Chapter 12

Transfer RNA-Dependent Aminolevulinic Acid Formation: Structure and Function Of Glutamyl-tRNA Synthetase, Reductase and Glutamate-1-Semialdehyde-2,1-Aminomutase

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Summary

In plants, green algae, archaea and in most bacteria the common precursor of all tetrapyrroles, 5-aminolevulinic acid, is formed by three enzymes. The initial substrate glutamate is converted to glutamyl-tRNA by glutamyl-tRNA synthetase for use in both protein and tetrapyrrole biosynthesis. During the first committed step an NADPH-dependent glutamyl-tRNA reductase reduces glutamyl-tRNA to form glutamate-1-semialdehyde, which is subsequently transaminated by glutamate-1-semialdehyde-2,1-aminomutase to yield 5-aminolevulinic acid. The enzymatic mechanisms deduced from biochemical investigations and recently solved crystal structures are described for all three enzymes. A potential pathway for metabolic channeling of the reactive aldehyde between glutamyl-tRNA reductase and the aminomutase is outlined.

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I. Two Pathways for 5-Aminolevulinic Acid Biosynthesis

Tetrapyrroles, such as hemes, chlorophylls, vitamin B_{12} and coenzyme F_{430} are widely distributed among all living organisms. The common precursor of all tetrapyrroles, 5-aminolevulinic acid (ALA), may be synthesized by two alternative, unrelated biosynthetic routes: in mammals, fungi and the α-group of the proteobacteria, condensation of succinyl coenzyme A and glycine is catalyzed by 5-aminolevulinic acid synthase to form ALA by the so-called 'Shemin pathway' (Shemin and Russel, 1953; Gibson et al., 1958; Kikuchi et al., 1958). The alternative C_5-pathway found in plants, archaea and most bacteria, incorporates the C_5-skeleton of glutamate (Beale and Castelfranco, 1973; Jahn et al., 1992) and involves the reduction of glutamyl-tRNA to glutamate-1-semialdehyde (GSA) catalyzed by NADPH-dependent glutamyl-tRNA reductase (GluTR) (Jahn et al., 1992; Vothknecht et al., 1996). In a following reaction pyridoxamine 5´-phosphate (PMP)-dependent glutamate-1-semialdehyde-2,1-aminomutase (GSAM) transaminates GSA to form ALA (Grimm et al., 1992; Ilag and Jahn, 1992; Smith et al., 1992; Chapter 11, Beale). This chapter focuses on the structure and function of the enzymes of the C_5-pathway. A detailed description of 5-aminolevulinic acid synthase function has previously been published (Ferreira and Gong, 1995; Chapter 11, Beale).

II. Glutamyl-tRNA Synthetase Forms Glutamyl-tRNA for Protein and Tetrapyrrole Biosynthesis

The precursor of the C_5 pathway for ALA formation is glutamate which is converted by glutamyl-tRNA synthetase (GluRS) to glutamyl-tRNA (Huang et al., 1984; Schön et al., 1986). Glutamyl-tRNA simultaneously participates in protein and tetrapyrrole biosynthesis (O'Neil and Söll, 1990; Jahn et al., 1992). The properties of GluRS and its substrate, $tRNA^{Glu}$, are discussed below only with relevance to ALA biosynthesis.

Abbreviations: ALA – 5-aminolevulinic acid; *C.* – *Chlamydomonas*; DAVA – diaminovalerate; *E.* – *Escherichia*; GluRS – glutamyl-tRNA synthetase; GluTR – glutamyl-tRNA reductase; GSA – glutamate-1-semialdehyde; GSAM – glutamate-1-semialdehyde-2,1-aminomutase; *M.* – *Methanopyrus*; PLP – pyridoxal 5´-phosphate; PMP – pyridoxamine 5´-phosphate; *S.* – *Salmonella*

III. Glutamyl-tRNA

Investigation of barley chloroplast, *Chlamydomonas* (*C.*) *reinhardtii* and *Chlorella* extracts first indicated that a tRNA molecule could be essential for the synthesis of ALA from glutamate (Huang et al., 1984; Kannangara et al., 1984; Weinstein and Beale, 1985). Sequence analysis of the active tRNA species in barley and *C. reinhardtii* confirmed that a $tRNA^{Glu}$ is involved (Schön et al., 1986; O´Neill and Söll, 1990; Chapter 11, Beale). A variety of in vitro and in vivo approaches established the identity elements of *Escherichia* (*E.*) *coli* $tRNA^{Glu}$ for recognition by GluRS (Fig. 1a). The identity set consists of U34, U35, C36 and A37 in the anticodon loop, G1:C72, U2:A71 and C4:G69 in the acceptor stem, U11:A24, U13:G22::A46 and C12:G23::C9 in the augmented D helix formed of the D-stem helix with several neighboring residues and the variable loop (N:N and N::N denote secondary and tertiary nucleotide base pairings). In addition, A46 and the absence of nucleotide 47 (D47) in the short variable loop were found to stabilize U13:G22::A46, a tertiary base pairing which is essential for tRNA-GluRS interaction (Sekine et al., 1996, 1999). The relevance to recognition of the 2-thio groups, in methylaminomethyl-2-thiouridine (mnm5s2U) in the wobble position U34, by *E. coli* GluRS in vitro was challenged by in vivo experiments with hypomodified $tRNA^{Glu}$ (Sylvers et al., 1993; Kruger and Sorensen, 1998; Madore et al., 1999).

