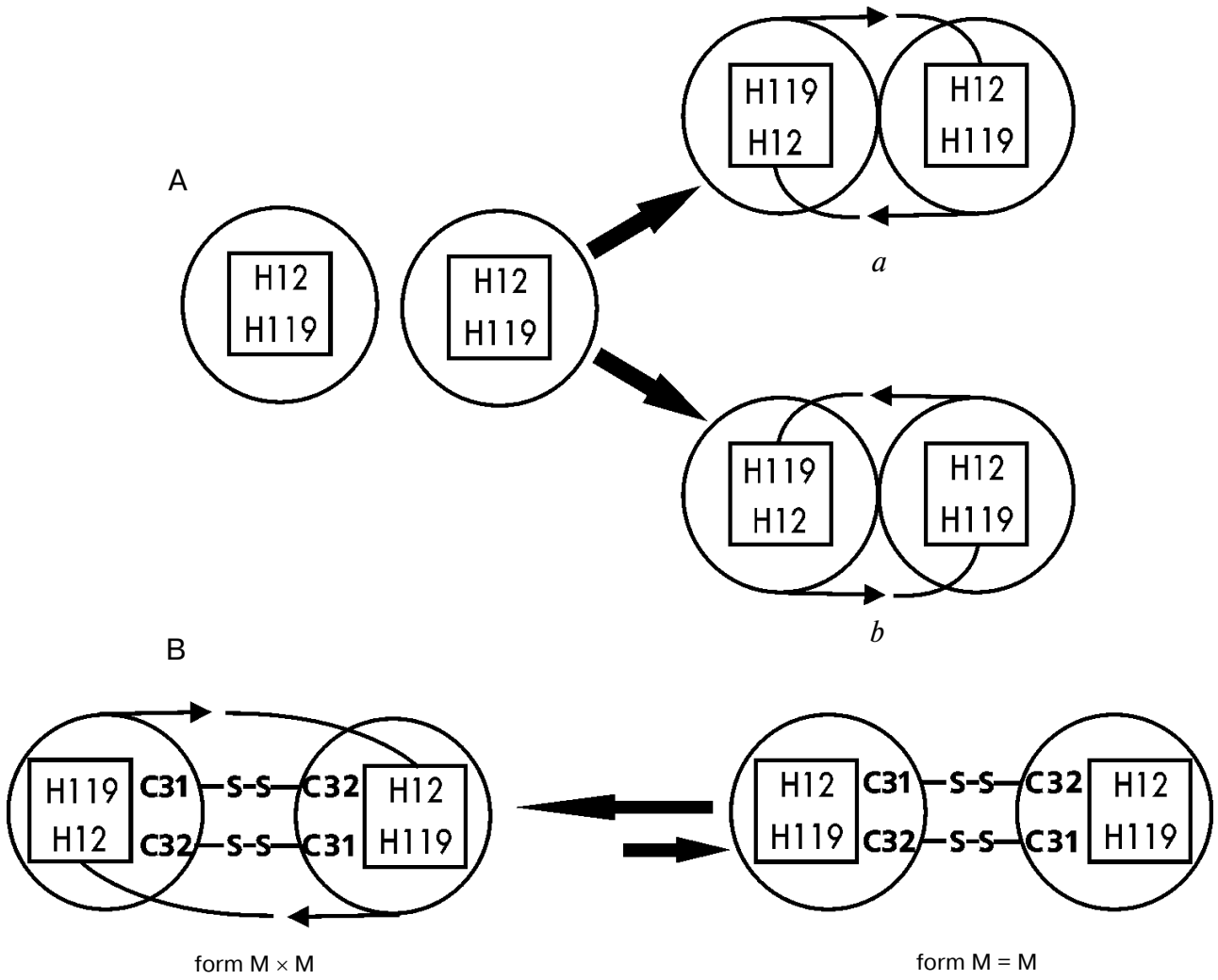


**Fig. 4.** Schematic structures of different RNase forms. A) Pancreatic ribonuclease. The active center of the monomeric enzyme form (depicted as a square) comprises His12 and His119. Under certain conditions, the N-terminal α-helices containing His12 swap between two monomers to form a dimer with composite active centers (*a*). Alternatively, C-terminal β-strands containing His119 can swap between two monomers (*b*). B) Bovine seminal ribonuclease. See text for details.

