Phytochemistry, 1979, Vol. 18, pp. 498–499. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0301-0498 $02.00/0

SHIKIMATE PATHWAY REGULATION IN SUSPENSIONS OF INTACT SPINACH CHLOROPLASTS

HORST BICKEL* and GERNOT SCHULTZ†

*Botanisches Institut and †Institut für Tierernährung, Arbeitsgruppe für Phytochemie und Futtermittelkunde, Tierärztliche Hochschule, D 3000 Hannover 1, West Germany

(*Revised received* 26 *September* 1978)

Key Word Index—*Spinacia oleracea*; Chenopodiaceae; regulation of biosynthesis; shikimate pathway; phenylalanine:tyrosine; tryptophan; plastoquinone; α-tocopherol.

INTRODUCTION

In microorganisms, regulation of the shikimate pathway by a feedback mechanism has been elucidated (see e.g. [1–3]; for reviews see [4, 5]), but little is known about this mechanism in higher plants [6–10]. To study the regulation in higher plants suspensions of intact spinach chloroplasts isolated according to [11] were illuminated with $^{14}CO_2$ or shikimate-[1,6-^{14}C] as substrates in the presence of phenylalanine, tyrosine and tryptophan, respectively. The chloroplasts used in this study were not purified [12, 13]. The shikimate pathway takes place, at least partially, in chloroplasts [9, 14, 15].

RESULTS AND DISCUSSION

The incorporation of ^{14}C from $^{14}CO_2$ (via CO_2-fixation) into aromatic amino acids and prenylquinones in the presence of phenylalanine, tyrosine or tryptophan (each 5 mM) indicates that the shikimate pathway in spinach is subject to feedback control by the end products (Table 1). Phenylalanine and tyrosine exert feedback control over their own rates of synthesis, whereas tryptophan controls the rate of synthesis of all 3 aromatic amino acids. Based on the known mechanism of the shikimate pathway, this indicates an attack on a step before the synthesis of chorismate. The synthesis of prenylquinones, both plastoquinone (PQ) and α-tocophenol (αT), is regulated largely by the concentration of tyrosine ([14, 15]; see also [16]).

To determine the point of attack of tryptophan more exactly, shikimate-[1,6-^{14}C] was fed as a more direct precursor. As can be seen in Table 2, the synthesis of aromatic amino acids is strongly inhibited by adding tryptophan. This indicates that tryptophan attacks a step between the synthesis of shikimate and chorismate. The addition of phenylalanine and tyrosine gave the same results as in the $^{14}CO_2$ experiment.

The present results demonstrate that the feedback regulation of the shikimate pathway in higher plants differs from that in microorganisms. In microorganisms the regulation takes place according to species either by end-product inhibition of 2-keto-3-deoxy-D-arabino-heptonic acid-7-phosphate (KDHAP) synthetase isoenzymes by the corresponding aromatic amino acid [1, 2] or by inhibition by chorismate and prephenate [3]. Furthermore, amino acids can control their own rate of synthesis by feedback inhibition [5].

In spinach, tryptophan inhibits the synthesis of all 3 aromatic amino acids. This was not only verified in the case of $^{14}CO_2$ incorporation but also for shikimate-[1,6-^{14}C] as substrate. This indicates that the point of inhibition is between shikimate and chorismate synthesis and not at the step of KDHAP formation. In cauliflower the KDHAP synthetase behaves similarly [6]; the enzyme is influenced neither by aromatic amino acids nor by chorismate and prephenate. However, there may be differences between plant species. In mung bean (*Phaseolus aureus*) the activity of the CM-1 form [7] but not the CM-2 form [8] of the chorismate mutase is enhanced by tryptophan but it is decreased by phenylalanine and tyrosine. From the results in spinach, a scheme for the feedback control of the shikimate pathway is proposed (Fig. 1). In the case of prenyl-

Table 1. ^{14}C-Incorporation from $^{14}CO_2$ in the light into aromatic amino acids and prenylquinones of chloroplast suspensions in the presence of phenylalanine, tyrosine and tryptophan, respectively

	Control	+5 mM Phenylalanine	+5 mM Tyrosine	+5 mM Tryptophan
	dpm/mg chlorophyll (in parentheses: % of control)			
Water phase*	82100000	75800000	82900000	70300000
Petrol phase*	2710000	3110000	3110000	3070000
Alanine	5500	14000	6200	8900
		(254)	(112)	(62)
Phenylalanine	9300	7600	15200	1700
		(82)	(163)	(18)
Tyrosine	1600	5800	450	550
		(386)	(25)	(37)
Tryptophan	5800	32000	12300	720
		(545)	(212)	(12)
Plastoquinone	600	970	1300	330
		(162)	(217)	(55)
α-Tocopherol	850	1900	1400	600
		(220)	(160)	(70)

Each expt; vol. 0.7 ml; 1.1 mg chlorophyll; 11 μmol $NaH^{14}CO_3$ (= 50 μCi); medium C according to [11] as modified in [18]. Temp = 20 ± 2°; light intensity $2 \cdot 10^6$ erg/cm^2/sec. Time = 30 min.

* Compounds in water phase and petrol (bp 60–80°) phase, respectively.

