Pergamon 0031-9422(95)00010-O Phytochemistry, Vol. 39, No. 2, pp. 263 276, 1995
Elsevier Science Ltd
Printed in Great Britain
0031 9422/95 S29.50 + 0.00

REVIEW ARTICLE NUMBER 102

ANTHRANILATE SYNTHASE IN MICROORGANISMS AND PLANTS

R. M. ROMERO,*† M. F. ROBERTS*‡ and J. D. PHILLIPSON*

*Department of Pharmacognosy, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, U.K.; †CIPRONA, Escuela de Quimica, Universidad de Costa Rica/CONICIT, San Jose, Costa Rica

(*Received* 8 *December* 1994)

Key Word Index—Anthranilate synthase; genes; reaction mechanism.

Abstract—The enzymology of anthranilate synthase (EC 5.4.99.6) in microorganisms and plants is reviewed. Aminoacid sequences of the enzyme subunits in different species are compared, and the mechanism of reaction is discussed.

INTRODUCTION

Tryptophan is utilized in microorganisms and in plants as a substrate for protein biosynthesis; however, in some plants it also has an important role as a precursor of secondary metabolites such as the endogenous auxin indoleacetic acid and other molecules which help to protect the plant against pathogens and herbivores. Synthesis of tryptophan from chorismate involves five reactions and the organization of genes encoding for the necessary enzymes varies considerably among prokaryotes. While *Escherichia coli* contains five genes in a single operon [1], *Bacillus subtilis* has six in a single operon [2] and *Acinobacter calcoaceticus* contains seven genes in three unlinked clusters [3, 4]. Unlinked clusters of various *trp* genes have been found in *Pseudomonas aeruginosa* [5, 6] and *P. putida* and *Rhizobium melitoti* [7].

Anthranilate synthase (EC 4.1.3.27) catalyses the first committed step in the sequence of reactions which lead to the biosynthesis of tryptophan from chorismate. In almost all microbial species, anthranilate synthase (AS) is an oligomer of nonidentical subunits designated AS α-subunit (ASI or component I) and AS β-subunit (ASII or component II). In some organisms the subunits are associated to give an $\alpha\beta$ dimer and in others an $\alpha_2\beta_2$ tetramer [8].

The AS α-subunit is encoded by the genes *trpE* in prokaryote (bacteria and blue–green algae) and, *TRP2* and *ASA1*/*ASA2* in eukaryote (fungi and plants, respectively), but since the β-subunit of the AS enzyme complex sometimes contains other enzymes of tryptophan biosynthesis, other genes, besides *trpG* (prokaryote) and *TRP3* and *ASB* (eukaryote), can encode the multifunctional subunit [9].

In plants although the enzyme has two subunits, the enzyme complex does not contain other functionalities. The genes for these subunits have been isolated from *Arabidopsis thaliana* [10, 11]. AS isoenzymes have been detected in cell cultures of tobacco and potato, whereas in microorganisms only the cyanobacterium *Pseudomonas aeruginosa* has these isoenzymes.

In all organisms the AS α-subunit catalyses an NH_3-dependent synthesis of anthranilate (Fig. 1, route a) and AS β-subunit binds glutamine (Fig. 1, route b) facilitating the transfer of the amide function of glutamine to AS α-subunit. The utilization of glutamine by anthranilate synthase therefore requires both subunits AS α-subunit and AS β-subunit, whereas the use of ammonia requires only the AS α-subunit [12].

OCCURRENCE OF ANTHRANILATE SYNTHASE

Microorganisms

AS enzymes have been purified and characterized from a number of microorganisms and in many cases an enzyme complex catalyses other reactions of tryptophan biosynthesis (Table 1, Table 2). The anthranilate synthase β-subunit can be associated with anthranilate phosphoribosyltransferase (PRT) in bacteria, or with indole-3-glycerol phosphate synthase (InGPS) in yeast and may be found with phosphoribosylanthranilate isomerase:indole-3-glycerol phosphate synthase (PRAI:InGPS) in other fungi. For example, in *Neurospora crassa*, AS is fused to InGPS and PRAI, where the AS subunits associate to form an $\alpha_2\beta_2$ tetramer with a relative molecular

‡Author to whom correspondence should be addressed.

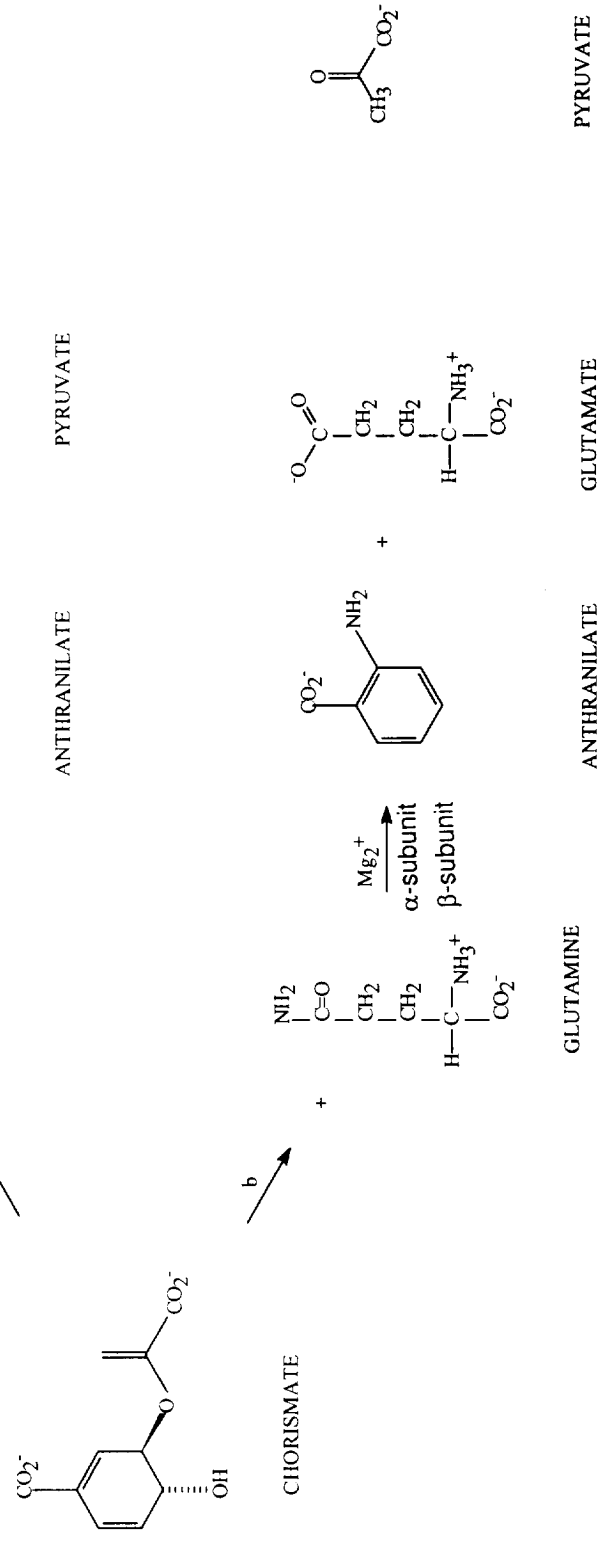

Fig. 1. Formation of anthranilate from chorismate.

