Enzymes with Molecular Tunnels

FRANK M. RAUSHEL,*† JAMES B. THODEN,¶ AND HAZEL M. HOLDEN*‡

Department of Chemistry, Texas A&M University, College Station, Texas 77843-3012, and Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706-1544

Received January 29, 2003

ABSTRACT

As a result of recent advances in molecular cloning, protein expression, and X-ray crystallography, it has now become feasible to examine complicated protein structures at high resolution. For those enzymes with multiple catalytic sites, a common theme is beginning to emerge: the existence of molecular tunnels that connect one active site with another. The apparent mechanistic advantages rendered by these molecular conduits include the protection of unstable intermediates and an improvement in catalytic efficiency by blocking the diffusion of intermediates into the bulk solvent. Since the first molecular tunnel within tryptophan synthase was discovered in 1988, tunnels within carbamoyl phosphate synthetase, glutamine phosphoribosylpyrophosphate amidotransferase, asparagine synthetase, glutamate synthase, imidazole glycerol phosphate synthase, glucosamine 6-phosphate synthase, and carbon monoxide dehydrogenase/acetyl-CoA synthase have been identified. The translocation of ammonia, derived from the hydrolysis of glutamine, is the most abundant functional requirement for a protein tunnel identified thus far. Here we describe and summarize our current understanding of molecular tunnels observed in various enzyme systems.

Introduction

A revolution in biological chemistry has occurred within the last two decades due in part to significant advances in molecular cloning, protein expression, and X-ray crystallography. Perhaps only a few structural biologists could have predicted how rapidly our understanding of complicated protein complexes has evolved in such a short time frame as evidenced by the recent structural analysis of the ribosome.1 The excitement that accompanied the first X-ray analysis of an enzyme, namely, that of hen egg white lysozyme, was significant.2 For the first time, it was possible to examine an enzyme active site on a detailed chemical level. In recent years, more complicated enzyme structures are being solved at an ever increasing pace. It was once envisioned that enzymes with multiple active sites would fold in such a manner as to juxtapose the catalytic centers quite close to one another. This naive assumption was discarded when the molecular architecture of tryptophan synthase was determined by the laboratory of David Davies.3 Amazingly, the two active sites of tryptophan synthase are separated by ~25 Å and connected to one another by a molecular tunnel. Since that first elegant analysis of a multiple active site enzyme, other more complicated protein structures have been solved to high resolution via X-ray crystallography, and the presence of molecular tunnels is becoming a recurring theme in structural biology. Here we describe, in a historical context, those enzymes containing molecular tunnels and discuss how our understanding of substrate channeling within these complex protein systems has progressed.

Tryptophan Synthase

The last two steps in the biosynthesis of L-tryptophan, as outlined in Scheme 1, are catalyzed by tryptophan synthase. In bacteria such as S. typhimurium, these two distinct reactions are catalyzed by separate polypeptide chains, referred to as the α- and β-subunits, which form a stable (αβ)2 heterotetramer. The α-subunit catalyzes the cleavage of indole-3-glycerol phosphate (IGP) to yield an indole intermediate and glyceraldehyde-3-phosphate (G3P) while the β-subunit, which contains pyridoxal phosphate, is responsible for the condensation of the intermediate indole with L-serine. In 1988, the first detailed X-ray structure of the enzyme from S. typhimurium was reported to 2.5 Å resolution and revealed several remarkable features.3 The quaternary structure of the enzyme was shown to be a nearly linear arrangement of the individual subunits in an αβββ/αααα order, resulting in an elongated molecule of ~150 Å in length. The α-subunit adopts a β(βα)5-barrel fold referred to as a TIM barrel motif. This type of three-dimensional architecture was first identified in triose phosphate isomerase and has since been observed in various other enzymes.4 In all TIM-barrel containing enzymes examined to date, the active sites are invariably located at the C-terminal end of the β-barrel. In tryptophan synthase, indole propanol phosphate, a competitive inhibitor bound to the α-subunit, is located in such a position (Figure 1). The larger β-subunit folds into two motifs of roughly equal size with the N-terminal domain core formed by four strands of parallel β-sheet and the C-terminal domain containing six strands of mixed β-sheet, five running parallel, and one orienting in an antiparallel arrangement. The pyridoxal phosphate cofactor is located at the interface between the two domains of the β-subunit and is attached to Lys 87.

