EVOLUTIONARY CONSIDERATION ON 5-AMINOLEVULINATE SYNTHASE IN NATURE

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Abstract. 5-Aminolevulinic acid (ALA), a universal precursor of tetrapyrrole compounds can be synthesized by two pathways: the C_5 (glutamate) pathway and ALA synthase. From the phylogenetic distribution it is shown that distribution of ALA synthase is restricted to the α subclass of purple bacteria in prokaryotes, and further distributed to mitochondria of eukaryotes. The monophyletic origin of bacterial and eukaryotic ALA synthase is shown by sequence analysis of the enzyme. Evolution of ALA synthase in the α subclass of purple bacteria is discussed in relation to the energy-generating and biosynthetic devices in subclasses of this bacteria.

1. Introduction

Tetrapyrrole biosynthesis is initiated by 5-aminolevulinic acid (ALA) formation either by condensation of glycine with succinyl-CoA, catalyzed by ALA synthase or by the C_5 (glutamate) pathway (Jordan, 1991). The latter includes three enzymes. Glutamate is esterified to $tRNA^{Glu}$ at the expense of ATP. The resulting product is reduced with NADPH to glutamate 1-semialdehyde which is rearranged to ALA by a specific aminomutase. The last reaction is inhibited by gabaculine (Hoober *et al*., 1988). The C_5 pathway has been shown to occur in the plastids of plants and algae and in cyanobacteria (Castelfranco and Beale, 1983). In more recent studies, this pathway has also been found in eubacteria and archaebacteria (reviewed in Avissar *et al*., 1989; Oh-hama *et al*., 1993). In the latter work distribution of ALA synthase was proposed to be restricted to the α subclass of purple bacteria or, to use its other name, *Proteobacteria* (Stackebrandt *et al*., 1988).

Since there has been a general acceptance of the view that mitochondria are of monophyletic origin with an endosymbiotic ancestor of the α purple bacteria though there is a controversy (see Section 3; Gray *et al*., 1989; Yang *et al*., 1985), one would be convinced of the broad distribution of ALA synthase in eukaryotes. Variation in its molecular size (peptide length) and control of the enzyme expression are followed in prokaryotes and mitochondria in yeast, chicken and mammals. Speculating on the evolution of ALA synthase, two bacterial enzymes which may be evolutionarily related to it and have CoA bound substrate are described. Possible implications for the existence of different types of ALA biosynthetic pathways in subclasses of purple bacteria are considered in relation to their bioenergetic and biosynthetic devices.

2. Distribution of ALA Synthase in α Purple Bacteria

In 1993 Oh-hama *et al.*, proposed that the C_5 pathway is the ancestral form of

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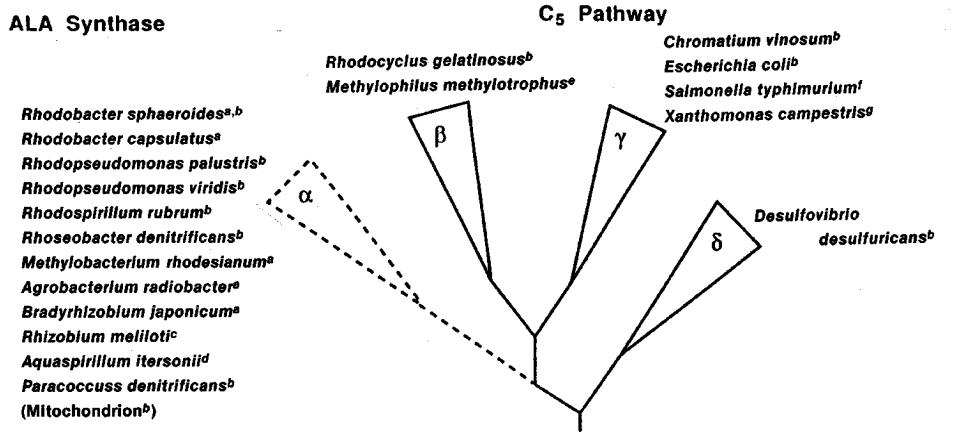

Figure 1. Distribution of the two ALA biosynthetic pathways among the bacteria belonging to α, β, γ and δ subclasses of purple bacteria classified by Woese (1987). For references [a] see Table I; [b] Oh-hama *et al.* (1993); [c] Leong *et al.* (1985); [d] Lascelles *et al.* (1969); [e] Lloyd *et al.* (1993); [f] Elliott *et al.* (1990) and [g] Murakami *et al.* (1993).

