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Clues from a halophilic methanogen about aromatic amino acid biosynthesis in archaebacteria

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Abstract. Extensive diversity in features of aromatic amino acid biosynthesis and regulation has become recognized in eubacteria, but almost nothing is known about the extent to which such diversity exists within the archaebacteria. *Methanohalophilus mahii*, a methylotrophic halophilic methanogen, was found to synthesize L-phenylalanine and L-tyrosine via phenylpyruvate and 4-hydroxyphenylpyruvate, respectively. Enzymes capable of using L-arogenate as substrate were not found. Prephenate dehydrogenase was highly sensitive to feedback inhibition by L-tyrosine and could utilize either $NADP^+$ (preferred) or NAD^+ as cosubstrate. Tyrosine-pathway dehydrogenases having the combination of narrow specificity for a cyclohexadienyl substrate but broad specificity for pyridine nucleotide cofactor have not been described before. The chorismate mutase enzyme found is a member of a class which is insensitive to allosteric control. The most noteworthy character state was prephenate dehydratase which proved to be subject to multimetabolite control by feedback inhibitor (L-phenylalanine) and allosteric activators (L-tyrosine, L-tryptophan, L-leucine, L-methionine and L-isoleucine). This interlock type of prephenate dehydratase, also known to be broadly distributed among the gram-positive lineage of the eubacteria, was previously shown to exist in the extreme halophile, *Halobacterium vallismortis*. The results are consistent with the conclusion based upon 16S rRNA analyses that *Methanomicrobiales* and the extreme halophiles cluster together.

Key words: Archaebacteria – Halophile – Methanogen – *Methanohalophilus mahii* – Aromatic amino acid biosynthesis – Metabolic interlock

Archaebacteria, which thrive under extreme environmental conditions that do not favor most eubacteria, possess many features (e.g., such as cell-wall and cell-membrane architecture, coenzymes, metabolic patterns, protein structure, genome organization, processes of transcription and translation, and evolutionary behavior) that distinguish them from eubacteria (Woese 1987). In contrast, few comparisons have been made of the extent and overlap of possible differences in biosynthetic pathways such as those which form amino acids.

Metabolic diversity of aromatic amino acid biosynthesis in eubacteria and eukaryotes has proven to be quite extensive with respect to such character-state features as: *(i)* alternative enzymic steps, *(ii)* cofactor specificities of pathway dehydrogenases, *(iii)* allosteric control patterns, *(iv)* presence or absence of regulatory isozymes, *(v)* multifunctional proteins, and *(vi)* genetic organization of pathway genes (Byng et al. 1982; Ahmad and Jensen 1988). These character states have been useful in deducing the most probable ancestral state for an enzyme within a particular phylogenetic cluster (Jensen 1985). This is exemplified by the dehydratase reaction in phenylalanine biosynthesis which exhibits distinctive patterns of evolution within the gram-positive (Berry et al. 1987), gram-negative (Ahmad and Jensen 1988), and cyanobacterial divisions (Hall et al. 1982) of the eubacterial kingdom.

The extreme halophile, *Halobacterium vallismortis*, was the first archaebacterium for which aromatic-pathway diversity was examined. *H. vallismortis* was found to possess a complex type of prephenate dehydratase subject to activation by a number of aromatic and non-aromatic amino acids. This class of enzyme had been denoted as the interlock type of prephenate dehydratase in the low-G+C gram-positive *(Bacillus-Lactobacillus-Clostridium-Streptococcus)* line of descent (Jensen et al. 1988). Relationships between the extreme halophiles and the halophile-*Methanomicrobiales* have been weakly suggested by oligonucleotide signatures (Yang et al. 1985) and have been more conclusively defined by 16S rRNA sequence comparisons (Woese and Olsen 1986). As a beginning step in the systematic expansion of comparative studies within the archaebacterial kingdom, we initiated an evaluation of aromatic amino acid biosynthesis in *Methanohalophilus mahii*. *M. mahii* (Paterek and Smith 1985, 1988), a methanogenic moderately halophilic

