Phytochemistry, Vol. 27, No. 6, pp. 1571–1574, 1988.
Printed in Great Britain.

0031-9422/88 $3.00+0.00

REVIEW ARTICLE NUMBER 32

ORNITHINE BIOSYNTHESIS, AND ARGININE BIOSYNTHESIS AND DEGRADATION IN PLANT CELLS

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(*Received* 23 *October* 1987)

Key Word Index—Ornithine; arginine; enzymes; metabolic regulation; organelles; compartmentation.

Abstract—The biosynthesis of ornithine, and the biosynthesis and metabolism of arginine, are complex processes that have been shown to be strictly compartmented within plant cells. Recent work carried out on the compartmentation of the enzymes involved in these pathways, together with earlier work on the way in which their activity is regulated, has enabled us to derive a reasonably simple but coherent scheme for the operation of the interdependent pathways. In this scheme arginine is seen to play a key role as a feedback regulator of the activity of certain key enzymes. In order for the two pathways to function in an interdependent manner, certain key intermediates must be transported across organellar barriers. Knowledge of the nature and mode of operation of such transport systems unfortunately appears to be lacking.

INTRODUCTION

The amino acids ornithine and arginine are important to plants for a variety of reasons. Ornithine is a required intermediate for the biosynthesis of arginine, and also is utilized in the biosynthesis of other plant secondary products such as alkaloids and polyamines [1]. Arginine is of course an important constituent of proteins, but in addition is thought to serve under some circumstances as a store of nitrogen [2] and for the biosynthesis of secondary products such as polyamines [1].

A previous review article [3] had documented much of the evidence available by 1980, for the nature of the reactions involved in the biosynthesis and metabolism of ornithine and arginine. This article did not however discuss the compartmentation of intermediates and enzymes involved in these pathways.

The biosynthesis of ornithine from glutamate proceeds in all organisms via a pathway that involves *N*-acetylated intermediates. The particular type of pathway that operates in plants is shown in Fig. 1. This pathway is common to non-enteric bacteria, fungi, and green algae, as well as a variety of higher plants [3, 4]. In this pathway, the enzyme ornithine acetyltransferase [EC 2.3.1.35] plays a key role in transferring an acetyl group from acetylornithine to glutamate, thus conserving the acetyl group with the formation of acetyl glutamate and ornithine. The Enterobacteriaceae have a pathway that is similar to that shown in Fig. 1, but which differs in the possession of the enzyme acetylornithinase [EC 3.5.1.16] in place of ornithine acetyltransferase. Thus in the Enterobacteriaceae the acetyl group of acetylornithine is not conserved by being transferred to glutamate, but is released as acetate. Arginine appears to be synthesized from ornithine in all organisms via the reactions of the urea cycle, originally proposed by Krebs and Henseleit [5].

Arginine catabolism occurs in plants via the enzyme arginase [EC 3.5.3.1], to yield ornithine and urea. In ureotelic organisms (terrestrial mammals and adult amphibia), the ornithine produced during the hydrolysis of arginine by arginase is recycled in the production of more arginine, the urea produced is excreted. In plants, the ornithine produced via arginine degradation is thought to be separated in some way from that produced via synthesis from glutamate [3]. The degradative pool of ornithine is thought to be utilized in the production of glutamate and proline [3]. Urea produced by plants is hydrolysed to ammonia and carbon dioxide via urease [EC 3.5.1.5] and these products reutilized in biosynthesis [2].

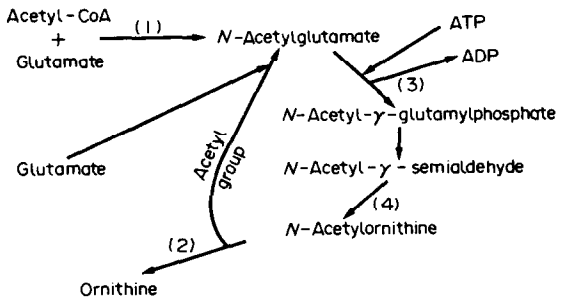

Fig. 1. Reactions involved in ornithine biosynthesis. Enzyme key: (1) acetylglutamate synthase, (2) ornithine acetyltransferase, (3) acetylglutamate kinase, (4) acetylornithine transaminase.