Cell-cycle regulation in budding yeast *Saccharomyces cerevisiae* requires highly regulated transitions that are driven by the activation of the Cdc28 cyclin-dependent kinase (Cdk) [26-28]. *In vivo*, Cdk interacts with a number of proteins, including Cks1, which associates with cyclin–Cdc28 kinase complexes [29]. The biological function of Cks1 can be modulated by switching between two distinct forms: the monomer and the intersubunit β-strand-interchanged dimer, whose crystal structure was determined recently [30]. Comparison of this Cks1 domain-swapped dimeric structure with that of CksHs2 (see above) reveals that β-strand swapping between domains is a conserved attribute in this essential cell cycle protein and, as such, is likely to be functionally important for regulating interactions with cyclin-dependent kinases or other protein targets.

There is reason to think that three-dimensional domain swapping may be involved in the realization of other mechanisms regulating cellular activity. Such a possibility is considered, for example, in a study on the properties of the Spo0A protein, the key regulator of development (sporulation) in *Bacillus* [31].

## THREE-DIMENSIONAL DOMAIN SWAPPING AS A POSSIBLE MECHANISM FOR THE FORMATION OF LINEAR POLYMERS AND AMYLOIDS

The examples of three-dimensional domain swapping discussed above illustrate the mechanism involved in

the formation of closed structures where all interdomain contact areas specifically interact with their partners. At the same time, a number of observations suggest that under certain conditions three-dimensional domain swapping can give rise to "open-ended" linear oligomers, i.e., oligomers with an interdomain contact area exposed to the medium. As shown in Fig. 5A, they can serve as nucleation sites for the elongation of the polymer chain.

Eisenberg and coworkers, who proposed a mechanism of open-ended oligomer formation by three-dimensional domain swapping [2, 3], considered the possibility of it being realized under conditions favoring the production of large protein aggregates and amyloid structures. This idea is supported by the fact that three-dimensional domain swapping and amyloid fiber formation share common features: 1) both are highly specific in that they involve a single type of protein; 2) both amyloid-forming proteins and domain-swapping proteins have two stable forms, separated by a high-energy barrier; and 3) both amyloid structures and domain-swapped oligomers can form linear aggregates [17].

Evidence for three-dimensional domain swapping as a mechanism of amyloidogenic protein oligomerization was obtained in a recent study on human cystatin C [32]. The polypeptide chain of this monomeric protein (120 amino acid residues) is folded in two subdomains connected by a short linker: the N-terminal subdomain which consists of a short strand $\beta_1$, a long helix $\alpha_1$, and a long strand $\beta_2$, and the C-terminal subdomain comprising three antiparallel strands $\beta_3$–$\beta_5$. The physiological function of native monomeric cystatin C is to reversibly inhibit cysteine proteases. In pathological processes, it gains the capacity to oligomerize; the formation of stable oligomeric forms is most pronounced in the event of "conformational diseases" associated with the L68Q mutation which causes an aggregation of the protein in the brain arteries of elderly people with amyloid angiopathy.

