Substrate channelling

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Substrate Channelling

Nature's Own Tunnels

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The fascinating process of channelling of metabolic intermediates through enzymes is discussed in this article. Often, the active sites of two enzymes that catalyse sequential reactions in a metabolic pathway are connected through an intra-molecular 'void' – a sort of tunnel, lined by amino acids through which intermediates pass. Synergy between the subunits leads to a phenomenal increase in the overall rate of catalysis. There are enzymes with a single channel, some with multiple channels and some without any discrete channel/s, yet achieving high catalytic rates. Unravelling details about these natural tunnels of angstrom dimensions has evoked great interest and curiosity in biochemists all over the world.

Enzymes are by far the most important agents of biological function. Ever since James B Sumner obtained octahedral crystals of urease (1916) and convincingly proved it to be a crystallizable 'globulin' (protein) with catalytic ability, the study of enzymes has yielded fascinating details about the chemistry of life. All the information required by a protein to achieve its secondary and tertiary levels of structure is contained within its primary structure and a protein structure as such is complete at the tertiary level. Only some have a quaternary or oligomeric organization which greatly increases the genetic economy as well as efficiency of an organism. The catalytic sites of such enzymes are often brought together in an alignment that favours transfer of 'metabolic intermediates'[1] between the active sites.

Metabolite Channelling (MC) thus acts to facilitate quick movement of intermediates, thereby not only just decreasing their transit time but also preventing their loss by diffusion into the surrounding environment. In the process, labile intermediates are blocked from solvent as well as their entry into any competing metabolic pathway. This gives rise to a phenomenon known as 'catalytic enhancement' whereby the overall rate of a reaction is considerably enhanced. Now, the nature of binding between such enzymatic associations is non-covalent and thus held by weaker forces. Hence, the isolation of such complexes is not feasible, and this has generated considerable debate as to whether metabolic channelling actually occurs or not, although now it is an established fact that the process indeed takes place. This incredible process is explained here with the help of a few examples.

Keywords: Substrate channelling, enzyme, reaction, tryptophan synthase, formylglycinamide ribonucleotide amidotransferase, thymidylate synthase-dihydrofolate reductase.

[1] Metabolic intermediates are nothing but products of one biochemical reaction that serve as reactants for another.

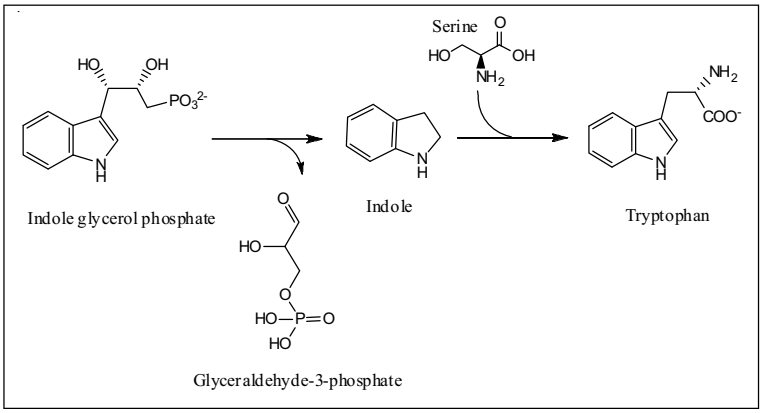


Figure 1. Reaction catalysed by tryptophan synthase enzyme.

The discovery of an intramolecular tunnel in tryptophan synthase, by Miles and co-workers [3], unravelled for the first time the molecular basis of substrate channelling [7]. Tryptophan synthase is an oligomeric enzyme that catalyses the last two reactions in the *L*-tryptophan biosynthetic pathway through its two subunits – α and β. Significantly, humans lack tryptophan synthase; so they cannot synthesize tryptophan and hence tryptophan is an essential amino acid [8]. Tryptophan synthase has been reported in bacteria, yeast and moulds among others [10]. While the α subunit catalyses the reversible formation of indole and glyceraldehyde-3-phosphate from indole-glycerol-phosphate, the β subunit catalyses the irreversible condensation of indole with *L*-serine to form tryptophan and water in a pyridoxal phosphate (PLP) catalysed reaction (see *Figure* 1). In bacteria such as *Salmonella typhimurium*, the α and β subunits combine to form a stable multienzyme complex $(\alpha\beta)_2$, whereas in yeasts and moulds, the α and β domains are located on a single polypeptide chain forming a bifunctional enzyme $(\alpha–\beta)_2$ [7,10].

Kinetic studies on the enzyme have shown rapid transfer of indole (>1000 s^{-1}) as well as rapid and irreversible reaction of indole at the β site [9]. Furthermore, there is some sort of inter-subunit communication that maintains kinetic synergy between the subunits such that there is a smooth and efficient channelling of indole without any concomitant accumulation in the tunnel.