In contrast to the detailed analysis of $tRNA^{Glu}$ for GluRS recognition, the identity elements of glutamyl-tRNA for GluTR were mainly subject to theoretical considerations (Jahn et al., 1991; Willows et al., 1995). The isolation of an *Euglena gracilis* plastidic $tRNA^{Glu}$ gene mutant, impaired in chlorophyll biosynthesis but capable of protein biosynthesis, revealed the only verified glutamyl-tRNA identity element in position C56 (Stange-Thomann et al., 1994).

IV. Glutamyl-tRNA Synthetase

GluRS (glutamyl-tRNA synthetase or glutamic acid $tRNA^{Glu}$ ligase, EC 6.1.1.17) esterifies glutamate with the 2´-terminal hydroxy group of tRNA which is specific for this amino acid. A two step mechanism including the activation of the amino acid by ATP and its subsequent transfer to the tRNA is generally accepted (Freist et al., 1997) (Fig. 2a):

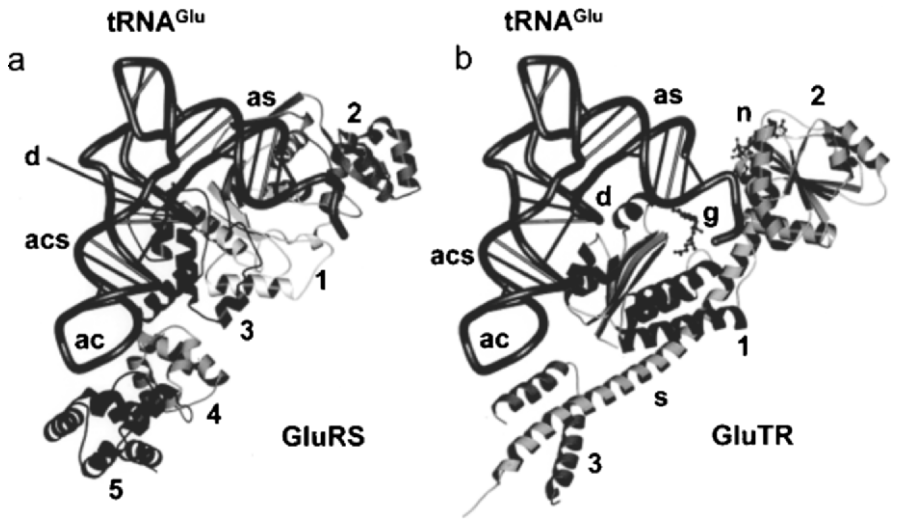

Fig. 1. A ribbon diagram of the complexes of (a) $tRNA^{Glu}$/glutamyl-tRNA synthetase (b) $tRNA^{Glu}$/glutamyl-tRNA reductase monomer (modeled). The complexes have been oriented to provide the equivalent view of the $tRNA^{Glu}$. Individual domains are numbered. as – acceptor stem of $tRNA^{Glu}$, ac – anticodon, acs – anticodon stem, d – D-stem, g - Glutamycin, n – NADPH (modeled), s – spinal helix. Note the similarity in binding modes in both proteins. Generated using *MOLSCRIPT* (Kraulis, 1991) rendered with *POVRAY* (www.povray.org) as implemented in *GL_RENDER* (www.hhmi.swmed.edu/external/Doc/Gl_render).

$$\text{GluRS} + \text{Glu} + \text{ATP} (+ \text{tRNA}^{Glu}) \rightarrow \text{GluRS} + \text{Glu-AMP} + \text{PP}_i (+ \text{tRNA}^{Glu}) \quad (1)$$

$$\text{GluRS} + \text{Glu-AMP} + \text{tRNA}^{Glu} \rightarrow \text{GluRS} + \text{Glu-tRNA}^{Glu} + \text{AMP} \quad (2)$$

GluRS is one of ten class I aminoacyl-tRNA synthetases: its ATP-binding domain bears a variant (HVGG) of the characteristic HIGH motif (Cusack et al., 1990; Eriani et al., 1990). Evolutionarily and sequence-wise, GluRS is closely related to glutaminyl-tRNA synthetase (Breton et al., 1986). Many bacteria, archaea and organelles lack glutaminyl-tRNA synthetase. Here GluRS glutamylates both $tRNA^{Glu}$ and $tRNA^{Gln}$. The misacylated Glu-$tRNA^{Gln}$ is converted to the required Gln-$tRNA^{Gln}$ (glutaminyl-tRNA) by an amidotransferase (Schön et al., 1988; Jahn et al., 1990; Curnow et al., 1997; Tumbula et al., 2000).

