

## The Molecular Basis of Substrate Channeling\*

Edith Wilson Miles<sup>‡§</sup>, Sangkee Rhee<sup>¶</sup>,  
and David R. Davies<sup>¶</sup>

From the <sup>‡</sup>Enzyme Structure and Function Section,  
Laboratory of Biochemistry and Genetics and the  
<sup>¶</sup>Laboratory of Molecular Biology, NIDDK, National  
Institutes of Health, Bethesda, Maryland 20892

Substrate channeling is the process of direct transfer of an intermediate between the active sites of two enzymes that catalyze sequential reactions in a biosynthetic pathway (for reviews see Refs. 1 and 2). The active sites can be located either on separate domains in a multifunctional enzyme or on separate subunits in a multienzyme complex.

Substrate channeling has been proposed to decrease transit time of intermediates, prevent loss of intermediates by diffusion, protect labile intermediates from solvent, and forestall entrance of intermediates into competing metabolic pathways (2). Loss of an intermediate by diffusion may be especially important in the case of a neutral species, such as indole, which could escape from the cell by passive diffusion across cell membranes (2–5). Nevertheless, there has been considerable debate over whether channeling actually occurs and whether it is advantageous (5, 6).

X-ray crystallographic studies on several enzyme complexes have revealed two molecular mechanisms for channeling. The discovery of an intramolecular tunnel in tryptophan synthase (7) provided the first molecular mechanism for channeling. For a long time this was a unique example, but recent structure determinations have revealed plausible evidence for tunneling of ammonia and carbamate in carbamoyl-phosphate synthase (CPS)<sup>1</sup> (8) and of ammonia in phosphoribosylpyrophosphate amidotransferase (GPATase) (9).

The structure of dihydrofolate reductase-thymidylate synthase, however, showed no evidence for a tunnel between the two active sites (10). Neither are the two active sites adjacent to one another. Instead, there are positively charged residues along the surface between the active sites that form an electrostatic highway sufficient to channel the negatively charged dihydrofolate with high efficiency (10, 11).

The focus of this minireview will be on the structural basis of channeling in the four enzyme structures cited above and on solution studies of these systems.<sup>2</sup> Scheme I summarizes the reactions catalyzed by the four enzymes. A comparison of the structural and kinetic results reveals that these enzymes frequently exhibit allosteric interactions that synchronize the reactions to prevent the build-up of excess intermediate (12–16).

### Tunneling

**Tryptophan Synthase**—Tryptophan synthase catalyzes the last two reactions in the biosynthesis of L-tryptophan (Scheme I, A) (for reviews see Refs. 14 and 17–19). In bacteria, the two reactions are catalyzed by separate  $\alpha$  and  $\beta$  subunits, which combine to form a stable multienzyme complex,  $(\alpha\beta)_2$ . In yeast and molds, the two

reactions are catalyzed by separate  $\alpha$  and  $\beta$  sites in a single bifunctional polypeptide chain,  $(\alpha\beta)_2$ .<sup>3</sup> Evidence that indole is not liberated as a free intermediate in the overall conversion of indole-3-glycerol phosphate and L-serine to L-tryptophan suggested that indole passes directly from the  $\alpha$  site to the  $\beta$  site without release to the surrounding solvent (20–23). The 2.8-Å resolution crystal structure of tryptophan synthase from *Salmonella typhimurium* showed that the active sites of the  $\alpha$  and  $\beta$  subunits are about 25 Å apart (7). The structure also revealed that the  $\alpha$  and  $\beta$  sites are connected by a largely hydrophobic intramolecular tunnel with dimensions sufficient to accommodate up to four molecules of indole (Fig. 1). Further refinement of the native structure and determination of several structures in the presence of different ligands have revealed a number of ligand-induced conformational changes that may be important for channeling indole and also for allosteric communication between the  $\alpha$  and  $\beta$  subunits (24–28). In the absence of ligands, the active sites of both the  $\alpha$  and  $\beta$  subunits are accessible to solvent. Dramatic conformational changes occur upon addition of ligands that bind to both  $\alpha$  and  $\beta$  subunits (25, 28). These changes include ordering of disordered loop structures in the vicinity of the active site of the  $\alpha$  subunit together with movement of a  $\beta$  subunit subdomain (residues 93–189). These conformational changes restrict the access of solvent to the two active sites and to the tunnel and may also prevent escape of the indole intermediate into bulk solvent (14, 25, 28).

High resolution studies of the native enzyme showed that the tunnel was blocked by the side chain of  $\beta$ Phe-280. However, crystal structures of the enzyme in the presence of different cations showed that exchange of  $K^+$  or  $Cs^+$  for  $Na^+$  results in movement of the side chain of  $\beta$ Phe-280 out of the tunnel (24), suggesting that  $\beta$ Phe-280 can act as a gate to control passage through the tunnel. This change in structure is accompanied by movements of many tunnel-associated residues that link the  $\alpha$  and  $\beta$  active sites.

