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SHORT COMMUNICATION

# O-METHYLTRANSFERASE ACTIVITY FROM YOUNG FLOWER PETALS OF *IMPATIENS BALSAMINA**

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**Abstract**—Petals from stage 3 flower buds of the purple ($LLhhP^rP^r$) genotype of *Impatiens balsamina* were examined for *O*-methyltransferase activity. Acetone powders of the tissue gave active preparations which would methylate caffeic acid to ferulic acid in the presence of *S*-adenosyl-L-methionine. The biosynthetic role of this enzyme is discussed.

## INTRODUCTION

IN A PREVIOUS paper we described the quantitative and qualitative distribution of hydroxycinnamic acids in mature petals of the red, white, and purple genotypes of Impatiens balsamina.[1] In this study it was noted that *p*-coumaric and ferulic acids were present in relatively large quantities but caffeic acid was present in extremely low amounts. Furthermore, it was observed that the red genotype ($HHllP^rP^r$) which produces pelargonidin also produced a higher ratio of *p*-coumaric acid to ferulic than did the purple malvidin-producing genotype ($LLhhP^rP^r$), in which this ratio was reversed. These results seemed to indicate that the purple genotype had a higher methylating activity than either of the other two genotypes since ferulic acid concentration is highest in this genotype and malvidin is produced. This paper presents the preliminary results in isolating *O*-methyltransferase activity from this tissue.

## RESULTS AND DISCUSSION

The presence of *O*-methyltransferase has now been reported from a variety of plant tissues, and this activity shows a broad specificity for *vicinal*-polyphenols.[2–4] We have examined acetone powders of young flower petals of the purple genotype of *Impatiens balsamina* for *O*-methyltransferase activity using caffeic acid as the substrate. This hydroxycinnamic acid has previously been shown to be a precursor of ferulic acid[5] which can then serve as a precursor for the methylated anthocyanins.[6] We have been able to prepare active preparations which methylate caffeic acid to ferulic acid and this activity can be destroyed by boiling the enzyme or omitting caffeic acid from the reaction mixture.

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† Contribution No. 63 from the Botanical Laboratories, University of South Florida, U.S.A.

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*Effects of Reducing Agents*

Earlier studies by Hess[4] have shown that a reducing agent is required for activity in both *Petunia* and *Triticum*, whereas Finkle[2] has demonstrated activity in the absence of reducing compounds. Table 1 shows the results of the reducing agents tested in our preparations. After 3 hr, incorporation is nearly the same in all tubes, with the possible exception of that containing glutathione where there appears to be a slight inhibition during the early stages of incubation. After 10 hr, however, there is a marked difference between the tubes with reducing agent and the one without. The reaction mixtures without reducing agent show an actual decrease in the amount of incorporation. This decrease has been observed repeatedly in reaction mixtures incubated about 6 hr or longer without a reducing agent and it would appear that the reducing compounds are not required for activity but may be necessary to minimize substrate oxidation and product degradation during incubation.

TABLE 1. EFFECT OF REDUCING COMPOUNDS ON *O*-METHYLTRANSFERASE ACTIVITY*

| Reaction conditions† | Incubation period (hr) | Counts/min × $10^4$ per mg protein | Incorporation (%) |
|---|---|---|---|
| Control— no caffeate | 3 | 0·7 | 0·02 |
| | 10 | 0·8 | 0·03 |
| No reducing compound | 3 | 192·9 | 9·24 |
| | 10 | 91·5 | 4·38 |
| Glutathione | 3 | 125·3 | 5·62 |
| | 10 | 201·9 | 9·05 |
| β- Mercaptoethanol | 3 | 188·6 | 9·01 |
| | 10 | 204·2 | 9·76 |
| Sodium dithionite | 3 | 200·8 | 10·37 |
| | 10 | 217·3 | 11·23 |

* See experimental for reaction mixture.

† 10 μmoles of each reducing compound was used per reaction mixture.