ALA biosynthesis, while the ALA synthase pathway evolved later in the α purple bacteria adapted to aerobic growth during oxygenation of the Earth's atmosphere. The reliability of the previous proposal was strengthened by the listing of seven other examples on the phylogenetic tree of the 16S ribosomal RNA of purple bacteria (Woese, 1987, Figure 1). *Pseudomonas denitrificans*, which possesses ALA synthase (cited in Lloyd *et al.*, 1993) and ubiquinone (UQ)-10 as major isoprenoid quinones (see Section 5) would also belong to the α subclass.

3. Expression of ALA Synthase Genes in Prokaryotes and Eukaryotes

3.1. PROKARYOTES

Since 1958 the occurrence and properties of ALA synthase in bacteria have been studied in detail (Jordan, 1991). The enzyme consists of homodimer with subunits varying from 40 to 60 kDa requiring pyridoxal 5' phosphate (PLP) for activity. The complete nucleotide sequence coding for ALA synthase and the derived amino acid sequence, ranging from 401 to 408, have been reported for *Rhodobacter sphaeroides*, *R. capsulatus*, *Agrobacterium radiobacter* and *Bradyrhizobium japonicum* (Table I).

R. sphaeroides can rapidly adapt from non-photosynthetic aerobic growth to photosynthetic anaerobic growth and vice versa. The complex regulation of tetrapyrrole biosynthesis in *R. sphaeroides* led to a view of a bacterochlorophyll specific ALA synthase (Lascelles, 1978). Supporting this, two ALA synthase genes, *hemA* and *hemT*, were found and characterized by Neidle and Kaplan (1993a,b).

Table I
Characterization of ALA synthase in prokaryotes and eukaryotes

Organism	End. products of the pathway	Gene symbol	Peptide chain length[a] [ ][c]–[ ][b]	Reference
Rhodobacter sphaeroides	Bchl[d]; heme vitamin B_{12}	*hemA* *hemT*	407 407	Neidle and Kaplan (1993a and 1993b)
Rhodobacter capsulatus	Bchl; heme vitamin B_{12} siroheme	*hemA*	401	Hornberger *et al*. (1990)
Agrobacterium radiobacter	heme vitamin B_{12}	*hemA*	405	Drolet and Sasarman (1991)
Bradyrhizobium japonicum	heme	*hemA*	408	McClung *et al*. (1987)
R. sphaeroides subsp. *denitrificans*	Bch1 heme			Michalski and Nicholas (1987)
Methylobacterium rhodesianum	Bchl; heme vitamin B_{12}			Sato *et al*. (1985)
Saccharomyces cerevisiae	heme	*HEM1*	548 70(30–35)–478	Urban-Grimal *et al*. (1986)
Chicken Housekeeping	heme	*ALASN*	635 191(56)–444	Borthwick *et al*. (1983 and 1985) Riddle *et al*. (1989)
Erythroid	heme	*ALASE*	513 83(18[e])-430	Riddle *et al*. (1989)
Rat Housekeeping	heme		642 197(56)-445	Yamamoto *et al*. (1988)
Human Housekeeping	heme	*ALAS1*	640 197(56)-443	Bawden *et al*. (1987) Bishop (1990)
Erythroid	heme	*ALAS2*	582 137 –445	Bishop (1990)

[a] Including N-terminal presequence of eukaryotic enzymes; [b] Chain length of the C-terminal peptide, which in homologous with bacterial ALA synthase; [c] Chain length of the N-terminal peptide including the peptide of presequence shown in round brackets; [d] Bacteriochlorophyll; [e] Calculated from difference between precursor and mature enzyme masses.

However, transcription of *hemT* was not detected in wild type cells under the physiological growth conditions tested, but in a mutant strain in which the *hemA* gene had been inactivated. A consensus sequence that might bind the oxygen-sensitive transcriptional regulator has been noted upstream of both *hemA* gene and *puc* operon which encodes structural polypeptides of the light-havesting pigment complex (Lee and Kaplan, 1992). A common transcriptional regulator helps to couple the synthesis of ALA and *puc*-encoded proteins (Neidle and Kaplan, 1993b).

Two types of ALA synthase, Fr.-I and Fr.-II, have been reported in the cells of *R. sphaeroides* (Tuboi *et al*., 1970), *R. sphaeroides* subsp. *denitrificans* and *Methy-*

lobacterium rhodesianum (references in Table I). It seems that one enzyme was formed constitutively, while the other inducibly for bacteriochlorophyll formation. Characterization of ALA synthase gene expression in *R. sphaeroides* as described above will be one step in understanding the formation of the two ALA synthases in these bacteria. No operation of the C_5 pathway on bacteriochlorophyll biosynthesis was observed in *R. sphaeroides* (Oh-hama *et al*., 1985), suggesting that the enzymes of the C_5 pathway do not exist in the bacterium.