Kinetic studies have established that the tunnel actually functions to channel indole between the two sites (12, 29, 30). Transient kinetic analysis shows that the rate of indole transfer through the tunnel is very rapid ( $>1000\text{ s}^{-1}$ ), that the reaction of indole at the  $\beta$  site is fast and irreversible, and that the reaction of L-serine at the  $\beta$  site to form aminoacrylate increases the  $\alpha$  site cleavage of indole-3-glycerol phosphate by approximately 30-fold (12). These three features of the reaction kinetics promote efficient channeling of indole and prevent accumulation of the indole intermediate. However, a mutation ( $\beta$ E109D) that reduces the rate of reaction of indole at the  $\beta$  site (12) and another mutation ( $\beta$ C170F or  $\beta$ C170W) that partially blocks the indole tunnel (31) result in the accumulation of indole.

The kinetic results also indicate that intersubunit communication keeps the reactions catalyzed by the wild-type  $\alpha$  and  $\beta$  subunits in phase so that the indole intermediate does not accumulate (12). These kinetic results and others (32, 33) argue that the reaction of L-serine at the  $\beta$  site to form a pyridoxal phosphate-aminoacrylate intermediate produces a conformational change that is transmitted to the  $\alpha$  site and enhances the rate of indole formation from indole-3-glycerol phosphate. Thus, indole is produced at the  $\alpha$  site with a significant rate only when the  $\beta$  site is ready to receive it.

The crystallographic data suggest mechanisms for the allosteric interactions between the two active sites. The ligand-induced closure and ordering of the  $\alpha$  subunit loop 6 is accompanied by enhanced interactions with  $\alpha$  loop 2. Specifically, interactions between the loop 6 residue,  $\alpha$ Thr-183, and the loop 2 residue,  $\alpha$ Asp-60, as well as main chain interactions decrease the flexibility of loop 2 and are accompanied by increased interactions of loop 2 with

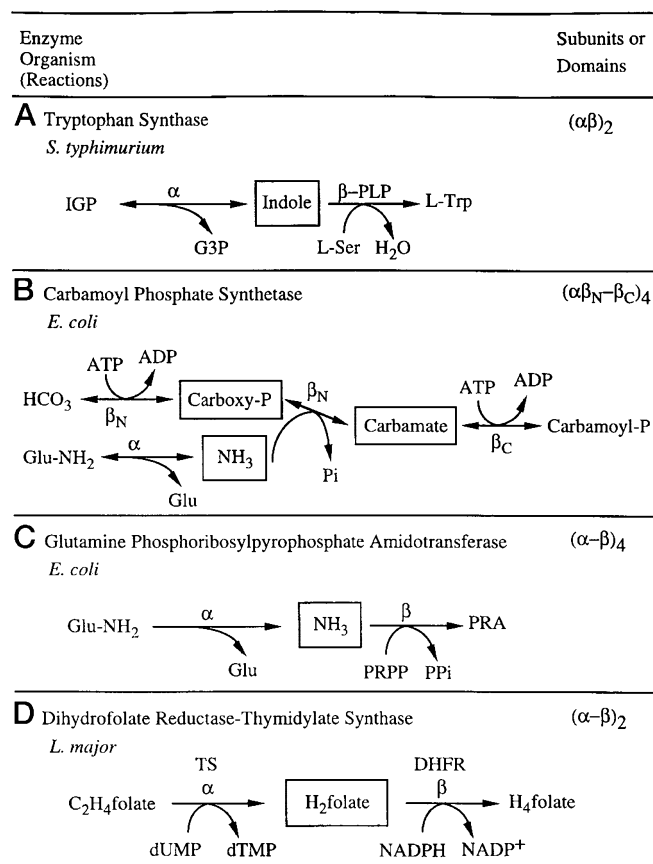
\* This minireview will be reprinted in the 1999 Minireview Compendium, which will be available in December, 1999.

§ To whom reprint requests should be addressed: Laboratory of Biochemistry and Genetics, NIDDK, Bldg. 8, Rm. 225, 8 Center Dr. MSC 0830, Bethesda, MD 20892-0830. Tel.: 301-496-2763; Fax: 301-402-0240; E-mail: EdithM@intra.nidk.nih.gov.

