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CHORISMATE MUTASE IN MICROORGANISMS AND PLANTS

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Key Word Index—Plants; microorganisms; chorismate mutase; genes; reaction mechanisms; active site.

Abstract—The enzymology of chorismate mutase in plants and microorganisms is reviewed to include occurrence, gene–enzyme relationships, reaction mechanisms and details of active site studies.

INTRODUCTION

Chorismate mutase (EC 5.4.99.5) catalyses the conversion of chorismate into prephenate and plays a key role in the biosynthesis of the essential aromatic amino acids tyrosine and phenylalanine. The catalysed reaction is unique in nature because it is the only example in primary metabolism of a pericyclic process, a Claisen rearrangement [1].

Microorganisms are diverse in the arrangement and variety of enzymes comprising the terminal pathways of tyrosine and phenylalanine. They can synthesize these amino acids via phenylpyruvate or arogenate for phenylalanine or via 4-hydroxyphenylpyruvate or arogenate for tyrosine, utilizing either bifunctional chorismate mutases (CM-P, CM-T, CM-DAHP synthase) or monofunctional chorismate mutases (CM-F, CM-R) (Fig. 1) [2, 3]. In plants, only monofunctional forms of chorismate mutase have been isolated and they differ from one another in their regulation by tyrosine and phenylalanine. It is possible to find from one to three chorismate mutase isoenzymes (CM-1, CM-2 and CM-3) [4].

OCCURRENCE OF CHORISMATE MUTASE

Microorganisms

Chorismate mutase has been isolated and characterized from many microorganisms (Table 1) and in all the cases investigated so far, except for *Bacillus subtilis* [5] and *Streptomyces aureofaciens* [6], the enzyme is a dimer with similar or identical subunits and molecular weights between 23 000 and 91 000 (*Acinobacter calcoaceticus*) [7, 8].

Different isoenzymes can be found in microorganisms, three bifunctional, CM-P, CM-T and CM-DAHP synthases, which contain besides the chorismate mutase activity, prephenate dehydratase, prephenate dehydrogenase and 3-deoxy-D-arabinoheptulosonate 7-phosphate synthase activities, respectively. There are also two monofunctional enzymes, CM-F and CM-R, which differ in their allosteric regulation by the aromatic amino acids phenylalanine and tyrosine: CM-F is unregulated, and CM-R is regulated [3].

Plants

Chorismate mutase has been characterized from many plants, and the presence of three isoenzymes has been detected in several species (Table 2). The molecular weights of the enzymes range from 36 000 (CM-2, *Vigna radiata*) to 84 000 (CM-1, *Papaver somniferum*). Besides the data in Table 2, a comparison of chorismate mutase isoenzyme patterns in a variety of plants was made after separating the isoenzymes by disc electrophoresis [9]. All anthophyta tested, except some members of the Lotoideae subfamily of the Leguminosae, contained three isoenzymes, while vascular but primitive plants such as pine contained two isoenzymes. Most algae, both blue–green and green, contain one chorismate mutase [10–12].

ISOENZYMES

Microorganisms

The five chorismate mutase isoenzymes that are found in microorganisms provide alternative enzymic routes for the biosynthesis of phenylalanine and tyrosine (Fig. 1). The chorismate mutase: prephenate dehydratase (P-protein) catalyses the formation of prephenate via the