Table 2. ^{14}C-Incorporation from shikimate-[1,6-^{14}C] in the light into aromatic amino acids of chloroplast suspensions in the presence of phenylalanine, tyrosine and tryptophan, respectively

	Control	+5 mM Phenylalanine	+5 mM Tyrosine	+5mM Tryptophan
	dpm/mg chlorophyll (in parentheses: % of control)			
Water phase	18300000	14900000	18100000	14900000
Petrol phase	10000	120000	110000	70000
Phenylalanine	13600	726	20700	923
		(5)	(152)	(7)
Tyrosine	1490	2190	1250	154
		(147)	(84)	(10)
Tryptophan	1820	2190	3780	697
		(120)	(208)	(38)

Each expt: vol. 0.7 ml; 0.7 mg chlorophyll; 10 μmol $NaHCO_3$; 8 μCi shikimate-[1,6-^{14}C] (sp. act. 12.5 μCi/μmol); for further details see Table 1.

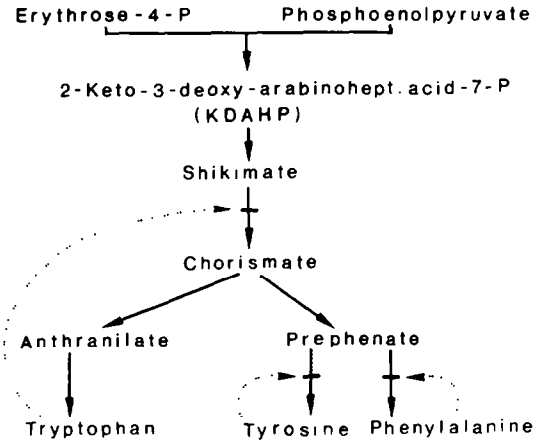

Fig. 1. Feedback regulation of the shikimate pathway by phenylalanine, tyrosine and tryptophan in suspensions of intact spinach chloroplasts.

quinone synthesis it may be suggested that it is regulated by supply of tyrosine, as it is for tryptophan in the formation of IAA [17].

EXPERIMENTAL

Shikimate-[1,6-^{14}C] was obtained from CIS, Gif-sur-Yvette, France.

Chloroplasts were isolated from spinach according to ref. [11] modified as described in ref. [18], and illuminated by the procedure of ref. [19].

Amino acids were isolated and determined as the dansyl derivatives [19].

Prenylquinones were isolated and determined as described in ref. [20], except that tocopherol was not oxidized.

Acknowledgements—Financial support by the Deutsche Forschungsgemeinschaft is thankfully acknowledged.

REFERENCES

1. Brown, K. D. and Doy, C. H. (1963) *Biochim. Biophys. Acta* **77**, 170.
2. Wallace, B. J. and Pittard, J. (1967) *J. Bacteriol.* **93**, 237; **94**, 1279.
3. Jensen, R. A. and Nester, E. W. (1965) *J. Mol. Biol.* **12**, 468.
4. Lingens, F. (1968) *Angew. Chem.* **80**, 384.
5. Haslam, E. (1974) *The Shikimate Pathway*. Butterworths, London.
6. Huisman, O. C. and Kosuge, T. (1974) *J. Biol. Chem.* **249**, 6842.
7. Gilchrist, D. G. and Kosuge, T. (1974) *Arch. Biochem. Biophys.* **164**, 95.
8. Gilchrist, D. G. and Kosuge, T. (1975) *Arch. Biochem. Biophys.* **171**, 36.
9. Feierabend, J. and Brassel, D. (1977) *Z. Pflanzenphysiol.* **82**, 334.
10. Rothe, G. (1973) *Biochem. Physiol. Pflanz.* **164**, 475.
11. Jensen, R. G. and Bassham, J. A. (1966) *Proc. Natl. Acad. Sci. U.S.A.* **56**, 1095.
12. Larsson, C., Collin, C. and Albertsson, P. Å. (1971) *Biochim. Biophys. Acta* **245**, 425.
13. Larsson, C., Andersson, B. and Roos, G. (1977) *Plant Sci. Letters* **8**, 291.
14. Schultz, G. and Bickel, H. (1977) *Proc. 5th Hungarian Bioflavonoid Symp.* Mátrafüred 1977 (Farkas *et al.*, eds.) pp. 271–284. Akadémiai Kiadó, Budapest.
15. Bickel, H. and Schultz, G. (1976) *Phytochemistry* **15**, 1253.
16. Goodwin, T. W. (1965) in *Biosynthetic Pathways in Higher Plants* (Pridham, J. B. and Swain, T., eds.) pp. 57–71. Academic Press, London.
17. Belser, W. L., Murphy, J. B., Delmer, D. P. and Mills, S. E. (1971) *Biochim. Biophys. Acta* **237**, 1.
18. Bickel, H. (1978) Thesis, Technical University, Hannover.
19. Bickel, H., Palme, L. and Schultz, G. (1978) *Phytochemistry* **17**, 119.
20. Schultz, G., Huchzermeyer, Y., Reupke, B. and Bickel, H. (1976) *Phytochemistry* **15**, 1383.