

## Minireview

# Interdomain interactions in oligomeric enzymes: creation of asymmetry in homo-oligomers and role in metabolite channeling between active centers of hetero-oligomers

Natalya K. Nagradova\*

*A.N. Belozerski Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, Russia*

Received 9 October 2000; revised 23 November 2000; accepted 27 November 2000

First published online 5 December 2000

Edited by Vladimir Skulachev

**Abstract** Interdomain interactions play an important role in the structural organization of many enzymes and the conformational flexibility of their molecules. In this review, the role of intrasubunit and intersubunit domain–domain interactions in the origins of pre-existent asymmetry of homo-oligomeric D-glyceraldehyde-3-phosphate dehydrogenase and tryptophanyl-tRNA synthetase is discussed on the basis of recent X-ray data and other available information about the properties of these and related enzymes. In addition, a novel key function of interdomain interactions is considered: their potential contribution to intramolecular channeling of intermediates between active centers located on different subunits of a hetero-oligomeric enzyme ( $\alpha,\beta$ -heterodimeric carbamoyl phosphate synthetase). © 2001 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.

**Key words:** Pre-existent asymmetry in oligomeric protein; Domain–domain interaction; Half-of-the-sites reactivity; Conformational flexibility; Metabolite channeling; D-Glyceraldehyde-3-phosphate dehydrogenase; Tryptophanyl-tRNA synthetase; Carbamoyl phosphate synthetase

## 1. Introduction

It has long been recognized that proteins exhibit a molecular architecture consisting of globular and compact substructures (structural domains) that may be autonomous folding units [1–3].

Rossmann and Liljas were the first to demonstrate that different domains of some proteins have different functions (e.g. NAD-binding and catalytic functions in a number of dehydrogenases [4]). Since that time, a large body of structural information has accumulated on multidomain enzymes. It became clear that one of the most important consequences of a monomer's multidomain organization is that the active center is typically located at the interface between the domains, which provides the structural flexibility needed for the enzyme to function. The binding of a substrate to one of the domains induces appreciable (sometimes large-scale) structural changes, which involve domain displacements and result in the closure of the interdomain cleft to shield the substrate

from water by surrounding it with catalytic residues [5]. Consequently, domain motions provide a mechanism by which the induced fit phenomenon described by Koshland [6] can be accomplished. Structural mechanisms for domain motions in proteins are delineated and analyzed in the excellent review by Gerstein et al. [7].

Association of identical monomers into oligomers considerably increases the complexity of the system of interdomain interactions: in addition to intrasubunit interactions involved in the formation of the active center of each monomer, intersubunit contacts arise between domains belonging to different monomers. In this way, a certain region of each domain becomes involved in the formation of the active center, whereas another region provides the basis for contact with the neighboring monomer. This is rather important given the nature of domains as relatively independent globular structures which are able to change conformation cooperatively and to move around as 'rigid bodies' [7]. Thus, a movement of a domain induced, for instance, by the binding of substrate in the active center, may in a certain way affect the intersubunit contact region. On the other hand, even minor changes in the intersubunit contact area formed by domains may bring about significant alterations in the active centers which are formed at the interface between other regions of the same domains. This creates the structural basis for a stringent correlation between the state of intersubunit contacts in an oligomer and the functional state of the active centers.

In the case of homo-oligomers, the above effect may manifest itself, for example, in the interconversion between the symmetric and asymmetric states of the macromolecule: the active centers may become conformationally non-equivalent. In the first part of the present review, data on three cases of pre-existing asymmetry of homo-oligomers are summarized and an attempt is made to characterize the molecular mechanisms underlying the manifestation of this property. The second part focuses on the functional consequences of interdomain interactions in a hetero-oligomeric enzyme, in order to highlight a role played by domain movements in the channeling of intermediates between active centers located at considerable distances from each other in the enzyme molecule.