Abbreviation: DAHP, 3-deoxy-D-arabino-heptulosonate-7-phosphate

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bacterium, represents a novel genus which has been placed in the order *Methanomicrobiales* [as defined by Balch et al. (1979)], the phylogenetically nearest of the three methanogen orders to the extreme halophiles (Woese 1987). *M. mahii* is considered to be a unique organism and is not thought to be a marine methanogen which has adapted to a hypersaline environment (Paterek and Smith 1988).

Materials and methods

Growth of Methanohalophilus mahii

The moderate halophilic methanogen (ATCC 35705), named *M. mahii* by Paterek and Smith (1988), was isolated, characterized and originally designated as *Halomethanococcus mahii* (type strain SLP) by Paterek and Smith (1985). Cultures for this investigation were grown under optimum conditions of salt (2 M NaCl), pH (7.5) and temperature (35 °C) according to the procedure of Paterek and Smith (1985). The cells were harvested by low-speed centrifugation and stored as whole cell pellets at −90 °C.

Preparation of crude extracts

Cell pellets, resuspended in an equal volume of 50 mM potassium phosphate buffer (pH 7.5) containing 2 M KCl and 1 mM dithiothreitol, were disrupted at 4 °C by sonication with four 15-s bursts at an intensity of 100 W from a Lab-Line Ultratip Labsonic System (Lab-Line Instruments, Inc., Melrose Park, Ill., USA). The disrupted cell suspension was ultracentrifuged at 145,000 × *g* for 60 min at 4 °C to remove cell debris. The resulting supernatant was then dialyzed in a continuous-flow microdialysis system (Gibco BRL, Gaithersburg, Md., USA) at 4 °C against the buffer indicated. These preparations, stored at 4 °C, served as the protein extract for this investigation.

Analytical procedures

Arogenate dehydrogenase (Bonner and Jensen 1987), arogenate dehydratase (Fischer and Jensen 1987a), prephenate dehydrogenase (Fischer and Jensen 1987b), prephenate dehydratase (Fischer and Jensen 1987c), chorismate mutase (Cotton and Gibson 1965), shikimate dehydrogenase (Berry et al. 1987), and DAHP synthase (Jensen and Nester 1966) activities were assayed by methodologies as referenced. Standard reaction mixtures were assayed at 37 °C and contained 50 mM potassium phosphate buffer (pH 7.5) plus the following substrates for each enzyme reaction as indicated: DAHP synthase (2 mM phosphoenolpyruvate and 2 mM erythrose-4-phosphate); shikimate dehydrogenase (2 mM shikimate and 2 mM $NADP^+$); chorismate mutase (2 mM chorismate); prephenate dehydrogenase (2 mM prephenate and 2 mM $NADP^+$); arogenate dehydrogenase (1.5 mM L-arogenate and 2 mM $NADP^+$); prephenate dehydratase (2 mM prephenate); and arogenate dehydratase (1 mM L-arogenate). Unless otherwise indicated, standard assay conditions for prephenate dehydratase also included the presence of 0.5 M KCl and 2 mM L-tyrosine to ensure full activation of the enzyme. Protein concentrations were determined according to the method for Bradford (1976) using bovine serum albumin as the reference protein.

Biochemicals and chemicals

All biochemicals and chemicals unless otherwise indicated were obtained from Sigma Chemical Co. (St. Louis, Mo., USA). Chorismate was prepared from culture supernatants of *Klebsiella pneumoniae* 62-1 (Gibson 1964). L-Arogenate and prephenate were prepared by the method of Bonner et al. (1990).