Perhaps most striking in this first structural analysis of tryptophan synthase was the ~25 Å distance between the indole propanol phosphate bound to the α-subunit and
the pyridoxal phosphate cofactor residing in the \( \beta \)-subunit of the \( \alpha,\beta \)-functional unit. A largely hydrophobic tunnel, with appropriate dimensions for the passage of indole, was observed connecting these individual active sites. In keeping with the need to maintain the structural integrity of the molecular tunnel, the dimeric interface between the \( \alpha \)- and \( \beta \)-subunits is quite extensive. Specifically \( \sim 1190 \text{ Å}^2 \) and \( 1110 \text{ Å}^2 \) of surface areas of the \( \alpha \)- and \( \beta \)-subunits, respectively, are buried at the dimeric interface.3

Three amino acid residues partially block the substrate tunnel, namely, Tyr 279 and Phe 280 of the \( \beta \)-subunit and Leu 58 of the \( \alpha \)-subunit.5 Leu 58 resides in a region of polypeptide chain with relatively higher temperature factors, thus suggesting that this region is fairly mobile. In the structure of tryptophan synthase solved in the presence of sodium ions, Tyr 279 and Phe 280 partially block the tunnel.5 Strikingly, when the sodium ions are replaced with potassium or cesium ions, these aromatic side chains move to a position lining rather than blocking this molecular pathway.

X-ray structures of tryptophan synthase complexed with a variety of substrate analogues have demonstrated that when the active sites of both the \( \alpha \)- and \( \beta \)-subunits are occupied, a disordered surface loop in the \( \alpha \)-subunit, Arg 179 to Asn 187, becomes ordered and clamps down over the \( \alpha \)-subunit active site. Additional conformational changes that occur upon substrate binding include a rigid body rotation of the \( \alpha \)-subunit with respect to the \( \beta \)-subunit and movements of the \( \beta \)-subunit in the region defined by Gly 93 to Gly 189. The net effect of these conformational changes is to restrict access of the solvent to both the \( \alpha \)- and \( \beta \)-subunit active sites and also to the tunnel.5,7

The catalytic mechanism of tryptophan synthetase has been probed by steady state and transient kinetic analyses.8 In single turnover experiments, the concentration of the intermediate indole is extremely low, indicating that once formed it is rapidly translocated from the site of production within the \( \alpha \)-subunit to the site of utilization in the \( \beta \)-subunit.9 Tryptophan synthase also exhibits a high degree of synchronization between the two active sites. It is clear that the allosteric control of activity is governed by chemical events initiated within the \( \beta \)-subunit. Thus, formation of the amino-acrylate intermediate from L-serine stimulates the cleavage of indole glycerol phosphate by 30-fold at the \( \alpha \)-subunit. This rate enhancement must
be modulated via conformation changes propagated from one active site to another.

**Carbamoyl Phosphate Synthetase**

The most complex structure to be solved of a protein containing a substrate channel has been that of carbamoyl phosphate synthetase, hereafter referred to as CPS. This enzyme catalyzes the formation of carbamoyl phosphate, which is subsequently employed in the biosynthesis of arginine and pyrimidines in eukaryotes and prokaryotes and in the urea cycle in most terrestrial vertebrates. The enzyme has attracted significant attention in part because of its important metabolic role, its large size, and its interesting catalytic mechanism, which is outlined in Scheme 2. As obtained from *Escherichia coli*, the enzyme consists of two polypeptide chains, referred to as the small and large subunits. The small subunit is responsible for the hydrolysis of glutamine to glutamate and ammonia, while the large subunit provides the binding sites for the two molecules of ATP required for the reaction. As indicated in Scheme 2, the reaction mechanism of CPS proceeds through three separate intermediates: carboxy phosphate, ammonia, and carbamate. CPS belongs to the class I amidotransferases that employ a Cys–His catalytic couple for the hydrolysis of glutamine. All of the biochemical data on CPS suggest that there is no uncoupling of the separate chemical reactions, as outlined in Scheme 2.