## 2. Homo-oligomers

### 2.1. D-Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

Despite the fact that this tetrameric enzyme is composed of

\*Fax: (7)-095-9393181.  
E-mail: serene@cityline.ru

chemically identical subunits, and all its active centers are equivalent in terms of catalyzing the physiological reaction, under certain conditions (reaction with bulky substrate analogs or modification of the essential cysteine residues by some reagents) the active centers exhibit a pairwise non-equivalence (half-of-the-sites reactivity). Another characteristic property of GAPDH isolated from many sources is negative cooperativity upon binding of the coenzyme [8–12]. To explain this behavior, Bernhard, MacQuarrie and Seydoux [8,9] have offered the pre-existing asymmetry model, whereby the holo-tetramer exists in an equilibrium between the symmetric and the asymmetric forms, which can be shifted towards the asymmetric form by the binding of a specific ligand. An alternative model elaborated by Koshland et al. was based on the assumption that asymmetry could be induced upon substrate-binding to the holo-form which is symmetric [10].

Long-term experimental studies performed in succeeding years have yielded a considerable body of information on subunit non-equivalence in GAPDH [13–20], but the principal question concerning the structural basis of this phenomenon remained unanswered. It was not sufficiently clarified even by a direct crystallographic demonstration of molecular asymmetry in an abortive ternary complex, in which a lobster muscle GAPDH–NAD complex was covalently linked with the substrate analog, 3,3,3-trifluorobromoacetone [21]. The results did not lend themselves to an unambiguous interpretation in view of the possibility that the asymmetry might have been a ligand-induced effect.

One of the first demonstrations of pre-existent asymmetry of the apo-GAPDH observed with the physiological substrate was obtained in the studies by our laboratory, which showed that selective chemical modification of Arg 231 in all four active centers of rabbit muscle GAPDH stabilizes the tetramer in the asymmetric state where only two active centers are able to catalyze the physiological reaction, albeit with lower activity (about 5% of the activity of the unmodified enzyme) [22,23]. Since all four arginine residues had been equally accessible to modification, one could assume that the observed asymmetry had not been induced by the attachment of the modifying agent, but resulted from the stabilization of one of the pre-existing states of the apoenzyme. Evidence was also obtained that stabilization of GAPDH in the asymmetric state does not affect the NAD-binding properties of the enzyme, and the half-of-the-sites reactivity and negative cooperativity in the binding of NAD are therefore probably generated by different mechanisms [24].

The similarity of results obtained with GAPDHs from different sources (rabbit muscle and *Escherichia coli* [25,26]) has made it possible to analyze them on the basis of structural information available for GAPDH from *E. coli* [27]. Fig. 1 shows the structural elements of the GAPDH monomer involved in contacts between the NAD-binding domain (residues 1–147, as well as the C-terminal  $\alpha_3$  helix), and the catalytic domain (residues 149–311). It is seen that interdomain interactions of the ‘intrasubunit’ type are largely due to hydrogen bonds between amino acid residues which are constituents of the  $\alpha_3$  helix of the coenzyme-binding domain, on the one hand, and of the loop 275–290 of the catalytic domain, on the other. Of considerable importance for the formation of the active center (which incorporates Cys 149, His 176, Arg 231 and several other residues) is a network of interactions developed by Asp 312 and Asn 313 with a group of amino

acid residues located in the  $\beta$ -strands  $\beta_1$ ,  $\beta_2$  and  $\beta_3$  or nearby (Asn 236, Val 237, Ser 238). All these residues are strictly conservative.

Furthermore, the figure shows that another part of the NAD-binding domain, i.e. the  $\alpha_C$  helix and the loop connected to it, is also involved in contacts with the catalytic domain, but in this case the contacts are of the ‘intersubunit’ type, namely between the coenzyme-binding domain of one subunit and the catalytic domain of the other. Filled triangles mark the amino acid residues involved in intersubunit contacts of the *r* type. It is seen that the hydrogen bonds in this contact region are formed almost exclusively between amino acid residues located in the NAD-binding domain of one subunit and in the so-called S-loop (residues 177–201) of the catalytic domain belonging to the neighboring subunit. This deserves particular attention because the amino acid sequence of the S-loop is one of the less conservative regions in the polypeptide chain and varies significantly in GAPDHs from different sources. Thus, even though the three-dimensional structure of *E. coli* GAPDH is very close to that of GAPDH from *Bacillus stearothermophilus* [29], the S-loops of these two enzymes contain very dissimilar amino acid sequences [30].