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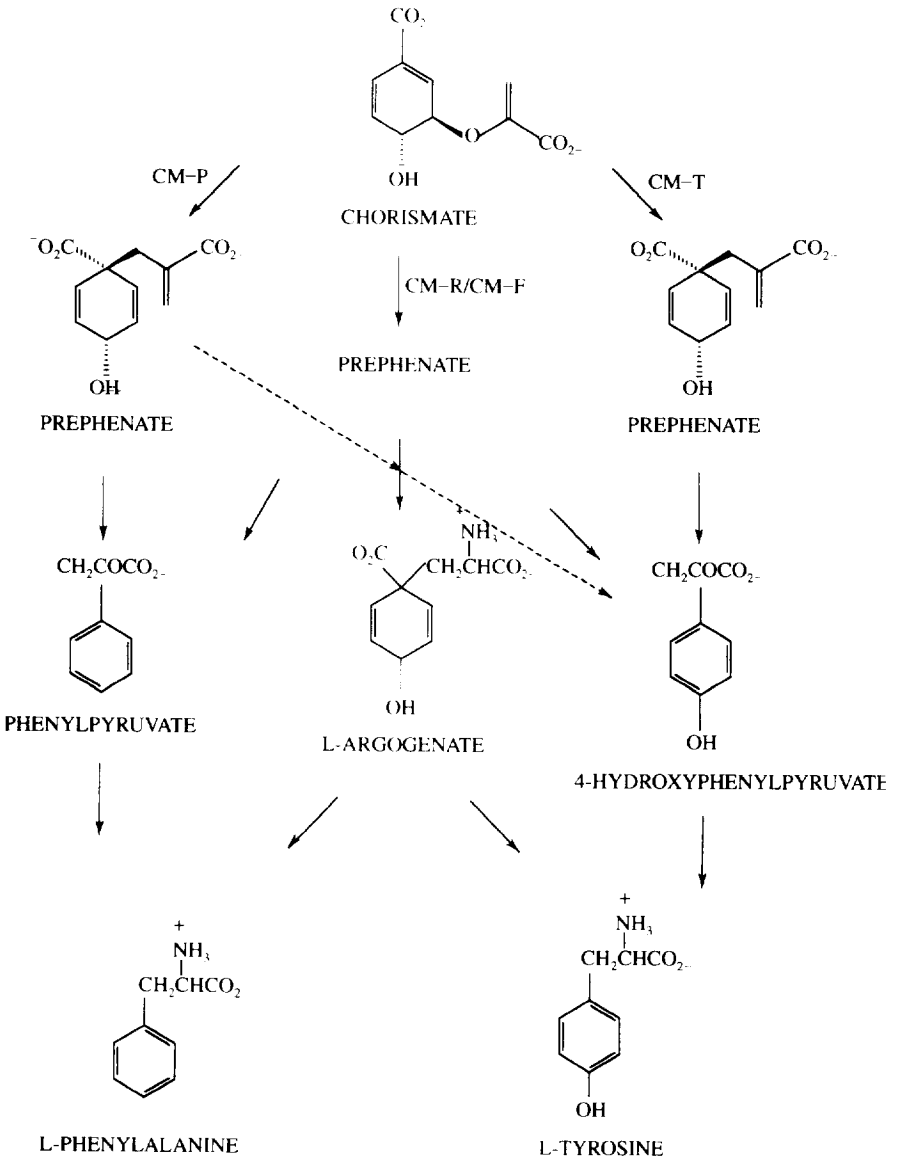

Fig. 1. Different arrangements of phenylalanine and tyrosine biosynthesis in microorganisms. (CM-DAHP synthase is not included.)

cm-p domain, and the prephenate is transformed into phenylpyruvate through the action of the prephenate dehydratase component. The phenylpyruvate is converted by a separate enzyme into L-phenylalanine, which inhibits the two activities of the P-protein. This bifunctional P-protein is present in two of the three major superfamilies, A, B and C, of gram-negative bacteria. Superfamily A includes, for example, *Alcaligenes eutropus*, and superfamily B includes enteric genera, *Oceanospirillum*, *Zanthomonas*, fluorescent pseudomonads and *Acinetobacter*) [8, 13–15].

Chorismate mutase: prephenate dehydrogenase (T-protein) is involved in the formation of prephenate, which is utilized preferentially for the biosynthesis of L-tyrosine. Prephenate is transformed into 4-hydroxyphenylpyruvate via the action of the cyclohexadienyl dehydrogenase component of the same bifunctional enzyme. The CM-T is inhibited by tyrosine and it has been detected only in the enteric bacteria *Aeromonas* and *Alteromonas* [13]. The CM-DAHP synthase is present in gram-positive bacteria *Bacillus* [16] and *Brevibacterium* (*Corynebacterium*) [17].

The monofunctional chorismate mutase, CM-F, catalyses the formation of prephenate, which is utilized for biosynthesis of L-tyrosine or L-phenylalanine, via either 4-hydroxyphenylpyruvate or L-arogenate, on the one hand, or via either phenylpyruvate or L-arogenate on the other. This enzyme lacks allosteric control and is