Table 1. Occurrence of monofunctional anthranilate synthase in microorganisms

Organism	M_r	Structure	Separation/purity	Refs
Aeromonas formicans	220000		Sephadex G-200	18
Proteus morganii	140000	$\alpha_2\beta_2$	Sephadex G-200	18
Proteus vulgaris	140000	$\alpha_2\beta_2$	Sephadex G-200	18
Erwinia carotavora	140000	$\alpha_2\beta_2$	Sephadex G-200	18
Enterobacter liquefaciens	140000	$\alpha_2\beta_2$	Sephadex G-200	18
Bacillus subtilis	840000	$\alpha\beta$	$(NH_4)_2SO_4$/Sephadex G-100	19 23
Bacillus licheniformis	α: >100000 β:24000		$(NH_4)_2SO_4$/Sephadex G-100	23
Bacillus coagulans	α:80000 β:18000		$(NH_4)_2SO_4$/Sephadex G-100	23
Bacillus pumilis	86000	$\alpha\beta$	$(NH_4)_2SO_4$/Sephadex G-100	23 112
Bacillus mascerans	α:62000 β:24000		$(NH_4)_2SO_4$/Sephadex G-100	23
Bacillus alvei	90000	$\alpha\beta$	$(NH_4)_2SO_4$/Sephadex G-100	23 112 113
Chromobacterium violaceum	86000		Crude	20
Staphylococcus aureus	65000		Crude	21
Serratia marcescens	141000	$\alpha_2\beta_2$	Purified to homogeneity: protamine sulphate/$(NH_4)_2SO_4$/DEAE-cellulose/ECTEOLA-cellulose/hydroxylapatite	24 114 115
Pseudomonas putida	75000	$\alpha\beta$	Purified to homogeneity*	8 28
P. aeruginosa	79000	$\alpha\beta$	$(NH_4)_2SO_4$/Sephadex G-100	25
P. actifovorans	155000	$\alpha_2\beta_2$	Biogel P-150/DEAE cellulose	26
P. multivorans	150000	$\alpha_2\beta_2$	$(NH_4)_2SO_4$/Sephadex G-100	25
P. testosteroni	155000		Biogel P-150/DEAE cellulose	26
Acinetobacter calcoaceticus	86000	$\alpha\beta$	$(NH_4)_2SO_4$/Sephadex G-100	22 4
Clostridium butyricum	84000	$\alpha_2\beta_2$	$(NH_4)_2SO_4$/Sephadex G-100	27 116
Bacillus caldotenax	α:54000		Partially purified†	100
Serratia marinorubra	140000	$\alpha\beta$	Sephadex G-200	18
Euglena gracilis	80000		Protamine sulphate/$(NH_4)_2SO_4$/DEAE-cellulose/Sephadex G-200	29

*ASI: $(NH_4)_2SO_4$/Sephadex G-100/DEAE-cellulose/preparative electrophoresis. ASII: $(NH_4)_2SO_4$/Sephadex G-100/DEAE-cellulose/calcium phosphate chromatography.

†Streptomycin sulphate/$(NH_4)_2SO_4$/DE-5PW chromatography.

mass (M_r) of 310000 [13]. The trifunctional β-subunit of the AS complex of *N. crassa* has been purified from a mutant which produces no detectable α-subunit and a M_r of 200000 was determined using gel filtration [14]. The activity of the glutamine-dependent anthranilate synthase was measured adding a partially purified preparation of free α-subunit.

Another apparent three enzyme complex containing AS, PRAI and InGPS was partially purified from a *Claviceps* sp., strain SD 58, and showed a M_r of 400000 estimated using gel filtration. The PRAI and InGP activities were also detected in a multifunctional protein which did not contain AS activity and had an apparent M_r of 165000 [15]. However, anthranilate synthase from *Saccharomyces cerevisiae* is a bifunctional enzyme with InGPS activity and shows an $\alpha\beta$ structure with a M_r of 130000. Further purification indicated that InGPS activity resides with the AS β-subunit [16].

In species of the genera *Escherichia*, *Salmonella*, *Klebsiella*, *Citrobacter* and *Enterobacter*, it has also been found that AS and PRT activities reside in a single, large complex protein [12]. An example is the *Escherichia coli* enzyme which has a small subunit with M_r of 20000 (AS β-subunit) and another with M_r of 45000 (AS α-subunit), and these subunits, associate to form an $\alpha_2\beta_2$ tetramer with M_r of 260000 [17].

In some species of genera *Pseudomonas*, *Erwinia*, *Bacillus*, *Proteus* and *Serratia*, and in *Aeromonas formicans*, *Enterobacter liquefaciens*, *Chromobacterium violaceum*, *Staphylococcus aureus*, *Acinobacter calcoaceticus*, and *Clostridium butyricum*, AS is not associated with other enzymes of tryptophan biosynthesis [18–23]. The AS

Table 2. Occurrence of multifunctional anthranilate synthase in microorganisms

Organism	M_r	Structure	Separation/purity	Refs
Salmonella typhimurium	280000	$\alpha_2\beta_2$	$(NH_4)_2SO_4$/Matrix gel Orange A	117 118 119 120
Enterobacter aerogenes	170000	$\alpha_2\beta_2$	Partial purification†	121
Citrobacter freundii	250000	$\alpha_2\beta_2$	Sephadex G-200	18
Citrobacter ballerupensis	250000	$\alpha_2\beta_2$	Sephadex G-200	18
Enterobacter cloacae	250000	$\alpha_2\beta_2$	Sephadex G-200	18
Erwinia dissolvens	250000	$\alpha_2\beta_2$	Sephadex G-200	18
Saccharomyces cerevisiae	130000	$\alpha_2\beta_2$	Purified to homogeneity‡	16
Escherichia coli	260000	$\alpha_2\beta_2$	*$(NH_4)_2SO_4$/DEAE-cellulose	17
Neurospora crassa	α: 76000 β: 84000		Purified to homogeneity§‖	122 123 13
Claviceps spec. SD58	400000	$\alpha\beta$	Purified to homogeneity¶	15

*Purified to homogeneity.

†Protamine sulphate/$(NH_4)_2SO_4$/Sephadex G-200/DEAE-cellulose.

‡Protamine sulphate/$(NH_4)_2SO_4$/ethylamino-Sepharose/hydroxyapatite/DEAE-Sepharose.

§$(NH_4)_2SO_4$/Sephadex G-25/DEAE-cellulose/Sephadex G-75/DEAE-cellulose/Sephadex G-200.

‖$(NH_4)_2SO_4$/Anthranilic acid–agarose chromatography/DEAE-cellulose/gel filtration/preparative electrophoresis.

¶Protamine sulphate/$(NH_4)_2SO_4$/DEAE cellulose/hydroxyapatite/Sephadex G-200/DEAE-cellulose.

Table 3. Occurrence of anthranilate synthase in plants

Organism	M_r	Structure	Separation/purity	Refs
*Catharanthus roseus**	143000	$\alpha_2\beta_2$	Purified to homogeneity‡	48
Pisum sativum†	95300		Partially purified§	32
Zea mays†	95500		Partially purified§	32
Nicotiana tabacum†	200000		$(NH_4)_2SO_4$/Sephacryl S-200	34
*Ruta graveolens**	220000		Partially purified‖	47

*Cell cultures.

†Plant.

‡Polyethylethyleneglycol/Q-Sepharose/Orange A/Mono-Q/Superose 6.

§Protamine sulphate/$(NH_4)_2SO_4$/DEAE-cellulose/Sephadex G-150.

‖$(NH_4)_2SO_4$/DEAE-Sephacel/Sephadex G-200.

subunits are associated in a tetramer in *Serratia marcescens* (AS α-subunit M_r 600000 and AS β-subunit M_r is 21000, AS M_r 141000 [24]), *Pseudomonas multivorans* and *P. testosteroni* (AS tetramer $M_r \sim 150000$ [25, 26]) and *Clostridium butyricum* (M_r 89000) [27]).

Both AS subunits isolated from *Pseudomonas putida* have been obtained in pure form: AS α-subunit has a M_r of 63400 and AS β-subunit of 18000 [28]. Later the M_r of AS β-subunit was redetermined using sodium dodecyl sulphate–gel electrophoesis giving a M_r of 21800 and the amino acid sequence determination gave a value of 21684 [8]. Less purified preparations of AS from *P. aeruginosa* and *P. acifovorans* have also been studied, and the AS enzymes found to have relative molecular weights of 79000 and 155000 [25, 26], and these values suggest $\alpha\beta$ and $\alpha_2\beta_2$ structures, respectively. *Acinetobacter calcoaceticus* also has an AS with $\alpha\beta$ structure and M_r of 86000 [4]. The eukaryote *Euglena gracilis* has the AS α and β-subunits fused (M_r = 80000 [29]).

Plants

AS has been investigated mainly in crude extracts, and in some cases it has only been partially purified (Table 3). The enzyme has been detected in leaves of wheat (*Triticum aestivum*) [30], corn (*Zea mays*) [31, 32], pea (*Pisum sativum*) [32], potato (*Solanum tuberosum*) [33], tobacco (*Nicotiana tabacum*) [34] and *Datura innoxia* [35], and from the seeds of walnut (*Juglans regia*) [36]. AS has been studied in cell cultures of carrot (*Daucus carota*) [37–40], tobacco [37–39, 41, 42], rice (*Oryza sativa*), tomato (*Lycopersicon esculentum*), soybean (*Glycine max*) [38], *Dioscorea deltoidea* [43], *Cinchona succirubra* [44], and *Ruta graveolens* [45, 46].

