# PIGMENT BIOSYNTHESIS AND PRECURSOR METABOLISM IN RED BEET SEMI-CONTINUOUS SUSPENSION CULTURES.

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## INTRODUCTION

Most chemical and biochemical studies on the biosynthetic pathways leading to betanin in red beet (Beta vulgaris L.}, have been carried out in whole plant organs or tissue slices (1,2,3). The problem of high dilution encountered with such tracer-precursor experiments can be avoided by using cell suspension cultures (4); we used such a system to follow up the bio-transformation of tyrosine and dihydroxyphenylalanine (DOPA) in red beet and test the proposed biosynthetic schemes (Fig. 1).

Induction of friable callus and subsequent isolation of cells, usually led to a derepression of the controls exerted on the biosynthetic pathways, therefore allowing the appearance of variant cell lines. In red beet cell cultures, at least two, more or less stable, variants may be isolated by visual selection. We decided to study the comparative biochemistry of these two cell lines (BVR-red cells / BVW-white cells) in order to understand the mechanism by which betanin synthesis might be switched on and off in the plant cell.

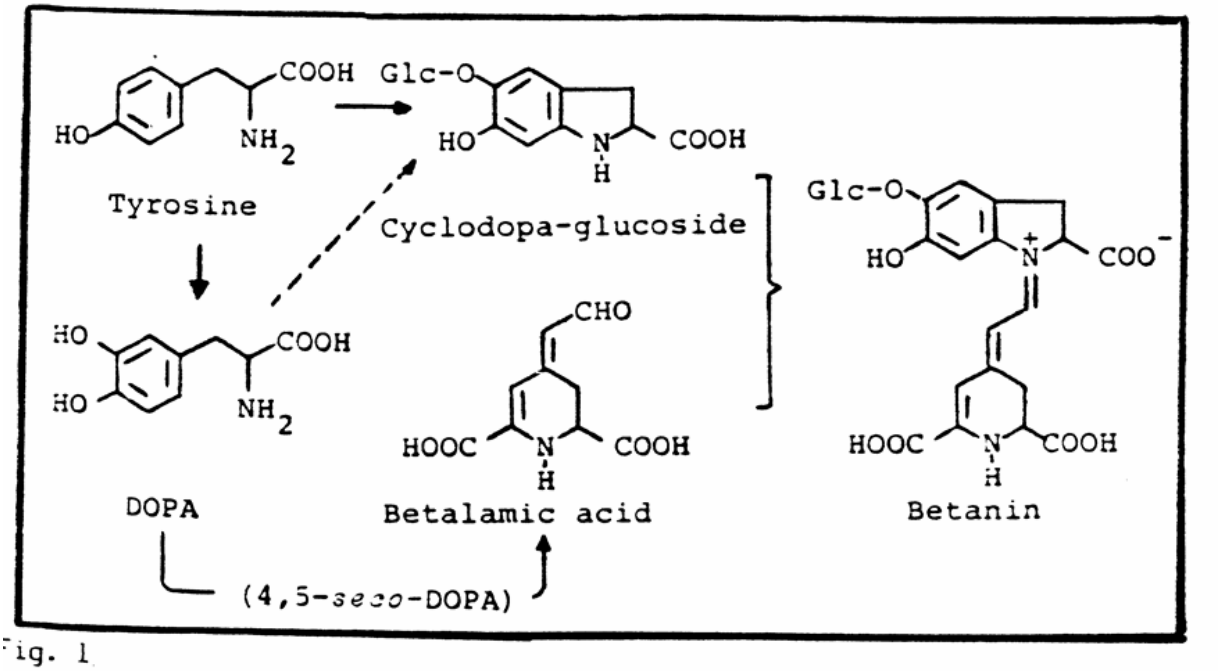


Fig. 1

## MATERIAL AND METHODS

Red beet habituated cultures were a gift from Prof. M.H. Zenk. They have been maintained on 85 medium with glucose as sole carbon source and no hormone complement. At least three consecutive transfers at internals of 84 h. were made in liquid medium before each experiment. The aqueous crude extract (pH 3} was analysed after centrifugation and microfiltration by Reverse-Phase-HPLC coupled to a UV(230nm) detector, followed by continuous scintillation counting (Radiomatic Instruments: Flo One HP).