Results

Activities of aromatic amino acid pathway enzymes

Table 1 shows the specific activities of key enzymes of aromatic amino acid biosynthesis in *M. mahii*. DAHP synthase was not found although an extensive series of extract preparations and assays were carried out under alternative conditions. Shikimate dehydrogenase, assayed in the backwards direction, was present at high activity. This enzyme was incapable of substituting quinate for shikimate, or NAD^+ for $NADP^+$. Chorismate mutase, the last common enzymatic step before the terminal branches of L-tyrosine and L-phenylalanine synthesis, was also found at high activity. Activities of prephenate dehydrogenase and prephenate dehydratase were present, but not those of arogenate dehydrogenase or arogenate dehydratase, indicating that tyrosine and phenylalanine biosynthesis occurs solely through the hydroxyphenylpyruvate and phenylpyruvate routes, respectively. This also precludes the possibility that *M. mahii* might possess dehydratase and dehydrogenase enzymes of the cyclohexadienyl type that are able to use prephenate and arogenate as alternative substrates. Prephenate dehydrogenase exhibited a preference for $NADP^+$, although NAD^+ was also accepted as coenzyme.

Properties of aromatic amino acid pathway enzymes

As shown in Fig. 1A–D none of the enzymes required high salt (KCl) for activity. In fact, the activities of shikimate dehydrogenase, chorismate mutase and pre-

Table 1. Specific activities of aromatic amino acid pathway enzymes in extracts of *Methanohalophilus mahii*

Enzyme	Specific activity[a] (nmol/min · mg protein)
DAHP synthase	Not found
Shikimate dehydrogenase	
NAD^+	Not found
$NADP^+$	18.00
Chorismate mutase	18.33
Prephenate dehydrogenase	
NAD^+	0.19
$NADP^+$	0.41
Arogenate dehydrogenase	
NAD^+	Not found
$NADP^+$	Not found
Prephenate dehydratase	0.90
Arogenate dehydratase	Not found

[a] Specific activities were determined employing standard assay conditions. These results are representative of one of six independent experiments

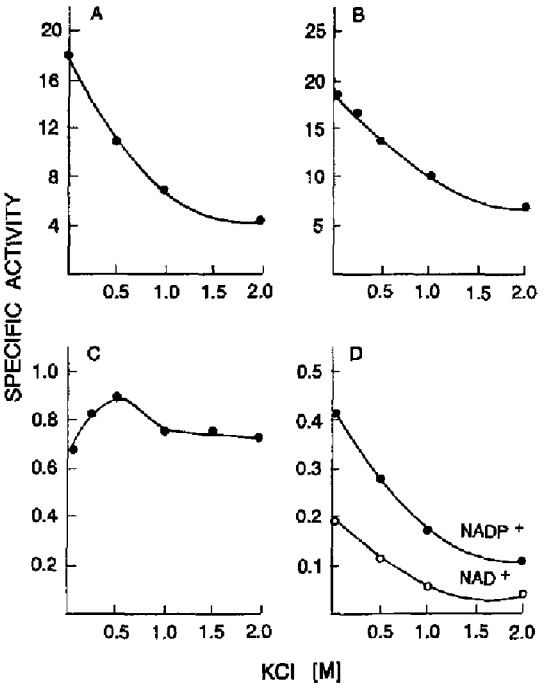

Fig. 1. Effect of salt concentration upon activities of shikimate dehydrogenase **A**, chorismate mutase **B**, prephenate dehydratase **C**, and prephenate dehydrogenase **D** from *Methanohalophilus mahii*. Specific activities (nmol/min · mg protein) were determined employing standard assay conditions in the presence of the indicated concentration of KCl. Prephenate dehydratase was assayed in the presence of 2 mM L-tyrosine. These results are representative of one of two independent experiments