In GAPDH from *B. stearothermophilus*, the character of the amino acid sequence in the region of residues 187–191 precludes the formation of a hydrogen bond between Arg 231 and Asp 192 in any of the four subunits of the tetramer. In all of them, Arg 231 is hydrogen-bonded to Thr 179. On the other hand, the *E. coli* GAPDH subunits are non-equivalent: two of them (the so-called O and Q subunits) can form a hydrogen bond in two alternative ways: either Arg 231–Thr 179 or Arg 231–Asp 192 [27]. In the first case, all four subunits become conformationally identical, and the symmetric tetramer is formed. In the second case, the asymmetric structure is stabilized. These structural data provide an explanation of why chemical modification of Arg 231 locked the tetramer in a state where only two of the four active centers became accessible to the physiological substrate, glyceraldehyde-3-phosphate [22–26]. This might occur owing to stabilization of one of the two alternative conformational states of the tetramer, the asymmetric one, with different environment of the arginine in the two pairs of subunits.

A question arises as to whether the ability of the oligomer to exist in an equilibrium between the symmetric and the asymmetric states has any functional significance. At the present level of our knowledge, no definite answer can be given yet. Under steady-state conditions, all four active centers of the *E. coli* GAPDH are catalytically equivalent, which suggests that the binding of glyceraldehyde-3-phosphate shifts the equilibrium towards the symmetric state. On the other hand, some observations apparently indicate that a switching from ‘all-of-the-sites’ to ‘half-of-the-sites’ activity may occur under specific conditions [16]. A hypothetical possibility also exists that stabilization of the tetramer in the asymmetric state might serve as a mechanism regulating the number of simultaneously functioning active centers.

## 2.2. Homo-oligomeric aminoacyl-tRNA synthetases

### 2.2.1. Tryptophanyl-tRNA synthetase (*TrpRS*) from *B. stearothermophilus*.

This enzyme, catalyzing tryptophan activation by ATP with subsequent aminoacylation of tRNA<sup>Trp</sup>, belongs to the class I of aminoacyl-tRNA synthetases [31]. The N-terminal domain (~200 residues) of the enzyme mol-