The enzyme from *E. coli* readily interconverts from an \( \alpha_{\beta}\)-heterodimer to an \( (\alpha_{\beta})_4 \)-heterooctamer, depending upon the presence or absence of various effector molecules including ornithine and UMP. Before 1997, it was tacitly assumed that the three active sites of CPS would be quite close to one another. Surprisingly, however, when the structure of the \( (\alpha_{\beta})_4 \)-heterooctamer from *E. coli* was solved, the three active sites were farther apart than ever envisioned, as indicated in Figure 2. Specifically, the active site within the small subunit is positioned ∼45 Å from the first active site of the large subunit, which in turn is located ∼35 Å from the second active site within the large subunit. The overall fold of the small subunit is distinctly bilobal with the C-terminal half dominated by a 10-stranded mixed β-sheet. The topology of the mixed β-sheet is exceedingly similar to that observed in GMP synthetase, another class I amidotransferase. The large subunit contains two so-called ATP-grasp motifs that cradle the required nucleotides.

Visual inspection of this model of CPS, coupled with a computational search with the software package “GRASP”, revealed a molecular tunnel connecting the three active sites as indicated in Figure 2. This tunnel extends from...
the base of the small subunit active site, toward the first active site of the large subunit, and is lined, for the most part, with unreactive side chains and backbone atoms. The portion of the tunnel lying between the two active sites of the large subunit is somewhat less hydrophobic. In support of the molecular tunnel, many of the residues lining the pathway are absolutely conserved, and those residues that are not strictly conserved are typically replaced with amino acid residues of comparable chemical reactivities.\(^{20}\) The average minimum radius of this molecular passageway is \(\sim 3.4\ \text{Å}\), which is sufficient for the passage of the reactive intermediates from one active site to the next.

Recently, as a test of the validity of the molecular passageway mapped out in Figure 2, a series of site-directed mutant proteins were created within the first half of the tunnel leading from the small subunit to the large subunit.\(^{21,22}\) Specifically, Gly 359 of the small subunit, which forms an integral part of the interior wall of the tunnel, was targeted for investigation. These studies demonstrated a direct correlation between the size of the substituted amino acid and the extent of uncoupling of the partial reactions catalyzed by CPS, specifically, glutamine and ATP hydrolysis. Subsequently, the structure of the G359F mutant of CPS was determined and indicated that in the case of this particular protein, the uncoupling of the separate chemical reactions was a direct function of creating an escape route for the ammonia intermediate.\(^{23}\) Taken together, these site-directed mutagenesis experiments and structural analyses further support the existence and importance of the tunnel highlighted in Figure 2.

The catalytic mechanism for the synchronization of the active sites within CPS has been addressed by rapid quench investigations.\(^{24}\) It has been shown that the hydrolysis of glutamine within the small subunit is enhanced about 500-fold when bicarbonate is phosphorylated by ATP to form the carboxy phosphate intermediate. In contrast, there is essentially no effect on the rate of carboxy phosphate formation upon the hydrolysis of glutamine. Thus, it appears that the phosphorylation of bicarbonate serves as the trigger for the hydrolysis of glutamine. Ammonia is therefore released into the tunnel only when the reactive intermediate is waiting to form carbamate. A small number of ordered water molecules have been observed within the tunnel that has evolved for the passage of ammonia.\(^{20}\) These water molecules apparently do not react with the carboxy phosphate intermediate at an appreciable rate in competition with ammonia since the net stoichiometry of the overall reaction is maintained as presented in Scheme 2. This is consistent with the significantly greater nucleophilicity for ammonia relative to water. The specific conformational changes that are propagated from the large subunit to the small subunit have not, as yet, been identified. The extent of interaction and coupling between the two active sites within the large subunit of CPS has not been elucidated.

<table>
<thead>
<tr>
<th>Scheme 3. Reaction Catalyzed by Glutamine Phosphoribosylpyrophosphate Amidotransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Scheme 3" /></td>
</tr>
</tbody>
</table>