A. Biochemical Characterization

The copy number of GluRS molecules per *E. coli* cell is between 300 and 900 depending on the growth medium (Pedersen et al., 1978). The molecular ratio of GluRS to ribosomes and elongation factors is roughly balanced at different growth conditions. Bacterial and green algal enzymes were found to be active as monomers with a M_r of ~50,000 (Freist et al., 1997). In *E. coli* and *Bacillus subtilis,* GluRS forms a complex with adenylosuccinate AMP-lyase (EC 4.3.2.2.). This protects GluRS against heat denaturation, increases its affinity for glutamate and ATP (Lapointe and Söll, 1972; Proulx et al., 1983; Gendron et al., 1992) and coordinates purine metabolism and protein biosynthesis. Chloroplast, mitochondrial and the cytoplasmic wheat GluRS are dimeric (Ratinaud et al., 1983). The K_m of GluRS is in the range of 10^{-4} and 10^{-5} M for ATP, about 10^{-5} M for glutamate and 10^{-7} M for $tRNA^{Glu}$ (Freist et al., 1997). In common with other aminoacyl-tRNA synthetases, GluRS activity is Mg^{2+}-dependent. *E. coli* GluRS enzyme activity is also Zn^{2+}-dependent (Liu et al., 1993). Extended X-ray absorption fine structure (EXAFS) studies identified a non-planar coordination site involving three sulfur atoms and one nitrogen (Cys98, 100, 125, His127). ATP inhibits zinc removal, suggesting that the binding site is near the active site. Analogous studies indicate that ATP-recognition involves the adenine base, two hydroxyl groups of the ribose moiety and the phosphate chain. Only bacterial and plastidic enzymes provide appropriate precursor for tetrapyrrole biosynthesis.