Table 1. Occurrence of chorismate mutase in microorganisms

Microorganism	Enzyme	M_r	Structure	Separation/purity	References
Candida maltosa	CM-F	63000		G-25/DEAE cellulose	94
Pichia guilliermondii	CM-F		—	DEAE cellulose	95
Saccharomyces cerevisiae	CM-R	—	Homodimer	Mono-Q/Mono-Q/Sephadex G-75	73
Bacillus subtilis ATCC 6051	CM-F	44000	Homotrimer	DEAE-cellulose/DEAE-cellulose/gel filtration/Mono-Q/gel filtration $(NH_4)_2SO_4$/ ethylamino sulphate/ethylamino Sepharose	19
*Enwinia herbicola**	CM-F	34000	Homodimer	DEAE cellulose/hydroxyapatite/gel filtration/ DEAE cellulose	37
Streptomyces aureofaciens	CM-F	63000		$(NH_4)_2SO_4$/Sephadex G-200/hydroxyapatite/Sephadex G-100/preparative disc electrophoresis/sucrose gradient electrophoresis	6 96
Arxula adeninivorans SBUG 724 *Brettanomyces anomalous* SBUG 289 *Candida albicans* SBUG 182 *Candida parapsolosis* SBUG 523 *Candida tropicalis* SBUG 590 *Candida utilis* SBUG 576 *Cryptococcus uniguttulatus* SBUG 305 *Debaryomyces hansenii* SBUG 528 *Hansenula bimundialis* SBUG 117 *Hansenula fabianii* SBUG 131 *Hansenula henricii* SBUG 155 *Hansenula polymorpha* SBUG 500 *Kluyveromyces marxianus* SBUG 702 *Rhodotorula aurantiaca* SBUG 244 *Rhodotorula glutinis* SBUG 63 *Saccharomycopsis capsularis* SBUG 241 *Schizosaccharomyces octosporus* SBUG 575 *Sporobolomyces salmonicolar* SBUG 549 *Trigonopsis variabilis* SBUG 703 *Yarrowia lipolytica* SBUG 130	CM		—	DEAE cellulose	25
Serpens flexibilis ATCC 29606	CM-P	–	—	DEAE cellulose	93
Azomonas insignis ATCC 29360 *Azotobacter paspali* ATCC 23833 *Azotobacter vinelandii* ATCC 17962	CM-P	—		DEAE cellulose	97

Continued overleaf

Table 1. *Continued*

Microorganism	Enzyme	M_r	Structure	Separation/purity	References
Neisseria gonorrhoeae ATCC 27630	CM-P			DEAE cellulose	98
Aerobacter aerogenes	CM-T	76000	Dimer	Protamine sulphate/$(NH_4)_2SO_4$/AMP-Sepharose/hydroxyapatite	79
Acinobacter calcoaceticus	CM-P	91000	Homodimer	Streptomicin sulphate $(NH_4)SO_4$ DE52 DE52 Phenylalanine Sepharose/Sephadex G-150/hydroxyapatite	8
	CM-F	23000		DEAE-cellulose/gel filtration	7
Escherichia coli	CM-P	40000	Homodimer	Streptomycin sulphate/phenyl-Sepharose/hydroxyapatite/Sepharosyl-phenylalanine	99 100
	CM-T	88000	Dimer	streptomycin sulfate/$(NH_4)_2SO_4$/Sepharose-AMP/DEAE cellulose/hydroxyapatite	101‡
Citrobacter freundii ATCC 29935			—		
Shigella dysenteriae ATCC 11456a			—		
Enterobacter aerogenes ATCC 13048		—	—		
Enterobacter agglomerans ATCC 29915 (aerogenic strain)		—	—		
Cedecae davisae ATCC 33431		—	—		
Kluyvera ascorbata ATCC 33433	CM-P	—		DEAE cellulose	18
Hafnia alvei ATCC 13337	CM-T	—			
Edwardsiella tarda ATCC 15947		—	—		
Yersinia enterocolitica ATCC 9610			—		
Proteus vulgaris ATCC 29905		—	—		
Providencia alcalifaciens ATCC 9886		—	—		
Morganella morganii ATCC 2583		—	—		
Proteus mirabilis ATCC 29906	CM-P	—	—		
Aeromonas hydrophila ATCC 14715	CM-T	—	—	DEAE cellulose	2
Alteromonas putrefaciens ATCC 8071		—	—		
Salmonella enteritidis ATCC 13076	CM-F	—	—		
Enterobacter cloaceae ATCC 13047	CM-P	—	—	DEAE cellulose	18
Enterobacter agglomerans ATCC 27155 (anaerogenic strain)	CM-T	—	—		
	CM-F	—	—		
Serratia marcescens ATCC 13880	CM-P	—	—	DAAE cellulose	2
	CM-T	—	—		
Serratia rubidaea ATCC 27614	CM-F	40000	Homodimer	DEAE cellulose/hydroxylapatite/DEAE cellulose/Bio-Gel P150	3
	CM-P	—	—	DEAE cellulose	18
	CM-T	—	—	DEAE cellulose	18

Erwinia herbicola ATCC 33243	—	—			
E. carotovora ATCC 15713	CM-F	—			
E. amylovora ATCC 15580	CM-P		DEAE cellulose/hydroxyapatite	2	
E. milletiae ATCC 33261	CM-T				
E. chrysanthemi ATCC 11663	CM-T	—			
E. tracheiphila ATCC 33245					
	CM-F				
Salmonella typhimurium ATCC 15277	CM-P		DEAE cellulose	2	
	CM-T				

