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SHORT COMMUNICATION

AN ANTHOCYANIN-DECOLORIZING SYSTEM IN FLORETS OF *CICHORIUM INTYBUS*

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Abstract—The anthocyanins of the chicory floret were decolorized by an enzyme system prepared from the florets. The activity of this enzyme and the anthocyanin content of the florets decreased through the day. This enzyme required the presence of catechol, or a similar substance, in the reaction mixture, and decolorized delphinidin glycosides more readily than other anthocyanins.

INTRODUCTION

ANTHOCYANIN-decolorizing systems have been described for fungi,[1] and the leaves,[2,3] and fruit[4–6] of higher plants. We report the presence of a similar system in the florets of chicory. This plant flowers daily from June to September in many parts of the northern hemisphere.[7,8] The heads open about daybreak revealing bright blue florets whose color fades through the morning and early afternoon. By 5.00 p.m. the florets are dead. The next day other heads open and the process is repeated. Todt,[9] has studied the rhythm of flower-opening and associated anthocyanin content variations in this plant under various regimes. The rapid loss of anthocyanin from the florets during the day under natural conditions has not been investigated previously.

RESULTS AND DISCUSSION

Absorption spectra in the visible region of a 1 per cent methanolic extract of florets showed that the major pigment was anthocyanin (λ_{max} = 540 nm; E_{max}/E_{440} 18 per cent). The pH optimum of the enzyme from the florets in McIlvaine's buffer (pH 2·2–8·0) and borate buffer[10] (pH 7·4–9·0) was found to be in the range of pH 6·6–7·0. This is characteristic of other plant decolorizing enzyme systems,[3,5,6] but unlike the fungal system.[1] At pH 7·0

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[3] E. BAYER and K. WEGMAN, *Z. Naturforsch.* **12b**, 37 (1957).
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[7] M. L. FERNALD, *Gray's Manual of Botany*, American Book Company, New York (1950).
[8] A. R. CLAPHAM, T. G. TUTIN and E. F. WARBURG, *Flora of the British Isles*, Cambridge University Press, Cambridge (1962).
[9] D. TODT, *Z. Botany* **50**, 1 (1962).
[10] R. M. C. DAWSON, D. C. ELLIOTT, W. H. ELLIOTT and K. M. JONES, *Data for Biochemical Research*, Oxford University Press, London (1959).

(25°) a linear response of reaction rate against enzyme concentration was observed over the range 0·2 mg to 2 mg per a final volume of 4 ml. The properties of the enzyme would suggest that it is of the catecholase rather than of the glycosidase type.[11]

The ability of floret acetone powders to catalyse the decolorization of anthocyanins in crude extracts of chicory florets sampled at 9.00 a.m. decreased through the day while the anthocyanin content of the florets decreased (Table 1). The color disappeared first from the upper end of the corolla and proceeded towards the ovary. A short time later a progressive browning of the florets in the same direction was observed. On bright, hot days the color was lost much more rapidly than on dull, cold days.

In some of the decolorizing systems so far described it has been found necessary to add catechol to the reaction mixtures to obtain maximum activity.[3,5] We found that when catechol (0·3 ml of 0·1 M) was added the decolorizing ability was decreased by as much as 40 per cent. However, when the crude chicory anthocyanin (a delphinidin glycoside)[11] was partially purified by paper chromatography and used as substrate, the activity of the decolorizing

TABLE 1. ACTIVITY OF THE ANTHOCYANIN-DECOLORIZING ENZYME AND ANTHOCYANIN CONTENT OF FLORETS AT VARIOUS TIMES OF THE DAY (22 AUGUST 1968)

Time of day	Enzyme units†	Anthocyanin content*
6.00 a.m.	107·5	1·47
8.30	92·5	0·94
10.00	75	0·97
11.30	60	0·75
1.30 p.m.	53·5	0·37
3.30	57·5	0·25

* Absorbance at 515 nm of five florets in 3 ml 0·1 N HCl in methanol.
† Reaction mixture contained 0·4 mg enzyme in a final volume of 4 ml. pH 7·0, 25°.

system was low. The addition of catechol (0·3 ml 0·1 M) to the reaction mixture allowed decolorization at a rate comparable to that obtained with crude chicory anthocyanin. From this we would suggest that catechol, or some similar necessary promotor, is present in the crude anthocyanin preparation. Addition of catechol to the crude preparation probably increased the concentration of promotor to a non-optimal level.[5]

The quantity of anthocyanin and the activity of the decolorizing system in the florets decreased concurrently throughout the day (Table 1). The degree of corolla browning also increased basipetally throughout the day denoting the breakdown of cellular structure and ultimate death of the cells. The simultaneous loss of color and enzyme activity of florets may have been due to the breakdown of membrane integrity permitting mixing of enzyme and vacuolar substrate resulting in decolorization and subsequently in the inactivation of the enzyme by noxious substances.

The enzyme from chicory flowers did not decolorize the crude anthocyanins from apple skin (cyanidin-3-galactoside) or from pelargonium petals (pelargonidin and peonidin

[11] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, London and New York (1967).

3,5-diglucosides) with or without catechol, but did decolorize the crude anthocyanins from mung bean seedling (which are based on delphinidin).[12] Partially purified extracts of mung anthocyanins were not easily decolorized. This ability of the chicory-decolorizing enzyme to decolorize delphinidin glycosides more readily than the other anthocyanins is interesting in view of similar data which has already been reported for a related system in the egg-plant, *Solanum melongena*.[6]

EXPERIMENTAL

The anthocyanin-decolorizing enzyme and substrate were prepared as follows. 2 g of fresh florets were homogenized briefly in 50 ml of cold acetone (−25°) and the homogenate poured into a cold Büchner funnel. The acetone was removed by vacuum filtration and the residue air and vacuum dried. The resulting acetone powder, about 200 mg, was stored at −25°. The enzyme could then be suspended in buffer at the desired pH. The anthocyanin substrate was prepared from florets collected at 9·00 a.m. by macerating them in 0.01 N HCl and centrifuging at 2000 rev/min. The substrate concentration was adjusted so that the absorbance at 515 nm was 1·00 when diluted with 10 vol. of N HCl.

The activity of the anthocyanin-decolorizing system was assayed at 25° by adding the acetone powder (0·4 mg in a final volume of 4 ml) in McIlvaine's buffer[10] (pH 7·0) to the neutralized anthocyanin substrate and withdrawing 0·5 ml aliquots at intervals. Each aliquot was delivered into 3 ml of N HCl and the absorbance at 515 nm determined. The rate of loss of absorbance at 515 nm was used as a measure of enzyme activity. A unit of decolorizing enzyme was defined as the amount of enzyme which caused a change of 0·01 units of absorbance at 515 nm in 10 min at 25°. The relative activity was expressed in enzyme units per mg acetone powder. Control reaction mixtures were prepared by replacing the enzyme solution in the reaction mixture with an enzyme solution inactivated by heating in boiling water for 5 min.

[12] J. T. A. PROCTOR, unpublished.