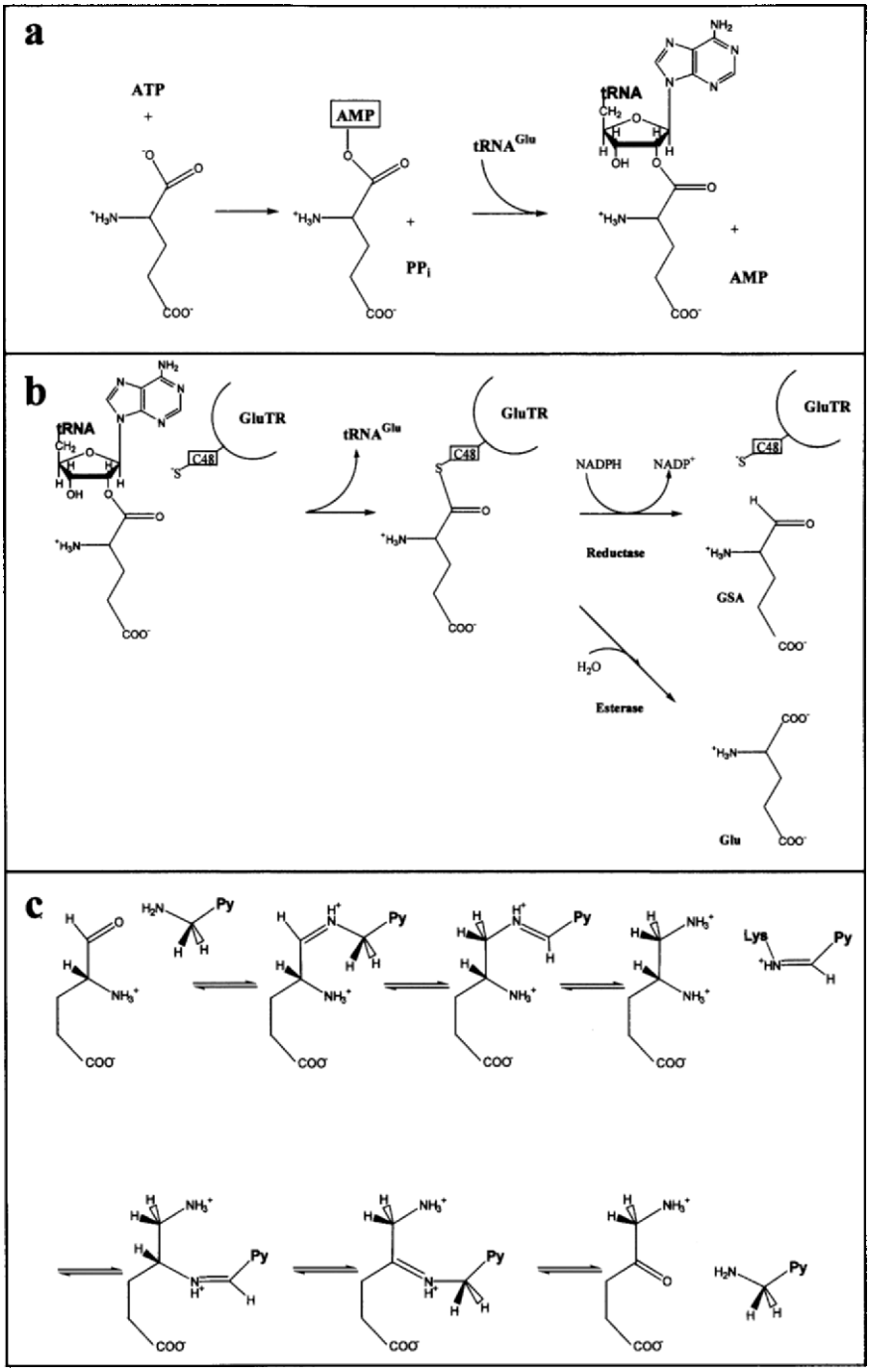

Fig. 2. The chemical reactions catalyzed by a) glutamyl-tRNA synthetase (GluRS), b) glutamyl-tRNA reductase (GluTR) and c) glutamate-1-semialdehyde-2,1-aminomutaseamino (GSAM). a) GluRS specifically binds ATP and glutamate, allowing the amino acid carboxyl oxygen of glutamate to displace pyrophosphate from ATP. In a second step the 2′-OH of glutamyl-tRNA attacks the carbonyl carbon of glutamate-AMP displacing AMP and generating glutamyl-tRNA^{Glu}. b) The thiol group of the catalytic cysteine 48 of GluTR initiates a nucleophilic attack at the tRNA-bound carbonyl carbon of glutamyl-tRNA, covalently binding glutamate to the enzyme and displacing glutamyl-tRNA. In a second step hydride transfer from NADPH releases glutamate-1-semialdehyde (GSA) from GluTR, restoring the latter. In the absence of NADPH, GluTR functions as an esterase, transferring a hydroxyl group from water, producing glutamate. c) GSA from GluTR is transferred to GSAM. Here the pyridoxamine-5-phosphate (PMP) binds the aldehyde of GSA forming an aldimine. Proton transfer and Schiff's base formation producing 5-pyridoxyl phosphate form of GSAM, releases the intermediate 4,5-diaminovalerate (DAVA). Aldimine formation at the former amino group of GSA, proton transfer and release of PMP produces the final 5-aminolevulinic acid (ALA).

B. Crystal Structure

The crystal structure of GluRS from the extreme thermophile *Thermus thermophilus* has been solved for both the uncomplexed protein (Nureki et al., 1995) and in complex with $tRNA^{Glu}$ (Sekine et al., 2001b). The monomeric enzyme (468 residues) consists of five distinct domains arranged to form an elongated, slightly curved molecule (Fig. 1a). Domain 1, a Rossmann ATP-binding fold, is conserved in all class-I tRNA synthetases. It bears the two characteristic ATP-binding motifs, HIGH (HVGG here) and KMSKS. Adjacent regions of domains 2 and 3 are also structurally conserved. Located on either side of the ATP-binding pocket of domain 1, they create a conspicuous groove that accommodates the tRNA acceptor arm. The remaining conserved α-helices of domain 3 interact with both the D-stem region at the concave elbow angle of the tRNA as well as the anticodon stem. The binding mode of the tRNA in class I tRNA synthetase complexes is thus conserved. Regions unique to each synthetase allow for specificity. Such regions of domains 2 and 3 create the glutamate recognition pocket. Domains 4 and 5, structurally unique to GluRS in their entirety, are reserved for $tRNA^{Glu}$-anticodon recognition and discrimination. Arginine residues are crucial for anticodon recognition in GluRS. Arg417 and Arg435, respectively, bind the phosphate and base of C34 while Arg358 hydrogen bonds C36 in a Watson-Crick-like pattern. Only U35 is recognized by a planar main-chain segment (Thr444-O and Pro445-N).

V. Glutamyl-tRNA Reductase

The discovery that $tRNA^{Glu}$ is involved in the biosynthesis of ALA prompted the formulation of the three-step-pathway: In the first step, glutamate is activated by ligation to the cofactor $tRNA^{Glu}$. Then tRNA-bound glutamate is reduced to GSA. Finally in the third step GSA is transaminated to ALA (Huang et al., 1984; Kannangara et al., 1984; Weinstein and Beale, 1985; Schön et al., 1986). The enzyme catalyzing the first committed step of tetrapyrrole formation is glutamyl-tRNA reductase (GluTR), the second enzyme is glutamate-1-semialdehyde-2,1-aminomutase (GSAM).

A. Biochemical Characterization

Early investigations of bacterial and plant GluTRs resulted in controversy regarding cofactor requirements, relative molecular masses and potential catalytic mechanisms (Chapter 11, Beale). In retrospect, the extended structure of GluTR (Moser et al., 1999, 2001), solubility problems due to unspecific disulfide bridge formation and hydrophobic interaction (Schauer et al., 2002) may partly explain these discrepancies.

A combined biochemical and crystallographic approach using recombinant GluTR from the extreme thermophilic archaeon *Methanopyrus* (*M.*) *kandleri* finally established the catalytic mechanism and its structural basis (Moser et al., 1999, 2001). The gene *hemA* encoding GluTR from *M. kandleri* was expressed in *E. coli*. The recombinant enzyme was purified to homogeneity. Using *E. coli* glutamyl-tRNA as substrate, the specific activity was determined to be 0.75 nmol h^{-1} mg^{-1} at 56 °C. Intensive spectroscopic and biochemical analysis of *M. kandleri* GluTR demonstrated that neither heme, flavins nor metal ions are required for catalysis. Reduction and inhibition ability of NADPH analogues were investigated. NADH, lacking the 2′-phosphoryl group, does not substitute for NADPH nor does it inhibit *M. kandleri* GluTR. Reduced β-nicotinamide mononucleotide corresponding to removal of the adenosine phosphate moiety does, by contrast, inhibit NADPH-binding. Removing the adenine amino group (reduced nicotinamide hypoxanthine dinucleotide phosphate) similarly inhibits GluTR. Interestingly, both 2′-NADPH and 3′-NADPH are equally active. Thus, all the major determinants of NADPH are required for efficient recognition and utilization by GluTR. Recently, soluble *E. coli* GluTR was expressed and purified through co-expression with chaperones. Most biochemical characteristics of *M. kandleri* GluTR could be confirmed for the enzyme from *E. coli*. A major difference is a requirement of Mg^{2+} for enzymatic activity in *E. coli* GluTR (Schauer et al., 2002).