3.2. EUKARYOTES

In the phylogenetic tree of eukaryotic (nuclear) lineage, the earliest diverged protists possess neither mitochondria nor chloroplasts (Sogin *et al*., 1989). These primitive eukaryotes have been considered plausible hosts in which mitochondria evolved from a symbiotic α purple bacterium (Cavalier-Smith, 1992). *Gigardia* in the group also lacks catalase (Cavalier-Smith, 1990). It is probable that they do not have the C_5 pathway of ALA synthesis but no evidence yet exists for this. Most mitochondrial proteins including ALA synthase are coded to nuclear genes, synthesized as precursor proteins and imported posttranslationally into mitochondria to be processed inside of the organelles to mature proteins (Hay *et al*., 1984).

a) Yeast

ALA synthase in *Saccharomyces cerevisiae* (Table I) differs from bacterial and animal enzymes in a 22 amino acid insertion near the lysine residue to which PLP is bound (Dierks, 1990). A mutant strain of the *HEM 1* gene required ALA for growth (Labbe-Bois and Labbe, 1990), indicating that ALA is produced solely via ALA synthase in *S. serevisiae*.

b) Avians and Mammals

The complete nucleotide sequences coding for two ALA synthase isozymes and the derived amino acid sequences have been reported for chicken, rat and human (Table I). One enzyme is responsible for general tetrapyrrole biosynthesis in cell tissues (housekeeping enzyme), while the second is expressed exclusively in erythroid cells. Alignments to the amino acid sequences of the prokaryotic and eukaryotic enzymes (Urban-Grimal *et al*., 1986; McClung *et al*., 1987; Riddle *et al*., 1989; Neidle and Kaplan, 1993a) indicated that the animal enzyme precursors are composed of at least two portions: the C-terminal 430-445 amino acid segments of the isozymes are very similar to each other in primary sequence and resemble the bacterial enzymes, while the N-terminal portion varies in length and no homology is recognized between the isozymes. Bacterial ALA synthase has been shown to lack the amino acids corresponding to this N-terminal portion. These observations suggest that the N-terminal portion of the enzymes is not necessary for enzymic activity and may have evolved independently acquiring additional functions during that evolution (Riddle *et al*., 1989).

Inducers of hepatic ALA synthase such as barbiturates are substrates for the microsomal cytochrome P450-associated monooxygenase, and induce cytochrome P450 in liver (Whitlock, 1986). The inducing drugs were shown to stimulate chicken hepatic ALA synthase gene transcription (Hamilton *et al*., 1991). On the other hand, in the steroidogenic tissues (adrenal cortex, testis and ovary), the cytochrome P450s present in both endoplasmic reticulum and mitochondria catalyze monooxygenation reaction in steroid biosynthesis and are not inducible by xenobiotics but by specific pituitary hormones (Whitlock, 1986). Erythroid ALA synthase is also not induced by porphyrinogenic drugs (Wada *et al*., 1967). Dierks (1990) proposed a translational control of the erythroid ALA synthase gene expression by iron based on the structural and sequence similarity of a segment of the erythroid ALA synthase mRNA with that of the two proteins, ferritin and transferrin receptor both of which are concerned with iron metabolism and are biosynthetically controlled by iron.

c) Plants and Algae

Plants and algae are expected to contain two types of ALA synthetic pathways since they contain both chloroplast and mitochondrion. Consistent with this view it was shown that in *Euglena gracilis* heme *a* was formed by ALA synthase, while chlorophyll was formed exclusively by the C_5 pathway (Weinstein and Beale, 1983). Reports of the existence of ALA synthase in green and non-green plant tissues, however, are viewed as too preliminary to be firmly accepted (Beale and Weinstein, 1990). In achlorophyllus artichoke tuber, ALA synthesis and cytochrome P450 induction were effectively blocked by gabaculine, which was regarded as an indication of cytoplasmic and microsomal heme synthesis by the C_5 pathway in the tuber (Werck-Reichart *et al*., 1988).

4,5-Dioxovaleric acid (DOVA) transaminase fraction has been obtained in maize leaf extracts together with the enzyme catalyzing NADH-dependent reduction of 2-oxoglutarate to DOVA, both of which support ALA formation from 2-oxoglutarate (Lohr and Friedmann, 1976). The enzymes were more active in dark-grown tissues than in greening ones and ATP-independent (Harel *et al*., 1978). Recently McKinney and Ades (1991) reported that the hepatic DOVA transaminase was inhibited by gabaculine. These results suggest that aplastidic hemes in artichoke may be formed by aplastidic enzymes, an alternate C_5 pathway of ALA synthesis. There is a view that the rRNA genes of the plant mitochondion were acquired from the second (endo)symbiont at a relatively late stage in the evolution of a primitive green alga, and that plant mitochondrial DNA is characterized as an evolutionary mosaic (Gray *et al*., 1989). Further work is needed to establish the biosynthetic pathway of ALA which supports aplastidic heme formation in plants.