In order to grasp completely the significance of any biochemical pathway, we must appreciate first of all, the sequence of intermediates and enzymes involved. Secondly, we must understand the compartmentation of these enzymes and intermediates, and how they are regulated in a metabolic fashion. The first objective of this article is to review available evidence for the compartmentation and regulation of the activity of the enzymes involved in the biosynthesis of ornithine and arginine in plant cells. A second concern is to try and point out some gaps that remain to be filled before our knowledge of the exact way in which the pathways work is complete.

Ornithine biosynthesis, its regulation and compartmentation

Early experiments [4] which studied some of the enzymes involved in ornithine biosynthesis [Fig. 1], in various plant tissues suggested that acetylglutamate synthase [EC 2.3.1.1] is present in quantities far lower than those of ornithine acetyltransferase. These data, together with the order in which the sequence of reactions involved in ornithine biosynthesis is known to occur, lead to the emphasis placed in Fig. 1 on a cyclical series of reactions. In this scheme, acetylglutamate synthase is visualized as occupying an essentially anaplerotic role, serving to top up the levels of acetylglutamate as required. The main reaction involved in acetylglutamate formation appears to be that catalysed by ornithine acetyltransferase.

Recent experiments have been directed towards getting a clearer picture of the relative importance of the synthase and transferase enzymes to the overall pathway [6, 7]. These experiments utilized an active site directed analogue of *N*-acetylornithine, namely *N*-bromoacetylornithine [NBAO]. This analogue was shown to be a very effective inhibitor of ornithine acetyltransferase, but not to inhibit acetylglutamate synthase. Soybean cell suspension cultures were grown in a concentration of NBAO [6 mM] that permitted *ca* 40% of the normal growth found in untreated controls. Over a 144 hr period, levels of ornithine acetyltransferase activity, and the concentration of free ornithine were both reduced by greater than 40%, compared with control experiments. By contrast, levels of acetylglutamate synthase were unaffected. This result appeared to confirm the idea that ornithine acetyltransferase plays a major role in the transfer of acetyl groups to acetylglutamate, while acetylglutamate synthase plays an essentially anaplerotic role.

Other work [8, 9] using enzymes isolated from cotyledons of germinating peas, was concerned with the regulation of acetylglutamate synthase, and acetylglutamate kinase [EC 2.7.2.8] activities. Partially purified synthase was found to be inhibited by arginine [8]. The kinase enzyme was purified to homogeneity from pea cotyledons, and shown to be a complex oligomeric enzyme [9]. It can exist in dimeric or tetrameric forms, the shift from dimer to tetramer being initiated by increasing the concentration of either *N*-acetylglutamate or arginine. Arginine was found to strongly inhibit the enzyme activity, this inhibition being relieved by *N*-acetylglutamate which activated the enzyme. The activity of ornithine acetyltransferase was unaffected by arginine [8]. Thus acetylglutamate kinase appears to be a key regulatory point for metabolic control of the pathway. The inhibitory effects of arginine described above appear to be similar to its effect on the corresponding enzymes isolated from green algae [3]. For arginine biosynthesis, ornithine can be considered to be the key intermediate, playing the role of a carbon skeleton on which the complete arginine molecule is built up [5]. Thus it is of obvious strategic importance to plant cells to regulate the activity of acetyl glutamate kinase, an enzyme that is committed early to the overall pathway of ornithine biosynthesis. The feedback inhibitor used for this purpose is arginine, the end product of the two linked pathways.

The compartmentation of some of the key enzymes shown in Fig. 1 has recently been investigated using plant cell suspension cultures [10]. It has been shown that acetylglutamate synthase and acetylglutamate kinase are located in the cytoplasm of soybean cells. Ornithine acetyltransferase and acetylornithine aminotransferase [EC 2.6.1.11] are both located in the plastid fraction. It is noteworthy that the cytoplasmic location found in plant cells for acetylglutamate kinase, is one suggested to occur some time ago by Taylor and Stewart [11]. This suggestion was made on the basis of early reports of the feed-back inhibition by arginine, and the finding that argininosuccinate lyase is a cytoplasmic enzyme.

Arginine biosynthesis and metabolism, its regulation and compartmentation

Arginine is synthesized in plants from ornithine, via reactions which are common to the urea cycle of ureotelic animals [3, 5]. Certain key enzymes have been shown to be regulated. Thus carbamyl phosphate synthetase [EC 6.3.5.5] is subject to feed-back inhibition by nucleotides such as UMP, while this inhibition is relieved by ornithine [12]. Argininosuccinate synthetase [EC 6.3.4.5] was shown to be regulated by energy charge, with arginine acting as a modifier of this energy regulation [13].