phenate dehydrogenase were progressively inhibited by increasing concentrations of salt. At 2 M salt, the activities of these enzymes were 20 to 35% of the initial activities. In contrast, prephenate dehydratase was tolerant of salt concentrations up to 2.0 M, and activity was even stimulated in the presence of about 0.5 M salt. At salt concentrations greater than 2 M prephenate dehydratase was also subject to significant inhibition, retaining only 30% of optimum activity when assayed in the presence of 3 M salt (data not shown). Results obtained with NaCl were similar to those shown in Fig. 1A–D, except that prephenate dehydratase activities were about 25% greater with KCl. Moderately halophilic methanogens, requiring 1.5 to 3 M NaCl for growth, have been shown to accumulate high intracellular K^+ concentrations (up to 1.1 M) which were as much as 40-fold greater than the extracellular K^+ concentration (Lai et al. 1991). Thus, KCl was used throughout the course of this study.

In view of the contrasting effects of salt on the various enzyme activities, we determined the ability of salt to stabilize enzyme activities. Cell-free extracts of *M. mahii* were routinely prepared in 2 M KCl. Microdialysis to remove the salt, followed by time-course assays to assess enzyme stability in extracts prepared with various concentrations of salt, revealed that only prephenate dehydratase needed significant concentrations of salt for stability. Maximal stabilization (>90%) of enzyme activity required 1.5 M KCl. Concentrations of 1 M and 0.1 M KCl resulted in retention of 53% and 21% of maximal enzyme stability, respectively, after storage at 4 °C for 40 h. Such differing effects of salt concentration upon enzyme stability compared to effects upon enzyme activity have been reported for other enzymes from halophiles (Zaccai and Eisenberg 1990). NaCl was as effective as KCl in stabilizing prephenate dehydratase activity. In contrast to prephenate dehydratase, neither shikimate dehydrogenase, chorismate mutase, nor prephenate dehydrogenase required salt for stability. The preparation and incubation (at 4 °C for up to 40 h) of *M. mahii* extracts in concentrations of KCl which were inhibitory to activity appeared to have negligible impact on the stabilities of these three enzymes, provided that the salt was removed or sufficiently diluted prior to assay. Stabilities of these enzymes beyond this time-frame were not investigated.

The optimal conditions for enzyme activity given in Table 2 generally parallel the optimal conditions (e.g., NaCl = 2 M; pH = 7.5; and temperature = 35 °C) required by *M. mahii* for growth and methanogenesis. However, only the prephenate dehydratase required K^+, which is known to be intracellularly high (0.6–1.1 M) in moderately halophilic methanogens (Lai et al. 1991), for optimal activity.

Table 2. Properties of aromatic amino acid pathway enzymes from *M. mahii*

Enzyme	Optimum[a]			K_m(mM)[b]	
	KCl [M]	pH	Temp [°C]	Cofactor	Substrate
Shikimate dehydrogenase					
NADP+	−0−	8.0	40	0.50	0.71
Chorismate mutase	−0−	7.5	35		2.22
Prephenate dehydrogenase					
NAD+	−0−	8.0	30−40	1.25	0.57
NADP+	−0−	8.0	30−40	0.08	0.56
Prephenate dehydratase	0.5	7.4	45		0.21

[a] Optimal of KCl and temp. (C) were determined using standard substrate concentrations and the appropriate variable parameter. The pH profiles were determined using standard substrate concentrations in Bistris-propane buffer adjusted to the required pH. These results are representative of one of two independent experiments

[b] The K_m values, determined from double-reciprocal plots, are the means of two repetitions obtained employing optimum assay conditions of KCl, pH, and temperature

The pH optimum of each enzyme in this study was found to be within the narrow range of 7.4–8.0. The prephenate dehydratase exhibited the narrowest pH profile, yielding only 50% of optimum activity at pH values which were less than 7 or greater than 8. Only 5% of optimum activity was measured when assays were performed at pH 9. Shikimate dehydrogenase, chorismate mutase and prephenate dehydrogenase were active over a broad range of pH (6.5–9.5). Of the three enzymes, chorismate mutase retained the greatest activity over the pH range tested, showing 71% and 57% of optimum activity at pH 6.5 and 9.5, respectively.