X-ray crystallography data of the tryptophan synthase crystals from *S. typhimurium* also enabled determination of 3-D structures which revealed the tunnel to be 25Å in length, lined by predominantly hydrophobic amino acid residues and capable of accommodating as many as four indole molecules [7].

Formylglycinamide Ribonucleotide-Amidotransferase (FGAR-AT) is another example of enzyme-exhibiting substrate channelling [5]. It catalyses the fourth step of the *de novo* purine biosynthesis pathway from FGAR and glutamine (*Figure* 2). This pathway produces purines[2] which represent the building blocks for DNA and RNA synthesis.

[2] Purines (adenine and guanine) and pyrimidines (thymine, cytosine and uracil) make up the two groups of nitrogenous bases as well as nucleotide bases and are thus building blocks of DNA and RNA.

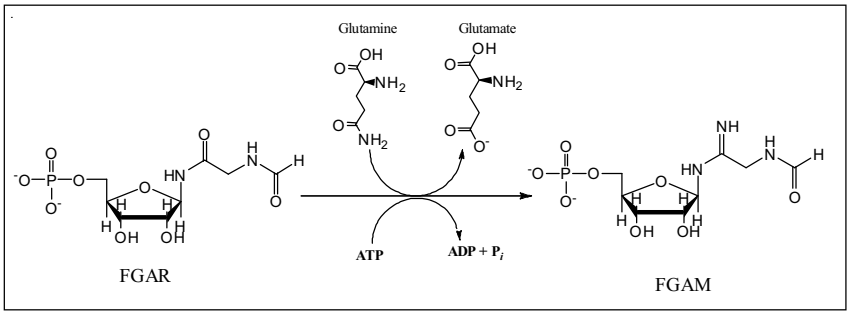


Figure 2. Reaction catalysed by FGAR-AT enzyme.

The kind of distinction which we saw in tryptophan synthase between the multienzyme complex in bacteria, and a single bifunctional enzyme in yeasts is represented here too, albeit on a finer scale. In eukaryotes and Gram-negative bacteria, the FGAR-AT is a single 140 kDa protein having four functional segments or 'domains': the N-terminal domain (referring to the beginning of protein chain). FGAM synthetase domain, a linker domain and the C-terminal glutaminase domain (referring to the end of protein chain). In contrast, in archea and Gram-positive bacteria, the FGAR-AT is formed from three separately formed proteins that work together to perform the same function as eukaryotic FGAR-AT [13].

At the glutaminase domain, glutamine, an amino acid, is hydrolysed and the product, ammonia gas is released. This ammonia gas is postulated to be channelled through a 'tunnel' to the FGAR/ATP binding site [4]. While it may be fanciful to assume the tunnel to be a straight or a curved path, it is neither! It is, in fact, just an empty space – a void, through the enzyme, the walls of which are formed by the amino acids making up the protein (*Figure* 3) [5,13].

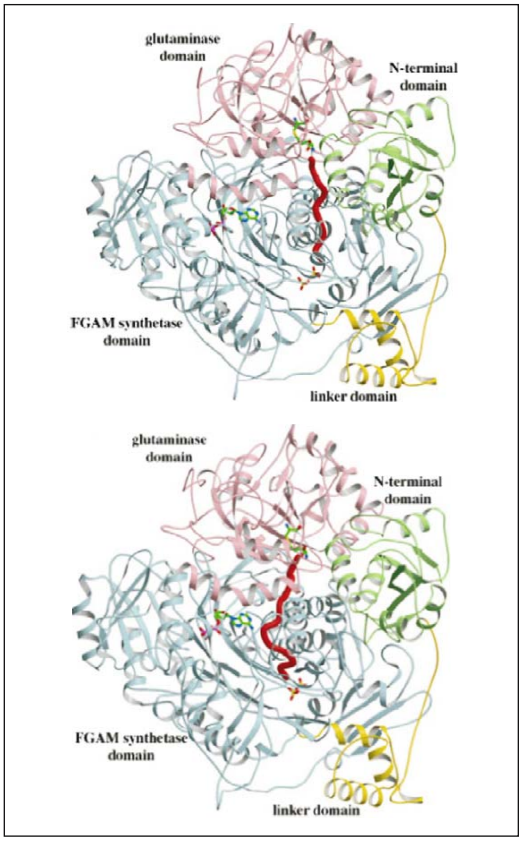


Figure 3. The red tubes in these figures illustrate the two possible ammonia channels in FGAM synthetase enzyme in *Salmonella typhimurium*.
(Reprinted (adapted) with permission from Anand *et. al.*, Domain Organization of *Salmonella typhimurium* Formylglycinamide Ribonucleotide Amidotransferase Revealed by X-ray Crystallography. *Biochemistry,* Vol.43, pp.10328–10342, 2004. Copyright (2004) American Chemical Society.)