* Expressed in *E. coli*.
† The enzyme can be a mixture of dimer, tetramer and probably octamer because it undergoes a concentration-dependent self-association [102].
‡ Other purification procedures have been reported: streptomycin sulphate, $(NH_4)_2SO_4$, Blue Dextran-Sepharose, DEAE-Sepharose [Hudson *et al.*, 1984], streptomycin sulphate $(NH_4)_2SO_4$, Blue A gel/Sepharose-AMP [Turnbull *et al.* 1990].
G25 and DEAE cellulose are both Sephadex products.

found in gram-negative bacteria (although it is not present in half of the genera of enteric bacteria) [18], in gram-positive *B. subtilis* [19] and *S. aureofaciens* [6] and in cyanobacteria. Organisms from gram-negative superfamily B possess either a monofunctional CM-F or the CM-T protein, but usually not both [20]. However, CM-F and CM-T co-exist in some enteric bacteria [3].

The monofunctional CM-R exhibits allosteric inhibition by L-phenylalanine and/or L-tyrosine and allosteric activation by tryptophan, and is characteristic of eukaryotic microorganisms such as *Saccharomyces cerevisiae* [21], *Euglena gracilis* [22], *Neurospora crassa* [23] and *Claviceps paspali* [24]. Chorismate mutase activity was investigated in several other yeast species (Table 1) where the detected enzymes were localized exclusively in the cytosol and were not classified as CM-F or CM-R, although they would fit the definition of CM-R. All the enzymes were activated by tryptophan, but the enzyme from *Brettanomyces anomalous*, *Candida albicans*, *Candida tropicalis*, *Cryptococcus uniguttulatus*, *Debaryomyces hansenii* and *Hansenula henricii* was not inhibited by either phenylalanine or tyrosine. The other enzymes that were also detected after separation using DEAE cellulose were inhibited by phenylalanine and/or tyrosine excepting *Yarrowia lipolytica*, which showed a small activation in the presence of phenylalanine and tyrosine (Table 1) [25].

Plants

In plants it is possible to find from one to three monofunctional chorismate mutase isoenzymes. (Table 2). For example, two CM isoenzymes have been obtained from pea (*Pisum sativum*) [26, 27]. The presence of only one CM isoenzyme was reported for potato (*Solanum tuberosum*) [28]. However, later, these researchers detected three isoenzymes [29]. It is possible that the absence of a second CM isoenzyme in those plants where only one CM enzyme has been reported may be due to instability of the second isoenzyme or to a lack of separation when the enzymes have been partially purified. Nevertheless, it is possible also that the presence of only one enzyme means only one active pathway for aromatic amino acids.

Where two isoenzymes have been detected, which is the case for most plants, one of them is activated by tryptophan and regulated by tyrosine or phenylalanine, and the other one is not regulated. When three isoenzymes are present the situation is more complex because the isoenzymes show different patterns of regulation. For example, in alfalfa, all the three forms are inhibited by *p*-coumarate; CM-1 and CM-3 are inhibited by tyrosine and phenylalanine and activated by tryptophan; CM-1 and CM-2 are inhibited by other secondary metabolites such as caffeate; and chlorogenate and CM-3 is activated by 3,4-dimethoxycinnamate and inhibited by ferulate [30].

Cell cultures developed from *Brassica juncea* yield some interesting results in that the undifferentiated callus showed the presence of two enzymes of chorismate mutase, as well the presence of two isoenzymes for

Table 2. Occurance of chorismate mutase in plants

Plant	Enzymes	M_r	Structure	Separation/purity	References
Brassica juncea (callus)*	CM-1			G-25/DEAE cellulose/$(NH_4)_2SO_4$	31
	CM-2				
Brassica rapa (fleshy roots)	CM			Crude	32
Spinacia oleracea (roots)	CM-1	59000		G-25/DEAE Sepharose	32
	CM-2	48000			
Papaver somniferum	CM-1	84000	Dimer	DEAE cellulose/Sephadex G200/	33
(seeds and seedlings)	CM-2	80000	Dimer	hydroxyapatite	
Sorghum bicolor (seedlings)	CM-1	56000		G-25/DEAE cellulose/ω-amino hexyl-agarose/ Red A gel/L-tryptophan agarose	34
	CM-2	48000		G-25/DEAE cellulose/Blue A gel/ ω-Aminohexyl agarose/Synchropak AX-310	
Medicago sativa (plant and seedlings)	CM-1	46000		Protamine sulphate/$(NH_4)_2SO_4$/Sephadex	30
	CM-2	58000		G-100/electrophoresis	
	CM-3	69000			
Pisum sativum (seedlings)	CM-1			DEAE cellulose	26
Vigna radiata (beans)	CM-1	50000		$(NH_4)_2SO_4$/hydroxyapatite/ DEAE cellulose/ Sephadex G-100/ultrafiltration	103
	CM-2	36000		$(NH_4)_2SO_4$/DEAE cellulose/ ultrafiltration/ Sephadex G-100	
Nicotiana silvestris	CM-1	52000		G-25/DEAE cellulose/	104, 105
(Leaf tissue and cell suspension)	CM-2	65000		hydroxyapatite/gel filtration	
Daucus carota (cell suspension)	CM			Crude extract	106
Solanum tuberosum	CM-1			Blue A/tryptophan agarose	28
(tubers and leaves)	CM-1	55000		G-25/DEAE cellulose	29
	CM-2	52000			107
Quercus pedunculate (leaves)	CM-1	45000		$(NH_4)_2SO_4$/Sephadex G-50/hydroxyapatite gel	108
	CM-2	—			
Ruta graveolens L	CM-1	56000		$(NH)_2SO_4$/DEAE Sephacel/Sephadex G-150	109
(Suspension cultures)	CM-2	45000			