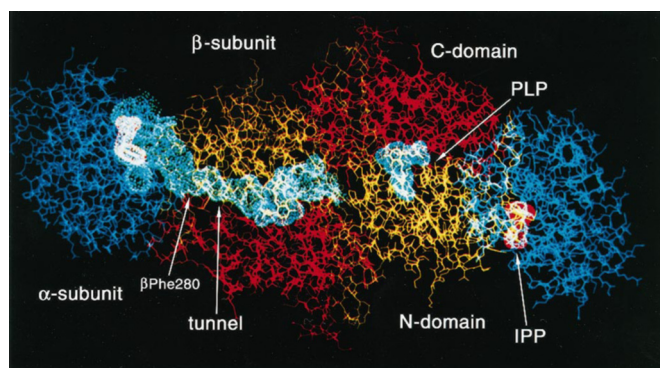
<sup>1</sup> The abbreviations used are: CPS, carbamoyl-phosphate synthase; GPATase, glutamine phosphoribosylpyrophosphate amidotransferase; DHFR, dihydrofolate reductase; TS, thymidylate synthase; PRPP, phosphoribosylpyrophosphate.

<sup>2</sup> We will not consider enzymes in which the intermediate is covalently bound (e.g. pyruvate dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase).

<sup>3</sup> We use the hyphen to indicate that two different catalytic domains (e.g.  $\alpha$  and  $\beta$ ) are located on a single polypeptide chain in a bifunctional or multifunctional enzyme.



**SCHEME I. Enzymes that exhibit substrate channeling in their reactions.** Channeled intermediates in reactions are boxed. Domains in multifunctional enzymes are separated by *hyphens*. Additional abbreviations used are: *IGP*, indole-3-glycerol phosphate; *PLP*, pyridoxal phosphate; *G3P*, D-glyceraldehyde 3-phosphate; *H<sub>2</sub>folate*, dihydrofolate; *H<sub>4</sub>folate*, tetrahydrofolate; *C<sub>2</sub>H<sub>4</sub>folate*, methylene tetrahydrofolate; *Glu-NH<sub>2</sub>*, glutamine; *PRA*, phosphoribosylamine.

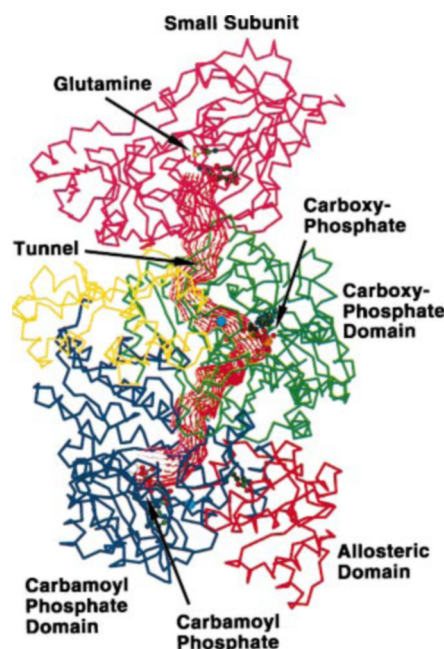


**FIG. 1. Tryptophan synthase  $\alpha_2\beta_2$  complex.** A substrate analog, indole-3-propanol phosphate (*IPP*), locates the  $\alpha$  subunit active site, and pyridoxal phosphate (*PLP*) locates the  $\beta$  subunit active site. The indole tunnel between the active sites of the  $\alpha$  subunit (blue) and  $\beta$  subunit is shown in the left part of the complex (7). This tunnel passes between the N-domain (yellow) and the C-domain (red) of the  $\beta$  subunit.

residues in the  $\beta$  subunit mobile subdomain (residues 93–189) (25, 28). These interactions could provide a route of communication from the  $\alpha$  site to the  $\beta$  site and vice versa.

The combined structural and kinetic results provide a model for the allosteric properties of tryptophan synthase (14, 32). Allosteric signals derived from covalent transformations at the  $\alpha$  and  $\beta$  sites are proposed to switch the enzyme from an open, low activity state to which ligands bind to a closed high activity state, which prevents the escape of indole.

**CPS**—A remarkable example of channeling occurs in the case of CPS, which catalyzes the synthesis of carbamoyl phosphate from bicarbonate, glutamine, and two molecules of ATP (Scheme I, B)



**FIG. 2. CPS.** The upper small subunit (red) catalyzes the release of ammonia from glutamine. The large subunit contains the carboxyphosphate and the carbamoyl phosphate active sites. A tunnel, 96 Å long, connects these three sites (8).

(for recent reviews see Refs. 34–36). Earlier solution studies (37) provided evidence that the synthesis involves a series of four separate reactions that generate three reactive and unstable intermediates:  $\text{NH}_3$ , carboxyphosphate, and carbamate (Scheme I, B). These intermediates must be efficiently channeled and the reactions effectively coupled because the reaction stoichiometry is precisely 2 mol of ADP and 1 mol of glutamate for every mole of carbamoyl phosphate (37). The  $\text{NH}_3$  formed from glutamine must be channeled because the  $K_m$  for free  $\text{NH}_3$  is 3 orders of magnitude greater than that of glutamine. In addition, recent results using  $^{13}\text{C}$  NMR to measure isotopic oxygen exchange are consistent with a mechanism that requires channeling of the carbamate intermediate (38).