The molecular weights of the AS enzyme from crude extracts from corn and pea separated on Sephadex G-100 were 95 500 and 95 300, respectively [32]. In tobacco, the form of AS which is resistant to tryptophan inhibition (trp-resistant), had a M_r of 200 000, and the M_r of the tryptophan-sensitive (trp-sensitive) AS was 150 000 [34]. Subsequently the M_r of the enzyme from corn was redetermined and a value of 150 000 was reported [34] which is in contrast with that previously found. All the molecular weights were determined using gel filtration chromatography. The M_r of AS isolated from cell cultures of *Ruta graveolens* was found to be 220 000 [47].

Anthranilate synthase from cell suspension cultures of *Catharanthus roseus* has been purified to a single protein, which had optimum activity at between pH 7.5 and 8.3. This AS enzyme was found to be a tetramer consisting of two large and two small subunits with molecular weights of 67 000 ± 500 and 25 500 ± 500, respectively. The M_r of the AS tetramer determined by gel filtration was 143 000 ± 500 [48].

ISOENZYMES

Microorganisms

Two anthranilate synthase enzymes have been found in *Pseudomonas aeruginosa* and both have conventional α and β subunits [49], each enzyme having a different role in biosynthesis. One participates in tryptophan biosynthesis and is strongly inhibited by tryptophan and the genes for this enzyme have been designated as *trpE* (for α subunit) and *trpG* (for β subunit), being similar to the genes isolated from *P. puptida*. The second AS, which was not inhibited by tryptophan, is encoded by the genes designated *phnA* (α submit) and *phnB* (β subunit). This latter enzyme participates in the synthesis of the blue–green phenazine pigment, pyocyanin, which may be classified as a secondary metabolite [6]. A hypothetical scheme for pyocyanin biosynthesis is shown in Fig. 2 [50].

It has been reported that AS from an alkaloid-producing strain of *Claviceps paspali* was not inhibited by tryptophan, but later the AS enzyme from *Claviceps* species strain SD58 was reported to be tryptophan inhibited [51]. It is not clear from this work whether the cells from which the enzyme was isolated produce alkaloid. Subsequent research with the same strain, SD58, indicated an alkaloid-producing *Claviceps* which yielded only AS which was inhibited by tryptophan and there was no evidence to support the presence of a second isoenzyme which was not tryptophan inhibited [15].

Tryptophan feedback-resistant mutants of AS have been obtained in microorganisms, for example in *E. coli* K-12, *E. coli* W and *S. typhimurium*. In *E. coli* K-12 mutants, it was established that the presence of the trp-resistant AS was the result of a single mutation [15, 52].

Plants

AS isoenzymes appear to occur more commonly in plants and these isoenzymes are mostly tryptophan inhibited. However, in *Solanum tuberosum* cell suspension cultures [33] which were resistant to growth inhibition by D,L-5-methyltryptophan, two AS isoforms were separable by preparative polyacrylamide gel electrophoresis: one form was feedback-sensitive and the other feedback-resistant to tryptophan. The AS isoforms from the normal cell lines were also examined and the trp-sensitive form was found to predominate. In *Nicotiana tabacum* L. cells, two forms of AS (trp-sensitive and trp-resistant) were detected and separated by gel filtration. When protoplasts were fractionated by differential centrifugation, the trp-sensitive form was found in the particulate fraction and the trp-resistant in the cytosol. When cells were selected for resistance to growth inhibition by D,L-5-methyltryptophan (5MT) they were found to contain predominantly the trp-resistant form, which was only present in small amounts in the wild-type cells. Plants regenerated from both normal and 5MT resistant cells lack detectable quantities of trp-resistant AS. Overexpression of the trp-resistant AS reappeared when callus was initiated from leaves of these plants [53, 34].

Altered AS enzymes less sensitive to tryptophan feedback-inhibition have been reported in *Datura inoxia*, *Dioscorea deltoidea*, *Daucus carota* and other species, but only one AS isoenzyme was detected [31, 35, 38, 40, 43, 54, 55].

The presence of two AS enzymes in *Pseudomonas aeruginosa* and in cell cultures of potato and tobacco, may support the hypothesis that two complete pathways exist for the biosynthesis of aromatic amino acids for primary and secondary metabolism [56].

GENE ENZYME RELATIONSHIP AND FUNCTIONALITY

Microorganisms

The gene–enzyme relationships in microorganisms up to date have been extensively studied in comparison with plants, in particular the genes and enzymes of tryptophan pathway. Genes for the seven enzymatic functions of the pathway in prokaryotes are represented by the letters *A–G*, for example *trpE*, and in eukaryotic organisms by numbers and capital letters, for example *TRP2* (Fig. 3, Table 4) [9].

In bacteria the AS α-subunit is encoded by the single gene *trpE*, however the situation for the AS β-subunit is more complex. In some bacteria the AS β-subunit is encoded by a single gene, *trpG*, as is found in *Serratia marcescens* [57], and in *Thermus thermophilus* [58]. In other microorganisms such as *E. coli*, *Salmonella typhimurium* and *Shigella dysenteriae*, *trpG* is fused to the *trpG* gene, which codes for anthranilate phosphoribosyl transferase, the second enzyme in the tryptophan pathway [59, 1]. However *Rhizobium melitoti* presents a special case in that a single gene encodes both the AS α-subunit and the AS β-subunit activities [60]. The product of this unusual *trpE(G)* gene, for anthranilate synthase, is feedback inhibited by tryptophan, as in the case for all known microbial anthranilate synthases with the exception of *P. aeruginosa* [61].

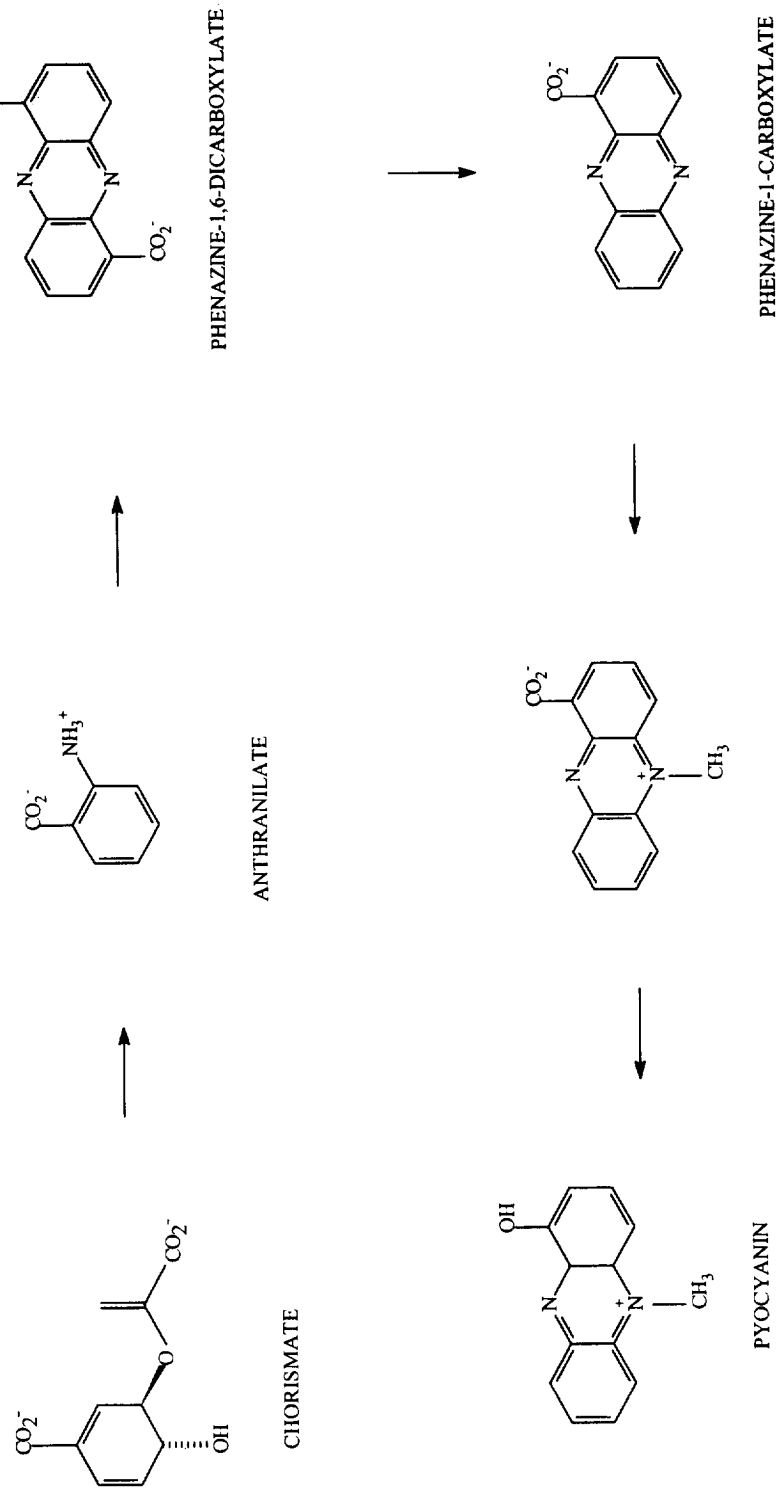

Fig. 2. Hypothetical scheme for pyocyanin biosynthesis (after Leisinger and Margraff, 1979).