*CM-1 is absent in root-forming callus.

DAHP synthase [31], whereas the root-forming callus only showed the presence of one enzyme for CM and one for DAHP synthase. These enzymes were also unregulated as the isoenzymes in normal *B. juncea* callus. An explanation may be that, in the phase of rapid cell division and proliferation of undifferentiated callus, the presence of both isoenzymes correlates with a higher requirement for amino acids. On the one hand, the inactivation of one of the isoenzymes might be attributed to a higher content of products of phenoloxidase reactions and other tanning products [32]. In *Papaver somniferum* seedlings the CM-2 activity decreased with age, and for five-day-old seedlings only CM-1 activity was detected [33].

Besides the information in Table 2, there has been an examination of the CM isoenzymes patterns in other plants [34]. Only one enzyme was found after protein separation on DEAE-cellulose in *Avena sativa*, *Brassica oleracea*, *Eschscholtzia california* and *Pennisetum typhoides*. The enzyme from these plants was always activated by tryptophan and inhibited by phenylalanine and tyrosine. *Amaranthus hypochondriacus*, *Hordeum vulgare*, *Hoya carnosa*, *Medicago sativa*, *Sorghum bicolor*, *Spinacia oleracea*, *Xerosicyos danguyi* and *Zea mays* contained two isoenzymes, the regulated CM-1 and the unregulated CM-2.

GENE ENZYME RELATIONSHIP AND FUNCTIONALITY

Microorganisms

Only few genes encoding chorismate mutase activity have been cloned and sequenced, and maybe this is because the chorismate mutases comprise a set of functionally related proteins that show little sequence similarity to each other. This absence of similarity stands in

contrast to other enzymes of the aromatic amino acid biosynthesis, especially to other chorismate-utilizing enzymes [19].

The genes *ARO7*, *aroH* and *aroQ* encode for the monofunctional chorismate mutases and *pheA* and *tyrA* encode the bifunctional enzymes having catalytic domains for CM-P and CM-T, respectively. The *ARO7* gene from *Saccharomyces cerevisiae*, which encodes CM-R, has been cloned and sequenced [35, 21], but no significant homology between the monofunctional yeast chorismate mutase and the corresponding domains of the two bifunctional *Escherichia coli* enzymes was found. In other yeast enzymes *ARO3*, *ARO4* and *TRP4*, gene products share significant homology with their *E. coli* counterparts.

The CM-F enzyme from *B. subtilis* is encoded by the gene *aroH*, which has been cloned and sequenced [19], but the derived amino acid sequence has no significant similarity to any sequence in the GenBank and EMBL databases. For example, the *aroH* gene was found to be slightly similar to a short region of the amino-terminal (*N*-terminal) portion of the two *E. coli* bifunctional enzymes [36], but no similarity was detected with the monofunctional enzyme of *S. cerevisiae* [21].

The gene *aroQ* for CM-F from *Erwinia herbicola* was also cloned and exhibited no obvious resemblance to the gene for the *B. subtilis* monofunctional chorismate mutase protein [37]. The *pheA* amino acid sequence, which encodes the bifunctional CM-P from *Pseudomonas stutzeri*, showed homology in the *N*-terminal portions with CM-P and CM-F from *E. coli*. From the group of three amino acid residues reported to be associated with the activities of the CM-P from *E. coli*, the cysteine residue 216 and threonine residue 278 have been conserved, while the cysteine residue 374 was substituted by serine [38]. The amino acid sequences of *tyrA* and *pheA* have significant similarities in their *N*-terminal portions only, with 22 of the first 56 residues being identical [39]. This homologous region reflects not only the chorismate mutase domain but also a common ancestry [40, 41].

