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REVIEW

FUNCTION OF SEROTONIN IN SEEDS OF WALNUTS

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Key Word Index—Spermatophyta; seeds; ammonia detoxification; enzyme localization; tryptophan biosynthesis; protein bodies; secondary plant product; monoamine; serotonin; review.

Abstract—In plants serotonin (5-hydroxytryptamine) may function as a hormone and as a protective agent against predation. A role for serotonin as a secondary plant product involved in ammonia detoxification in seeds of walnuts (*Juglans regia*) is now also proposed. Serotonin is formed from tryptophan synthesized via the constitutive enzymes of the shikimate pathway localized in the plastids, and is stored in protein bodies developed in the cotyledons during maturation. By the accumulation of serotonin in these protein bodies, the seeds, which have no vacuoles for storage or excretion of hydrophilic secondary plant products, are able to detoxify ammonia by the synthesis of serotonin.

INTRODUCTION

Serotonin is a well known biogenic monoamine in vertebrates. It is also synthesized in plants where it occurs in a wide range of families [1]. Other secondary plant products have evolved due to a selectional advantage [2] but unfortunately in the case of serotonin little is known about its function in plants.

In its possession of auxin-like activity [3] serotonin may be a plant growth hormone. However the accumulation of serotonin in the plant greatly exceeds the level needed for this hormonal function. Most of the accumulated serotonin is assumed to be localized in the vacuoles [4], which serve as storage or excretion compartments of various hydrophilic secondary products in plant cells [5].

Serotonin has also been reported to be one of the physiologically active compounds of the poison accumulated in the stinging nettle of *Urtica dioica* [6, 7] and trichomes which are found on the pods of *Mucuna pruriens* [8]. In this way serotonin may have a protective role, like tannins [9] or alkaloids [10] which are synthesized by plants to provide protection against predation. However since serotonin also occurs in fruits or seeds, e.g. pineapple [11], bananas [12], tomatoes [13], and walnuts [14], which are edible to man and digestible to vertebrates, this would suggest a function beside that protective role.

In walnut seeds serotonin is accumulated mainly during the period after abscission of the fruits [15]. During this period no exchange of precursors or end products with the whole plant occurs. Therefore these seeds are very suitable for the study of the biosynthesis, regulation, and function of a compound like serotonin.

During maturation a so-called trophophase–idiophase development takes place in the seeds, which is characteristic of many microbial cultures [16–20] and which has also been reported for higher plants with respect to the formation and ripening of pollen grains [21–24].

In walnut seeds the phase of cell division and growth [25, 26] is followed by a phase of differentiation, when the cotyledons become storage organs. The cells of the cotyledons lose their vacuoles and protein bodies along with lipid bodies are developed [27]. At this time the synthesis of serotonin begins, an event which can be followed in the isolated cotyledons. Also to be noted is that the synthesis of serotonin is stimulated by exogenous tryptophan, the precursor of serotonin [15]. Changes in the content of lipid, protein and amino acids after abscission suggest that proteolysis takes place during this period and that the synthesis and accumulation of serotonin serves for the detoxification of ammonia derived from deamination of amino acids. The conditions for this special form of metabolic excretion are realized in walnut seeds.

ENZYMES OF TRYPTOPHAN AND SEROTONIN METABOLISM

Cell free extracts from walnut seeds contain levels of glutamine synthetase with $K_m(NH_4^+) = 6.8 \times 10^{-5}$ M and K_m(glutamate) $= 2.7 \times 10^{-3}$ M [28] comparable with the K_m of the glutamine synthetase from peas [29, 30]. Walnut seeds contain glutamate at *ca* 6 mM [15] and therefore they are able to assimilate low concentrations of ammonia by the synthesis of glutamine.