4. Comparison of ALA Synthase with *bioF* and *K1b* Gene Products

Two PLP enzymes have been found to have homology with ALA synthase. Otsuka

et al. (1988) found that 7-keto-8-aminopelargonic acid synthase, the product of *bioF* gene which is involved in biotin biosynthetic operon (*bio*) of *Escherichia coli*, consists of 384 amino acids, and shares 116 amino acid identities with a bacterial ALA synthase. The second is the *K1b* gene product, 2-amino-3-ketobutyrate CoA ligase, which may be involved in threonine metabolism. Mukherjee and Dekker (1990) isolated a peptide of 23 amino acids from the enzyme of *E. coli* containing the lysine residue to which PLP is bound, and found that the segment of 10 amino acids around the lysine residue has a high level of homology with that of ALA synthase from chicken liver. In a comparison of complete sequence of the two genes in *E. coli* with that of *hemA* gene in *R. sphaeroides*, conservation was noted in 65 amino acid residues and in two glycine-rich regions which could be involved in CoA and PLP binding (Neidle and Kaplan, 1993a). ALA synthase enzyme could have arisen by modification of substrate specificity in an ancestor gene duplicate encoding a PLP enzyme having CoA-bound substrate.

5. Bioenergetic and Metabolic Perspective on the Evolution of ALA Synthase

The purple bacteria class includes the enteric, chemolithotrophic, methylotrophic and other familiar eubacterial groups in addition to the purple photosynthetic bacteria. Now there is evidence showing that these non-photosynthetic bacteria arose from photosynthetic ancestors by loss of the photosynthetic apparatus and adaptation to new functions of the photosynthetic electron transport chain.

Woese (1987) has described that the β subgroup may ultimately be shown to be a subgroup within the γ subgroup (cf. Figure 1). This branching order of the purple bacteria has also been proposed in the distribution of cytochrome c_2 and c_{551} (Dickerson, 1980), UQ-10 and UQ-8 though there are exceptions (Collins and Jones, 1981; Lane *et al*., 1985) and of *pheA* (chorismate mutase-prephenate dehydrogenase) gene product which is involved in the biosynthetic pathway of phenylalanine (Ahmad and Jensen, 1988). In the phylogenetic tree constructed from F_1F_0ATP synthase subunit c/III/9 gene (Recipon *et al*., 1992), three lineages were shown to stand out: α purple bacteria together with mitochondria, cyano-bacteria together with chloroplasts and other eubacteria including *E. coli* (γ subclass). These facts from various sources indicate the distinctive characteristic of α subclass in purple bacteria evolution.

As for the evolution of the citric acid cycle, a pathway of terminal substrate oxidation, the insertion of oxoglutarate dehydrogenase oxidizing 2-oxoglutarate to succinyl-CoA was thought to complete it (Gest, 1987). The known obligate chemolithotrophs do not belong to α subclass but to β and γ subclasses of purple bacteria and are characteristic in their lack of 2-oxoglutarate dehydrogenase in the citric acid cycle (Matin, 1978). *Methylophilus*, a methylotroph lacking 2-oxoglutarate dehydrogenase (Lloyd *et al*., 1993) belongs to β subclass, while

Methylobacterium having the complete citric acid cycle (Colby *et al*., 1979) is in α subclass (Figure 1). In *E. coli* (γ subclass) the three enzymes in the citric acid cycle including 2-oxoglutarate dehydrogenase are repressed during the anaerobic growth. Expression of the anaerobic fumarate respiration system in *E. coli* is controlled by the *fnr* gene product (Spiro and Guest, 1990). *Chromatium* cells grown in acetate medium seem to lack 2-oxoglutarate dehydrogenase activity (Nicolay *et al*., 1983), while those grown in malate medium contain this activity (Beatty and Gest, 1981). Thus, in γ purple bacteria the operation of the citric acid cycle is differently controlled in accord with their individual metabolic demands. ALA synthase would have evolved in the purple bacteria in which aerobic metabolism was most markedly developed among the subclasses of phototrophic purple bacteria. Development of the aerobic metabolism is thought to be correlated with the increase of O_2 in the environment (Gest, 1987) due to abiotic O_2 formation and the appearance of oxygenic photosynthesis (Berkner and Marshall, 1965; Towe, 1985).

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