The temperature optima of the enzymes in this study were clustered in the range of 30 to 45 °C. Chorismate mutase and prephenate dehydratase exhibited the lowest (35 °C) and the highest (45 °C) thermal optima, respectively. All the enzymes except prephenate dehydratase, which was inactive, exhibited 60% or greater of their optimum activities at 25 °C. None of the enzymes were active at 65 °C. Both shikimate dehydrogenase and prephenate dehydrogenase possessed broad ranges of activity, retaining 50% or greater of their optimum activity over the temperature range of 25–55 °C.

K_m values determined for the cofactors and/or substrates of the enzymes of this study are also listed in Table 2. Chorismate mutase possessed a low affinity for substrate. The K_m of 0.08 mM determined for $NADP^+$, compared to a value of 1.25 mM for NAD^+, demonstrates a perference for $NADP^+$ which is not obvious when comparing specific activities at saturating substrate concentrations (Table 1). A K_m of 0.21 mM for prephenate was determined for prephenate dehydratase when the enzyme was assayed under conditions of activation by 2 mM L-tyrosine.

Allosteric control of aromatic biosynthesis in M. mahii

Activation of prephenate dehydratase. M. mahii possessed a prephenate dehydratase which was allosterically activated by both aromatic and non-aromatic hydrophobic amino acids. As shown in Fig. 2, the activity of prephenate dehydratase was quite low in the absence of allosteric effectors. Activation by L-tyrosine was very dramatic (greater than 13-fold at 2 mM L-tyrosine). L-Tryptophan, L-leucine and L-methionine were moderate activators of enzyme activity. At concentrations of 2 mM or less, these effectors were several-fold less efficient than L-tyrosine. L-Isoleucine proved to be only a weak activator while L-valine was ineffective as an activator. The activation (reaction velocity versus effector concentration) of prephenate dehydratase was hyperbolic with each effector. The activation of prephenate dehydratase appears to result from the lowering of the K_m for substrate. For example, the K_m values of 0.21 mM and 1.18 mM were determined under conditions of activation produced by 2 mM and 0.25 mM L-tyrosine, respectively. The influence of salt concentration upon allosteric activation by amino acid effectors appears to be minimal since significant differences were not noted between enzyme activations determined at KCl concentrations between 0.1–2 M.

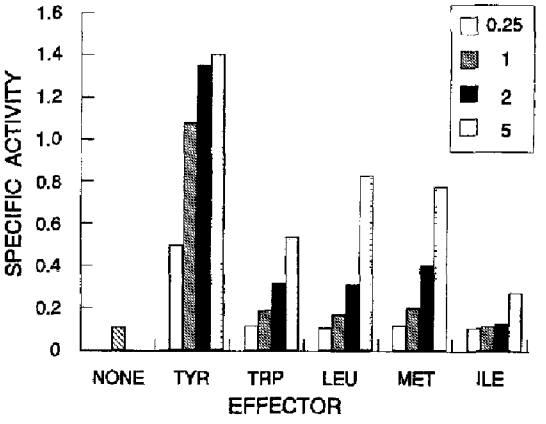

Fig. 2. Allosteric activation of prephenate dehydratase. Specific activities (nmol/min · mg protein) were determined employing standard assay conditions in the presence of 0.5 M KCl and the indicated concentration of effector. Effector concentrations (mM) are given in the figure inset. These results are representative of one of three independent experiments

Feedback control of prephenate dehydratase and prephenate dehydrogenase. Activities of the prephenate-specific enzymes of the terminal branches of L-tyrosine and L-phenylalanine biosynthesis were tested for feedback inhibition by their respective endproducts. L-Phenylalanine was an exceedingly potent inhibitor of the basal activity of prephenate dehydratase (causing complete inhibition at only 13 μM), as well as an effective antagonist of L-tyrosine activation. The ability of L-phenylalanine to antagonize activation of prephenate dehydratase by 2 mM L-tyrosine is shown in Fig. 3A. Under these conditions of assay, 40 μM L-phenylalanine produced 50% inhibition. Prephenate dehydrogenase was quite sensitive to inhibition by L-tyrosine when K_m levels of prephenate were used (Fig. 3B).