The ester bond linking the 2′ end of $tRNA^{Glu}$ and glutamate is by necessity labile. To generate a stable substrate analogue representing the last adenosine residue of the tRNA the bridging O was replaced by N. This analogue was synthesized from the structurally related puromycin aminonucleoside and named glutamycin. *M. kandleri and E. coli* GluTR are competitively inhibited by glutamycin (Moser et al., 1999; Schauer et al., 2002). Other *E. coli* GluTR inhibitors were identified by a cell-based, high-throughput screening method but not further characterized (Loida et al., 1999).

The expression of the gene for GluTR, *hemA,* was intensively studied in *E. coli*, *Salmonella* (*S.*) *typhimurium* and *Pseudomonas aeruginosa*. The level of transcriptional regulation is low and influenced by oxygen tension, cellular heme concentration and the presence of alternative electron acceptors for anaerobic growth like nitrate (Darie and Gunsalus, 1994; Hungerer et al., 1995; Choi et al., 1996; McNicholas et al., 1997; Verderber et al., 1997; Krieger et al., 2002; Schobert and Jahn, 2002). Changes in cellular GluTR concentration were explained by heme-induced proteolytic degradation of the enzyme (Wang et al., 1997; Wang et al., 1999a). The N-terminal region of *S. typhimurium* GluTR was found to bind heme causing structural re-arrangements and leading to proteolytic turnover (Wang et al., 1999b).

B. Enzyme Mechanism

Treatment of GluTR from *M. kandleri* and *E. coli* with iodoacetamide or 5,5´-dithiobis(2-nitrobenzoic acid) abolishes enzyme activity implicating a nucleophilic cysteine in catalysis. All cysteines of both GluTRs were individually replaced by serines. Mutants Cys48Ser (*M. kandleri*) and Cys50Ser (*E. coli*) were completely inactive confirming that these cysteines are the active site nucleophiles. In fact, these residues correspond to the only cysteine conserved in all GluTRs. Surprisingly, both enzymes efficiently turn over glutamyl-tRNA in the absence of NADPH liberating glutamate. The rate of this GluTR-dependent glutamyl-tRNA esterase activity is comparable to GluTR reductase activity in the presence of NADPH. Similar esterase activity is also observed for other enzymes such as glycerinaldehyde-phosphate dehydrogenase, thiol proteinases and aldehyde dehydrogenases that form a covalent acyl-enzyme intermediate involving an active site cysteinyl residue. GluTR mutants Cys48Ser and Cys50Ser, correspondingly, did not possess any esterase activity. A catalytic mechanism for GluTR was proposed whereby the sulfhydryl group of Cys-48 (Cys-50 in *E. coli*) nucleophilically attacks the α-carbonyl group of tRNA-bound glutamate forming an enzyme-bound thioester intermediate with the concomitant release of $tRNA^{Glu}$ (Fig. 2b). This reaction intermediate was isolated and visualized for *E. coli* GluTR. Finally, hydride transfer from NADPH to the thioester-bound glutamate produces GSA. In the absence of NADPH, a water molecule takes its place, hydrolyzing the reactive thioester bond and releasing glutamate (Moser et al., 1999, 2001, Schauer et al., 2002).

C. Crystal Structure

Instead of the anticipated protein tetramer, the crystal structure of *M. kandleri* GluTR revealed an extended V-shaped dimer (Moser et al., 2001) (Fig. 3). Each monomer consists of three distinct domains arranged along an extended curved 'spinal' α-helix. The first, N-terminal domain consists of two subdomains: a small βαββααβ and a larger, three α-helix subdomain. These three helices roughly align with the spinal helix to form a 4-helix bundle. A short loop links domain 1 to domain 2, a classical NAD(P)H-binding fold (Carugo and Argos, 1997). This domain is followed by the 110-Å spinal helix of 18 α-helical turns. N-terminally, the spinal helix reinforces the loop between domain 1 and 2, then passes domain 1, and C-terminally extends into, and forms part of the dimerization domain. This domain 3 is composed of a symmetric six-helix bundle, three helices deriving from each of two interacting monomers.

D. Structural Basis of Catalysis

The crystal structure of GluTR was solved in a complex with glutamycin (Moser et al., 2001). The inhibitor binds within a deep pocket at the interface of the subdomains of domain 1 and is specifically recognized by an array of strictly conserved amino acids (Figs. 1b, 3, 4). A bidentate salt-bridge, between the carboxylate group of glutamycin and Arg50 at the bottom of the pocket, represents the most discriminating interaction. This resembles the carboxylate recognition mode of aspartyl-tRNA synthetase (Eiler et al., 1999) and presumably of glutamyl-tRNA synthetases (Sekine et al., 2001a). Further, the enzyme specifically recognizes the α-amino group and less specifically the ribose moiety of the inhibitor. The structure of the enzyme-inhibitor complex supports the proposed catalytic mechanism for the enzyme (Moser et al., 1999) (Figs. 3, 4). Cys48 is particularly sensitive to oxidation. To obtain crystals of GluTR it had to be replaced by serine resulting in an inactive enzyme (Moser et al., 1999). In the crystal structure, Ser48 (Cys48) is located at the edge of the glutamate-binding pocket and its hydroxyl (sulfhydryl) group is in close proximity to the α-carbonyl carbon atom of glutamycin, ready to nucleophilically attack the activated α-carboxylate of glutamyl-tRNA. This would result in the expected covalent thioacyl intermediate and the release of $tRNA^{Glu}$. In a second step, the thioacyl intermediate would be reduced to the product GSA by hydride transfer form NADPH. Although the