Glutamine Phosphoribosylpyrophosphate Amidotransferase

The X-ray crystallographic analyses of tryptophan synthase and CPS provided the first direct evidence for preformed molecular tunnels between active sites within multiple polypeptide chains. However, the structural study of glutamine phosphoribosylpyrophosphate amidotransferase (GPATase) revealed a new feature in substrate channeling: the formation of a tunnel only when both active sites are occupied by the appropriate ligands. GPATase catalyzes the first committed step in the de novo pathway for the biosynthesis of purine nucleotides as outlined in Scheme 3, where phosphoribosylpyrophosphate (PRPP) is converted to phosphoribosylamine (PRA). The enzyme, which like CPS employs glutamine as the direct source of ammonia, belongs to the class II amidotransferases.\(^ {12}\) In the class II amidotransferases, an N-terminal cysteine residue serves as the active site nucleophile required for glutamine hydrolysis. Each subunit of the dimeric GPATase from \(E.\ coli\) contains two active sites surrounded by a polypeptide chain of 504 amino acid residues. The first structures solved of GPATase from \(E.\ coli\) were those of the wild-type protein, the protein complexed with the feedback inhibitor AMP, and the enzyme with bound 6-diazo-5-oxonorleucine, a glutamine analogue.\(^ {25}\) These models were essentially identical to one another and demonstrated that the binding site for the PRPP moiety was exposed to solvent. Additionally, these structures revealed that the overall fold of the enzyme contained two motifs per subunit: an N-terminal domain delineated by Cys 1 to Ile 230 and the C-terminal domain defined by Tyr 231 to Tyr 465. The N-terminal domain contained two antiparallel \(\beta\)-sheets flanked on the outer edges by \(\alpha\)-helices, while the C-terminal region was dominated by a five-stranded parallel \(\beta\)-sheet (Figure 3).

When Smith and co-workers determined the structure of GPATase in the presence of diazo-5-oxonorleucine and a stable carbocyclic analogue of PRPP, a largely hydrophobic tunnel of \(\sim 20\ \text{Å}\) in length and connecting the two active sites was formed as indicated in Figure 3.\(^ {26}\) The formation of the tunnel resulted from a kinking of the C-terminal helix, a restructuring of the loop between Arg 73 to Ser 79, and an ordering of a flexible surface loop formed by Val 325 to Arg 354. Interestingly, 14 of the 17 amino acid residues lining the molecular conduit in GPATase are invariant in the 24 reported amino acid sequences.\(^ {26}\)
Asparagine Synthetase

Following the successful X-ray analysis of GPATase, a second enzyme belonging to the class II amidotransferases, asparagine synthetase from *E. coli*, was determined.27 As the name implies, this enzyme catalyzes the production of asparagine from glutamine, ATP, and aspartate (Scheme 4). The reaction mechanism is thought to proceed through a β-aspartyl-AMP intermediate. Like GPATase, it is a dimeric enzyme with each subunit containing two active sites: one responsible for the binding and hydrolysis of glutamine and the other for the positioning of ATP and aspartate, the formation of the β-aspartyl-AMP intermediate, and the ultimate synthesis of asparagine. Only one structure of the enzyme is known to date, that of a site-directed mutant protein whereby the active site Cys 1 was changed to an alanine residue. This structure was solved in the presence of both glutamine and AMP. As can be seen in Figure 4, the N-terminal domain of asparagine synthetase (Cys 1 to Asp 194) is strikingly similar to that of GPATase. The C-terminal domain of asparagine synthetase contains a five-stranded parallel β-sheet that positions the AMP moiety across its C-terminal edge. Interestingly, the last 37 residues are disordered in this structure and may prove to be important in binding the aspartate substrate. A molecular tunnel of ~19 Å in length connects the two active sites and is formed primarily by backbone atoms and hydrophobic side chains (Figure 4).

Glutamate Synthase

The NADPH-dependent glutamate synthase from *Azospirillum brasilense* is a complex iron–sulfur protein that catalyzes the reductive transfer of ammonia from L-glutamine to the C-2 carbon of 2-oxoglutarate to yield two molecules of L-glutamate, as indicated in Scheme 5.28 Its physiological role is to provide a pathway for nitrogen metabolism in plants and microorganisms. The overall reaction catalyzed by glutamate synthase has been shown to take place in five steps.28 The enzyme functions as an α,β-heterodimer with the α- and β-subunits having molecular weights of ~150 000 and 50 000, respectively. The α-subunit harbors the binding sites for 2-oxoglutarate, the FMN cofactor, and a 3Fe–4S center and additionally provides the catalytic machinery required for the hydrolysis of glutamine to glutamate. It is in the β-subunit where NADPH is oxidized with the subsequent reduction of FAD.29 The reducing equivalents are then transferred to FMN through the iron–sulfur clusters. At one active site in the α-subunit, the side chain carboxamide group of glutamine is hydrolyzed to produce the first molecule of L-glutamate and ammonia. The ammonia then adds to...
C-2 of 2-oxoglutarate to generate the 2-iminoglutarate intermediate. This intermediate is reduced by FMN to generate the second molecule of L-glutamate. In short, the α-subunit functions to convey electrons to the R-subunit, which is the actual functional core of the enzyme. Indeed, the isolated R-subunit can produce L-glutamate from 2-oxoglutarate and glutamine in the presence of an artificial electron donor such as methyl viologen.\(^3\)\(^0\) Additionally, the isolated α-subunits, but not the β-subunits, form dimeric species.\(^3\)\(^1\)

