GLUCOSINOLATE BIOSYNTHESIS IN OILSEED RAPE - RECENT PROGRESS IN BIOCHEMISTRY AND MOLECULAR BIOLOGY

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ABSTRACT

The enzymes involved in the early stages of glucosinolate biosynthesis in oilseed rape have been identified and characterised. Two microsomal enzymes are present in the leaf, catalysing the conversion of amino acids to aldoximes. These NADPH- and O_2-dependent enzymes appear to be related to the flavin-linked monooxygenases found in animal tissues, and cDNA probes based on the animal gene sequences have been used to identify similar genes in rape tissues. Both enzymes have strict substrate specificity, and thus control the range of amino acid precursors able to enter the biosynthetic pathway. Conversion of aldoximes to thiohydroximates is thought to proceed via a cysteine conjugate, and C-S lyases which cleave such conjugates have been studied. The glucosinolate biosynthetic system is regulated both developmentally and in response to external factors. Light is necessary for the expression of the aldoxime-forming enzymes, which are active in young and expanding leaf tissues but which are absent from older mature leaves. The enzymes specific for the biosynthesis of alkenyl and aromatic glucosinolates are not present in cotyledons, whereas the peroxidases involved in indole glucosinolate formation are found in such tissue.

INTRODUCTION

Any attempt to manipulate the glucosinolate content of oilseed rape through biotechnology, whether to alter the nutritional qualities of the seed or enhance the crop's resistance to pests and diseases, requires a sound understanding of the biosynthetic pathway(s) involved. Althought the sequence of chemical changes has been identified through feeding studies, until recently very little was known of the enzymes involved, especially in the crucial early steps of the pathway. Based on a superficial similarity with the biosynthesis of cyanogenic glucosides, it had been assumed that similar enzymes would be involved in glucosinolate synthesis - particularly that cytochrome P450-type enzymes would be responsible for the conversion of amino acids to aldoximes, known intermediates in both cyanogenic glucoside and glucosinolate biosynthesis. However, all attempts to demonstrate the presence of such cytochromes P450 in rape tissues have been unsuccessful. Many such studies used etiolated cotyledons or leaves, and looked for a membrane-bound and channelled biosynthetic complex as has been demonstrated for cyanogenic glucoside biosynthesis (these studies are reviewed in Poulton & Møller, 1993).

Taking a rather different approach, we have looked for the enzymes of glucosinolate biosynthesis in young green leaves, tissues in which rapid glucosinolate accumulation has been shown (Porter *et al.* 1991). As previously reported, we were able to demonstrate the presence of aldoxime-forming monooxygenase activities in microsomes extracted from young leaves (Bennett *et al.*1993; Dawson *et al.* 1993), with characteristics similar to the flavin-linked monooxygenases (FMOs) found in animal tissues (Ziegler,

1988).

We will be reporting on the characteristics of these enzymes, their developmental regulation, and our initial attempts to identify the genes which code for them. In addition some preliminary studies of thiohydroximate formation will be presented.

EXPERIMENTAL

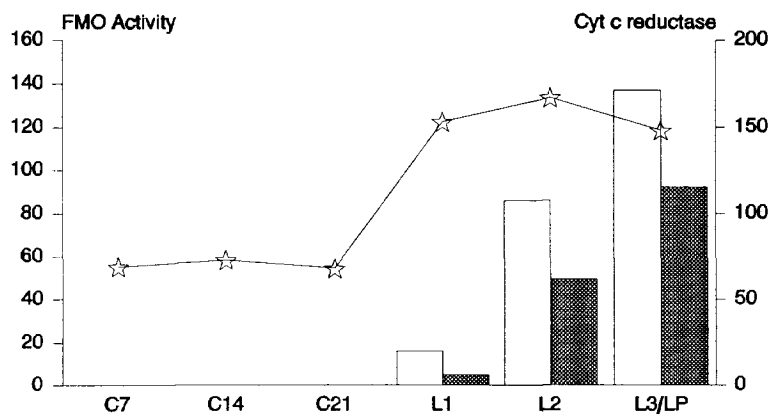
Methods used for the growth of oilseed rape plants (*Brassica napus* cv. Bienvenu), preparation of leaf microsomes and the assay of monooxygenase activities, were previously described (Bennett *et al.*1993; Dawson *et al.* 1993). C-S lyase activity was assayed using cystathionine, cystine, or SBC. Northern blots of rape leaf mRNA were probed with cDNA corresponding to conserved sequences from mammalian FMO genes (cDNA kindly provided by Dr.C.Dolphin, London). Aldoxime-forming peroxidase activity was assayed using the methods of Ludwig-Muller & Hilgenberg (1988).

RESULTS & DISCUSSION

Two aldoxime-forming monooxygenases are present in oilseed rape leaves. Both are dependent on NADPH and molecular oxygen, and are inhibited by DTT and Cu^{2+}, but not by CO or any other cytochrome P450 inhibitor. One enzyme is specific for homophenylalanine - it is inactive with Phe, Tyr, & Trp. The other enzyme is active with higher homologues of Met, from dihomoMet upwards, but not with Met, homoMet, or any other amino acid. Modification of the sulphur atom in Met homologues (removal, replacement with O, oxidation) abolishes the ability to act as a substrate.

Both activities follow a similar developmental pattern in leaves (Table 1). Neither is present in emerging cotyledons, but high activity is found in young true leaves and leaf primordia. The activities decline as the leaves age and are absent from mature leaves prior to senescence. Both activities are sensitive to light, as they rapidly decline in leaves of plants transferred to the dark, and are not found in etiolated tissue. The indole aldoxime-forming peroxidase activity, thought to be involved in the biosynthesis of indole glucosinolates, follows a similar developmental pattern, but is also found in cotyledons.

FIGURE 1. FMO activities (nmol g^{-1} FW h^{-1}) in cotyledons (C) of 7, 10 & 21-d old plants, and leaves (L) 1, 2 & 3 (+ primordia - LP) of 14-d old plants. Open bar - HPhe FMO; filled bar - DHMet FMO; line - cyt c reductase (nmol g^{-1} FW min^{-1}).



Oilseed rape leaves contain at least one active C-S lyase which can utilise cystathionine, cystine, or the artificial substrate SBC. This enzyme (or enzymes) is pyridoxal phosphate-dependent. Attempts to separate the different activities by a variety of chromatographic procedures have been unsuccessful, and work is now concentrating on studies with a synthetic aldoxime-cysteine conjugate, the putative intermediate in glucosinolate biosynthesis. This step in the pathway introduces the S for the glucose-thioester link, and is one of the key points where glucosinolate and S metabolism interact.

Using cDNA based on highly conserved sequences of mammalian FMO genes, we have probed N-blots of mRNA extracted from rape (and other Brassica) leaves. We are currently examining which (if any) of the bands which hybidise are likely to represent the aldoxime-forming FMO genes. Cloning and sequencing of these genes will lead to the possibility of manipulating the glucosinolate content of rape tissues in a directed way.

From our knowledge of the biochemistry, it is clear that a relatively simple antisense approach should enable us to eliminate a whole class of glucosinolates (alkenyl, aromatic, indolyl) in a give tissue without affecting the other classes. Such changes are likely to have dramatic effects on pest and pathogen host recognition. Alternatively, the key genes could be inserted with altered promoters, to boost the production of glucosinolates in response to predation or infection and so enhance crop protection against non-specialist organisms.

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