Plants

The chorismate mutase cDNA from *Arabidopsis thaliana* is the only chorismate mutase cDNA isolated from a plant. The identity of the deduced amino acid sequence shows 41% homology with the CM sequence from *S. cerevisiae*, but the *N*-terminal portion shows no homology. Hardly any similarity was found to known sequences of bacterial chorismate mutases [42].

REACTION MECHANISM

The chorismate to prephenate rearrangement has been extensively studied mechanistically because it is a rare example of an enzyme-catalysed pericyclic reaction; however, there is still no consensus in the details of the mechanism of the enzymic process [43].

Several mechanisms have been proposed for the chorismate mutase-catalysed reaction utilizing the chorismate mutase enzyme from *E. coli*, *B. subtilis*, *Streptomyces aureofaciens* and *Aerobacte aerogenes*, and for the analogous thermal reaction based on isotope effects [44, 43], molecular orbital calculations [45–47], kinetic studies [46, 48], reactivities of substrate analogues [49–51] and transition state analogues [52–54]. The mechanism where C_3–O bond breakage precedes C_9–C_1 bond formation is supported by the data available (Fig. 2). The non-enzymic process is believed to proceed via a concerted, asynchronous reaction [55] in which bond breaking is far in advance of bond making at the transition state [44]. In contrast, for the mechanism of the enzyme-catalysed rearrangement it can be either through an asynchronous concerted rearrangement or through a dissociate mechanism involving the formation of discrete intermediates.

It has also been established that, for both enzymic and non-enzymic processes, the chorismate mutase catalysis proceeds via a chair-like transition state rather than a boat-like transition state (Fig. 2) [47, 56, 57]. Analysis by ^{1}H NMR in aqueous solution at 25° established that, while the pseudodiequatorial conformer of chorismate predominates, as would be expected, some 10–40% of the molecules exist in the pseudodiaxial conformer, that is, the species required for the 3,3-rearrangement [58].

One mechanism which has been proposed involves acid catalysed protonation of the vinyl ether oxygen with attack by an enzyme nucleophile at C-5 to give a transient intermediate. The enolpyruvate fragment attacks at the C-1 position by a possible *SN2'* route [59]. The existence of the nucleophile has not been established (Fig. 3).

It is important to mention that while the non-enzymic rearrangement is unusually fast in aqueous solution ($t_{1/2}$ = 15 hr for chorismate at 30°), chorismate mutase accelerates the process 2×10^6 fold (37°, pH 7.5) [46]. The structural features that are essential for enzyme catalysis have been established. Neither the 5,6-olefinic nor the 4-OH group is absolutely necessary, but the enzyme requires besides the allyl vinyl ether, the two carboxylate groups for active site binding and catalysis

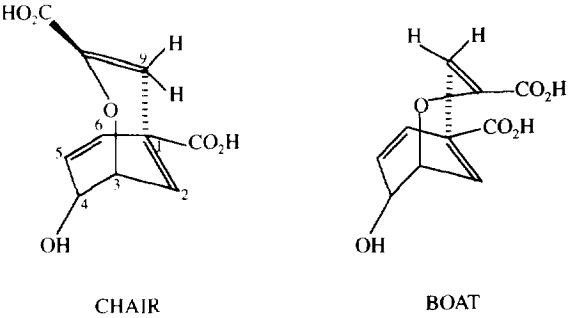

Fig. 2. Possible transition states in the chorismate mutase reaction.