*Effect of Magnesium*

In studies of *O*-methyltransferase from *Nerine*, Mann *et al.*[7] reported that divalent cations did not stimulate the reaction, but magnesium is usually included in the reaction mixtures by other workers. We tested for the requirement of magnesium in our reaction mixture and the results are presented in Table 2. It is apparent that magnesium was not required for activity in our preparations but the presence of this metal in the reaction mixture indicates that it, like the reducing agents, might help to stabilize the reaction mixtures over a long incubation period possibly by inhibiting product degradation.

After the effect of magnesium and reducing agents had been established, we chose to include them both in reaction mixtures to minimize substrate oxidation and product degradation. A complete reaction mixture was then tested and the conversion of caffeic acid to ferulic was found to be linear for approximately 6 hr before beginning to decline. It has not yet been ascertained whether the decline in incorporation is due to the degradation of product or to limiting concentrations of *S*-adenosyl-L-methionine.

[7] J. D. MANN, H. M. FALES and S. H. MUDD, *J. Biol. Chem.* **238**, 3820 (1963).

TABLE 2. EFFECT OF MAGNESIUM ON THE ACTIVITY OF O-METHYLTRANSFERASE ACTIVITY*

| Reaction conditions | Incubation time (hr) | Counts/min $\times 10^4$ per mg protein | Incorporation (%) |
|---|---|---|---|
| Boiled enzyme | 5 | 0·39 | 0·01 |
| | 9 | 0·36 | 0·01 |
| No magnesium | 5 | 200·0 | 6·30 |
| | 9 | 162·0 | 5·10 |
| Plus 10 μmoles magnesium | 5 | 179·0 | 5·80 |
| | 9 | 206·0 | 6·70 |

* See Experimental for reaction conditions

The presence of O-methyltransferase is of interest in plant tissue which produces methylated anthocyanins and hydroxycinnamic acids. It will be important to determine whether the absence of methylated anthocyanins and low levels of ferulic acid in the red genotype and the methylated anthocyanins and high levels of ferulic acid in the purple genotypes can possibly be correlated with the O-methyltransferase activities of these tissues. Further purification of this enzyme and a quantitative analysis of other genotypes is underway and will be reported at a later date.

## EXPERIMENTAL

### *Enzyme Preparation*

Flower petals from stage 3 buds of the purple ($LLhhP^rP^r$) genotype were removed by dissection and homogenized in cold acetone, centrifuged at 30,000 *g* and re-extracted with cold acetone. The residue was collected by filtration and air-dried at room temp. The dried powder was stored at $-10°$ and showed no loss of activity over several months.

### *Enzyme Assay*

Fifty mg of acetone powder was extracted with 1 ml of 0·1 M $NaHCO_3$ at 4° for 10 min. The suspension was centrifuged at 30,000 *g* for 30 min and the supernatant removed. The reaction contained 0·5 ml of enzyme (0·23 mg protein); 2·5 μmoles of freshly prepared sodium caffeate, 200 μmoles $KH_2PO_4$, 10 μmoles $MgCl_2$, 10 μmoles glutathione (reduced form), and 0·35 μc S-adenosyl-L-methionine (methyl $C^{14}$) specific activity 55 μc/μm (International Chemical and Nuclear Corp.) in a total volume of 1 ml. The final pH of the reaction mixture was 8 and the incubations were done at 25°. Controls were made by heating the enzyme in a boiling water bath for 5 min or omitting sodium caffeate from the reaction mixture. At various intervals, 100 μl fractions were removed, acidified with 10 μl of 6 N HCl and extracted 5 times with 200 μl aliquots of anhydrous ethyl ether. The ether solubles were spotted on Whatman No. 1 chromatograph paper and 5 μg of unlabelled ferulic acid was added as carrier. The papers were developed descending in benzene–acetic acid–water (10:7:3) and ascending in 2% formic acid. The ferulic acid spot was located in UV light, cut out, eluted with 95% EtOH into a scintillation vial and counted in a liquid scintillation spectrometer.

Protein determination were done using Pottys'[8] modified Lowry method.

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[8] V. H. POTTY, *Anal. Biochem.* **29**, 535 (1969).