CPS from *Escherichia coli* is composed of two subunits: a small monofunctional glutamine amidotransferase ( $\alpha$ ), which belongs to the Triad class, one of the two classes of amidotransferases (34), and a large, bifunctional subunit ( $\beta$ ) in which the N- and C-terminal halves are homologous, each having an ATP binding site. The N-terminal half ( $\beta_N$ ) catalyzes the production of carboxyphosphate, which reacts with  $\text{NH}_3$  to form carbamate, whereas the C-terminal half ( $\beta_C$ ) catalyzes the phosphorylation of carbamate.

The 2.8-Å resolution crystal structure<sup>4</sup> (Fig. 2) of the enzyme in the presence of ADP,  $\text{Mn}^{2+}$ , phosphate, and ornithine (an allosteric effector) (8, 35) revealed that the three active sites are very far apart; the glutamine binding site of the small subunit ( $\alpha$ ) is 45 Å from the ADP binding site of the carboxyphosphate domain ( $\beta_N$ ), which is 35 Å from the ADP site in the carbamoyl phosphate domain ( $\beta_C$ ). Examination of the structure reveals a tunnel with a length of at least 96 Å by which intermediates might pass from the active site of the small subunit to the carbamoyl phosphate site (Fig. 2). Although the average minimum radius (3.2–3.5 Å) of the proposed tunnel is sufficient for passage of the  $\text{NH}_3$  and carbamate intermediates, constrictions (2.1–2.5 Å) are observed in at least two places. Movements of residues in the tunnel wall or ligand-induced conformational changes may enlarge the dimensions of the tunnel. Interestingly, the proposed  $\text{NH}_3$  tunnel between the glutamine and carboxyphosphate sites is lined with hydrophilic residues and is thus quite different from the hydrophobic  $\text{NH}_3$  tunnel in GPATase. In contrast, in the proposed tunnel between the carboxyphosphate and carbamoyl phosphate sites, there are few charged side chains that might hydrolyze the labile carbamate intermediate.

<sup>4</sup> Currently at 2.1-Å resolution (50).



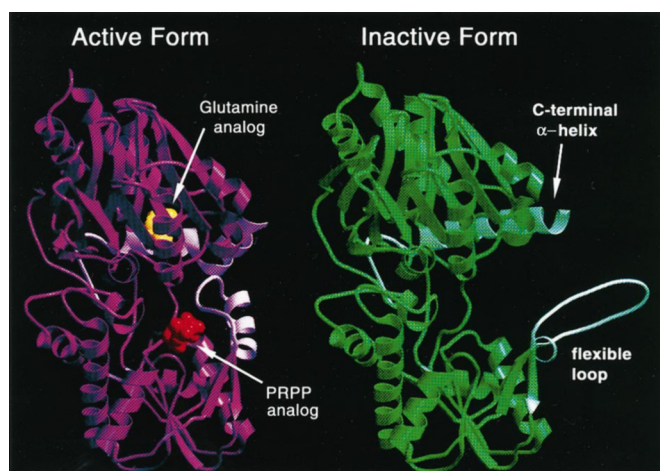


FIG. 3. Structure of GPATase showing the conformational changes that occur when substrate analogs are bound to the two active sites. The changes result in closing off the active sites from solvent and in formation of a 20-Å hydrophobic tunnel for ammonia transfer (9).

The coordination of the three active sites of CPS to work synchronously in the synthesis of carbamoyl phosphate must result from allosteric communication between these distant sites (16). However, the mechanism of communication has yet to be determined. CPS catalyzes a very slow hydrolysis of glutamine in the absence of bicarbonate and ATP. Addition of these substrates increases the rate of glutamine hydrolysis by 3 orders of magnitude.

**GPATase**—GPATase catalyzes the transfer of the amide nitrogen from glutamine to PRPP, producing phosphoribosylamine, pyrophosphate, and glutamate (Scheme I, C) (for a recent review, see Ref. 34). GPATase from *E. coli* is a bifunctional enzyme that has two separate catalytic domains on a single polypeptide chain: an N-terminal glutaminase domain ( $\alpha$ ) and a C-terminal acceptor domain ( $\beta$ ). The glutaminase domain belongs to a second class of amidotransferases (Ntn) that have a catalytic N-terminal nucleophile. The enzyme is a homotetramer, ( $\alpha\beta$ )<sub>4</sub>, and is regulated by nucleotides and by allosteric control.