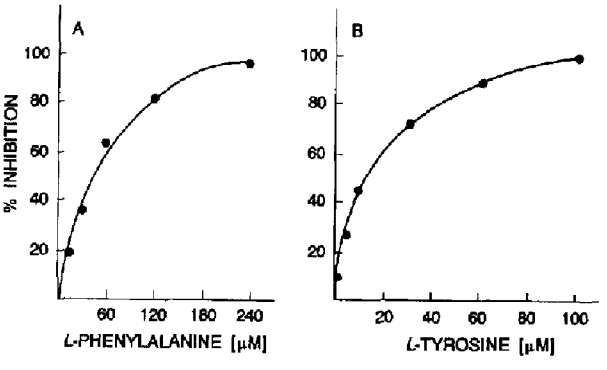

Fig. 3. Feedback control of L-tyrosine-activated prephenate dehydratase by L-phenylalanine **A** and prephenate dehydrogenase by L-tyrosine **B**. The specific activities of prephenate dehydratase and prephenate dehydrogenase ($NADP^+$) obtained in the absence of inhibitor molecules were 1.29 and 0.52 nmol/min · mg protein, respectively. Assays were performed employing standard conditions where substrate concentrations were approximately K_m. These results are representative of one of two independent experiments

Shikimate dehydrogenase and chorismate mutase. Shikimate dehydrogenase and chorismate mutase were not subject to allosteric control by any of the aromatic amino acids or hydrophobic amino acid effectors utilized in this study.

Discussion

Enzymic construction and regulation of aromatic amino acid biosynthesis in Methanohalophilus mahii

Enzyme activities for prephenate dehydratase and prephenate dehydrogenase (but not arogenate dehydratase or arogenate dehydrogenase) were detected in extracts of *M. mahii*, suggesting that phenylalanine and tyrosine synthesis occurs exclusively via the phenylpyruvate and hydroxyphenylpyruvate routes, respectively. Both the phenylalanine and tyrosine terminal branches in *M. mahii* were tightly controlled by feedback inhibition, as is typical of this pathway arrangement in nature.

The mode of L-phenylalanine biosynthesis exhibited in *M. mahii*, characterized by specificity of prephenate dehydratase for prephenate, activation by remote effectors, and feedback inhibition of prephenate dehydratase by L-phenylalanine, respresents a widely distributed pathway arrangement found in *Bacillus* (Rebello and Jensen 1970), mycoplasmas (Berry et al. 1987), and extreme halophiles (Jensen et al. 1988).

The allosteric control of prephenate dehydratase of *M. mahii* by remote effectors (i.e., effectors originating from other pathways) has been demonstrated in the organisms cited above, as well as in the coryneform bacteria (Fazel and Jensen 1980). This distinctive pattern of allostery, exemplified by the enzyme in *Bacillus subtilis*, is one of several examples in nature which have been termed "metabolic interlock" to describe the regulatory effects of amino acids upon enzymes operating in separate pathways (Jensen 1969). These effects were demonstrated in vivo as well as in vitro. It was subsequently shown that the positive and negative allosteric effectors of the *B. subtilis* prephenate dehydratase modulate enzyme activity by mediating interconversions between active (octamer) and inactive (dimer) forms of the enzymes (Pierson and Jensen 1974; Riepl and Glover 1979). The possibility that the *M. mahii* prephenate dehydratase may undergo similar molecular-mass transitions in response to allosteric effectors seems appealing although this phenomenon has yet to be confirmed in this organism.