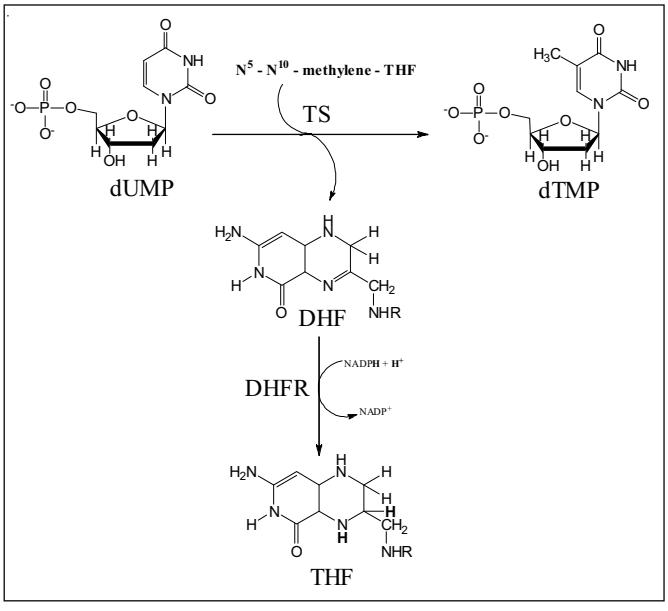
The glutaminase and FGAM synthetase active sites are situated about 30Å apart, but interestingly, unlike tryptophan synthase that has one tunnel/channel, two prospective paths have been postulated for ammonia channelling in FGAM synthetase based on X-ray crystallographic studies [13]. Thus, ammonia generated at one active site of the enzyme travels to another active site through one or both the channels. However, the exact mechanism by which both the active sites work in concert and the exact path traversed by ammonia still remains elusive.

The reason why substrate channelling needs to be understood in great detail is the potential importance of utilizing such information for drug development. For example, the dihydrofolate reductase (DHFR) and thymidylate synthase (TS) enzymes are of critical importance in *de novo* biosynthesis of pyrimidine.

Incredible as the enzyme is in its function, so it is in its mechanism too. Although DHFR and TS are separately translated and have their own separate existence in animals, fungi and eubacteria, plants and euglenozoas have a bifunctional fusion gene with both enzyme activities combined onto a single polypeptide chain with the DHFR domain at N-terminal and TS domain in C-terminal. Among the plants and euglenozoas, *Leishmania major* DHFR-TS has been studied in detail [6,11].

Remarkably, this enzyme does not have any intramolecular tunnel or channel, yet the rate of transfer of dihydrofolate (the metabolic intermediate in this case) between the two sites is as rapid as that of indole in tryptophan synthase (>1000 s^{-1})! This incredible feat is achieved through a unique mechanism of 'electrostatic channelling' [12]. It has been proposed that the negatively charged dihydrofolate 'slides' along a positively charged electrostatic 'highway' that links up the TS and DHFR domains which are situated about 40Å away[6]. The two domains at each site are activated upon binding of ligand to the other domain. Such reciprocal communication leads to a synergistic coupling of the reaction at the two sites [11,12]. The fact that there is an unusually high degree of conservation of positively charged residues in TS and

Figure 4. Reactions catalysed by TS and DHFR.



DHFR enzymes in organisms where they are separately translated indicates that organization of two domains in a single polypeptide chain might just have been an evolutionary design aimed at enhancing the efficiency and genetic economy of the organism in which they are present.

As mentioned earlier, armed with the information we obtain by understanding the structure – function correlation of these enzymes, we can design novel drugs or rationally engineer an enzyme, say to enhance its efficiency, to find cures for specific diseases. For example, cancerous cells multiply rapidly, hence they need a continuous supply of nucleotides for DNA synthesis. Therefore, continued DNA biosynthesis is necessary for cancerous cells to proliferate or increase in number. The strategic position of thymidylate synthase in DNA biosynthesis pathway has led to the clinical use of 5-fluro deoxyuridylate monophosphate (Fd-UMP) as an anti-cancer drug. Fd-UMP, in fact, acts as a *suicide inhibitor*[3] – being an irreversible inhibitor of TS, it undergoes the first two steps of normal enzymatic reaction, but is blocked at the third step (catalysed by thymidylate kinase). Since abundant supply of dTMP is essential for rapidly growing cancerous cells and Fd-UMP limits their availability, it acts as a powerful specific inactivator [14].

Drugs developed with the help of this knowledge are used in cancer chemotherapy as well as in certain autoimmune disorders. Moreover, study of enzymes like these gives us a great deal of information about their structure–function relationship as well.

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Suggested Reading

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[3] Suicide inhibitors are also known as mechanism-based inhibitors and Trojan Horse substrates. Such inhibitors participate in a catalytic mechanism that ends up irreversibly inhibiting itself. These inhibitors are substrates that have been modified. Since they are derivatives of the enzyme's actual substrate, the enzyme begins processing it as such. However, as the reaction proceeds, modifications of the substrate result in the formation of a reactive intermediate that forms covalent bonds with the enzyme that irreversibly inactivates it. Hence, they prevent further reaction and are considered suicidal.

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