GPATase can utilize either glutamine or free NH<sub>3</sub> for the biosynthesis of phosphoribosylamine (Scheme I, C). Early studies provided evidence that glutamine and free NH<sub>3</sub> bind at distinct sites (39). First, free NH<sub>3</sub> inhibits glutamine hydrolysis and competes with glutamine as an ammonia source. Second, alkylation of an essential catalytic residue by a glutamine analog or by a sulfhydryl reagent inhibited the glutamine-dependent activity but not the NH<sub>3</sub>-dependent activity. These results support a mechanism for glutamine utilization that involves amide transfer (*i.e.* channeling) to the NH<sub>3</sub> site.

Crystal structures have been reported for GPATase from *E. coli* in an open, inactive form (13, 40) and in a closed, active form containing analogs of glutamine and PRPP at the two active sites (9) (Fig. 3). Comparison of these structures reveals that activation of GPATase results in the formation of a 20-Å tunnel that connects the two active sites (9). This tunnel is created by the ordering of a flexible loop in the C-terminal domain, which closes over the space between the active sites, effectively sequestering both sites from bulk solvent. This change is accompanied by the kinking of the adjacent C-terminal  $\alpha$ -helix. In contrast to CPS, the walls of the tunnel are lined with conserved hydrophobic residues. The hydrophobic tunnel may function not only as a passageway for NH<sub>3</sub> but also to exclude water and ensure that NH<sub>3</sub> is the only nucleophile able to enter the tunnel and to react with PRPP. The conformational differences between the active and inactive forms provide a structural basis for understanding how PRPP binding in the acceptor domain stimulates glutaminase activity in the glutamine domain 200-fold (13).

#### Electrostatic Channeling

TS and DHFR catalyze sequential reactions in the thymidylate pathway, which supplies 2-deoxythymidylate for DNA synthesis (Scheme I, D). Although TS and DHFR are distinct monofunctional enzymes in most species, the two activities are found on a single

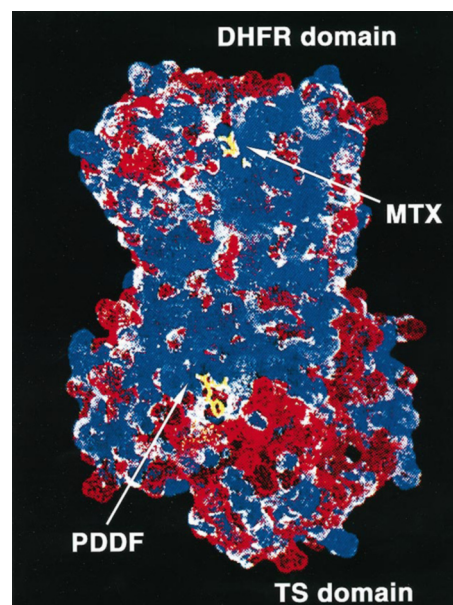


FIG. 4. The surface of the DHFR-TS showing the electrostatic potential distribution. Active site ligands are shown in yellow. The two sites are connected by a strong positive pathway along the surface (10). MTX, methotrexate; PDDF, 10-propargyl-5,8-dideazafofolate.

polypeptide chain in protozoa and some plants. DHFR-TS from *Leishmania major* is a homodimer, ( $\alpha\beta$ )<sub>2</sub>, with an N-terminal DHFR domain connected to the C-terminal TS domain. The 2.8-Å resolution x-ray structure of this bifunctional enzyme (10) suggests a novel mechanism of “electrostatic channeling” across the surface of the protein. The negatively charged dihydrofolate intermediate is proposed to move along a positively charged electrostatic “highway” that links the TS active site to the DHFR site 40 Å away (10, 11) (Fig. 4). The proposed channeling mechanism is supported by the conservation of positively charged patches across the surface of monofunctional TS and DHFR from other sources, suggesting that the monofunctional enzymes may also channel substrates *in vivo* (11).

Electrostatic channeling in DHFR-TS is also supported by Brownian dynamics simulation and experimental kinetic studies (41–44). A recent transient kinetic analysis of DHFR-TS from *L. major* (15) demonstrates that the dihydrofolate intermediate is channeled efficiently as the result of two features of the reaction kinetics: the rate of dihydrofolate transfer between the two sites is very rapid ( $>1000 \text{ s}^{-1}$ ) and each site is activated by ligand binding to the other site. Thus, reciprocal communication between the two sites leads to tight coupling of the reactions at the two sites.

It is predicted that the highly negatively charged polyglutamylated molecules of dihydrofolate, which occur in cells (45), would be even more efficiently channeled than the monoglutamylated folates (10). The crystal structure of DHFR-TS reveals that the negatively charged glutamate moieties of the folate analogs at the two sites lie in a groove along the electropositive highway between the two sites. The possibility that the bifunctional enzyme has evolved to enhance channeling is supported by the finding that the DHFR domain in the bifunctional enzyme has six extra positively charged residues located between the two sites, which may function in binding the polyglutamate tail.