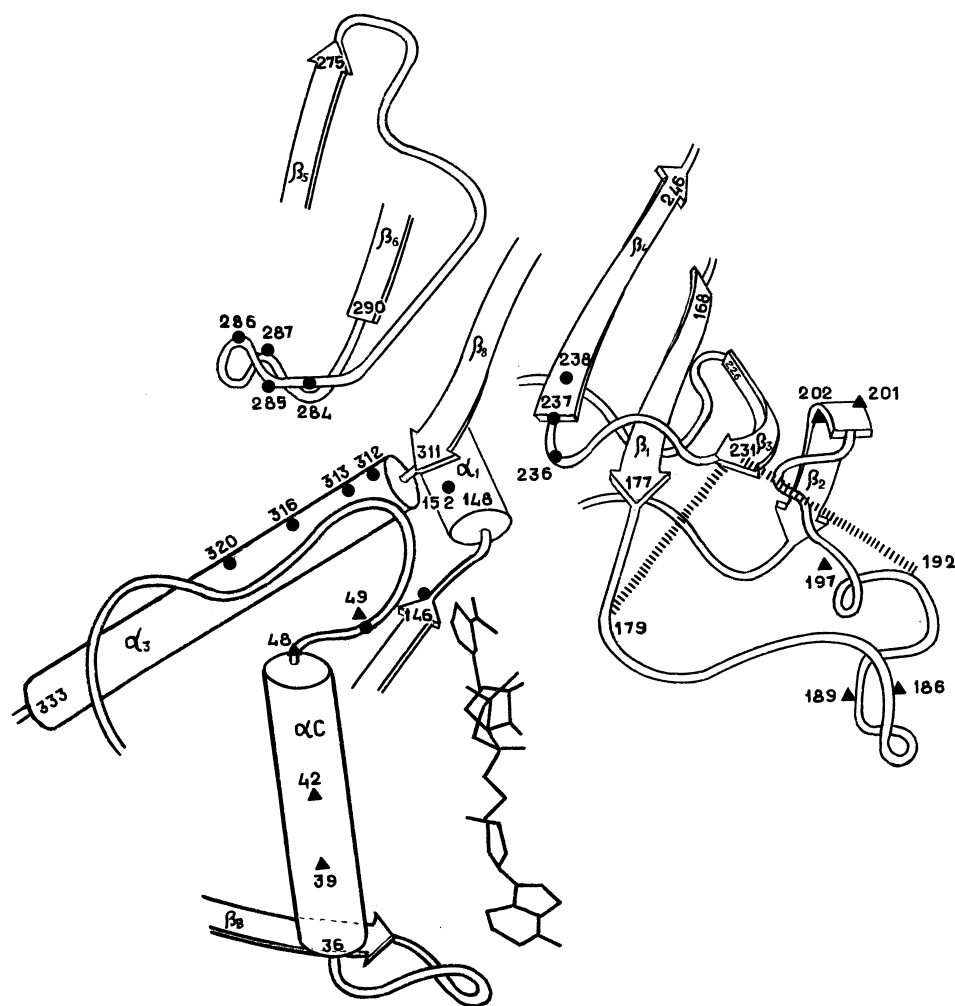


Fig. 1. Fragments of the three-dimensional structure of GAPDH. Bold lines are used to draw the structural elements of the coenzyme-binding domain, and thin lines, the structural elements of the catalytic domain. Filled circles mark the amino acid residues which form hydrogen bonds between the domains inside a monomer of *E. coli* GAPDH. The hydrogen bonds are formed between amino acid residues 49 and 284, 146 and 152, 312 and 236, 312 and 285, 313 and 237, 313 and 238, 313 and 236, 316 and 286, 320 and 269, and 320 and 287. Filled triangles mark the amino acid residues which form hydrogen bonds between neighboring subunits (contacts of the *r* type). These hydrogen bonds are formed between residues 13 and 186, 48 and 186, 39 and 189, 48 and 197, 42 and 197, 49 and 201, and 48 and 202. Two alternative possibilities of a hydrogen bond formation between Arg 231 and either Thr 179 or Asp 192 in *E. coli* GAPDH are also shown (see text for details). The picture has been taken from [28], with alterations.

ecule contains TIGN and KMSKS sequence motifs which occur at each end of a supersecondary structure called the Rossmann fold, but are brought close to each other in the tertiary structure by a pronounced twist of the four-stranded parallel  $\beta$ -sheet, to form much of the adenine–nucleotide binding site [32]. The second domain is a small four-helix bundle of about 95 residues containing a putative binding site for the tRNA<sup>Trp</sup> anticodon.

The recent solving of the tertiary structure of this enzyme in the absence of ligands [33] and a comparison of the data obtained with the results of a study of the TrpRS–tryptophanyl-5' AMP complex [34] have made it possible to reveal an important role played by the movements and rearrangements of various domains in the process of organization of different regions of the active site. Thus, it was established that unexpected rearrangements occur in the structure of the nucleotide-binding fold, giving rise to various new kinds of interdomain interactions: the amino-terminal  $\alpha A$  helix, the TIGN and KMSK sequences, together with the distal helical domain

rotate as a single rigid body away from what remains of the Rossmann fold, opening the AMP-binding site, seen in the TrpRS–tryptophanyl-5' AMP complex [34], into two halves. In this way, a new form of the C-terminal domain is created; the functional boundary between this domain and the N-terminal domain (lacking the  $\alpha A$ ) now lies within the Rossmann fold domain itself. As particularly emphasized by the authors [33], the small domain defined by the domain motion contains both TIGN and KMSKS sequences, which have been shown to interact with the ribose and pyrophosphate moieties in the transition state of the tyrosine activation by TyrRS [35,36], and are therefore of central importance to the catalysis of amino acid activation.