Normally glutamine itself acts as a sink for assimilated ammonia [31] transported to the vacuoles to prevent inhibition of glutamine synthesis by the end product. In plant cells like those of walnut seeds lacking vacuoles for storage of glutamine, the equilibrium of the glutamine synthetase reaction results in a concomitant increase of glutamine and glutamate, as well as ammonia. Walnut seeds therefore appear to be unable to accumulate large amounts of glutamine. Thus the concentration of glutamine is five times lower than that observed in the leaves [32]. It is therefore postulated that serotonin is synthesized as a second compound for ammonia detoxification. In this way glutamine is first utilized in the anthranilate synthetase reaction with the formation of tryptophan by the five constitutive enzymes of the shikimate pathway (anthranilate synthetase to tryptophan synthetase) already available in young seeds [28, 33]. Tryptophan is then transformed to serotonin [15]. One of the two enzymes needed for this transformation is an aromatic amino acid decarboxylase which shows activity first in the later stage of seed maturation and is seen therefore to be an adaptive enzyme [34]. The substrate specifity of this enzyme, which is similar to that of the tryptophan decarboxylase from tomato shoots [35], and the stimulation of serotonin accumulation by exogenous tryptamine [Große, W., unpublished], indicate the role of tryptamine as an intermediate. These results show similarities to the accumulation of bufotenine in seeds of *Piptadenia peregrina* [36].

REGULATION OF SEROTONIN SYNTHESIS

In contrast to the enzymes of the shikimate pathway, aromatic amino acid decarboxylase is an adaptive enzyme extractable from walnut seeds at the time when serotonin becomes detectable [34] and the adult cotyledons take on the function of storage organs [27]. These results exclude the accumulation of serotonin by a blocked tryptophan dissimilation pathway, but indicate the formation of a new biosynthetic pathway for serotonin synthesis in maturing seeds. It is of interest that isopycnic centrifugation on RbCl density gradients of this enzyme extracted from H_2O and D_2O treated cotyledons respectively demonstrates *de novo* synthesis of the decarboxylase [37].

COMPARTMENTATION

The use of membranes in the spatial organization of a biosynthetic pathway to separate enzymes from inhibitory or toxic end products is a common principle in the metabolism of secondary plant products [5]. Metabolism of serotonin is a good example of the necessity for the compartmentation of the biosynthesis of monoamines.

Feedback inhibition of the anthranilate synthetase

From tobacco and carrot cell cultures [38], as well as walnut seeds *in vitro* [28] we know that tryptophan affects anthranilate synthetase by feedback inhibition. Plant cells seem to be unable to accumulate tryptophan by compartmentation except in the case of the excretion into the laticifers of *Euphorbia polychroma* [39]. Therefore tryptophan inhibits its further synthesis and is useless for ammonia detoxification and metabolic excretion. On the other hand 5-hydroxytryptophan, tryptamine, and serotonin are more useful in this respect, reaching the same strength of inhibition of the anthranilate synthetase in concentrations respectively 10 [28], 250 [Große, W., unpublished] and 1000 times [28] higher than that reached by tryptophan.

Localization of the biosynthetic enzymes

At a high concentration serotonin becomes inhibitory for anthranilate synthetase [28]. In order to detoxify large quantities of ammonia the tryptophan synthesizing enzymes and the serotonin must be spatially separated in different compartments. As shown by density gradient centrifugation the five enzymes, anthranilate synthetase to tryptophan synthetase, concerned with the shikimate pathway are localized in the proplastids of walnut seeds [28]. With respect to the localization of these enzymes in etioplasts from etiolated pea seedlings [40, 41] plastids are also seen to be able to synthesize tryptophan.

The localization of shikimate dehydrogenase in plastids of roots and shoots of pea seedlings [42] along with the ^{14}C incorporation from $^{14}CO_2$, [1-^{14}C]phosphoenolpyruvate, or [1,6-^{14}C]shikimate into aromatic amino acids following incubation of isolated intact spinach chloroplasts in the light [43–45], demonstrate that the complete shikimate pathway is operating in the plastids. Glutamine synthetase, like that found in pea roots [46], may also be localized partially in the proplastids of walnut seeds. Therefore that part of ammonia detoxification, leading from ammonia assimilation to tryptophan biosynthesis, may take place in the plastids.

