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THE DECOLORIZATION OF BETACYANIN BY POLYAMINES

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Key Word Index—*Beta vulgaris*; Chenopodiaceae; red beet; betacyanin; spermine; decolorization.

Abstract—The pigment betacyanin, the efflux of which has been used as a measure of membrane integrity in red beet discs, has now been shown to react with di- and polyamines with consequent decolorization.

INTRODUCTION

The diamine putrescine and the polyamines spermidine and spermine have been shown to stabilize the membranes of animals [1], algae [2] and higher plants (reviewed in ref. [3]). Some workers, attempting to demonstrate that polyamines stabilize membranes in red beet tissue, have monitored the effect of these and other compounds on betacyanin efflux [4–6]. While it is possible that polyamines will stabilize the red beet membrane, it now seems that much of this apparent reduction in betacyanin efflux can be attributed to a reaction between polyamines and the red pigment with consequent decolorization.

RESULTS AND DISCUSSION

Although control samples lost some colour on incubation in air-saturated pH 7.5, 0.1 M Tris buffer for 90 min (Table 1), this loss was greatly increased by spermine. Colour loss was greatest in oxygen-flushed solutions, as found by Attoe and von Elbe [7], though nitrogen flushing did not prevent the betacyanin decolorization in the presence of spermine. Relatively small losses were found with ammonium sulphate, EDTA and lysine, though a marked loss could be found with diaminopropane. Putrescine and spermidine were almost as effective as the spermine in causing decolorization (results not shown).

On incubation for 2 hr at 40° in citrate (pH 3–6) and Tris (pH 6–7.2) buffers the greatest loss on incubating with spermine was found at the highest pH. On incubating in the absence of spermine greatest stability was found at the highest pH. On treating the betacyanin with various spermine concentrations up to 10 mM, colour loss was correlated with, but was not proportional to, spermine concentration (Table 2). With 1 mM spermine, loss after 8 min in pH 7.5, 0.1 M Tris buffer was 76% while in pH 7.5, 0.1 M phosphate buffer the loss was 60%. The corresponding losses without the polyamine were 23% and 29% respectively. Proline, which is known to exchange with the cyclodopa [8], was ineffective in reducing the colour, even at 10 mM.

The changes in the spectrum in the visible range were monitored with and without addition of spermine (1 mM) on incubation at 40° in Tris buffer for up to 2 hr (Fig. 1). Changes in the absorption spectrum were rapid on addition of spermine. The peak at 540 nm decreases, while *A* at 300–430 nm increases. When the peak at 540 nm had been reduced by 85% after 2 hr, the *A* at 470 nm was half that found originally at 540 nm, having declined by only 20% in this period. After heating for 5 min at 100° in the absence of spermine, peaks were found at 430, 480 and 540 nm, in agreement with ref. [9], while in the presence of spermine, boiling for 5 min gave peaks only at 370 and 480 nm (Fig. 1).

Table 1. Percentage loss of colour (*A* 540 nm) from betacyanin solution after 90 min incubation at 40° with various compounds (all at 1 mM) in pH 7·5, 0·1 M Tris buffer

Treatment	Gas	% Loss
Control	Air	17
Control	Oxygen	34
$(NH_4)_2SO_4$	Air	25
Diaminopropane	Air	47
NaEDTA	Air	18
Lysine	Air	29
Spermine	Air	87
Spermine	Nitrogen	87
Spermine	Oxygen	93

Table 2. Effect of spermine concentration on loss of colour (*A* 540 nm) from betacyanin incubated for 2 hr in pH 7·5, 0·1 M Tris buffer at 37°

Spermine concentration (mM)	% Loss
Control	30
0·01	35
0·1	48
1	79
10	85

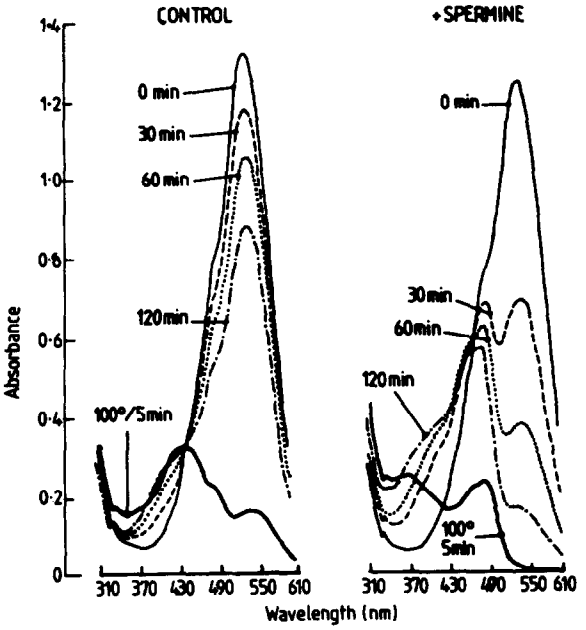

Fig. 1. Changes in absorption spectra of betacyanin from red beet incubated at 40° in pH 7·5, 0.1 M Tris buffer with or without 1 mM spermine. Also shown is the effect of heating for 5 min at 100° with and without this amine.

The mechanism of this decolorization is at present unknown. It is possible that spermine catalyses the decomposition of betacyanin by the mechanism proposed by von Elbe *et al.* [10] and Havlikova *et al.* [11] on interaction with the three carboxyl groups, causing the loss of cyclodopa from the betalamic acid. Since on electrophoresis spermidine and spermine appeared to give the same products after incubation with betacyanin, this mode of interaction is plausible. The major product of the reaction between spermine and betalains was yellow, and carried a neutral charge. It is therefore unlikely to be betalamic acid which, although yellow, would be strongly anodic [12, 13]. Since this product was ninhydrin negative it is unlikely that the reaction takes places by a direct exchange of the amino groups with the cyclodopa on the betalamic acid. The natural occurrence of di- and polyamines in food [14] may be a factor in determining the stability of betacyanins added as food colorants.

EXPERIMENTAL

Betacyanin from red beet (*Beta vulgaris* L.) was purified using Dowex 50 resin [15], freeze dried and stored at −15° until required. On being dissolved, the conc was adjusted to give an initial *A* 540 nm of 1 to 1.5. Unless stated otherwise loss of pigment was monitored at 540 nm. HVE was effected in pH 6.7 NaPi buffer at 2 kV for 75 min. Using this method the red beet extract was shown to be a mixture of at least 3 pigments, the main component being betanin. Lysine and the amines were used as the hydrochlorides.

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