Another interesting result obtained in the structural study of TrpRS in the absence of ligands was the finding that, unlike the TrpRS–tryptophanyl-5' AMP complex, which is a symmetric dimer [34], in the absence of ligands the dimer becomes asymmetric. The small domain assumes a different conformation in each monomer. It has been shown that differ-

ences between the monomers constituting the dimeric unit result almost exclusively from variations in the interdomain flexibilities exhibited during the outward motion of the small domain relative to its configuration in the TrpRS–tryptophanyl-5' AMP complex. Thus, the angle  $\Delta\alpha$  subtended by the centers of mass in the two domains in ligand-free TrpRS and in the enzyme–tryptophanyl-5' AMP complex on the axis around which they rotate is close to  $14^\circ$  in one monomer but only about  $11^\circ$  in the other. The changes within the ligand-free asymmetric unit are limited to short segments before and after the  $\alpha A$  helix (residues 3–17), and a longer segment at the end of the final  $\beta$ -strand in the Rossmann fold structure lacking the  $\alpha A$  helix (residues 170–190).

The most remarkable fact is that the loop Arg 175–Ile 183 following the final  $\beta$ -sheet of the N-terminal domain, immediately precedes the KMSKS sequence and is located at the interface between the two domains. This suggests that interdomain motions accompanying the formation of the enzyme–tryptophanyl-5' AMP complex might somewhat differ in the monomers constituting the asymmetric dimer, thus creating conformationally different active centers.

**2.2.2. Tyrosyl-tRNA synthetase (TyrRS) from *B. stearothermophilus*.** The crystallographic studies demonstrating the pre-existent asymmetry of TrpRS are in excellent agreement with the results of experiments performed on TyrRS, an enzyme which is closely analogous to TrpRS both structurally and functionally [31]. In this case, the asymmetry showed itself in the half-of-the-sites reactivity exhibited by the enzyme in solution: only 1 mol of tyrosine could be bound and only 1 mol of tyrosyl-adenylate was formed per two active sites. Furthermore, only 1 mol of tRNA was able to bind to a dimer [37,38]. The results of these solution studies appeared to contradict those of X-ray crystallography which had shown the enzyme to be a symmetric dimer with an electron density allowing for 2 mol of tyrosine bound/mol of dimer [39,40]. To reveal the structural basis of the asymmetry, a series of elegant experiments were performed [41–43], demonstrating clearly that pre-existing asymmetry had in fact been the cause of the effects observed. The experimental approach was based on the construction of heterodimers of TyrRS containing one full-length subunit and one truncated subunit (an isolated N-terminal domain). The absence of the small C-terminal domain abolished the binding of tRNA to the truncated subunit but left the kinetics of the activation reaction (the formation of tyrosyl-adenylate) unaffected. The catalytic properties of the unmodified full-length and truncated subunits in the activation reaction were identical. The construction of heterodimers obtained from parental full-length and truncated homodimers provided a way to 'tag' the individual subunits so that a mutation can be specifically introduced into one predefined subunit. Two populations of heterodimers were prepared. The dimers of one population contained the His45Asn mutation introduced into the isolated activation domain, whereas the dimers of the other population contained the His45Asn mutation introduced into the activation domain within the full-length subunit.

The kinetics of the activation reaction performed by the two populations of heterodimers were then measured with the following results. 50% of heterodimers were found to be active in one subunit, and 50% in the other. In the presence of the substrates of the aminoacylation reaction, heterodimers containing Asn 45 on one subunit formed the first 0.5 mol

of Tyr-AMP per mol of dimer rapidly, i.e. at the 'wild-type' rate ( $t_{1/2} = 20$  ms), while the next 0.5 mol was formed four orders of magnitude slower ( $t_{1/2} = 200$  s, a rate expected for a mutant). Had the half-of-the-sites activity been induced by the formation of the first mol of tyrosyl-adenylate, the wild-type site would have been the one occupied. Yet the experiment showed that only 50% of the wild-type subunits had participated in the reaction, which means that the other 50% were inaccessible to substrates under these conditions. In terms of asymmetry, one should expect a random mix of active wild-type subunit/inactive mutant and inactive wild-type site/active mutant.

