Cytokinin Biosynthesis and Metabolism in *Vinca rosea* Crown Gall Tissue

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1 Introduction

Progress in the understanding of the mechanisms and controls of plant hormone biosynthesis and metabolism has to a large extent been limited by the availability of suitable systems for study. Whilst the ultimate aims of studies of this type must be an understanding of the ways in which plants regulate their endogenous hormone complement and the physiological significance of this regulation, we are still very far from achieving these in practice. In general, biosynthetic and metabolic studies of plant hormones are severely hampered by the extremely low levels of these compounds in most plant tissues. Indeed, the recent spectacular progress in the area of gibberellin biosynthesis and metabolism has been very dependent upon the use of fungal and immature seed systems in which gibberellin levels are sufficiently high to permit the use of unambiguous physical techniques for these studies.

Although the sensitivity of physical techniques is continually increasing, as, unfortunately, is their cost, progress in the field of cytokinin biochemistry is sorely hampered by the lack of suitable systems for biosynthetic and metabolic studies, in which these processes can be studies in a physiologically relevant and chemically rigorous manner. Ideally, the following requirements should be met by any system used for studies into plant hormone biosynthesis and metabolism:

1. The endogenous hormones should be completely and unambiguously characterised, both qualitatively and quantitatively;
2. The levels of these compounds should be sufficiently high to allow the feeding of precursors and intermediates, in amounts high enough to permit identification of products by reliable physical techniques, without greatly exceeding the endogenous levels.

The above two points represent a minimum requirement for valid studies. In addition, the lack of information on compartmentation of endogenous compounds and the effects of this on their biosynthesis and metabolism presents considerable further difficulties to the interpretation of results.

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J. Guern et al. (eds.), *Metabolism and Molecular Activities of Cytokinins* © Springer-Verlag, Berlin Heidelberg 1981
In recent years at Aberystwyth we have been investigating the suitability of several systems for detailed studies of cytokinin biosynthesis and metabolism, bearing in mind the above comments. To date, two systems which, in part at least, fulfil the above requirements have been studied. These are rooted cutting and decapitated plants of *Phaseolus vulgaris*, and tissue cultures of *Vinca rosea* crown gall tumours.

This paper describes preliminary studies on cytokinin biosynthesis and metabolism in *Vinca rosea* crown gall and normal tissue. Although the studies described here fall short of the criteria outlined previously, they are directed towards the eventual fulfilment of these criteria. The direction of future work based on these preliminary studies will be discussed briefly.

The origin and nature of crown gall tissues have been extensively covered in the literature and will not be discussed further here. However, from the point of view of studies of cytokinin biosynthesis and metabolism, the relatively high levels of cytokinins in *V. rosea* crown gall tissue and the fact that the compounds have been unambiguously identified make it a suitable system for study. In addition, the fact that cytokinin production in this tissue is in some way under the control of the Ti plasmid adds an additional valuable genetic dimension to these studies.

2 Endogenous Cytokinins of *V. rosea* Crown Gall Tissue and Its Normal Counterpart

The initial reports on the nature of the endogenous cytokinins of *V. rosea* crown gall tissue are very confused and controversial. To the best of our knowledge, the original work on cell division factors in this tissue (Wood et al., 1969; Wood, 1970) has never been substantiated. However, Miller (1974) unambiguously established that the major endogenous cytokinin of *V. rosea* crown gall tissue was zeatin riboside. In addition, Peterson and Miller (1977) and Morris (1977) identified zeatin-O-glucoside and ribosyl zeatin-O-glucoside as major cytokinins when the tissue was grown on ammonium chloride as the nitrogen source.

Although in general we have confirmed these initial findings, our results do differ from those of Miller in some important aspects. Firstly, when care is taken to minimise the effects of phosphatase activity during extraction, by use of the extraction medium of Bieleski, a major portion of the extractable cytokinin activity is found in the nucleotide fraction. Further fractionation of this activity into mono-, di-, and tri-nucleotides by DEAE cellulose chromatography reveals that the activity is confined exclusively to the mono-phosphate components of the extract. Chemical and enzymatic degradations, and subsequent identification of the biologically active material as zeatin riboside, suggest that a major portion of the cytokinin activity of *V. rosea* crown gall tissue is due to zeatin riboside-5-monophosphate.

Secondly, HPLC fractionation of an extract of *Vinca* tissue, using a HPLC system specifically designed to separate cytokinin glucosides (see Fig. 1) revealed a large UV-absorbing peak at the elution position of zeatin-9-glucoside. UV and MS analysis of this component as the methylated, trimethylsilylated (TMS) and free compound confirmed its structure (Scott et al., 1980). Thus zeatin-9-glucoside must also be considered as a major component of the *Vinca* cytokinin complex. It was probably not observ-