The three-dimensional structure of the α-subunit from A. brasilense was recently solved to 3.0 Å resolution in complex with 2-oxoglutarate and L-methionine sulfone, a glutamine analogue (Figure 5).\(^3\)\(^2\) Each α-subunit folds into four distinct domains of approximately equal size. The N-terminal domain adopts the characteristic α/β/β/α architecture observed in the class II amidotransferases with the active site cysteine positioned at the N-terminus of the polypeptide chain. The central domain adopts an α/β topology with a parallel β-sheet flanked on one side by four α-helices and on the other side by a single helix. The third domain contains an (β/α)\(_8\) barrel that harbors both the binding site for FMN and the 3Fe–4S cluster. The final C-terminal domain adopts an α-helix topology. Biochemically, the N-terminal domain is responsible for the production of ammonia, while the FMN-binding domain catalyzes the reduction of the 2-iminoglutarate intermediate. The two active sites in glutamate synthase are separated by ~31 Å and connected by a tunnel that is constricted at one location by the main chain atoms of residues Thr 507 to Asn 508 and Ser 976 to Ile 977. The first half of ammonia conduit is formed by Tyr 211, Ser 212, Thr 213, Glu 233, Val 506, Thr 507, Asn 508, and Ile 977.
977 and is reasonably hydrophilic in character. Strikingly, it is not completely closed to the external solvent. The second half of the tunnel, on the other hand, is shielded from the solvent and is formed by loops from the FMN binding domain, and a long loop contributed by the central domain. The walls of this portion of the ammonia conduit are formed by backbone and side chain carbonyl oxygens and several aliphatic groups. Interestingly, six strictly conserved proline residues reside in this second half of the tunnel. It has been suggested that the constriction of the tunnel at residues Thr 507 to Asn 508 and Ser 976 to Ile 977 may function as a gating mechanism for proper signaling between active sites. The structure of a ferredoxin-dependent glutamate synthase, namely, that isolated from *Synechocystis* sp. PCC 6803, has also recently been solved, again indicating the presence of an ammonia tunnel that is ~30 Å in length.33

**Scheme 6. Reaction Mechanism Catalyzed by Imidazole Glycerol Phosphate Synthase**

Enzymes with Molecular Tunnels

![Diagram](image)

**FIGURE 6.** Ribbon representation of imidazole glycerol phosphate synthase. The glutaminase and cyclase domains are displayed in yellow and blue, respectively. The tunnel is indicated by the red wire drawing.

**Imidazole Glycerol Phosphate Synthase**

Imidazole glycerol phosphate synthase catalyzes the formation of imidazole glycerol phosphate (IGP) and 5′-5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) from N1-(5′-phosphoribosyl)-formimino-5-aminoimidazole-4-carboxamide ribonucleotide (PRFAR), thereby serving as a branch point leading to purine and histidine biosynthesis in some organisms (Scheme 6). The enzyme has attracted attention as a possible target for antifungal, antibacterial, and/or herbicide agents in light of the fact that the histidine biosynthetic pathway does not occur in mammals. As isolated from *Saccharomyces cerevisiae*, the monomeric enzyme contains 555 amino acid residues and two active sites: one required for glutamine hydrolysis and the other required for cyclization (Scheme 6). The first structure of imidazole glycerol phosphate synthase solved was that obtained from *S. cerevisiae* to 2.1 Å resolution.34 Since that initial X-ray crystallographic analysis, the three-dimensional structures of the enzymes from *Thermotoga maritima* and *Thermus thermophilus* have also been determined.35,36 All of these enzymes belong to the class I amidotransferase superfamily.