The study of the three-dimensional structure of different cystatin C forms has helped clear up the mechanism of this protein's dimerization and shed light on the question of how complex polymers and aggregates are

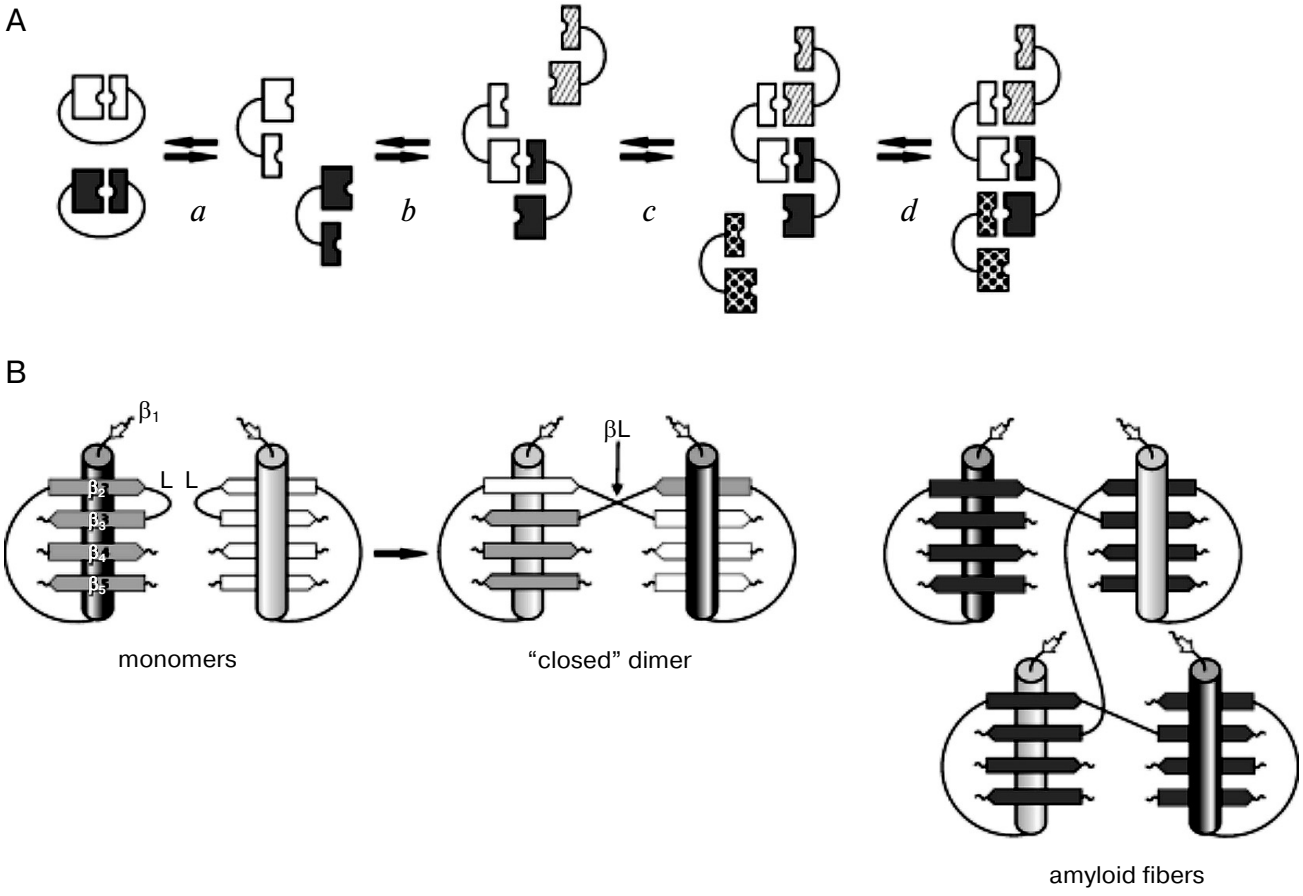


**Fig. 5.** A) Three-dimensional domain swapping as a mechanism of linear polymer formation. All monomers are identical; *a*) formation of open monomers requiring energy for the opening of two closed monomers; *b*, *c*, *d*) elongation of the chain, which can proceed autocatalytically. B) Proposed mechanism of cystatin C oligomerization resulting in the formation of stable dimers and amyloid structures. L, the loop-linker; βL, the β-sheet formed by two linkers, each of which connects $\beta_2$- and $\beta_3$-strands. See text for details.

generated [32]. A swapping of N-terminal subdomains between monomers, whereby a long $\beta_2$-strand of one subunit incorporates into the β-sheet formed by the $\beta_3$–$\beta_5$ strands of the C-terminal subdomain of another subunit, was found to constitute the structural basis of dimer formation. A dimer produced in this way becomes stabilized not only by the interaction between the N-terminal and C-terminal subdomains of the two subunits, but also by hydrogen bonds formed between two linker regions (amino acid residues 56-59), which create a short β-sheet (see Fig. 5B).