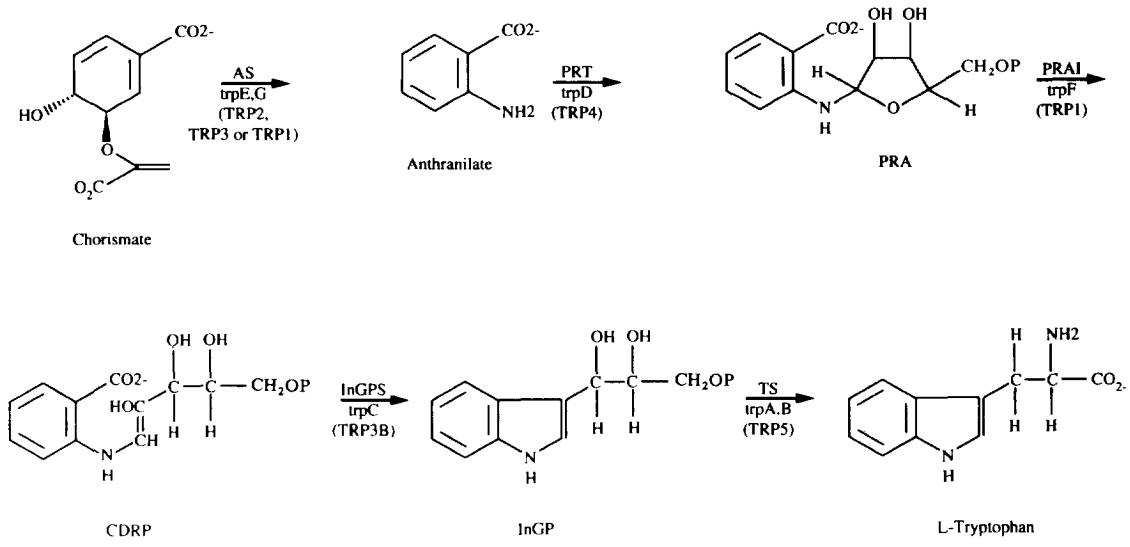

Fig. 3. Biosynthesis of tryptophan.

Table 4. Examples of gene-enzyme relationships and functional organization in plants and microorganisms with respect to anthranilate synthase

Organism	Gene encoding AS synthase (subunit encoded)	Functionality of the enzyme complex
Eukaryote:		
Neurospora crassa	*TRP2* (α-subunit) *TRP1* (β-subunit)	Trifunctional with PRT and InGP synthase
Saccharomyces cerevisiae	*TRP2* (α-subunit) *TRP3* (β-subunit)	Bifunctional with InGP
Euglena gracilis	*trpE(G)*(α and β subunits)	Monofunctional
Plants:		
Arabidopsis thaliana	*ASA1/ASA2*(α-subunit) *ASB1/ASB2/ASB3* (β-subunit)	Monofunctional?
Prokaryote:		
Escherichia coli	*trpE* (α-subunit) *trpG–trpD* (β-subunit)	Bifunctional with anthranilate phosphoribosyl transferase
Serratia marcescens	*trpE* (α-subunit) *trpG* (β-subunit)	Monofunctional
Rhizobium melitoti	*trpE(G)* (α and β subunits)	Monofunctional

Pseudomonas putida and *P. aeruginosa* possess two genes *trpE* and *trpG* that encode the AS α-subunit and the small AS β-subunit of anthranilate synthase [49]. In experiments where mutants of these organisms were created [62, 49] the DNA sequence analysis as well as growth and enzymes assays of the strains developed showed that *trpG* is the first gene in a three-gene operon that also contains *trpD* (phosphoribosylanthranilate transferase) and *trpC* (indole-3-glycerol phosphate synthase) while the gene *trpE* is solitary.

Pseudomonas aeruginosa also possesses a further gene pair encoding for AS: *phnA* and *phnB* [63]. These genes after being cloned, sequenced and inactivated *in vitro* by insertion of an antibiotic resistance gene, were reintroduced by homologous recombination into *P. aeruginosa*, thereby replacing the wild-type gene. The gene pair encoded by *phnA* and *phnB* participates in the synthesis of the phenazine, pyocyanin. Surprisingly, this latter gene pair is more closely related to *E. coli trpE* and *trpG* than *Pseudomonas trpE* and *trpG*, whereas *Pseudomonas trpE* and *trpG* are more closely related to *E. coli pabA* and *pabB*, which encode p-hydroxybenzoate synthase. Genes homologous to *phnA* and *phnB* were not found in *P. putida* PPG1 (a non-phenazine producer).

The AS enzymes in fungi are also composed of two subunits α and β encoded by the *TRP*2 and *TRP*3 or *TRP*1, respectively. The glutamine amidotransferase domain (β-subunit) is located on different multifunctional polypeptides so that the AS complex contains either AS, *N*-phosphoribosyl anthranilate isomerase and indole-3-glycerol phosphate synthase (*TRP*1 gene), or AS and InGP synthase (*TRP*3 gene) [64]. Biochemical studies have established that the first pattern of genetic control, where AS, PRA isomerase and InGP synthase domains reside in the same polypeptide, is present in *Neurospora crassa* [14, 65], *Aspergillus nidulans* [66, 67], *Coprinus radiatus* [68] and *Schizosaccharomyces pombe* [69, 70]. In general, the trifunctional gene encoding these activities has been widely observed in members of the classes Myxomycetes, Chytridiomycetes, Ascomycetes and some Basidiomycetes on the basis of classical purification techniques for protein and genetic studies.

Complexes of enzymes with AS and InGP synthase activities are found exclusively among genera of the order Endomycetales, e.g. *Dipodascus uninucleatus*, *Endomyces bisporus* and *Saccharomyces cerevisiae* [71–73]. In the alga *Euglena gracilis*, AS is encoded by only one gene *trpE(G)* because this enzyme has both subunits (AS α-subunit and AS β-subunit) fused [74].

Plants

Little work has been done with plants, generally because purification and characterization of AS is rather difficult due to its relative instability. Only the enzyme from *Catharanthus roseus* has been purified to a single ptotein but the genes for this enzyme in *C. roseus* have yet to be isolated [48]. This enzyme did not contain anthranilate-5-phosphoribosylpyrophosphate phosphoribosyltransferase activity.

Arabidopsis thaliana has two genes, *ASA*1 and *ASA*2, encoding the α-subunit of AS and the amino acid sequences of these genes are similar to their microbial counterparts [10]. *ASA*1 is induced by wounding and bacterial pathogen infiltration, suggesting an additional role for *ASA*1 in the production of tryptophan pathway metabolites as part of *Arabidopsis* defense response. This plant seems to have three very similar *ASB* genes which encode the β-subunit of AS and the most characterized of these genes, *ASB*1, encodes a β-subunit that can interact with either *ASA*1 or ASA2 in *E. coli* to provide functional glutamine-dependent AS activity. It is not known which *ASB* gene(s), if any is induced by pathogens [11].

Amino acid sequences

There have been 35 amino acid sequences published for the α and β subunits of AS enzyme and in most cases these have been reviewed and compared with those of *p*-aminobenzoate synthase (PABA) [75]. This latter enzyme, as well as isochorismate synthase, seems to have a reaction mechanism similar to AS [76]. PABA also has two dissimilar subunits although they are very similar in sequence, to their AS counterparts [77, 78]. An interesting fact is that species in three genera, *Acinetobacter*, *Bacillus* and *Pseudomonas*, have a single β-subunit that supplies the glutamine aminotransferase activity for both AS and PABA [3, 79, 80]. As these enzymes participate in both the conversion of chorismate to anthranilate and to *p*-aminobenzoate, they are called amphibolics. It is believed that species from the genus *Azospirillum* also carry an amphibolic protein [78].

To express quantitatively the degree of similarity of the amino acid sequences of AS and PABA, the method of Doolittle [82] was used. The normalized alignment score (NAS) is almost 10 times the per cent of identical residues less the gap penalty (gap penalty is 2.5 times the weight of a residue identity). For sequences 200 residues long, similarity values greater than 160 indicate that common ancestry is probable, and values above 280 are believed to make it certain.