The compartmentation of enzymes involved in arginine production was examined in soybean cells [14, 15] and in pea tissues [11]. Both groups of workers found that carbamyl phosphate synthetase and ornithine transcarbamylase [EC 2.1.3.3] occur inside plastids [proplastids and chloroplasts respectively]. The chloroplastic location of ornithine transcarbamylase was confirmed by De Ruiter [16]. Argininosuccinate synthetase and argininosuccinate lyase [EC 4.3.2.1] were shown to occur in the cytoplasm of plant cells [11, 14, 15]. In *Neurospora crassa* and in animals, carbamyl phosphate synthetase and ornithine transcarbamylase both occur inside the mitochondrion [17, 18]. Other enzymes involved in arginine biosynthesis are located in the cytoplasm. Similarly, enzymes involved in the synthesis of ornithine that are found inside plastids of plants, are found in the mitochondria of *Neurospora* [17, 18]. These results lend support to the earlier suggestion of Miflin and Lea [19], that enzymes of nitrite metabolism and many enzymes of amino acid metabolism, are located within the plastid fraction of plant cells.

In contrast to enzymes of arginine biosynthesis which increase significantly during seed formation in pea plants, enzymes of arginine metabolism such as arginase and urease are greatly increased during seed germination [16, 20, 21]. These data support the idea of arginine acting as a source of nitrogen during seed germination.

The subcellular distribution of arginase has been shown to be mitochondrial in broad bean [22], pea [11],

also jackbean and soybean [23]. Thus arginine degradation to ornithine occurring in the mitochondrion, appears to be distinctly separated from arginine synthesis occuring partly in plastids and partly in the cytoplasm. The mitochondrial and plastidial membranes would seem to correspond to the barrier postulated by Thompson [3] to separate ornithine produced by catabolism, and destined for utilization in glutamate (and to a lesser extent proline) production, from ornithine destined for arginine biosynthesis. In this context, it is noteworthy that Taylor and Stewart, located ornithine aminotransferase [EC 2.6.1.13] activity in the mitochondria of pea tissues. Ornithine aminotransferase is a key enzyme in glutamate production from arginine, and has been shown to increase in cotyledons of germinating seeds in response to increasing arginine levels, in a parallel fashion to arginase [3].

Compartmentation and metabolic regulation of steps involved in ornithine and arginine biosynthesis

The major steps involved in ornithine and arginine biosynthesis and their metabolic regulation are shown in Fig. 2. The key point for regulation of ornithine biosynthesis is at the synthesis of acetylglutamyl phosphate, where arginine plays a key role as an inhibitor, and acetylglutamate as an activator. Although acetylglutamate synthase and acetylglutamate kinase [Fig. 1.] are both inhibited by arginine, the kinase enzyme is far more sensitive to this inhibition [8]. Crude pea cotyledon extracts containing both enzymes showed only 56% inhibition of the synthase enzyme, but 100% inhibition of the kinase by 1 mM arginine. The physiological concentration of free arginine in germinating pea seeds was shown to be 1.15 μmol per seedling [8]. The enzyme assays utilized in this study contained enough enzyme protein to represent the physiological level of one seedling, plus 1.0 μmol of arginine. The greater sensitivity of the kinase enzyme may allow the synthase enzyme to function, while the kinase is inhibited. When the concentration of *N*-acetylglutamate reaches a significant level, the inhibition of the kinase enzyme by arginine is relieved, and synthesis of ornithine continues. This would ensure that in the cyclical pathway, a pool of *N*-acetylglutamate exists, which is maintained by the synthase enzyme acting as an anaplerotic enzyme feeding acetyl units from acetyl-CoA into the pathway as required.

In plants, carbamyl phosphate synthesized by the single carbamylphosphate synthetase of plastids, is utilized for ornithine and pyrimidine biosynthesis [3]. Thus carbamyl phosphate synthetase responds to inhibition by pyrimidines, [not shown in Fig. 2] this inhibition is relieved by ornithine. Arginine inhibits argininosuccinate synthetase, which is also regulated by energy charge [not shown in Fig. 2] [13]. Thus overall, ornithine biosynthesis is an integral part of arginine biosynthesis, and both pathways can be visualized as functioning as a cohesive whole; an attempt is made to portray this in Fig. 2.