These results support the suggestion that pre-existent asymmetry may be a common property of dimeric molecules in both TrpRS and TyrRS. Considering the similarity in the structural and functional properties of TrpRS and TyrRS, one is tempted to suggest that the basic principles underlying the development of dimer asymmetry might be similar in these two enzymes. Further studies are needed to test the validity of this proposal. At the same time, some progress has been made towards understanding the rationale behind the functional non-equivalence of TyrRS subunits associated in a dimer. Detailed kinetic analysis performed on heterodimers containing different mutations in each subunit has shown that for efficient catalysis to occur, two Tyr molecules must bind sequentially to the same active site. It was suggested that the second molecule of Tyr aids the dissociation of Tyr-tRNA by displacing the tyrosyl moiety from its binding site [42]. The authors also hypothesized that the functions of the catalytically inactive subunit are to bind tRNA and possibly to stabilize the active conformation of the dimer, since a monomeric enzyme does not activate Tyr.

In sum, the several examples considered above illustrate that an intriguing but poorly understood property of enzymes: the asymmetry which is intrinsic in the design of some homo-oligomers, is now becoming the subject of thorough structural analysis. From the data available, it might be assumed that a pairwise asymmetry is a result of minor changes in the character of interdomain interactions responsible for the stabilization of the active center in a catalytically competent conformation. One of the two subunits constituting the asymmetric unit seems to be more flexible (perhaps more unfolded) than the other, owing to the peculiarities of interdomain interactions of different types which determine the flexibility of the enzyme structure. Considering that transition between the symmetric and asymmetric states may turn out to be one of the regulatory mechanisms in oligomeric enzymes, the conditions under which it takes place and the factors capable of inducing it probably merit future study.

### 3. Hetero-oligomers

#### 3.1. Carbamoyl phosphate synthetase (CPS) from *E. coli*

As isolated from *E. coli*, this enzyme is an  $\alpha,\beta$ -heterodimer. The recently obtained crystallographic structure of CPS shows clearly that the active site on the small subunit, and two other active sites located on the large subunit are separated by nearly 100 Å in three-dimensional space [44]. The structural investigation revealed the relative location of the three independent active sites catalyzing the following reactions:

1. Hydrolysis of glutamine.

2. Synthesis of carboxyphosphate and displacement of phosphate by ammonia to produce carbamate.
3. Phosphorylation of carbamate to liberate carbamoyl phosphate.

The two active sites contained within the large subunit and catalyzing reactions 2 and 3 were found to be separated by a linear distance of  $\sim 35$  Å and connected by an intramolecular tunnel that runs through the interior of the entire protein [45]. On the other hand, the small subunit forms molecular contacts only with the N-terminal half of the large subunit. Furthermore, the active site identified in the small subunit for glutamine hydrolysis is  $\sim 45$  Å away from site 2 on the large subunit. Despite this, a direct transfer of ammonia from the small subunit to the large one was demonstrated without its appearance in solution [46]. These observations have raised the possibility of coupled synthesis of carbamoyl phosphate through the molecular tunnel from the small subunit to the large one. In order for this process to work efficiently, the catalytic activities within the three active sites must be coordinated with one another.

Experimental evidence has indeed been obtained for allosteric communication among the active sites [46]. Thus, the rate constants for the formation and hydrolysis of the thioester intermediate within the small subunit increase roughly 1000-fold when ATP is being hydrolyzed within the N-terminal half of the large subunit. A conformational change must therefore be transmitted from the large subunit to the small one that serves to optimize the orientation of specific active site residues. On the other hand, when glutamine is hydrolyzed by the small subunit, the steady-state rate of ATP hydrolysis increases by about one order of magnitude. It appears from these results that the phosphorylation of bicarbonate within the carboxyphosphate unit acts as a 'gate keeper' for the molecular tunnels in CPS. Only after the bicarbonate has been phosphorylated does the hydrolysis of glutamine become fast enough to inject a molecule of ammonia into the tunnel.