Values for the α-subunits of a wide range of microorganisms are given in Table 5, and because all these components have approximately 500 amino acid resi-

Table 5. Similarity (NAS) scores for AS α-subunits [75, 124]

Organism and gene	*E.c. trpE*	*V.p. trpE*	*P.a. phnA*	*B.l. trpE*	*R.m. trpE*	*S.a. trpE*	*P.a. trpE*	*B.s. trpE*	*S.c. trpE*
L. bif. trpE	187		248	117				233	180
E. coli trpE		580	456	426	238	269	252	240	224
V. par. trpE			431	415	208	275	252	230	215
P. aer. phnA				392	208	247	260	225	204
B. lac. trpE					198	222	216	205	208
R. mel. trpE						208	193	201	217
S. aur. trpE							213	261	248
P. aer. trpE								319	272
B. sub. trpE									255

Abbreviations: *V. par* and *V.p.*, *Vibrio parahaemolyticus*; *P. aer* and *P.a.*, *Pseudomonas aeruginosa*; *B. lac* and *B.l.*, *Brevibacterium lactofermentum*; *R. mel* and *R.m.*, *Rhizobium meliloti*; *S. aur* and *S.a.*, *Spirochaeta aurantia*; *B. sub.* and *B.s.*, *Bacillus subtilis*; *S.c.*, *Saccharomyces cerevisiae*; *E.c.* and *E. coli*, *Escherichia coli*; *L. bif.*, *Leptospira biflexa.*

dues, the values cited are within the range indicative of homology. There is remarkable similarity between the *trpE* amino acid sequences of *P. savastanoi*, *P. aeruginosa* and *P. putida* [83]. These sequences are on average 86% similar (values for *P. savastanoi* and *P. putida* were not included in Table 5).

The amino acid sequence of the AS α-subunit of *Bacillus caldotenax* has also been determined as have those of *B. subtilis* [2], *Clostridium thermocellum* [84], *Thermus thermophilus* [58], *E. coli* [59] and *Brevis lactofermentum* [85]. The conserved amino acids of the AS α-subunit of these species amount to 61 residues, most of them being found in the C-terminal half [86].

The *trpE* from *Haloferax volcanii* shows 29–30% amino acid sequence homology with *E. coli* and yeast enzymes, respectively, 34% homology with *Methanobacterium thermoautotrophicum*, and comparable values with the homologous *pabB* and *phnA* genes of *E. coli* and *P. aeruginosa* [87, 88].

Escherichia coli and *Shigella dysenteriae* show the highest similarity comparing their *trpG* sequences, where there were no gaps and only three nonidentical amino acids in the 195-residue polypeptide [59]. The fungi *Aspergillus nidulans* [89, 90], *Aspergillus niger* [89], *Penicillium chrysogenum* [91], *Phycomyces blakesleeanus* [92] and *N. crassa* have highly similar sequences (the NAS values are between 624 and 721 [75]).

Comparisons between other species are presented in Table 6 [75]. The *P. putida* AS β-subunit has a similar sequence to the NH_2-terminal end of *E. coli* and *S. typhimurium* AS β-subunit and the monofunctional AS β-subunit of *Serratia marcescens*. These results suggest that the glutamine amidotransferase region of the bifunctional AS of *E. coli* and *S. typhimurium* with the smaller subunit of S. marcescens and *P. putida* share the same evolutionary origin [8, 83]. This suggestion gives support to the idea that the bifunctional AS β-subunit polypeptides of *E. coli* and *S. typhimurium* arose by fusion of two separate genes.

The *trpG* from *H. volcanii* has 30–37% homology with the gene isolated from *E. coli* and yeast, respectively, and has 38% homology with *M. thermoautotrophicum trpG* [87].

The amino acid sequences for AS α-subunit predicted from DNA sequences of *A. thaliana ASA1* and *ASA2* were aligned with AS α subunits from *S. cerevisiae*, *E. coli* and *B. subtilus*, along with the PABA synthase α-subunit from *E. coli* and are 30–36% identical to these microbial subunits and 67% identical to each other [10]. The *ASB1* is 34–45% identical to the AS β-subunit of *S. cerevisiae* and *E. coli* AS and PABA synthase β-subunits [11].

THE REACTION MECHANISM

The availability of AS from organisms harbouring plasmids which contain the genes for the production of one or both AS subunits [12], has led to a series of mechanistic studies. The larger α-subunit of AS is involved with the binding of chorismate and the regiospecific amination/pyruvate elimination sequence [93]. This α-subunit can synthesize anthranilate directly from chorismate and a high concentration of ammonia. The low molecular weight β-subunit possesses the glutaminase activity that hydrolyses the co-substrate glutamine, via a γ-glutamyl-*S*-cysteinyl enzyme intermediate to release NH_3 for the amination sequence [76].

The glutamine binding site has been identified by using reactive analogues of glutamine as affinity labels which block the active site. An example is the inactivation of the multifunctional anthranilate synthase from *Neurospora crassa* that loses its glutamine-dependent anthranilate synthase activity on exposure to azaserine (*O*-diazoacetyl-L-serine) and DON (6-diazo-5-oxo-L-norleucine). The inactivation depends on the presence of the

Table 6. Similarity (NAS) score for anthranilate synthase β-subunits [75]

Organism and gene	*S.t trpG*	*S.m. trpG*	*V.p. phnB*	*P.a. trpG*	*B.l. trpG*	*B.s. trpG*	*P.p. trpG*	*P.a. trpG*	*A.c. trpG*	*R.m. trpG*	*S.c. trpG*	*N.c. trpG*
E. coli trp(G)	965	839	622	475	290	399	383	396	317	260	332	332
S. tym. trp(G)		834	627	470	290	384	363	381	302	265	330	322
S. mar. trpG			591	396	311	401	380	401	327	267	320	302
V. par trpG				415	347	369	367	379	362	347	342	296
P. aer. phnB					325	363	388	395	331	303	318	328
B. lac. trpG						225	270	316	278	206	265	281
B. sub. trpG							633	585	563	284	477	485
P. put. trpG								843	655	281	467	561
P. aer. trpG									649	297	472	500
A. cal. trpG										267	434	487
R. mel. trp(G)											305	251
S. cer. trp(G)												654

Abbreviations: *S. tym.* and *Salmonella typhimurium*; *S. mar.* and *S.m.*, *Serratia marcescens*; *V. par* and *V.p.*, *Vibrio parahaemolyticus*; *P. aer* and *P.a.*, *Pseudomonas aeruginosa*; *B. lac* and *B.l.*, *Brevibacterium lactofermentum*; *B. sub.* and *B.s.*, *Bacillus subtilis*; *P. put.* and *P.p.*, *Pseudomonas putida*; *A. cal.* and *A.c.*, *Acinetobacter calcoaceticus*; *R. mel.* and *R.m.*, *Rhizobium meliloti*; *S. cer.* and *S.c.*, *Saccharomyces cerevisiae*; *N.c. Neurospora crassa*.

substrate chorismate, which is an essential cofactor for the inactivation reaction, is enhanced by the cofactor Mg^{2+}, and is antagonized by glutamine. The ammonia-dependent anthranilate synthase activity is lost but at a slower rate, indicating that the maximum expression of the ammonia-dependent domain also depends on the interaction with an active glutamine amidotransferase site [94].

The most likely intermediate in the conversion of chorismate to anthranilate is an 'amino' analogue of isochorismate: *trans*-6-amino-5-[(1-carboxyvinyl)-oxy]-1,3-cyclohexadiene-1-carboxylate (Fig. 4). For the formation of this intermediate, it has been proposed that there is a formal *syn*-1,5 displacement of hydroxide by ammonia [95]. In these magnesium dependent enzymes, the most obvious role that Mg^{2+} may play is to chelate to the 4-hydroxyl group of chorismate, making it a better leaving group. The same mechanism is proposed for isochorismate synthase [96], but in this case the magnesium ion is bound to the enzyme with at least one ammonia molecule in its coordination sphere. The subsequent aromatization, when pure enzyme was used, consisted in a *cis* elimination/aromatization route (Fig. 4) [97]. However other researchers found that during aromatization of chorismate, the third methyl hydrogen in pyruvate comes from a *re*-face addition of a proton from the solvent [98].

The synthetic *trans*-aminoenolpyruvate is a chemically and kinematically competent intermediate in the biosynthesis of anthranilate but to date has not been detected as an accumulating intermediate during enzymatic processing. Both stepwise and concerted mechanisms are possible.