#### Concluding Remarks

A comparison of the structural and kinetic features of the channeling enzymes described above indicates some common features. These enzymes are either multifunctional proteins or stable multienzyme complexes. Because allosteric signals coordinate the activities of each of these enzymes (12–16), allosteric communication may be a general and essential feature of channeling enzymes. These allosteric signals coordinate activities at two or more sites and promote efficient channeling that prevents the buildup of intermediates and their loss into solution.

The four examples described above suggest that channeling may

be a more general feature of biochemical processes involving enzyme complexes. The once isolated example of indole channeling to prevent the escape of the neutral indole from the cell is now supported by examples of channeling of reactive intermediates. A large number of enzymes have been suggested to form stable or transient complexes and to exhibit channeling or direct transfer of intermediates (2). For example, the finding of  $\text{NH}_3$  tunnels in two families of amidotransferases (GPATase and CPS) suggests that other amidotransferases in these families may have similar mechanisms of  $\text{NH}_3$  transfer (34).

The products of these reactions may themselves be channeled to other enzymes. For example, there is kinetic and biochemical evidence for channeling between GPATase and glycylamide ribonucleotide synthetase (46). This evidence is supported by docking experiments between the crystal structures of GPATase and glycylamide ribonucleotide synthetase (47). Another example involves CPS, which catalyzes the first step in the pyrimidine biosynthetic pathway. In *Saccharomyces cerevisiae*, CPS is part of a larger polypeptide that catalyzes the first two steps in this pathway (CPS and aspartyl transcarbamoylase). In mammals, CPS is part of an even larger polypeptide, termed CAD, which catalyzes the first three steps in this pathway. The interaction of CPS and aspartyl transcarbamoylase has been proposed to promote effective coordination of the two activities and channeling of the labile carbamoyl phosphate intermediate in CAD (48) and in the enzyme from yeast (49).