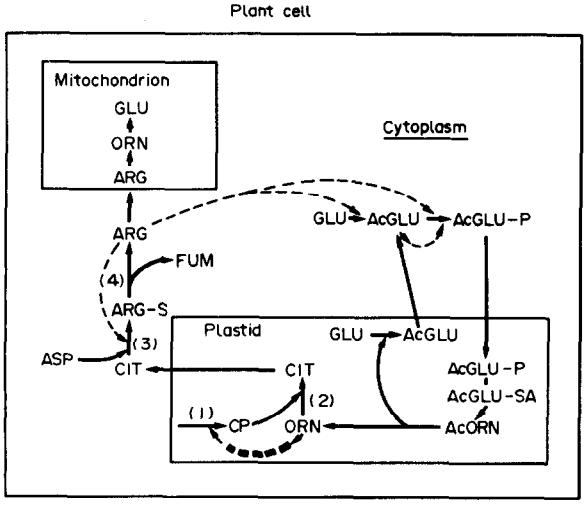

Fig. 2. A diagramatic summary of the compartmentation and metabolite regulation of ornithine and arginine biosynthesis discussed in the text. Solid lines indicate enzyme catalysed reaction. Dotted lines indicate inhibition or activation of enzyme reactions as shown below.

--------- = inhibition, ■■■■■ = activation. ARG = arginine, ARG-S = argininosuccinate, ASP = aspartate, AcGLU = acetylglutamate, AcGLU-P = acetylglutamyl-phosphate, AcGLU-SA = acetylglutamic-γ-semialdehyde, AcORN = acetylornithine, CIT = citrulline CP = carbamyl phosphate, FUM = fumarate, GLU = glutamate, ORN = ornithine

Enzyme key: (1) carbamyl phosphate synthetase, (2) ornithine transcarbamylase, (3) argininosuccinate synthetase, (4) argininosuccinate lyase.

Some areas needing further study

Transport of intermediates. Using mitochondria isolated from rat liver and heart, Gamble and Lehninger [24] studied the transport systems that exist for ornithine and citrulline. They showed that ornithine is transported into mitochondria by a specific electrogenic uniport carrier. Looking at Fig. 2, it would appear that such a system is not needed for mitochondria or plastids of plant cells. These workers also found that the efflux and influx of citrulline from mitochondria was non-energy dependent and may possibly utilize a neutral amino acid transporter. Conceivably such a system may also be utilized by plastids for citrulline transport. Figure 2 shows that plastids must also be capable of transporting acetylglutamyl phosphate and acetylglutamate. The authors are not aware of any work that has been carried out to characterize these transport systems, using organelles derived from plant cells.

Origin of pathway enzymes. Experiments utilizing cycloheximide and actinomycin-D, [7], showed that these compounds inhibited the biosynthesis of acetylglutamate synthase [a cytoplasmic enzyme] and ornithine acetyltransferase [a plastidic enzyme]. The result suggests that while both enzymes are synthesized in the cytoplasm, ornithine acetyltranferase may be synthesized as a precursor that is imported into plastids and modified to give a mature enzyme. Again, further more detailed studies with isolated organelles are needed in order to support this suggestion and also examine the origin of other plastidic enzymes mentioned here.

Biotechnological importance of pathway studies. Pseudomonas syringae pv. *phaseolicola* is the causative organism of the halo-blight disease of beans [*Phaseolus vul-*

garis L.]. The organism secretes a chlorosis inducing tripeptide (phaseolotoxin), that is cleaved in plant cells to produce *N*-[*N'*-sulphodiaminophosphinyl]-L-ornithine [PSorn], the major toxin present in diseased leaf tissues [25]. The compound PSorn is an analogue of the transition state intermediate of the ornithine transcarbamylase catalysed reaction, and is thus able to bind very readily to this enzyme, inactivating it in the process [25]. The most likely pathway for inactivation of ornithine transcarbamylase is for phaseolotoxin to be cleaved by a plant protease situated either in the cytoplasmic or vacuolar fractions of the cell. The PSorn must then be transported into chloroplasts by a transport system whose identity is as yet unknown. It is conceivable that if the nature of the transport system were known, a suitable defence mechanism could be devised to at least modify the effects of the toxin, if not eliminate them entirely. This example serves to point out the fact that more studies of a basic nature on biochemical pathways, are needed before benefits can accrue for applied use.

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