Active sites

Several experiments have been done to identify the active site on the anthranilate subunits. Different chemical methods were used to probe residues essential for AS α-subunit activity in *Serratia marcescens* [99]. For example, phenylglyoxal and 1,2-cyclohexanedione modified between two and five arginine residues and inactivated the subunit, but in the presence of chorismate, the rate of inactivation was reduced. Analysis of the data indicated that one arginine residue is essential for activity. The histidine residues were modified also with ethoxyformic anhydride and by photooxidation, and the enzyme was also inactivated, but the substrate prevented the inactivation. The enzyme lost activity when one cysteine residue was alkylated and a tryptic peptide containing the essential cysteine residue has been isolated.

Bacillus caldotenax was similar to *S. marcescens*, in that it showed that one arginine residue is crucial for AS α-subunit activity. The identity of this residue was established using site-directed mutagenesis and a comparative survey of amino acid sequences disclosed that six arginine residues were conserved in seven AS α-subunits from *B. caldotenax*, *Bacillus subtilis*, *Clostridium thermocellum*, *Thermus thermophilus*, *E. coli*, *Brevis lactofermentum* and *Saccharomyces cerevisiae* [100]. There was no conserved cysteine, but two histidines are conserved among AS of these microorganisms which suggest further studies to examine the effect of substitution of these histidine residues.

In the case of the AS β-subunit, the active site has been studied in *S. marcescens* [57, 101] and *Pseudomonas putida* [8]. The active site region for *S. marcescens* is virtually identical to that of *P. putida*. An essential cysteine residue of the active site was alkylated selectively in both microorganisms: in *P. putida* this active site residue is 79, and in *S. marcescens* it is residue 83. This essential residue is conserved in the AS β-subunits of *E. coli*, *S. typhimurium*, *S. dysenteriae*, *P. aeruginosa*, *Acinobacter calcoaceticus*. *N. crassa* and *S. cerevisiae* [71].

Later it was found that modification of a lysyl residue associated with the active site with pyridoxal 5'-phosphate and NaCN in *S. marcescens*, reduces the reactivity of the essential cysteinyl residue, resulting in the loss of

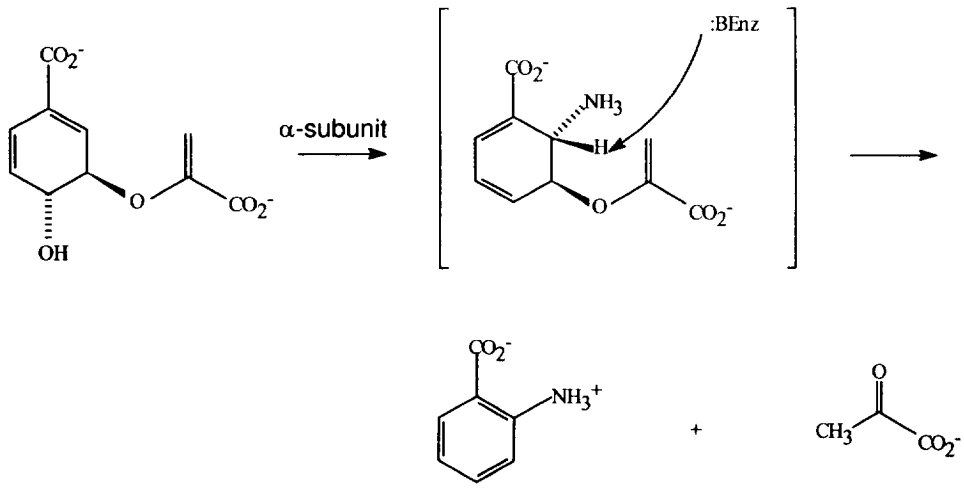

Fig. 4. *Cis* elimination/aromatization route for the formation of anthranilate from chorismate.

the amidotransferase activity. The rate of lysyl modification is enhanced by chorismate, providing evidence for the interaction of the cysteinyl and lysyl residues, and it has been suggested that the lysyl residue functions as a general acid–base to promote ionization of the cysteinyl residue [102]. The lysyl residue is conserved in all the organisms noted above [71].

The active site for tryptophan feedback inhibition has been studied in *Brevibacterium lactofementum*, where a 5-fluorotryptophan-resistant mutant was isolated and the mutation site was determined [85]. The mutant has an altered AS which was fully active even in the presence of 10 mM tryptophan, while the activity of the wild type AS under the same conditions was less than 1% in the absence of tryptophan. One adenine to cytosine single-base-pair substitution at codon Ser-38 (the Ser codon, AGC, was changed to an Arg codon, CGC) was the cause for the desensitization to feedback inhibition in the mutant. It was found also that the particular amino acid sequence from Leu-35 to Ser-38 (Leu–Leu–Glu–Ser), in *B. lactofermentum* α-subunit was conserved in the corresponding regions of *E. coli* [1], *Salmonella typhimurium* [103], *Haloferax volcanii* [87] and *B. subtilis* [2], suggesting that this particular amino acid region and Ser-38 are essential for the allosteric regulation of AS. Substitution of guanine to adenine in the attenuator region of the gene, appeared to enhance expression of enzymes in the tryptophan biosynthesis.

Another interesting study was done on a hybrid complex containing one catalytically active, feedback-insensitive to tryptophan and one catalytically inactive, feedback-sensitive mutant α-subunit from *Salmonella typhimurium*. The binding of a single inhibitor molecule to one of the two α-subunits, was sufficient for the propagation of a conformational change, which affected the active site of the companion α-subunit with the consequent reduction of AS activity [104].

UTILITY OF BIFUNCTIONAL ENZYMES FOR THE CLASSIFICATION OF BACTERIA

Since bifunctional proteins are the result of relatively infrequent genetic events that are faithfully conserved, they are reliable markers to define phylogenetic clusters [105]. The bifunctional enzyme anthranilate synthase–anthranilate phosphoribosyl transferase is present in the lineage shared by the genera *Escherichia*, *Salmonella*, *Citrobacter*, *Klebsiella* and *Enterobacter*, but is absent in other enteric bacteria from the genera *Erwinia*, *Serratia*, *Proteus*, *Morganella* and *Hafnia* [18, 106, 107]. The presence or absence of this enzyme has been used to separate two enteric clusters and it has been used to postulate that aerogenic and anaerogenic strains of *Enterobacter agglomerans* belong to different groups [108]. The strains assigned to *E. agglomerans* have been isolated mainly from plants, soil, foodstuffs, and human and animal sources [109], but it was proposed that the strains isolated from clinical sources be designated *E. agglomerans*, because of the conformity with the genus *Enterobacter*, and those isolated from plant lesions and surfaces be placed in the genus *Erwinia* [110]. It is difficult to distinguish strains from different sources due to the diversity in this group of organisms, that is why it has been suggested that the strains can be grouped with the genus *Erwinia* or *Enterbacter* depending upon whether bifunctional AS:PRT is absent or present [108]. For example, *Enterobacter agglomerans* ATCC 29915 (aerogenic) possesses the bifunctional enzyme, whereas *E. agglomerans* ATCC 27155 (anaerogenic) and *Erwinia* herbicola 33243^T lack AS:PRT. It was shown that the last two types of strains belong to the same genomic species [111] and together with the fact of the absence of the bifunctional enzyme, the exclusion of the *E. agglomerans* from the genus *Enterobacter* has been suggested [108].

SUMMARY

A number of interesting areas require further resolution. Plants in particular have not been investigated as extensively as microorganisms for anthranilate synthase. Nonetheless, important similarities have been found between AS in microorganisms and in plants and this holds true for all the enzymes of the chorismate pathways to aromatic amino acids suggesting that these pathways evolved once, thereby connecting yeasts with other fungi, prokaryotes and with plants.

Plants, unlike microorganisms, appear to contain isoenzymes and these are known to be spatially organized and may as a result have more specialized roles (56). For example, genes *ASA*1 and *ASA*2 encoding the AS α subunit in *Arabidopsis* both have putative chloroplast transit peptides at their amino acid terminal end and conserved amino acids involved in feedback inhibition by tryptophan. The cDNAs of the genes for the *ASA*1 and *ASA*2 α subunits of *Arabidopsis* complement those for AS in yeast and *E. coli* confirming that both genes in *Arabidopsis* encode for functional AS protein. Distribution of the mRNAs for *ASA*1 and *ASA*2 in various parts of *Arabidopsis* are overlapping but not identical and *ASA*1 mRNA is a lot more abundant in whole plants whereas *ASA*2 is only expressed at a constitutive basal level. *ASA*1 is induced by wounding and bacterial infiltration suggesting a novel role in defense systems. In plants therefore it would appear that there is differential expression of duplicated genes, and hence regulation of key steps in aromatic acid biosynthesis appears to involve differential expression of duplicated genes (10, 11).