Replacement of Leu68 by glutamine, as in the naturally occurring L68Q mutant, results in a weakening of subdomain interactions accompanied by the appearance of open monomeric forms. This in turn opens up possibilities for oligomerization to proceed by three-dimensional domain swapping. It is suggested that the resulting closed symmetric dimer is a dead-end product in the oligomerization pathway, not involved in the process of higher oligomerization. Unhampered chain-like oligomerization could start with the reconstruction of just one α-β-domain, leaving the other two α- and β-structures available for interaction with additional monomers, as shown in Fig. 5B.

A great stride toward elucidating the molecular mechanisms of neurodegenerative diseases was made in the recent crystallographic study of the human prion protein [33]. It is known that prion diseases, or spongiform encephalopathies, are associated with the conversion of a monomeric, protease-sensitive prion protein, $PrP^C$, into a protease-resistant, oligomeric form, $PrP^{Sc}$. Spectroscopic data show that $PrP^C$ is highly α-helical, whereas $PrP^{Sc}$ has a largely β-sheet structure [34]. Solution NMR studies of recombinant prion proteins from different species yielded similar, predominantly α-helical, monomeric structures. However, X-ray crystallography analysis of crystallized human prion protein unexpectedly showed it to be a dimer [33].

Each monomer comprised three long α-helices: helix 1 is at the dimer interface, and helix 2 is linked by an interchain disulfide bridge to the C-terminal helix 3 from the other polypeptide chain in the dimer. The existence of an interchain disulfide hydrogen bond has been confirmed by SDS-PAGE of dissolved crystals. It is interesting that a freshly prepared sample of the purified protein contained no covalent dimer. Incubation of the protein in solution at room temperature resulted in a time-dependent appearance of the dimeric form of the prion protein [33].

Having considered these data along with the fact that the crystallization process takes several weeks, the authors concluded that dimerization of the initially monomeric protein occurred during the crystallization process. A comparison of X-ray crystallography data obtained on the dimeric protein with the solution NMR structure of the human prion protein monomer revealed that the crystal structure can best be described as a three-dimensional domain-swapped dimer [33]. The data indicated that in the crystal structure α-helix 3 of one monomer packs against α-helix 2 of the other monomer in the dimer rather than against its own polypeptide chain. In other words, the monomers exchange their C-terminal α-helices.

The polypeptide chain region consisting of residues Val189-Phe198 crosses the dimer interface and connects the core of each monomer to the extended, swapped α-helix 3. Whereas in the NMR-derived structure of the monomer α-helix 2 ends at Gly195, in the crystal structure of the dimer the last turn of α-helix 2 has unwound to Val189. At the same time, α-helix 3 starts with Thr199 in both the monomer and dimer structures. The polypeptide chain segment between helices 2 and 3 (amino acid residues Thr190-Phe198) forms a short β-strand followed by a single helical turn in the dimer structure. The two β-strands in the switch regions of the two polypeptide chains come together at the dimer interface to form a two-stranded, antiparallel β-sheet with six main chain hydrogen bonds.

Dimerization of a prion protein via the domain swapping mechanism requires a dramatic conformational transition to occur: first, the intrachain disulfide bonds in the monomers (between helices 2 and 3) must be reduced; second, the two helices 3 must move across the dimer interface to swap and pack against the other half of the dimer, and third, the two disulfide bridges must re-form between polypeptide chains.