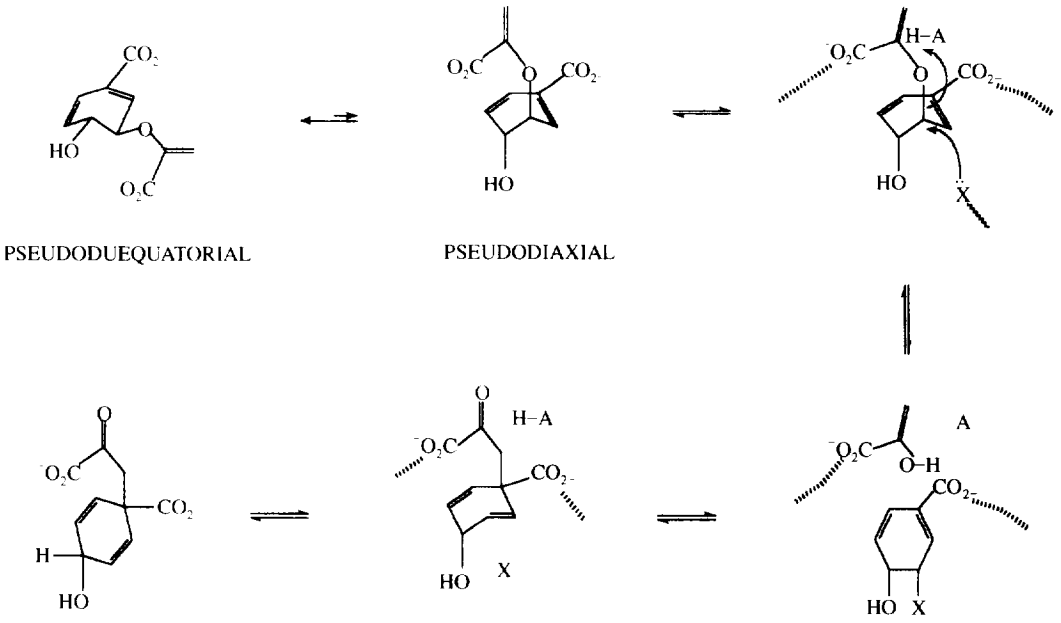

Fig. 3. Proposed mechanism for chorismate mutase catalysed 3,3-rearrangement of chorismate.

[43, 51, 60]. In summary, the structural requirements are indicated in the dotted box in Fig. 4.

Several inhibitors of chorismate mutase, mainly adamantane derivates and bicyclic diacids, have been synthesized [52–54, 61–63]. The oxabicyclic compound in Fig. 5, 3-*endo*-8-*exo*-8-hydroxy-2-oxabicyclo [3.3.1] non-6-ene-3,5-dicarboxylic acid, is the highest affinity inhibitor of chorismate mutase known [52] and it binds to the enzyme *ca.* 250 times more tightly than chorismate itself. This oxabicyclic compound was used to develop two antibodies, derived from mice, which were capable of catalysing the rearrangement of chorismate to prephenate. One antibody, 11F1-2E11, catalyses the reaction with a rate of acceleration of 10^4-fold over the uncatalysed reaction [64] and the other, 1F7, achieves 100-fold acceleration [65, 66]. Several experiments have been performed with these antibodies in order to elucidate how they function [64, 67–71].

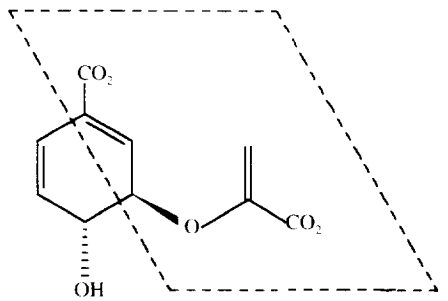

Fig. 4. Substrate recognition requirements for chorismate mutase.

Active sites

Several experiments have been performed to identify the active sites on the chorismate mutase enzyme. For example, in the yeast *S. cerevisiae* it is proposed that the tyrosine 234 residue (Tyr-234) is located at or near the allosteric activation site for tryptophan [72]. Also, for the same yeast, a single point mutation, in which threonine at position 226 has been replaced by isoleucine, generated a mutant that was not feedback-regulated by any of the aromatic amino acids and was permanently in the activated state [73]. Kinetic studies established that the site of allosteric activation is separate from the active site [73].

In the case of the bifunctional chorismate mutase enzymes, experiments to determine the active site have been performed, but it took some time to determine whether the two functionalities had a common active site. For example, for chorismate mutase: prephenate dehydrogenase (T-protein) from *E. coli* and *A. aerogenes*, chemical modification of sulphydryl groups of cysteine was associated with parallel loss of both enzyme activities [74, 75], suggesting that either the two activities were catalysed at a single site or that there was a close spatial relationship between the sites for the two functions [76–78]. The kinetic data obtained with CM-T from *A. aerogenes* were consistent with the two reactions occurring at a single site or at two separate sites with similar kinetic properties [79], and experiments with *E. coli* using substrate analogues indicated that the sites overlap [80]. The separability of T-protein functions remained in doubt until molecular genetic approaches established the genetic separability of mutase and dehydrogenase components in *E. coli* [39]. Also, the CM-T component of

T-protein from *E. herbicola* was removed by deletion of the 5′ terminus of the *tyrA* gene *in vitro* and a new monofunctional prephenate dehydrogenase was produced as a result [81]. Evidence in support of the molecular genetic approaches was obtained also from kinetic studies and experiments with substrate analogues [41]. It is noteworthy that a mutant enzyme from *E. coli*, lacking prephenate dehydrogenase but retaining chorismate mutase activity, was obtained some years before these findings. However, in this case the separability of the active sites still was not clear [82].