As shown in Table 3, the particular details of activation and inhibition of prephenate dehydratase by remote effectors vary considerably. A specific amino acid effector may activate or inhibit the enzyme of one bacterium but have no effect on another. When comparisons can be made, the relative efficiencies of an effector are often dissimilar.

An additional characteristic of the prephenate dehydratase of *M. mahii* was that the allosteric effects of L-phenylalanine (inhibition) prevailed over those of L-tyrosine (activation). Similar results were previously reported for the corynebacterium enzymes (Fazel and Jensen 1980) which, like the enzyme from *M. mahii* showed only negligible activity in the absence of effectors. In contrast, the type of prephenate dehydratase which is not allosterically affected by hydrophobic amino acids of non-aromatic origin but is strongly activated by L-tyrosine (e.g., in cyanobacteria) possesses a mode of L-tyrosine activation which dominates over L-phenylalanine inhibition (Hall and Jensen 1980).

Unlike the pathway for L-phenylalanine biosynthesis, the pathway arrangement and regulation indicated by the prephenate-specific tyrosine-pathway dehydrogenase of *M. mahii* is not widely conserved among the low G+C gram-positive lineage. For example, the prephenate dehydrogenase of *M. mahii* appears to be most similar to the class of enzyme found in *Bacillus* (Champney and Jensen 1970) while a cyclohexadienyl dehydrogenase (utilizes either prephenate or arogenate as substrate) and a substrate-specific arogenate dehydrogenase are found in mycoplasma (Berry et al. 1987) and coryneform bacteria (Fazel et al. 1980), respectively. However, the prephenate dehydrogenase of *M. mahii* differed from the *B. subtilis* enzyme in that it was not inhibited by L-phenylalanine and in its ability to utilize both NAD^+ and $NADP^+$ as cofactor. The *B. subtilis* prephenate dehydrogenase exhibits an absolute requirement for NAD^+. The broad specificity of cofactor recognition may be of interest in view of reports that a number of dehydrogenases from such archaebacterial species as the thermoacidophiles accept both cofactors (Danson 1988). Prephenate dehydrogenases having broad specificity for cofactor appear to be uncommon in all other phylogenetic groupings,

Table 3. Variable allosteric action by remote effectors of the interlock type of prephenate dehydratase

Bacterium	Remote effectors		Reference
	Activator[a]	Inhibitor	
Bacillus subtilis	MET = LEU > ILE > CYS > VAL	TRP	Pierson and Jensen 1974
Corynebacterium glutamicum	TYR > MET	TRP	Fazel and Jensen 1980
Synechocystis sp. 29108	TYR		Hall and Jensen 1980
Acholeplasma laidlawii	MET > ILE > VAL > TYR > TRP		Berry et al. 1987
Halobacterium vallismortis	ILE > TYR > MET > LEU	TRP[b]	Jensen et al. 1988
Methanohalophilus mahii	TYR > MET > TRP = LEU > ILE		This study

[a] Amino acid effectors are given in order of relative efficiencies

[b] At concentrations above 0.2 mM L-tryptophan, inhibition progressively declined, indicating a mixture of activating and inhibiting effects

although such breadth of cofactor specificity is known for some cyclohexadienyl dehydrogenases (Jensen 1992).

Neither shikimate dehydrogenase nor chorismate mutase of *M. mahii* showed regulation by any of the aromatic amino acids or remote effectors tested for allostery with the prephenate dehydratase. Lack of allosteric regulation is typical of eubacterial shikimate dehydrogenases. With one exception (Petzel and Hartman 1991), they are specific for NADPH as expected for reductive biosynthetic steps. Although some classes of chorismate mutase in nature exhibit complex multieffector allosteric control, chorismate mutases not subject to allosteric control have been reported in a broad range of organisms in nature including species of gram-negative bacteria, gram-positive bacteria, cyanobacteria, and higher plants (Jensen 1992).