Localization of serotonin accumulation

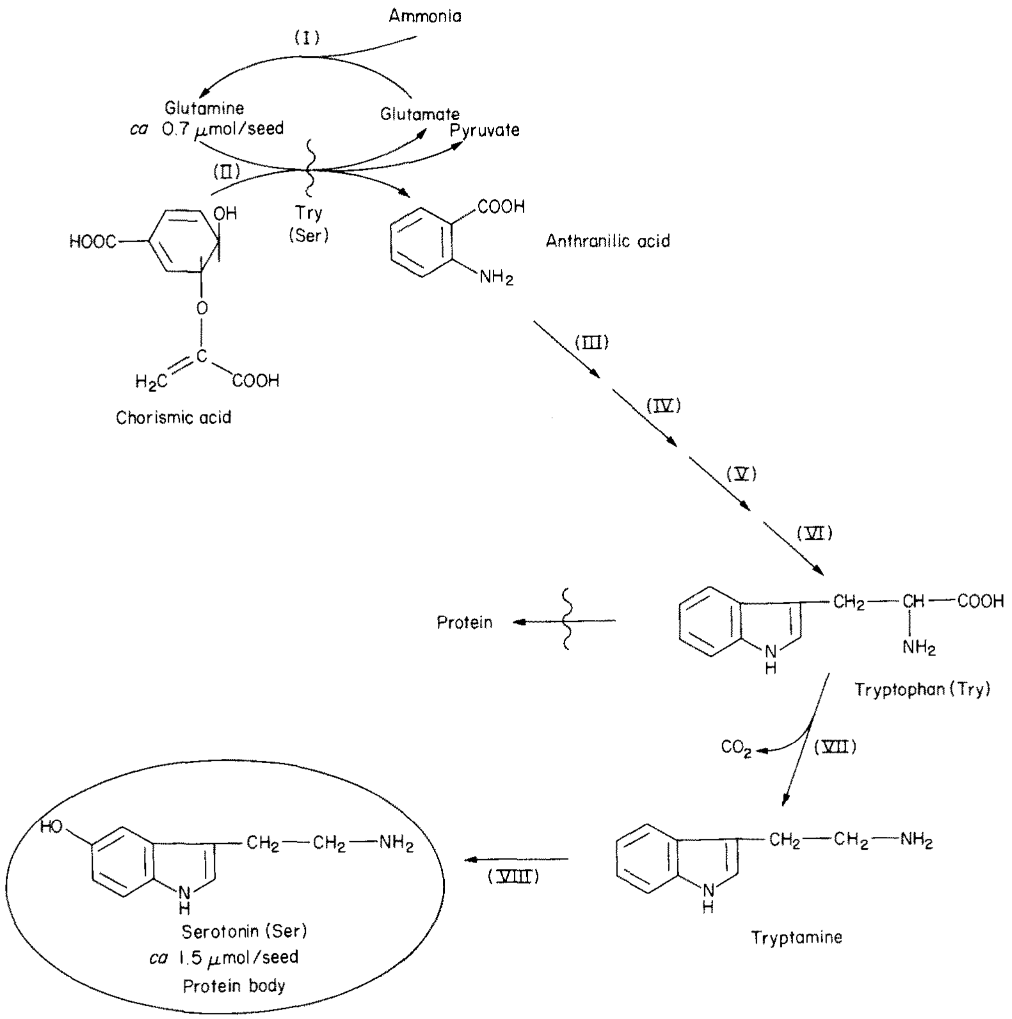
Due to their loss during maturation of the seeds, vacuoles are not available for storage of hydrophilic serotonin, the end product of the ammonia detoxification reaction in walnut seeds [27]. Histochemical analysis by the Falck–Hillarp method [47, 48] and non-aqueous density gradient centrifugation demonstrate the localization of serotonin in protein bodies [27]. In this way serotonin is separated from the anthranilate synthetase reaction, the reaction that controls flux through the biosynthetic pathway leading to tryptophan. On a higher level, serotonin did not inhibit its further synthesis and could accumulate as an ammonia detoxification product.