Another area which is still evolving is the investigation of the regulation of genes. The control of the genes of aromatic amino acid (tryptophan) biosynthesis has been investigated in yeast as a model system [73]. Studies of this pathway have led to some understanding of gene–enzyme relationships, promoters, protein–DNA interactions and translational control, enzyme structure and catalysis, protein–protein interaction and control of flow through the pathway. It has already been shown that whilst there are striking differences among various

species in the genetic organization of such activities that catalyse the reactions and their regulation, there are also many similarities.

In yeast, aromatic biosynthesis is controlled by regulation of enzyme synthesis by (i) the regulation of gene expression and (ii) by regulation of the enzyme activities that control the carbon flow. In the case of tryptophan this product controls the flow through the system by a feedback mechanism which moderates AS activity. In general, yeast organization and regulation of genes differs somewhat from prokaryotes but they have many features in common with eukaryotes. For example, the regulatory proteins GCN4 and PHO2 share similarities to other eukaryotic transcriptional factors and it seems likely that many components of the transcriptional machinery are interchangeable but many basal transcriptional factors remain to be identified and many aspects of the regulators of the regulators are completely unknown. There is, therefore, more information required on the transcriptional regulatory network if this is to be fully understood in both microorganisms and plants.

REFERENCES

1. Yanofsky, C., Platt, T., Crawford, I. P., Nichols, B. P., Christie, G. E., Horowitz, H., van Cleemput, M. and Wu, A. M. (1981) *Nucleic Acids Res.* **9**, 6647.
2. Henner, K. J., Band, L. and Shimotsu, H. (1984) *Gene* **34**, 169.
3. Sawula, R. V. and Crawford, I. P. (1972) *J. Bacteriol.* **112**, 797.
4. Sawula, R. V. and Crawford, I. P. (1973) *J. Biol. Chem.* **248**, 3573.
5. Holloway, B. W., Krishnapillai, V. and Morgan, A. F. (1979) *Microbiol. Rev.* **43**, 73.
6. Shinomiya, T., Shiga, E. and Kageyama, M. (1983) *Mol. Gen. Genet.* **189**, 382.
7. Johnson, A. W. B., Bibb, M. J. and Beringer, J. E. (1978) *Mol. Gen. Genet.* **165**, 323.
8. Kawamura, M., Keim, P. S., Goto, Y., Zalkin, H. and Heinrikson, R. L. (1978) *J. Biol. Chem.* **253**, 4659.
9. Hütter, R., Niederberger, R. and DeMoss, J. A. (1986) *Ann. Rev. Microbiol.* **40**, 55.
10. Niyogi, K. K. and Fink, G. R. (1992) *Plant Cell* **4**, 721.
11. Niyogi, K. K., Last, R. L., Fink, G. R. and Keith, B. (1993) *Plant Cell* **5**, 1011.
12. Zalkin, H. (1985) *Methods Enzymol.* **113**, 287.
13. Keesey, J., Paukert, J. and deMoss, J. A. (1981) *Arch. Biochem. Biophys.* **207**, 103.
14. Walker, M. S. and DeMoss, J. A. (1983) *J. Biol. Chem.* **258**, 3571.
15. Mann, D. F. and Floss, H. G. (1977) *Lloydia* **40**, 136.
16. Prantl, F., Strasser, A., Aebi, M., Furter, R., Niederberger, P., Kirschener, K. and Huetter, R. (1985) *Eur. J. Biochem.* **146**, 95.
17. Ito, J. and Yanofsky, C. (1969) *J. Bacteriol.* **97**, 734.
18. Largen, M. and Belser, W. L. (1975) *J. Bacteriol.* **121**, 239.
19. Kane, J. F. and Jensen, R. A. (1970) *Biochem. Biophys. Res. Commun.* **41**, 328.
20. Wegman, J. and Crawford, I. P. (1968) *J. Bacteriol.* **95**, 2325.
21. Procter, A. R. and Kloos, W. E. (1973) *J. Bacteriol.* **114**, 169.
22. Twarog, R. and Liggins, G. L. (1970) *J. Bacteriol.* **104**, 254.
23. Patel, N., Holmes, W. M. and Kane, J. F. (1974) *J. Bacteriol.* **119**, 220.
24. Zalkin, H. and Hwang, L. H. (1971) *J. Biol. Chem.* **246**, 6899.
25. Crawford, I. P. (1975) *Bacteriol. Rev.* **39**, 87.
26. Queener, S. F. and Gunsalus, I. C. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **67**, 1225.
27. Baskerville, E. N. and Twarog, R. (1972) *J. Bacteriol.* **112**, 304.
28. Queener, S. W., Queener, S. F., Meeks, J. R. and Gunsalus, I. C. (1973) *J. Biol. Chem.* **248**, 151.
29. Lara, J. C. and Mills, S. E. (1973) *J. Bacteriol.* **110**, 1100.
30. Singh, M. and Widholm, J. M. (1974) *Physiol. Plant.* **32**, 240.
31. Singh, M. and Widholm, J. M. (1975) *Biochem. Genet.* **13**, 357.
32. Hankins, C. N., Largen, M. T. and Mills, S. E. (1976) *Plant Physiol.* **57**, 101.
33. Carlson, J. E. and Widholm, J. M. (1978) *Physiol. Plant.* **44**, 251.
34. Brotherton, J. W., Hauptmann, R. M. and Widholm, J. M. (1986) *Planta* **168**, 214.
35. Ranch, J. P. and Widholm, J. M. (1983) *Basic Life Sci.* **22**, 381.
36. Grosse, W. (1977) *Z. Pflanzenphysiol.* **83**, 249.
37. Widholm, J. M. (1974) *Physiol. Plant.* **30**, 13.
38. Widholm, J. M. (1971) *Physiol. Plant.* **25**, 75.
39. Widholm, J. M. (1972a) *Biochim. Biophys. Acta* **261**, 44.
40. Widholm, J. M. (1972b) *Biochim. Biophys. Acta* **279**, 48.
41. Widholm, J. M. (1972c) *Biochim. Biophys. Acta* **261**, 52.
42. Belser, W. L., Baron Murphy, J., Delmer, D. P. and Mills, S. E. (1971) *Biochim. Biophys. Acta* **237**, 1.
43. Kutáček, M., Eder, J., Opatrný, Z., Vacková, K., Ahmad, M. M., Kefeli, V. I., Butenko, R. G., Karanova, S. L. and Karanova, R. V. (1981) *Biochem. Physiol. Pflanzen* **176**, 244.
44. Schmauder, H.-P., Gröger, D. and Koblitz, H. and Koblitz, D. (1985) *Plant Cell Rep.* **4**, 233.
45. Hertel, S. C., Schmauder, H.-P., Hieke, M. and Gröger, D. (1988a) *Biochem. Physiol. Pflanzen* **183**, 425.
46. Hertel, S. C., Schmauder, H.-P. and Gröger, D. (1988b) *Acta Biotechnol.* **8**, 553.
47. Hertel, S. C., Hieke, M. and Gröger, D. (1991) *Biochem. Physiol. Pflanzen* **187**, 121.
48. Poulsen, C., Bongaerts, R. J. M. and Verpoorte, R. (1993) *Eur. J. Biochem.* **212**, 431.
49. Essar, D. W., Eberly, L. and Crawford, I. P. (1990b) *J. Bacteriol.* **172**, 867.