It has been shown that all of the amino acids altered in human spongiform encephalopathies are concentrated in the prion protein in the swapped helix 3, the neighboring helix 2, and in the switch region between these helices. The mutations are located at the C-terminus of helix 2, next to the β-strand formed by the unwinding of the last helical turn that exists in the monomeric structure. This lends support to the idea that amino acid replacements in this region may affect the equilibrium between the monomer and the dimer.

The paper on human prion protein [33] discussed above presents the first high-resolution view of a structural transition in a prion protein associated with the appearance of its dimeric form. It is suggested that the presence of dimers could accelerate the emergence of a nucleus that acts as a seed for the formation of highly ordered $PrP^{Sc}$ aggregates. The discovery that dimerization of the prion protein proceeds by means of three-dimensional domain swapping is particularly interesting in view of the results obtained with human cystatin C. As discussed above, this amyloidogenic protein can also form a three-dimensional domain-swapped dimer, which suggests that a common hypothesis may be applied to explain the mechanisms of oligomerization of both proteins.

In the last few years a new concept has gained ground whereby the ability to form amyloids is not specific to the

small group of proteins involved in amyloid diseases [35-37]. This idea arose fortuitously from an NMR study of the SH3 module of phosphatidylinositol 3-kinase, which has no connection with any known disease [38]. At low pH, where the protein is partially unfolded, the solution turned into a viscous gel after several hours. When this gel was examined by electron microscopy and other techniques, it was found to contain well-defined fibrils with all the characteristics of those associated with the amyloid diseases [36]. The formation of similar fibrils has also been observed in studies on several other proteins under conditions favoring partial unfolding and at high protein concentrations.

On the basis of this information, Dobson and coworkers [37] proposed that every protein may form amyloid fibrils at high concentration under partially destabilizing conditions. To test this hypothesis, solution conditions were designed to see if fibrils could be deliberately formed from a soluble protein not associated with any disease [36, 37]. A small α-β protein, acylphosphatase, was incubated in a solution containing moderate concentrations of trifluoroethanol—a solvent known to denature proteins generating partially unfolded states, where hydrogen bonds between peptide groups are still stable. After several days under these conditions, the protein formed amyloid fibrils with all the characteristics of those associated with disease [35, 37].

Assuming that the molecular mechanism of amyloid fibril formation is associated with three-dimensional domain swapping, one is led to conclude that the potential ability of many proteins to form amyloid fibrils depends upon their capability of domain exchange. In one of his recent papers, Eisenberg states this idea as follows: "We propose that every protein may be domain-swapped at high concentration under partially destabilizing conditions" [17]. The possibility of this process occurring *in vivo* can depend, at least in part, on a breakdown of normal control and regulation mechanisms within a living organism.

In conclusion, the discovery of three-dimensional domain swapping has led to a breakthrough in the development of modern views on the general principles of protein structure. It shed light on one of the mechanisms involved in forming the homooligomeric structure of proteins and in the associated alteration of their functional properties.

The studies on three-dimensional domain swapping contributed to the understanding of interrelationships between the mode of subunit association within an oligomeric enzyme molecule and the ability of isolated subunits to serve as catalysts. Since the active centers of multidomain enzymes are usually located in interdomain regions, three-dimensional domain swapping results in the formation of composite active centers, as shown in the several examples cited in this review. Of particular interest are the cases (barnase, ribonuclease, glyoxalase) which demonstrate that a reversible transition from a monomer whose active center is located in the region of *intramonomeric* domain interactions, to an oligomer with active centers in the region of *intersubunit* domain interactions is not accompanied by enzyme inactivation. Hence it follows that a composite active center comprising amino acid residues from different subunits can be rearranged into a simple active center located within a single monomer.

The discovery of the three-dimensional domain swapping phenomenon has also contributed significantly to the development of several lines of research aimed in particular at elucidating the molecular mechanisms of diseases associated with the formation of complex aggregates and amyloids.

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