CONCLUSIONS

In walnut seeds the accumulation of serotonin serves for ammonia detoxification during maturation and the abscission of the fruits from the tree.

As shown by the accompanying scheme, ammonia is assimilated by glutamine synthetase (I). After the loss of vacuoles the seeds are unable to accumulate glutamine. Therefore the glutamine is utilized for the synthesis of tryptophan by the constitutive enzymes of the shikimate pathway: anthranilate synthetase (II), anthranilate phosphoribosyltransferase (III), *N*-(5′-phosphoribosyl) anthranilate isomerase (IV), indole 3-glycerol-phosphate synthase (V), and tryptophan synthetase (VI), all of which are localized in the plastids. In order to avoid serious feedback inhibition of the anthranilate synthetase reaction, tryptophan is transformed to the less inhibitory serotonin. The adaptive aromatic amino acid decarboxylase (VII), in company with a hydroxylase (VIII) needed for the formation of serotonin, is synthesized *de novo* at the onset of serotonin synthesis. Finally serotonin is withdrawn from the reaction by its deposition in the protein bodies.

It therefore appears that those seeds which have no



(I) Glutamine synthetase (EC 6.3.1.2)
(II) Anthranilate synthetase (EC 4.1.3.27)
(III) Anthranilate phosphoribosyltransferase (EC 2.4.2.18)
(IV) *N*-(5'-phosphoribosyl) anthranilate isomerase (see EC 4.1.1.48)
(V) Indole 3-glycerol-phosphate synthase (EC 4.1.1.48)
(VI) Tryptophan synthetase (EC 4.2.1.20)
(VII) Aromatic amino acid decarboxylase (EC 4.1.1 26/27)
(VIII) (Hydroxylase)

vacuoles for storage of hydrophilic secondary plant products, become able to accumulate serotonin as an ammonia-detoxification product.

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REFERENCES

1. Smith, T. A. (1977) *Phytochemistry* **16**, 171.
2. Bell, E. A. (1980) in *Encyclopedia of Plant Physiology* (Pirson, A. and Zimmermann, M. H. eds.), New Series, Vol. 8, p. 11. Springer, Berlin.
3. Niaussat, P.,Laborit, H., Dubois, C. and Niaussat, M. (1958) *C. R. Seances Soc. Biol. Paris* **152**, 945.
4. Schraudolf, H. (1969) *Naturwissenschaften* **9**, 462.
5. Luckner, M. (1980) in *Encyclopedia of Plant Physiology* (Pirson, A. and Zimmermann, M. H. eds.), New Series, Vol. 8, p. 23. Springer, Berlin.
6. Collier, H. O. I. and Chesher, G. B. (1956) *J. Pharmacol.* **11**, 186.
7. Schildknecht, H. (1977) in *Natural Products and the Protection of Plants.* Scripta Varia. (Marini-Bettolo, G. B., Ed.), Vol. 41, p. 59. Pontifical Academy of Science, Vatican City.
8. Bowden, K., Brown, B. G. and Batty, J. E. (1954) *Nature (London)* **174**, 925.
9. Feeny, P. P. (1970) *Ecology* **51**, 565.
10. Sturchow, B. and Low, I. (1961) *Entomol. Exp. Appl.* **4**, 133.