To explain the molecular mechanisms responsible for communication between the active sites, it has been postulated that they communicate via domain movements. This interesting proposal has been supported lately by detailed studies into the domain structure of the enzyme under different conditions. Both carboxyphosphate and carbamoyl phosphate synthase units were found to be composed of three domains: A, B and C domains. The recently obtained results [47] provided the first example of domain movement in CPS caused by the binding of a nucleotide triphosphate. This movement, triggered by hydrogen-bonding interactions between the  $\beta$  and  $\gamma$  phosphates of the ATP analog and the backbone amino groups, was accomplished by numerous small changes in the dihedral angles of the polypeptide chain backbone. The changes were propagated throughout one of the three domains, the B domain, with some atoms moving more than 7.0 Å. The whole body of information obtained by Thoden et al. [47] suggests that correlated openings and closings of these domains should influence both the catalytic activities of the active sites in the large subunit and the movements of substrates and products within this hetero-oligomeric enzyme. Detailed elucidation of the mechanism of these effects is of particular interest in the context of understanding the principles which can be used by a protein molecule to ensure a direct transfer of intermediates between different active centers.

**Acknowledgements:** This work was supported by Grant 00-15-97758 from the Program for the Maintenance of Scientific Schools in Russia and by Grant 99-04-48076 from the Russian Fund for Basic Research.

## References

- [1] Wetlaufer, D.B. (1973) *Proc. Natl. Acad. Sci. USA* 70, 697–701.
- [2] Janin, J. and Wodak, S.J. (1983) *Prog. Biophys. Mol. Biol.* 42, 21–78.
- [3] Jaenicke, R. (1987) *Prog. Biophys. Mol. Biol.* 49, 117–237.
- [4] Rossmann, M.G. and Liljas, A. (1974) *J. Mol. Biol.* 85, 177–181.
- [5] Sheng, X.R., Zhang, H.J., Pan, X.M., Li, X.F. and Zhou, J.M. (1997) *FEBS Lett.* 413, 429–432.
- [6] Koshland, D.E. (1959) in: *The Enzymes*, 2nd edn., Vol. 1, pp. 305–346, Academic Press, New York.
- [7] Gerstein, M., Lesk, A.M. and Chothia, C. (1994) *Biochemistry* 33, 6740–6749.
- [8] Bernhard, S.A. and MacQuarrie, R.A. (1973) *J. Mol. Biol.* 74, 73–78.
- [9] Seydoux, F., Malhotra, O.P. and Bernhard, S.A. (1974) in: *CRC Critical Reviews in Biochemistry* (Fasman, G.D., Ed.), Vol. 2, pp. 227–257, CRC Press, Cleveland, OH.
- [10] Stallcup, W.B. and Koshland Jr., D.E. (1973) *J. Mol. Biol.* 80, 41–62.
- [11] Dietz, G., Woenkhaus, C., Jaenicke, R. and Schuster, I. (1977) *Z. Nat.forsch.* 32C, 85–92.
- [12] Golovina, T.O., Muronetz, V.I. and Nagradova, N.K. (1978) *Biochim. Biophys. Acta* 524, 15–25.
- [13] Herzfeld, J. and Schlessinger, P.A. (1975) *J. Mol. Biol.* 97, 483–517.
- [14] Bode, J., Blumenstein, M. and Raftteery, M.A. (1975) *Biochemistry* 14, 1153–1160.
- [15] Mockrin, S.C., Byers, L.D. and Koshland Jr., D.E. (1975) *Biochemistry* 14, 5428–5437.
- [16] Kellershohn, N. and Seydoux, F. (1979) *Biochemistry* 18, 2465–2470.
- [17] Malhotra, O.P. and Bernhard, S.A. (1981) *Biochemistry* 20, 5529–5538.
- [18] Malhotra, O.P., Bernhard, S.A. and Seydoux, F. (1981) *Biochimie* 63, 131–141.
- [19] Cardon, J.W. and Boyer, P.D. (1982) *J. Biol. Chem.* 257, 7615–7622.
- [20] Malhotra, O.P. and Bernhard, S.A. (1989) *Biochemistry* 28, 124–128.
- [21] Garavito, R.M., Berger, D. and Rossmann, M.G. (1977) *Biochemistry* 16, 4393–4398.
- [22] Asryants, R.A., Kuzminskaya, E.V., Tishkov, V.I., Douzhenkova, I.V. and Nagradova, N.K. (1989) *Biochim. Biophys. Acta* 997, 159–166.
- [23] Kuzminskaya, E.V., Asryants, R.A. and Nagradova, N.K. (1991) *Biochim. Biophys. Acta* 1075, 123–130.
- [24] Kuzminskaya, E.V., Asryants, R.A. and Nagradova, N.K. (1992) *Biochim. Biophys. Res. Commun.* 187, 577–583.
- [25] Levashov, P.A., Schmalhausen, E.V., Muronetz, V.I. and Nagradova, N.K. (1995) *Biochem. Mol. Biol. Int.* 37, 991–1000.
- [26] Nagradova, N.K., Schmalhausen, E.V., Levashov, P.A., Asryants, R.A. and Muronetz, V.I. (1997) *Appl. Biochem. Biotechnol.* 61, 47–56.
- [27] Duee, E., Olivier-Deyris, L., Fanchon, E., Corbier, C., Branlant, G. and Dideberg, O. (1996) *J. Mol. Biol.* 257, 814–838.
- [28] Biesecker, G., Harris, J.I., Thierry, J.C., Walker, J.E. and Wonacott, A.J. (1977) *Nature* 266, 328–333.
- [29] Skarzynski, T., Moody, P.C.E. and Wonacott, A.J. (1987) *J. Mol. Biol.* 193, 171–187.
- [30] Martin, W. and Cerff, R. (1986) *Eur. J. Biochem.* 159, 323–331.
- [31] Arnez, J.G. and Moras, G. (1997) *Trends Biol. Sci.* 22, 211–216.
- [32] Carter Jr., C.W. (1993) *Annu. Rev. Biochem.* 62, 715–748.
- [33] Ilyin, V.A., Temple, B., Hu, M., Li, G., Yin, Y., Vachette, P. and Carter Jr., C.W. (2000) *Protein Sci.* 9, 218–231.
- [34] Doublet, S., Bricogne, G., Gilmore, C.J. and Carter, C.W. (1995) *Structure* 3, 17–31.
- [35] Fersht, A.R. (1987) *Biochemistry* 26, 8031–8037.
- [36] Fersht, A.R., Knill-Jones, J.W., Beduelle, H. and Winter, G. (1988) *Biochemistry* 27, 1581–1587.
- [37] Fersht, A.R. (1975) *Biochemistry* 14, 5–12.