50. Leisinger, T. and Margraff, R. (1979) *Microbiol. Rev.* **43**, 422.
51. Lingens, F., Goebel, W. and Uesseler, H. (1967) *Eur. J. Biochem.* **2**, 442.
52. Gibson, F. and Pittard, J. (1968) *Bacteriol. Rev.* **32**, 465.
53. Brotherton, J. E. and Widholm, J. M. (1985) *J. Chromatography* **350**, 332.
54. Widholm, J. M. (1977) *Planta* **134**, 103.
55. Sung, Z. R. (1979) *Planta* **145**, 339.
56. Hrazdina, G. and Jensen, R. A. (1992) *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **43**, 241.
57. Tso, J. Y., Hermodson, M. A. and Zalkin, H. (1980a) *J. Biol. Chem.* **255**, 1451.
58. Sato, S., Nakada, Y., Kanaya, S. and Tanaka, T. (1988) *Biochim. Biophys. Acta* **950**, 303.
59. Nichols, B. P., Miozzari, G. F., van Cleemput, M., Bennett, G. N. and Yanofsky, C. (1980) *J. Mol. Biol.* **142**, 503.
60. Bae, Y. M., Holmgren, E. and Crawford, I. P. (1989) *J. Bacteriol.* **171**, 3471.
61. Bae, Y. M. and Crawford, I. P. (1990) *J. Bacteriol.* **172**, 3318.
62. Essar, D. W., Eberly, L., Han, C.-Y. and Crawford, I. P. (1990a) *J. Bacteriol.* **172**, 853.
63. Essar, D. W., Eberly, L., Hadero, A. and Crawford, I. P. (1990c) *J. Bacteriol.* **172**, 884.
64. Hütter, R. and Niederberger, P. (1983) *Biotechnol. Adv.* **1**, 179.
65. Walker, M. S. and DeMoss, J. A. (1986) *J. Biol. Chem.* **261**, 16073.
66. Hütter, R. and DeMoss, J. A. (1967) *Genetics* **55**, 241.
67. Roberts, C. A. (1967) *Genetics* **55**, 233.
68. Henke, H., Guerdoux, J. L. and Hütter, R. (1973) *C. R. Acad. Sci. Ser. D* **276**, 2001.
69. Schweingruber, M. E. and Dietrich, R. (1973) *Experientia* **29**, 1151.
70. Thuriaux, P., Heyer, W.-D. and Strauss, A. (1982) *Curr. Genet.* **6**, 13.
71. Zalkin, H., Paluh, J. L., van Cleemput, M., Moye, S. and Yanofsky, C. (1984) *J. Biol. Chem.* **259**, 3985.
72. Mortimer, R. K. and Schild, D. (1985) *Microbiol. Rev.* **49**, 181.
73. Braus, G. (1991) *Microbiol. Rev.* **55**, 349.
74. Hankins, C. N. and Mills, S. E. (1976) *J. Biol. Chem.* **251**, 7774.
75. Crawford, I. P. (1989) *Ann. Rev. Microbiol.* **43**, 567.
76. Walsh, C., Liu, J., Rusnak, F. and Sakaitani (1990) *Chem. Rev.* **90**, 1105.
77. Goncharoff, P. and Nichols, B. P. (1984) *J. Bacteriol.* **159**, 57.
78. Kaplan, J. B. and Nichols, B. P. (1983) *J. Mol. Biol.* **168**, 451.
79. Buvinger, W. E., Stone, L. C. and Heath, H. E. (1981) *J. Bacteriol.* **147**, 62.
80. Kane, J. F., Holmes, W. M. and Jensen, R. A. (1972) *J. Biol. Chem.* **247**, 1587.
81. Zimmer, W., Aparicio, C. and Elmerich, C. (1991) *Mol. Gen. Genet.* **229**, 41.
82. Doolittle, R. F. (1986) *Of Urfs and Orfs: A Primer on How to Analyze Derived Amino Acid Sequences*, pp. 12–15. University Science Books, Mill Valley, CA.
83. Li, S. L., Hanlon, J. and Yanofsky, C. (1974) *Biochemistry* **13**, 1736.
84. Sato, S., Nakada, Y., Honnami, K., Yasui, K. and Shiratsuchi, A. (1989) *J. Biochem.* **105**, 362.
85. Matsui, K., Miwa, K. and Sano, K. (1987) *J. Bacteriol.* **169**, 5330.
86. Shiratsuchi, A. and Sato, S. (1991) *Biochim. Biophys. Acta* **1090**, 348.
87. Lam, W. L., Logan, S. M. and Doolittle, F. (1992) *J. Bacteriol.* **174**, 1694.
88. Meile, L., Stettler, R., Banholzer, R., Kotik, M. and Leisinger, T. (1991) *J. Bacteriol.* **173**, 5017.
89. Kos, T., Kuijvenhoven, A., Hessing, H. G. M., Puwels, P. H. and van den Hondel, C. A. M. J. J. (1988) *Curr. Genet.* **13**, 137.
90. Mullaney, E. J., Hamer, J. E., Roberti, K. A., Yelton, M. and Timberlake, W. E. (1985) *Mol. Gen. Genet.* **199**, 37.
91. Penalva, M. A. and Sanchez, F. (1987) *Nucleic Acids Res.* **15**, 1874.
92. Choi, H. T., Revuelta, J. L., Sadhu, C. and Jayaram, M. (1989) *Gene* **71**, 85.
93. Walsh, C. T., Erion, M. D., Walts, A. E., Delany, J. J. and Berchtold, G. A. (1987) *Biochemistry* **26**, 4734.
94. Paukert, J. L., Henkin, J., Keesey, J. and deMoss, J. A. (1982) *Arch. Biochem. Biophys.* **218**, 443.
95. Policastro, P. P., Au, K. G., Walsh, C. T. and Berchtold, G. A. (1984) *J. Am. Chem. Soc.* **106**, 2443.
96. Kozlowski, M. C. and Barlett, P. A. (1991) *J. Am. Chem. Soc.* **113**, 5897.
97. Teng, C.-Y. P. and Ganem, B. (1984) *J. Am. Chem. Soc.* **106**, 2463.
98. Asano, Y., Lee, J. J., Shieh, T. L., Spreafico, R., Kowal, C. and Floss, H. G. (1985) *J. Am. Chem. Soc.* **107**, 4314.
99. Tso, J. Y. and Zalkin, H. (1981) *J. Biol. Chem.* **256**, 9901.
100. Shiratsuchi, A. and Sato, S. (1992) *J. Biochem.* **112**, 714.
101. Tso, J. Y., Bower, S. G. and Zalkin, H. (1980b) *J. Biol. Chem.* **255**, 6734.
102. Bower, S. and Zalkin, H. (1982) *Arch. Biochem. Biophys.* **219**, 121.
103. Caligiuri, M. G. and Bauerle, R. (1991a) *J. Biol. Chem.* **266**, 8328.
104. Caligiuri, M. G. and Bauerle, R. (1991b) *Science* **252**, 1845.
105. Ahmad, S. and Jensen, R. A. (1986) *Trends Biochem. Sci.* **11**, 108.
106. Henderson, E. J., Nagano, H., Zalkin, H. and Hwang, H. (1970) *J. Biol. Chem.* **245**, 1416.
107. Zalkin, H. (1980) in *Multifunctional Proteins* (Bisswanger, H. and Schmincke-Ott, E., eds), pp. 124–149. John Wiley, New York.
108. Ahmad, S. and Jensen, R. A. (1989) *Int. J. Syst. Bacteriol.* **39**, 100.
109. Richard, C. (1985) in *Bergey's Manual of Systematic Bacteriology* (Krieg, N. R. and Holt, J. G., eds), Vol.

1, pp. 465–469. The Williams & Wilkins Co., Baltimore.
110. Ewing, W. H. and Fife, M. A. (1972) *Int. J. Syst. Bacteriol.* **22**, 4.
111. Beji, A., Meragaert, J., Gavini, F., Izard, D., Kersters, K., Leclerk, H. and De Ley, J. (1988) *Int. J. Syst. Bacteriol.* **38**, 77.
112. Hoch, S. O. and Crawford, I. P. (1973) *J. Bacteriol.* **116**, 685.
113. Catena, A. and DeMoss, R. D. (1970) *J. Bacteriol.* **101**, 476.
114. Robb, F., Hutchinson, M. A. and Belser, W. L. (1971) *J. Biol. Chem.* **246**, 6908.
115. Largen, M., Mills, S. E., Rowe, J. and Yanofsky, C. (1976) *Eur. J. Biochem.* **67**, 31.
116. Baskerville, E. N. and Twarog, R. (1974) *J. Bacteriol.* **117**, 1184.
117. Bauerle, R., Hess, J. and French, S. (1987) *Methods Enzymol.* **142**, 366.
118. Nagano, H. and Zalkin, H. (1970) *J. Biol. Chem.* **245**, 3097.
119. Henderson, E. J. and Zalkin, H. (1971) *J. Biol. Chem.* **246**, 6891.
120. Grieshaber, M. and Bauerle, R. (1974) *Biochemistry* **13**, 373.
121. Egan, A. F. and Gibson, F. (1972) *Biochem. J.* **130**, 847.
122. Gaertner, F. H. and deMoss, J. A. (1969) *J. Biol. Chem.* **244**, 2716.
123. Hulett, F. M. and DeMoss (1973) *Abstr. Annu. Meet. Amer. Soc. Microbiol.* **P47**, p. 148.
124. Brahamsha, B., Han, C.-Y., Crawford, I. P. and Greenberg, E. P. (1991) *J. Bacteriol.* **173**, 541.