A ribbon representation of the yeast enzyme is depicted in Figure 6. The N-terminal glutaminase domain (Met 1 to Gly 235) adopts a similar mixed ß-sheet topology to that observed in other members of the class I amidotransferase family including GMP synthetase and CPS.14,16 The catalytic cysteine residue, Cys 83, is located within hydrogen bonding distance to His 193. In the structure of the yeast enzyme, this cysteine residue is labeled with acivicin, a heterocyclic analogue of glutamine first isolated from *Streptomyces avicinus*. The cyclase domain (Leu 236 to Glu 552) folds into a classical ß/α TIM barrel. Even though the structure of the yeast enzyme was solved in the absence of a suitable analogue to label the cyclase active site, it can be assumed that the active site of the cyclase
domain is located at the C-terminal portion of the TIM barrel. Quite remarkably, in imidazole glycerol phosphate synthase, as opposed to tryptophan synthase and glutamate synthase, it is the back end of the \( \beta \)-barrel, farthest away from the active site, that abuts the glutaminase domain. As a result, the ammonia generated in the N-terminal domain must traverse a tunnel that occupies the entire length of the TIM barrel.

The location of the ammonia tunnel is depicted in Figure 6. The first half of the tunnel, leading from the glutaminase to the cyclase domains, is quite polar and is lined with conserved amino acid residues. There are four conserved and charged residues at the bottom of the \( \beta \)-barrel in the cyclase domain (Arg 239, Glu 293, Lys 360, and Glu 465) which serve as a “gate” between the two domains. The ammonia tunnel embedded in the cyclase domain is hydrophobic and formed by small aliphatic side chains. The manner in which the gate separating the two portions of the molecular tunnel opens to allow passage of the ammonia intermediate is not well understood. It has been suggested that rotation of the side chain of Lys 360 to form a hydrogen bond with \( \text{O}^- \) of Tyr 144 might open the gate wide enough for ammonia passage.

**Glucosamine 6-Phosphate Synthase**

Glucosamine 6-phosphate synthase, as indicated in Scheme 7, catalyzes the first committed step in the overall production of the N-acetylglucosamine, a key nitrogen-containing carbohydrate found in both bacterial and fungal cell walls. The reaction mechanism is thought to proceed via fructosimine 6-phosphate, which is isomerized to the product, glucosamine 6-phosphate. The enzyme from *E. coli* is a dimer, with each subunit containing 608 amino acid residues. Like GPATase and asparagine synthetase, glucosamine 6-phosphate synthase belongs to the class II amidotransferases. Recently, the three-dimensional structure of the bacterial enzyme was determined to 3.1 Å resolution, as indicated in Figure 7. For this particular analysis, the enzyme was crystallized in the presence of one of its substrates, fructose 6-phosphate. As indicated in Figure 7, a hydrophobic tunnel connects the glutaminase and isomerase domains. The diameter of this tunnel is \(~6\,\text{Å}\). Interestingly, the pathway is blocked by Trp 74. A mechanism whereby the binding of glutamine triggers the movement of the indole ring of Trp 74 out of the channel has been proposed.

**Conclusions**

A handful of proteins have now been identified that contain molecular tunnels for the passage of reactive intermediates from one active site to another. In addition to the seven proteins discussed above, a tunnel for the migration of carbon monoxide has recently been identified in the structure of carbon monoxide dehydrogenase/
Enzymes with Molecular Tunnels  Raushel et al.

acetyl-CoA synthase. The most common intermediate for passage through the molecular tunnels identified to date is ammonia. At this point it seems safe to assume that all proteins that hydrolyze glutamine to obtain ammonia as a biosynthetic ingredient will utilize a molecular tunnel for the translocation of ammonia from the site of production to the site of utilization. In a majority of the systems examined thus far, the structure of the tunnel is preformed in the absence of additional ligands bound to the individual active sites. However, in the case of at least one example, the tunnel is only formed after substrates have bound to their respective active sites. Within the glutamine amidotransferase family of enzymes the catalytic machinery seems to have been retained throughout evolution. However, the tunnel architecture is quite different in all of the examples identified to date is ammonia. At this point it seems safe to assume for passage through the molecular tunnels identified to date.

This research was supported in part by the NIH (Grants GM 55513 to H.M.H. and DK 30343 to F.M.R.) and the Robert A. Welch Foundation (Grant A-840 to F.M.R.).

References


AR020047K