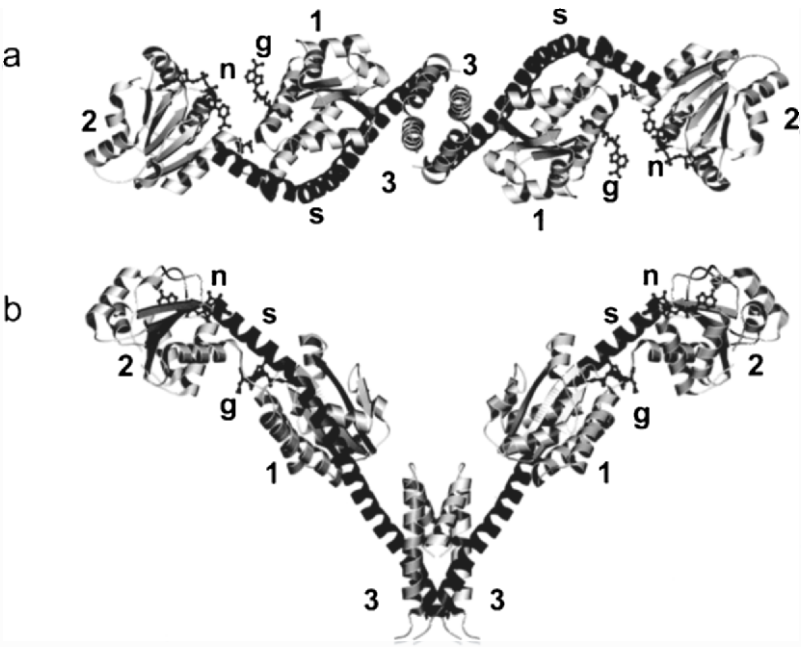

Fig. 3. A schematic diagram of the *Methanopyrus kandleri* GluTR dimer viewed (a) perpendicular to and (b) along the two-fold-axis. GluTR is composed of a catalytic domain (1), an NADPH-binding domain (2) and a dimerization domain (3) linked by a 'spinal' α-helix (s). Glutamycin (black) is recognized by the catalytic domain. Abbreviations and software as in Fig. 1, except rendering by *RASTER3D* (Merritt and Murphy, 1994).

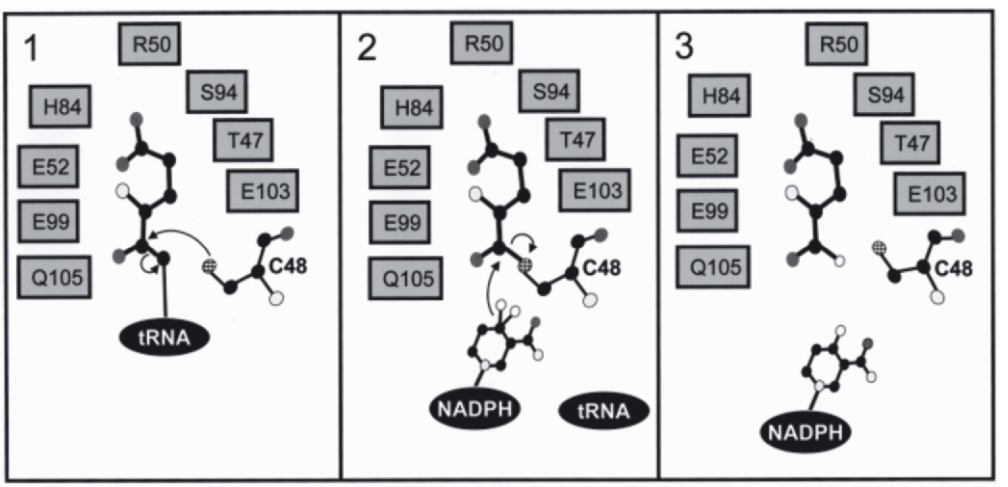

Fig. 4. Catalytic mechanism of GluTR. Conserved residues responsible for specific substrate recognition through an intricate hydrogen-bonding network are indicated. The reactive cysteine residue nucleophilically attacks the aminoacyl bond of glutamyl-tRNA (1). An enzyme-localized thioester intermediate is formed with the release of free $tRNA^{Glu}$ (2). The thioester is reduced by hydride transfer from NADPH leading to GSA (3).

canonical NADPH-binding site in domain 2 is not occupied in the crystal structure, a reliable position for NADPH may be inferred from structurally related NAD(P)H-binding domains. The resulting distance between the nicotinamide moiety and the glutamate-binding pocket of 21 Å indicates that domain 2 must rotate around the end of the spinal helix to close the active site and to place NADPH near the thioacyl-

bound glutamate for hydride transfer to occur. The present open structure of GluTR may, therefore, be described as a 'pre-active' state. To what extent the individual steps (binding of glutamyl-tRNA, tipping of domain 2, opening of the NADPH binding pocket and NADPH-binding) occur in concert or consecutively is presently not clear.

