Phytochemistry, 1975, Vol. 14, pp. 2435–2436. Pergamon Press. Printed in England.

BEHAVIOUR OF PHENYLALANINE AMMONIA-LYASE IN CARROT CELLS IN SUSPENSION CULTURES

N. SUGANO, T. TANAKA, E. YAMAMOTO and A. NISHI

Faculty of Pharmaceutical Sciences, University of Toyama, Gofuku 3190, Toyama, Japan

(*Revised received* 6 *June* 1975)

Key Word Index—*Daucus carota*; Umbelliferae; carrot; suspension culture; biosynthesis; phenylalanine ammonia-lyase; hydroxycinnamic acids.

Abstract—The high PAL activity in carrot cells in suspension culture was found at the linear and early stationary phases, with concomitant increases in phenylalanine and total amino acids. The biosynthesis of phenolic acids and liguin was linked to PAL activity and the phenylalanine pool from the late logarithmic to the linear phases. No close correlation was, however, observed at the early logarithmic and late stationary phases.

In the previous paper [1], we reported that the biosynthetic activity of phenolic acids in carrot cells in suspension culture was closely correlated to the cell growth and cultural conditions. In the present experiment, the activity of phenylalanine ammonia-lyase (PAL) was examined in relation to the pools of total amino acid and phenylalanine, and also lignin content in carrot cells as the culture proceeded. Total amino acid gradually increased from the linear to the late stationary phases. Phenylalanine also exhibited a similar but more pronounced change (Table 1). The high amount of total phenolic acid (caffeic, ferulic and *p*-hydroxycinnamic acids) was found at the early logarithmic and linear phases whereas lignin decreased linearly as the culture proceeded (Table 1). High PAL activity was found at the linear and early stationary phases (Table 1). Hahlbrock *et al.* have reported that PAL activity in parsley or soybean cells in suspension culture reached a maximum prior to the stationary phase and then drastically declined [2,3]. As previously shown, the incorporation of phenylalanine-[U-^{14}C] into phenolic acids was almost same in cells both at the late logarithmic and linear phases [1]. The pool size of phenylalanine was much higher per

Table 1. The contents of total amino acid, phenylalanine, total phenolic acid and PAL activity in carrot cells at the various growth phases

Growth phase	Total amino acid		Phenylalanine		Lignin	Total phenolic acid*	PAL activity	
	(μg/g fr. wt)	(pg/cell)	(μg/g fr. wt)	(pg/cell)	(pg/cell)	(pg/cell)	A_{290}/mg protein $\times 10^5$	$A_{290}/10^9$ cells
Early logarithmic (3-day culture)	587	11	10	0·18	43	0·39	36	26
Late logarithmic (6-day culture)	301	5	6	0·10	36	0·20	24	17
Linear (10-day culture)	584	15	25	0·66	22	0·26	194	68
Early stationary (14-day culture)	636	19	42	1·32	17	0·12	87	38
Late stationary (17-day culture)	810	24	60	1·73	—	—	71	22
Very late stationary (29-day culture)	149	14	26	0·59	—	—	0	0

* Calculated from the result in the previous paper [1].

cell at the linear than the late logarithmic phases. The actual synthesis of phenolic acids might therefore be increased at a similar extent during this period. From these results, it could be concluded that the biosynthesis of phenolic acids was enhanced at the linear phase by the increase in PAL activity. PAL has generally been described as a limiting factor in phenolic biosynthesis [4]. However, the high incorporation of phenylalanine-[U-^{14}C] into phenolic acid was found by us at the early logarithmic phase [1] when the PAL activity was rather low. Similarly, lignin and phenolic acid decreased at the early stationary phase despite the rather high activity of PAL and the increase in phenylalanine. These results suggest that phenolic biosynthesis in carrot cells does not always depend on PAL activity and phenylalanine biosynthesis.

EXPERIMENTAL

Cultured cells. The cell line GD-2 used in this experiment was derived from a storage root of red carrot cv. Kintoki [5]. The cell suspension was maintained, with light, in the liquid medium of Murashige and Skoog [6] containing 1 ppm 2,4-D. The detailed procedure of cell cultivation was as described earlier [7].

Quantitative assay of total amino acid. The extraction of total amino acid was performed as described previously [8]. The amino acids were separated on Amberlite GC-120, eluted with 5% ammonia–H_2O. The eluate was evaporated to dryness and the residue was dissolved in H_2O. To this aq. soln. ninhydrin reagent was added and the mixture was heated at 95° for 20 min. The total amino acid was determined by the absorbance at 570 nm. Leucine was used as the standard.

Quantitative assay of phenylalanine. Phenylalanine was separated by PC with BuOH–HOAc–H_2O (4:1:5). After the treatment with ninhydrin and then 15% $NaHCO_3$, a blue spot developed [9]. The blue pigment was eluted with MeOH and absorbance measured at 610 nm.

Assay of PAL. Extraction of PAL was principally performed by the method of Zucker [10]. The reaction was proceeded at 37° for 90 min. The measurement of PAL activity was spectrophotometrically determined as the increase in A at 290 nm. Protein content was determined by the method of Lowry *et al.* [11].

Quantitative assay of lignin. The assay was performed by the modified procedure [1] of Brauns *et al.* [12].

REFERENCES

1. Sugano, N., Iwata, R. and Nishi, A. (1975) *Phytochemistry* **14,** 1205.
2. Hahlbrock, K., Ebel, J., Ortman, R., Suteer, A., Wellmann, E. and Grisebach, H. (1971) *Biochim. Biophys. Acta* **244,** 7.
3. Hahlbrock, K., Kuhlen, E. and Lindl, T. (1971) *Planta* **99,** 311.
4. Camm, E. L. and Towers, G. H. N. (1973) *Phytochemistry* **12,** 961.
5. Sugano, N. and Hayashi, K. (1968) *Botan. Mag.* (Tokyo) **81,** 371.
6. Murashige, T. and Skoog, F. (1962) *Physiol. Plant.* **15,** 473.
7. Nishi, A. and Sugano, N. (1970) *Plant Cell Physiol.* **11,** 757.
8. Sugano, N. and Hayashi, K. (1967) *Botan. Mag.* (Tokyo) **80,** 440.
9. Pasicka, A. and Morgan, J. F. (1956) *Biochim. Biophys. Acta* **19,** 366.
10. Zucker, M. (1969) *Plant Physiol.* **44,** 912.
11. Lowry, O. H., Rosebrough, N. J., Farr, A. J. and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265.
12. Brauns, F. E. and Brauns, D. A. (1960) in *The Chemistry of Lignin* (suppl. Vol.), p. 127. Academic Press, New York.