- [38] Jakes, R. and Fersht, A.R. (1975) *Biochemistry* 14, 3344–3350.
- [39] Monteilhet, C. and Blow, D.M. (1978) *J. Mol. Biol.* 122, 407–417.
- [40] Blow, D.M. and Brick, P. (1985) in: *Biological Macromolecules and Assemblies: Nucleic Acids and Interactive Proteins* (Jurnak, F. and McPherson, A., Eds.), Vol. 2, pp. 442–469, Wiley, New York.
- [41] Ward, W.H.J. and Fersht, A.R. (1988) *Biochemistry* 27, 1041–1049.
- [42] Ward, W.H.J. and Fersht, A.R. (1988) *Biochemistry* 27, 5525–5530.
- [43] Fersht, A. (1999) in: *Structure and Mechanism in Protein Science: a Guide to Enzyme Catalysis and Protein Folding*, p. 449, W.H. Freeman and Co., New York.
- [44] Thoden, J.B., Holden, H.M., Wesenberg, G., Raushel, F.M. and Rayment, F. (1997) *Biochemistry* 36, 6305–6316.
- [45] Mullins, L.S. and Raushel, F.M. (1999) *J. Am. Chem. Soc.* 121, 3803–3804.
- [46] Miles, B.W., Banzon, J.A. and Raushel, F.M. (1998) *Biochemistry* 37, 16773–16779.
- [47] Thoden, J.B., Wesenberg, G., Raushel, F.M. and Holden, H.M. (1999) *Biochemistry* 38, 2347–2357.