11. Bruce, D. W. (1960) *Nature (London)* **188**, 147.
12. Waalkes, T. P., Sjoerdsma, A., Creveling, C. R., Weissbach, H. and Udenfriend, S. (1958) *Science* **127**, 648.
13. West, G. B. (1959) *J. Pharm. Pharmacol.* **11**, 319.
14. Kirberger, E. and Braun, L. (1961) *Biochim Biophys. Acta* **49**, 391.
15. Bergmann, L., Große, W. and Ruppel, H. G. (1970) *Planta* **94**, 47.
16. Bu'lock, J. D. (1975) *Dev. Ind. Microbiol.* **16**, 11.
17. Bu'lock, J. D., Hamilton, D., Hulme, M. A., Powell, A. J., Smalley, H. M., Shepherd, D. and Smith, G. N. (1965) *Can. J. Microbiol.* **11**, 765.
18. Nover, L. and Luckner, M. (1974) *Biochem. Physiol. Pflanz.* **166**, 293.
19. Luckner, M. (1977) in *Cell Differentiation in Microorganisms, Plants and Animals* (Nover, L. and Mothes, K. eds), p. 538. VEB Fischer & Elsevier, Jena and Amsterdam.
20. Luckner, M., Nover, L. and Böhm, H. (1977) *Secondary Metabolism and Cell Differentiation.* Springer, Berlin.
21. Shaw, G. (1971) in *Sporopollenin* (Brooks, J., Grant, P. R., Muir, M., Va Gijzei, P. and Shaw, G., eds.), p. 305. Academic Press, London.
22. Wiermann, R. (1973) *Planta* **110**, 353.
23. Quast, L. and Wiermann, R. (1973) *Experientia* **29**, 1165.
24. Sütfeld, R. and Wiermann, R. (1974) *Z. Pflanzenphysiol.* **72**, 163.
25. Nast, C. G. (1935) *Hilgardia* **9**, 345.
26. Nast, C. G. (1941) *Lilloa* **6**, 163.
27. Große, W. and Schmidt, U. (1980) *Ber. Dtsch. Bot. Ges.* **93**, 575.
28. Große, W. (1977) *Z. Pflanzenphysiol.* **83**, 249.
29. Varner, J. E. (1960) *Arch. Biochem. Biophys.* **90**, 7.
30. Pamiljans, V., Krishnaswamy, P. R., Dumville, G. and Meister, A. (1962) *Biochemistry* **1**, 153.
31. Givan, C. V. (1979) *Phytochemistry* **18**, 375.
32. Vickery, H. B., Pucher, G. W. and Clark, H. F. (1936) *Plant Physiol.* **11**, 413.
33. Große, W. and Witzsche, H. (1973) *Planta* **111**, 65.
34. Große, W. and Klapheck, S. (1979) *Z. Pflanzenphysiol.* **93**, 359.
35. Gibson, R. A., Barrett, G. and Wightman, F. (1972) *J. Exp. Botany* **23**, 775.
36. Fellows, L. E. and Bell, E. A. (1971) *Phytochemistry* **10**, 2083.
37. Klapheck, S. and Große, W. (1980) *Z. Pflanzenphysiol.* **100**, 325.
38. Widholm, J. M. (1972) *Biochim. Biophys. Acta* **261**, 44.
39. Mothes, K. and Meissner, L. (1964) *Forsch. Fortsch.* **38**, 328.
40. Große, W. and Eickhoff, F. (1974) *Planta* **118**, 25.
41. Große, W. (1976) *Z. Pflanzenphysiol.* **80**, 463.
42. Feierabend, J. and Brassel, D. (1977) *Z. Pflanzenphysiol.* **82**, 334.
43. Bickel, H. and Schultz, G. (1979) *Phytochemistry* **18**, 498.
44. Buchholz, B. and Schultz, G. (1980) *Z. Pflanzenphysiol.* **100**, 209.
45. Bickel, H., Palme, L. and Schultz, G. (1978) *Phytochemistry* **17**, 119.
46. Miflin, B. J. (1972) *Biochim. Biophys. Acta* **261**, 44.
47. Falck, B. (1962) *Acta Physiol. Scand.* **197**, 1.
48. Falck, B., Hillarp, N.-A., Thieme, G. and Torp, A. (1962) *J. Histochem. Cytochem.* **10**, 348.