## RESULTS AND DISCUSSION

### Amino-acid content of cells of the BVR and BVW lines.

In both cell lines, the maximum content of most amino-acid (in nmole per g FW) is observed on day 1 and 2 after transfert. Marked differences become

obvious within the first two days when amino acid content of the two cell lines is compared. We may distinguish 4 groups of amino acids: 1. DOPA alone, largely deficient in BVW cell line; 2. valine, alanine, glycine and GABA, much increased; 3. the majority of other amino acids, slightly increased; 4. phenyalanine, beta-alanine and ornithine, stable with time. In conclusion: regulation of DOPA synthesis (inhibition) is an important characteristic of the strain BVW.

Incorporation of tyrosine-Cl4 and DOPA-C14.

During a 3 h. feeding experiment with 3-day old BVR cells, tyrosine incorporation is slightly higher than DOPA incorporation. Tyrosine is transformed into DOPA, cyclodopa-glucoside and betanin. Although the overall incorporation of tyrosine and DOPA into betanin is of the same order, DOPA is, comparatively much less incorporated in cyclodopa-glucoside. Some "compartimentation" of tyrosine and DOPA may have to be taken into account.

High-Pressure-Liquid-Chromatographic-Analysis combined with flow scintillation counting of metabolites from labeled precursors.

This method for measurement of specific radioactivity is applied for the first time in this kind of analysis. From a comparison of BVR (fig. 2A) and BVW (fig. 2B) strains it follows that:

a) No DOPA is formed from tyrosine in the BVW cell line and we should therefore conclude that the block in betanin synthesis occurs at the site of DOPA synthesis from tyrosine.
b) Dopamine and tyramine in experiments using DOPA or tyrosine respectively accumulate in cells to a larger extent when betanin synthesis is inhibited.
c) Cyclodopa-glucoside is formed at a higher rate when tyrosine is given as precursor (in BVR strain).

Radiochromatoqraphic profiles of HPLC analysis:

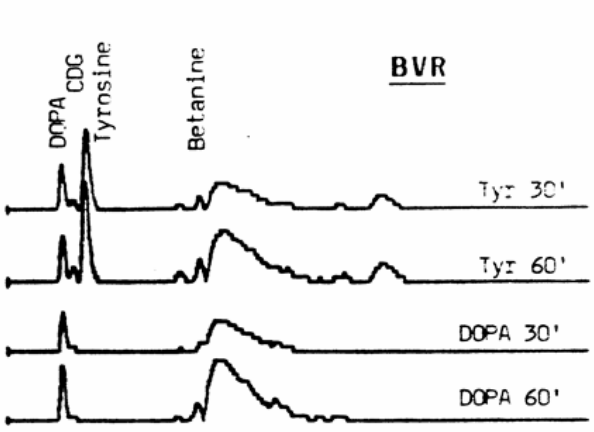


Fig 2A

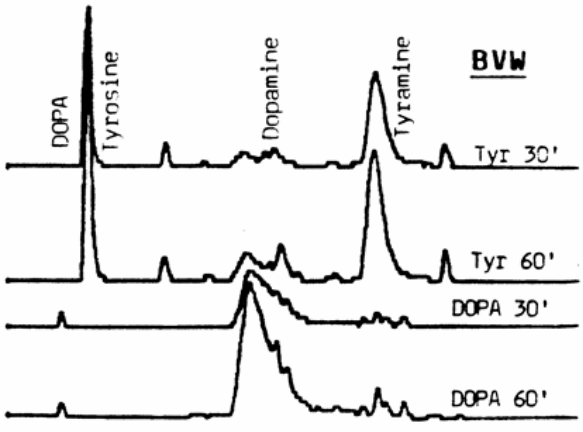


Fig. 2B

General conclusions

Our results confirm that the metabolisms of tyrosine and DOPA are distinct (and "compartmented";. Tyrosine is relatively better incorporated into the upper part of the betanin molecule (via cyclodopa-glucoside) than DOPA; DOPA, itself a product of tyrosine metabolism, is efficiently incorporated into the lower part of the molecule (in betalamic acid, see Fig.l).

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