#### REFERENCES

- Srere, P. A. (1987) Complexes of sequential metabolic enzymes. *Annu. Rev. Biochem.* **56**, 89–124
- Ovadi, J. (1991) Physiological significance of metabolite channeling. *J. Theor. Biol.* **152**, 1–22
- Davis, B. D. (1958) On the importance of being ionized. *Arch. Biochem. Biophys.* **78**, 497–509
- Manney, T. R. (1970) Physiological advantage of the mechanism of the tryptophan synthetase reaction. *J. Bacteriol.* **102**, 483–488
- Knowles, J. R. (1991) Calmer waters in the channel. *J. Theor. Biol.* **152**, 53–56
- Ovadi, J. (1991) Physiological significance of metabolite channeling: author's response to commentaries. *J. Theor. Biol.* **152**, 135–141
- Hyde, C. C., Ahmed, S. A., Padlan, E. A., Miles, E. W., and Davies, D. R. (1988) Three-dimensional structure of the tryptophan synthase  $\alpha_2\beta_2$  multienzyme complex from *Salmonella typhimurium*. *J. Biol. Chem.* **263**, 17857–17871
- Thoden, J. B., Holden, H. M., Wesenberg, G., Raushel, F. M., and Rayment, I. (1997) Structure of carbamoyl phosphate synthetase: a journey of 96 Å from substrate to product. *Biochemistry* **36**, 6305–6316
- Krahn, J. M., Kim, J. H., Burns, M. R., Parry, R. J., Zalkin, H., and Smith, J. L. (1997) Coupled formation of an amidotransferase interdomain ammonia channel and a phosphoribosyltransferase active site. *Biochemistry* **36**, 11061–11068
- Knighton, D. R., Kan, C. C., Howland, E., Janson, C. A., Hostomska, Z., Welsh, K. M., and Matthews, D. A. (1994) Structure of and kinetic channeling in bifunctional dihydrofolate reductase-thymidylate synthase. *Nat. Struct. Biol.* **1**, 186–194
- Stroud, R. M. (1994) An electrostatic highway. *Nat. Struct. Biol.* **1**, 131–134
- Anderson, K. S., Miles, E. W., and Johnson, K. A. (1991) Serine modulates substrate channeling in tryptophan synthase: a novel intersubunit triggering mechanism. *J. Biol. Chem.* **266**, 8020–8033
- Kim, J. H., Krahn, J. M., Tomchick, D. R., Smith, J. L., and Zalkin, H. (1996) Structure and function of the glutamine phosphoribosylpyrophosphate amidotransferase glutamine site and communication with the phosphoribosylpyrophosphate site. *J. Biol. Chem.* **271**, 15549–15557
- Pan, P., Woehl, E., and Dunn, M. F. (1997) Protein architecture, dynamics and allostery in tryptophan synthase channeling. *Trends Biochem. Sci.* **22**, 22–27
- Liang, P. H., and Anderson, K. S. (1998) Substrate channeling and domain-domain interactions in bifunctional thymidylate synthase-dihydrofolate reductase. *Biochemistry* **37**, 12195–12205
- Miles, B. W., Banzon, J. A., and Raushel, F. M. (1998) Regulatory control of the amidotransferase domain of carbamoyl phosphate synthetase. *Biochemistry* **37**, 16773–16779
- Yanofsky, C., and Crawford, I. P. (1972) In *The Enzymes* (Boyer, P. D., ed) Vol. 7, pp. 1–31, Academic Press, New York
- Miles, E. W. (1991) Structural basis for catalysis by tryptophan synthase. *Adv. Enzymol. Relat. Areas Mol. Biol.* **64**, 93–172
- Miles, E. W. (1995) Tryptophan synthase: structure, function, and protein engineering. In *Subcellular Biochemistry, Proteins: Structure, Function, and Protein Engineering* (Biswas, B. B., and Roy, S., eds) Vol. 24, pp. 207–254, Plenum Press, New York
- Yanofsky, C., and Rachmeler, M. (1958) The exclusion of free indole as an intermediate in the biosynthesis of tryptophan in *Neurospora crassa*. *Biochim. Biophys. Acta* **28**, 641–642
- DeMoss, J. A. (1962) Studies on the mechanism of the tryptophan synthetase reaction. *Biochim. Biophys. Acta* **62**, 279–293
- Creighton, T. E. (1970) A steady-state kinetic investigation of the reaction mechanism of the tryptophan synthetase of *Escherichia coli*. *Eur. J. Biochem.* **13**, 1–10
- Matchett, W. H. (1974) Indole channeling by tryptophan synthase of *Neurospora*. *J. Biol. Chem.* **249**, 4041–4049
- Rhee, S., Parris, K. D., Ahmed, S. A., Miles, E. W., and Davies, D. R. (1996) Exchange of  $\text{K}^+$  or  $\text{Cs}^+$  for  $\text{Na}^+$  induces local and long-range changes in the three-dimensional structure of the tryptophan synthase  $\alpha_2\beta_2$  complex. *Biochemistry* **35**, 4211–4221
- Rhee, S., Parris, K. D., Hyde, C. C., Ahmed, S. A., Miles, E. W., and Davies, D. R. (1997) Crystal structures of a mutant ( $\beta\text{K87T}$ ) tryptophan synthase  $\alpha_2\beta_2$  complex with ligands bound to the active sites of the  $\alpha$  and  $\beta$  subunits reveal ligand-induced conformational changes. *Biochemistry* **36**, 7664–7680
- Rhee, S., Miles, E. W., and Davies, D. R. (1998) Cryo-crystallography of a true substrate, indole-3-glycerol phosphate, bound to a mutant ( $\alpha\text{D60N}$ ) tryptophan synthase  $\alpha_2\beta_2$  complex reveals the correct orientation of active site  $\alpha\text{Glu49}$ . *J. Biol. Chem.* **273**, 8553–8555
- Rhee, S., Miles, E. W., Mozzarelli, A., and Davies, D. R. (1998) Cryo-crystallography and microspectrophotometry of a mutant ( $\alpha\text{D60N}$ ) tryptophan synthase  $\alpha_2\beta_2$  complex reveals allosteric roles of  $\alpha\text{Asp-60}$ . *Biochemistry* **37**, 10653–10659