The glutamate-binding pocket of GluTR clearly establishes the location of the 3´-terminal nucleotide of the glutamyl-tRNA. Placing the acceptor arm of the glutamyl-tRNA from the GluRS/glutamyl-tRNA complex from *E. coli* (Sekine et al., 2001b) near the catalytic domain, indicates that the concave elbow region of the tRNA is somewhat complementary to the external surface of the catalytic domain. The glutamyl-tRNA may thus be moved immediately adjacent to the GluTR, placing the acceptor arm into the cleft between the catalytic and the NADPH-binding domains (Fig. 1b). The elbow region of the tRNA and, in particular, the D-stem would interact with the catalytic domain, while the anticodon-region could interact with the α-helical, dimerization domain. Overall, this hypothetical model reveals a large degree of surface complementarity between tRNA and GluTR and, surprisingly, the regions of the tRNA interacting with the reductase are similar to those of the GluRS-tRNA complex: The acceptor arm lies within a large furrow between domains 1 and 2, allowing a multitude of specific interactions. The elbow region and the anticodon stem interact with domain 2 (domain 3 in GluRS). The anticodon itself appears to interact with domain 3, possibly allowing its specific recognition. Interestingly, in both GluRS and GluTR, the (proposed) anticodon-recognition region contains a high number of arginines, though not recognizably conserved. For GluRS, the tRNA complex was required to finally establish which arginines are responsible for anticodon recognition (Sekine et al., 2001b).

VI. Glutamate-1-Semialdehyde-2,1-Aminomutase

The synthesis of ALA from glutamyl-tRNA requires the exchange of amino and aldehyde groups between carbon 1 and 2 of GSA. This reaction is catalyzed by GSAM, EC 5.4.3.8 (Kannangara et al., 1984; Hoober et al., 1988; Jahn et al., 1992). The reaction differs from a classical aminotransferase reaction by its intramolecular nature. Nevertheless, GSAM represents a typical aminotransferase in structure and catalysis (Mehta and Christen, 1994).

A. Biochemical Features

In principle two catalytic routes of GSAM are possible: Starting with the pyridoxal 5´-phosphate (PLP) form of GSAM results in the formation of dioxovalerate, while the PMP form would lead to a diaminovalerate (DAVA) intermediate. Recombinant GSAM from *E. coli*, *Synechococcus* sp. and other sources was found to catalyze both reactions (Smith et al., 1991a; Ilag and Jahn, 1992). However, kinetic investigations of *Synechococcus* sp. and pea GSAM eventually identified diaminovalerate as the true intermediate (Smith et al., 1991b; Friedmann et al., 1992; Pugh et al., 1992). The active site lysine responsible for Schiff´s base formation with PMP was identified for various GSAMs (Grimm et al., 1992; Ilag and Jahn, 1992). The half-reactions of the PMP-dependent amino transfer are outlined in Fig. 2c. Spectroscopic analyses, including stop flow experiments, identified the reaction intermediates (Ilag and Jahn, 1992; Smith and Grimm, 1992; Brody et al., 1995; Smith et al., 1998). GSAM catalyzes an anomalous enantiomeric reaction discriminating between (S)-GSA and (R)-GSA. Interestingly, (R)-GSA is a substrate for the first half-reaction but the resulting (R)-DAVA is either inactive or a poor substrate for the second half-reaction (Friedmann et al., 1992; Smith et al., 1992). GSAMs are inhibited by gabaculine, an inhibitor of γ-aminobutyric acid aminotransferase and other aminotransferases (Hill et al., 1985; Hoober et al., 1988). Other inhibitors, 4-aminohex-5-ynoate, 4-aminohex-5-enoate and enantiomers of diaminopropyl sulfate have been developed (Tyacke et al., 1995; Contestabile et al., 2000).

B. Crystal Structure

GSAM is a member of an extensive family of structurally related, PLP-dependent proteins that include aminotransferases, racemases, decarboxylases, synthetases and mutases. GSAM shares the prototype aspartate aminotransferase fold and is structurally particularly similar to dialkylglycine decarboxylase and 4-aminobutyrate aminotransferase. Mechanistically GSAM differs from aminotransferases in that it exchanges amino and oxo groups in GSA without the need for additional cofactors other than PMP. It

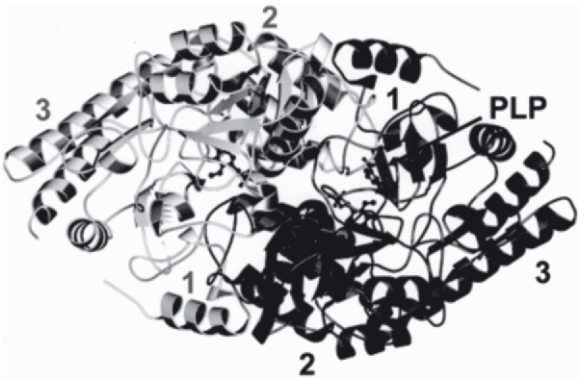

Fig. 5. Ribbon diagram of dimeric glutamate-semialdehyde-2,1-aminomutase (Hennig et al., 1997). One monomer is depicted in white the second in gray. Cofactors (PMP) and inhibitor (gabaculine) are shown as black ball and stick representations.

is a dimeric protein with an overall ellipsoidal shape when viewed along the dimer axis (Fig. 5, Hennig et al., 1997). The monomers interact through a large, convoluted interface. Morphologically distinct domains are not apparent. Instead, three domains may be rationalized based on three β-sheets: A three-stranded, antiparallel β-sheet in domain 1, a seven-stranded, parallel β-sheet (one strand is antiparallel) in domain 2 and a four-stranded, antiparallel β-sheet in domain 3. All domains, as well as interdomain connections, additionally include α-helices and long loops of low secondary structure content. Both cofactor and substrate are bound at the monomer-monomer interface. Domain 2 of one monomer accommodates the PLP/PMP head group, while the phosphate is additionally coordinated by a loop from domain 2 of the second monomer. Loops from all three domains participate in lining the substrate pocket. In the crystal structure, the GSAM dimer is observed to be imperfectly symmetrical. A loop consisting of residues 159–172 and laterally covering the substrate pocket is partly disordered in one monomer, while it is well structured in the second. This loop is essential for proper enzyme function (Contestabile et al., 2000). Presumably, GSAM oscillates between the two conformational states in which one monomer is in the closed, active state (with ordered active-site lid), while the second is in a relaxed state, allowing product and substrate to diffuse out of and in to the active site, respectively.