Significance of the interlock type dehydratase in archaebacterial genealogy

In the eubacteria extensive aromatic amino acid pathway studies have provided an appreciation of the diversity and conservation of pathway character states. A particular feature will cluster at a hierarchical level of phylogeny that cannot be predicted. For example, the bifunctional P-protein of phenylalanine biosynthesis is present in two of the three superfamilies of gram-negative bacteria, while the bifunctional T-protein of tyrosine biosynthesis evolved much later and is restricted to the enteric lineage, a small cluster within superfamily B (Ahmad et al. 1990).

The interlock type of prephenate dehydratase that is activated by hydrophobic amino acids of non-aromatic origin has proven to be a character state conserved throughout the gram-positive lineage but absent elsewhere among the eubacteria. Its discovery in the extreme halophile *Halobacterium vallismortis* (Jensen et al. 1988) and now in *M. mahii* establishes this feature as a focal point for perspective about aromatic pathway diversity in archaebacteria. Regulatory relationships of the interlock type of prephenate dehydratase have been interpreted in terms of a mechanism that maintains a balance of hydrophobic amino acids for optimal protein synthesis (Rebello and Jensen 1970; Pierson and Jensen 1974). The rudimentary translation scheme hypothesized for ancestral cells and the propensity of this system for errors has been discussed by Woese (1987), Yeas (1974), and Jensen (1976). In this context, it is of considerable relevance that erroneous substitution of hydrophobic amino acids for L-phenylalanine remains the most common translation error in both eubacterial and extreme-halophile systems (White and Bayley 1972).

The presence of the interlock type of prephenate dehydratase in both the extreme halophiles and halophilic methanogens but absence in all eubacteria except the gram-positive division suggests that the conservation of this ancient substrate-balancing mechanism for protein synthesis may be deeply rooted within the archaebacterial lineage. Perhaps the gram-positive eubacteria diverged earliest of the major eubacterial divisions from a common ancestor of the latter divisions which lost the balancing

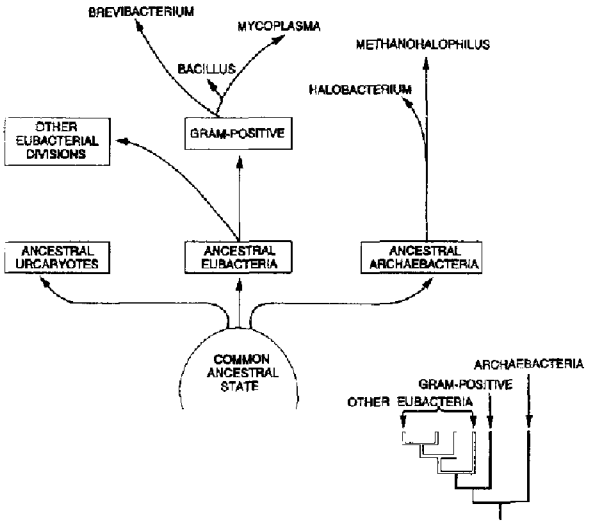

Fig. 4. Phylogenetic kingdoms defined by Woese (1987). The extreme halophiles and halophilic methanogens are members of the archaebacteria. Only the gram-positive grouping of the five major divisions of contemporary eubacteria is shown. The dendrogram at the lower right illustrates an order of branching for eubacterial division whereby the gram-positive lineage diverged from the archaebacteria (represented by the solid line on the far right) at the deepest phylogenetic position

mechanism in favor of other translational controls. This arrangement, whereby gram-positive eubacteria are conceptualized as the outlying eubacterial grouping closest to the archaebacteria, is illustrated by the dendrogram in the lower right portion of Fig. 4. The presence of the interlock type of prephenate dehydratase in both the halophilic methanogen *M. mahii* and in the extreme halophiles, provides further argument against the acceptance of the evolutionary placement of the halobacteria with the eubacteria, as proposed by Lake et al. (1985), rather than with the archaebacteria.

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