- Schneider, T. R., Gerhardt, E., Lee, M., Liang, P.-H., Anderson, K. S., and Schlichting, I. (1998) Loop closure and intersubunit communication in tryptophan synthase. *Biochemistry* **37**, 5394–5406
- Dunn, M. F., Aguilar, V., Brzovic, P. S., Drewe, W. F. J., Houben, K. F., Leja, C. A., and Roy, M. (1990) The tryptophan synthase holoenzyme complex transfers indole between the  $\alpha$ - and  $\beta$ -sites via a 25–30 Å long tunnel. *Biochemistry* **29**, 8598–8607
- Lane, A. N., and Kirschner, K. (1991) Mechanism of the physiological reaction catalyzed by tryptophan synthase from *Escherichia coli*. *Biochemistry* **30**, 479–484
- Anderson, K. S., Kim, A. Y., Quillen, J. M., Sayers, E., Yang, X.-J., and Miles, E. W. (1995) Kinetic characterization of channel impaired mutants of tryptophan synthase. *J. Biol. Chem.* **270**, 29936–29944
- Brzovic, P. S., Ngo, K., and Dunn, M. F. (1992) Allosteric interactions coordinate catalytic activity between successive metabolic enzymes in the tryptophan synthase holoenzyme complex. *Biochemistry* **31**, 3831–3839
- Banik, U., Zhu, D.-M., Chock, P. B., and Miles, E. W. (1995) The tryptophan synthase  $\alpha_2\beta_2$  complex: kinetic studies with a mutant enzyme ( $\beta\text{K87T}$ ) provide evidence for allosteric activation by an aminoacylate intermediate. *Biochemistry* **34**, 12704–12711
- Zalkin, H., and Smith, J. L. (1998) Enzymes utilizing glutamine as an amide donor. *Adv. Enzymol. Relat. Areas Mol. Biol.* **72**, 87–144
- Raushel, F. M., Thoden, J. B., Reinhart, G. D., and Holden, H. M. (1998) Carbamoyl phosphate synthetase: a crooked path from substrates to products. *Curr. Opin. Chem. Biol.* **2**, 624–632
- Holden, H. M., Thoden, J. B., and Raushel, F. M. (1998) Carbamoyl phosphate synthetase: a tunnel runs through it. *Curr. Opin. Struct. Biol.* **8**, 679–685
- Anderson, P. M., and Meister, A. (1966) Bicarbonate-dependent cleavage of adenosine triphosphate and other reactions catalyzed by *Escherichia coli* carbamyl phosphate synthetase. *Biochemistry* **5**, 3157–3163
- Raushel, F. M., Mullins, L. S., and Gibson, G. E. (1998) A stringent test for the nucleotide switch mechanism of carbamoyl phosphate synthetase. *Biochemistry* **37**, 10272–10278
- Messenger, L. J., and Zalkin, H. (1979) Glutamine phosphoribosylpyrophosphate amidotransferase from *Escherichia coli*. Purification and properties. *J. Biol. Chem.* **254**, 3382–3392
- Muchmore, C. R., Krahn, J. M., Kim, J. H., Zalkin, H., and Smith, J. L. (1998) Crystal structure of glutamine phosphoribosylpyrophosphate amidotransferase from *Escherichia coli*. *Protein Sci.* **7**, 39–51
- Elcock, A. H., Potter, M. J., Matthews, D. A., Knighton, D. R., and McCammon, J. A. (1996) Electrostatic channeling in the bifunctional enzyme dihydrofolate reductase-thymidylate synthase. *J. Mol. Biol.* **262**, 370–374
- Elcock, A. H., Huber, G. A., and McCammon, J. A. (1997) Electrostatic channeling of substrates between enzyme active sites: comparison of simulation and experiment. *Biochemistry* **36**, 16049–16058
- Meek, T. D., Garvey, E. P., and Santi, D. V. (1985) Purification and characterization of the bifunctional thymidylate synthase-dihydrofolate reductase from methotrexate-resistant *Leishmania tropica*. *Biochemistry* **24**, 678–686
- Trujillo, M., Donald, R. G., Roos, D. S., Greene, P. J., and Santi, D. V. (1996) Heterologous expression and characterization of the bifunctional dihydrofolate reductase-thymidylate synthase enzyme of *Toxoplasma gondii*. *Biochemistry* **35**, 6366–6374
- Kisliuk, R. L., Gaumont, Y., and Baugh, C. M. (1974) Polyglutamyl derivatives of folate as substrates and inhibitors of thymidylate synthetase. *J. Biol. Chem.* **249**, 4100–4103
- Rudolph, J., and Stubbe, J. (1995) Investigation of the mechanism of phosphoribosylamine transfer from glutamine phosphoribosylpyrophosphate amidotransferase to glycylamide ribonucleotide synthetase. *Biochemistry* **34**, 2241–2250
- Wang, W., Kappock, T. J., Stubbe, J., and Ealick, S. E. (1998) X-ray crystal structure of glycylamide ribonucleotide synthetase from *Escherichia coli*. *Biochemistry* **37**, 15647–15662
- Irvine, H. S., Shaw, S. M., Paton, A., and Carrey, E. A. (1997) A reciprocal allosteric mechanism for efficient transfer of labile intermediates between active sites in CAD, the mammalian pyrimidine-biosynthetic multienzyme polypeptide. *Eur. J. Biochem.* **247**, 1063–1073
- Penverne, B., Belkaid, M., and Herve, G. (1994) *In situ* behavior of the pyrimidine pathway enzymes in *Saccharomyces cerevisiae*. 4. The channeling of carbamylphosphate to aspartate transcarbamylase and its partition in the pyrimidine and arginine pathways. *Arch. Biochem. Biophys.* **309**, 85–93
- Thoden, J. B., Raushel, F. M., Benning, M. M., Rayment, I., and Holden, H. M. (1999) The structure of carbamoyl phosphate synthetase determined to 2.1 Å resolution. *Acta Crystallogr. Sec. D* **55**, 8–24