VII. Metabolic Channeling of Glutamate-1-Semialdehyde

The tRNA-dependent formation of ALA found in plants and most bacteria requires the concerted action of the two enzymes GluTR and GSAM. Metabolically they are linked by the aldehyde GSA. The chemical reactivity of GSA in the cellular medium means that an indirect transfer of the aldehyde from GluTR to GSAM is not probable, as formation of ALA would not be efficient. The three-dimensional shape of both GluTR and GSAM suggests an attractive solution to this metabolic problem. Placing the GSAM dimer from *Synechococcus* sp. (Hennig et al., 1997) alongside the extended and similarly dimeric GluTR, immediately, suggests that the open space delineated by the GluTR monomers could comfortably accommodate GSAM. The resulting model complex (Fig. 6 and Color Plate 1) displays a striking degree of surface complementarity between both enzymes. The most striking result of the proposed GluTR/GSAM complex is that the putative active site entrance of each GSAM monomer (Hennig et al., 1997) is positioned opposite a depression in domain 1 of GluTR. This depression and the glutamate recognition pocket are separated only by Arg50 and guarded by the conserved His84. The proposed complex may thus indicate that the GluTR-product GSA leaves the enzyme via this ‘back door‘ of the glutamate recognition pocket and is directly channeled into the active site of GSAM, a distance of about 26 Å, without exposure to the aqueous environment.

Both $tRNA^{Glu}$ (see above) and GSAM can, thus, be independently docked onto GluTR, each in a single plausible position. Though separately docked, the model of the ternary complex of GluTR, $tRNA^{Glu}$ and GSAM does not lead to steric clashes between GSAM and $tRNA^{Glu}$; instead, GSAM could laterally extend the interaction surface of GluTR for the tRNA.

VIII. Concluding Remarks

Recent decades, and especially the last few years, have witnessed the addition of significant detail to our picture of the tRNA-dependent ALA biosynthesis for tetrapyrrole formation in plants, archaea and most bacteria. The central biological functions of chlorophylls, hemes, vitamin B_{12} and other tetrapyrroles underscore the need for an efficient, robust system to reliably and continuously provide large

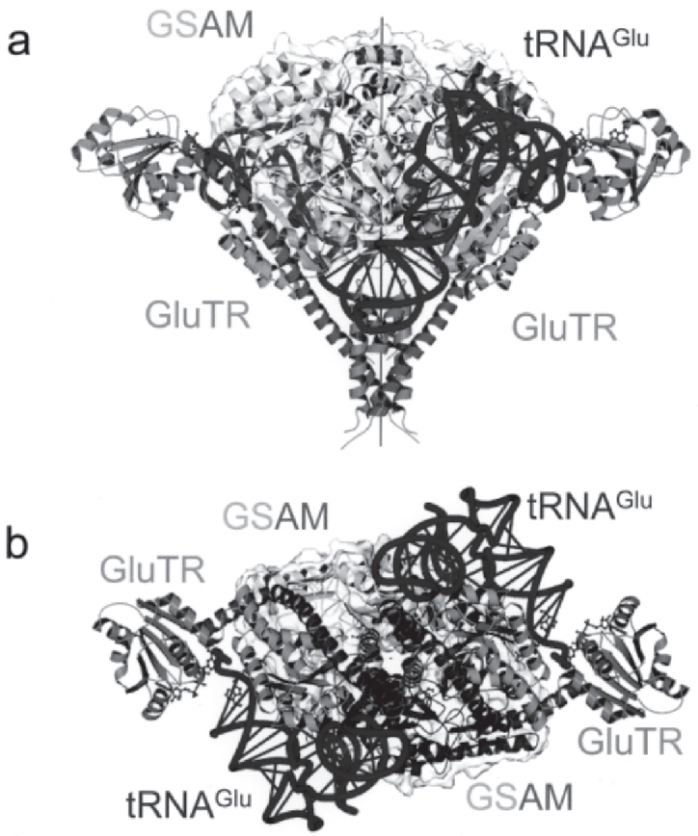

Fig. 6. The proposed ternary complex of GluTR/tRNA^{Glu}/GSAM viewed (a) perpendicular to the common two-fold axis and (b) along this axis from the dimerization domain of GluTR (opposite direction to Fig. 3a). The ribbon depiction of V-shaped GluTR is rendered in shades of gray. tRNA^{Glu} is represented as a backbone model (black), while GSAM is shown by a transparent surface covering a light-gray and white ribbon diagram. (Produced as Fig. 1 with the addition of *GRASP* (Nicholls et al., 1991). See also Color Plate 1.

amounts of this vital intermediate. Understanding the mechanisms involved and their regulation may help us to appreciate the underlying intricacies and adapt this knowledge to our specific needs in such fields as food production, vitamin synthesis, antibiotics and herbicide development.

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