The bifunctional chorismate mutase: prephenate dehydratase has been shown by a variety of procedures to have for the enzyme activities separate sites [83], which include a sulphydryl group that was concluded to be essential for the prephenate dehydratase activity [84, 85]. The active sites were vunerable to differential inhibition and selective chemical activation [86, 87]. Since mutants lacking only chorismate mutase or dehydratase activity have been isolated [86, 88–90], it is clear that the two catalytic functions of the CM-P enzyme are spatially distinct. Mutations in the *pheA* gene of *E. coli* and *Erwinia herbicola*, which encode a protein that was insensitive to phenylalanine inhibition, have also been obtained [91, 92].

UTILITY OF BIOCHEMICAL-PATHWAY CHARACTERISTICS TO FINE-TUNE THE PHYLOGENETIC POSITION OF CERTAIN MICROORGANISMS

Phylogenetic trees may be constructed using modern nucleic acid sequencing techniques, and characteristics of aromatic amino acid pathways may be used as a basis to fine-tune the phylogenetic position of certain organisms. These criteria has been used for investigating the phylogenetic position of the enteric bacteria. The method suggests three major clusters. Enterocluster 1, which possesses a gene fusion *trpG–trpD* encoding anthranilatesynthase: anthranilate 5-phosphoribosylpyrophosphate phosphoribosyltransferase and includes the genera *Escherichia*, *Shigella*, *Citrobacter*, *Salmonella*, *Klebsiella* and *Enterobacter*. Enterocluster 2 lacks the *trpG–trpD* gene fusion, but contains the overflow pathway to L-phenylalanine (a pathway which utilizes a monofunctional chorismate mutase for the formation of phenylalanine, Fig. 1), and consists of species of *Serratia* and *Erwinia*. Enterocluster 3 lacks both the *trpG–trpD* gene fusion and the overflow pathway to L-phenylalanine and includes the genera *Cedecea*, *Kluyvera*, *Edwardsiella*, *Hafnia*, *Yersinia*, *Proteus*, *Providencia* and *Morganella*. This method of classification was compared with that of the partial trees based upon sequences, and the results were generally consistent [18].

In a different phylogenetic grouping, this type of fine-tuning has indicated that *Serpens flexibilis* clusters with *Pseudomonas stutzeri*, *P. mendocina*, *P. alcaligenes* and *P. pseudoalcaligenes* within the group Ia pseudomonad cluster, on the basis of the fact that this organism also lacks the overflow pathway to phenylalanine [93].

CONCLUSIONS

The material presented indicates the extent of our knowledge of chorismate mutase and its role in shikimate metabolism. This review has set out to investigate some of the apparent differences between plant and microorganisms in this group of enzymes. Most of the work on the structure, function and reaction mechanism of this enzyme has come from studies with microorganisms.

In microorganisms, CM is multifunctional, while, to date, this has not been found to be the case in plants. Microorganisms have five identifiable isoenzymes which are multifunctional, whereas in plants only two isoenzymes with an occasional third isoenzyme appear to exist and these are monofunctional of the two identifiable isoenzymes, CM-1 and CM-2, the former is activated by tryptophan and feedback inhibited by phenylalanine and tyrosine whereas CM-2 is insensitive to both these amino acids. While CM-1 is a plastid isoenzyme, CM-2 is found in the cytosol. However, the ratio of CM-1 to CM-2 in different tissues varies, suggesting organ specific regulation of expression of these enzymes [29]. Of current interest is the recent isolation, with structural determination, of monofunctional CM from *B. subtilis* [110]. The structural detail outlined in this paper provides a basis for the biochemical studies which indicated a pericyclic mechanism for the conversion of chorismate into prephenate.

In microorganisms the isoenzymes for CM have little sequence similarity to each other and hence, to date, relatively few genes have been cloned and sequenced for this enzyme. In plants, work with the *Arabidopsis thaliana* cDNA library has so far yielded limited results since isolated cDNA for CM had only 41% similarity with cDNA for CM from a *S. cerevisiae* mutant. All cDNA for CM activity isolated from *A. thaliana* had an *N*-terminal region similar to known plastid transit peptides [80].

It is of interest that there are recent reports on the occurrence of other enzymes of the shikimate pathway, as well as CM, which are found to be active in non-photosynthetic plant tissues. This non-photosynthetic pathway appears to make complete use of the shikimate pathway to synthesize aromatic amino acids independent of light, unlike the pathway in photosynthetic tissues [32]. The role of these two pathways for shikimate metabolism continues to intrigue research groups, and the requirement of plants for two such pathways and their true role in the regulation of the flow of carbon through this pathway, which is substantial, remains to be fully elucidated [111].

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