The biosynthetic pathway of the S-alk(en)yl-L-cysteine sulfoxides (flavour precursors) in species of Allium

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Abstract

Pulse labelling experiments with 35SO42− fed for 24h to intact plants (shooted onion sets) of Allium cepa (onion) showed that > 70% of the label appeared in the S-alkenyl-L-cysteine sulfoxides within 18h, reached a maximum at 48h and thereafter decreased. The amount of label detected in the γ-glutamyl peptide fractions was below 20% of the total label at any time. It is concluded that in intact plants (at the growth stage used) the γ-glutamyl peptides are not the immediate precursors of the S-alkenyl-L-cysteine sulfoxides. The major S-alkenyl-L-cysteine sulfoxide in onion was found to be compartmentalized mainly within the endoplasmatic reticulum.

Abbreviations: AllCysSO – (+)-S-2-propenyl-L-cysteine sulfoxide; MeCysSO – (+)-S-methyl-L-cysteine sulfoxide; PrenCysSO – trans-(+)-S-1-propenyl-L-cysteine sulfoxide; ProCysSO – (+)-S-propyl-L-cysteine sulfoxide

Introduction

Allium species contain a high proportion (1–5% dry weight) of non-protein sulphur amino-acids (Lancaster & Shaw 1989). One class of these secondary metabolites, the S-alk(en)yl-L-cysteine sulfoxides give rise to the characteristic flavours associated with the Allium species. Four sulfoxides have been found to occur naturally in the Allium species: namely (+)-S-methyl-, (+)-S-propyl-, trans-(+)-S-1-propenyl-, and (+)-S-2-propenyl-L-cysteine sulfoxides (respectively Me-, Pro-, Pren- and AllCysSO). All Allium species are reported to contain MeCysSO (Lancaster & Shaw 1989), while ProCysSO predominates in chives, PrenCysSO in onions and AllCysSO in garlic (Edwards et al. 1994).

Flavour in Allium is only released when the tissue is cut or broken. Work on garlic (Cavellito & Bailey 1944; Stoll & Seebeck 1951) and on onion (Virtanen & Späre 1961; Schwimmer 1968) established that when the plant tissue was cut the S-alk(en)yl cysteine sulfoxides were hydrolysed by the enzyme alliinase. Lancaster & Collin (1981) provided evidence that the S-alk(en)yl cysteine sulfoxides were compartmentalised within the cytoplasm and the hydrolytic enzyme alliinase located within the vacuole. The initial products of the reaction between alk(en)yl cysteine sulfoxides and alliinase are ammonia, pyruvate and alk(en)yl sulphenic acids; the last of which breaks down non-enzymatically to form a variety of disulphides, which have a much milder flavour. The quantitative and qualitative difference in the alk(en)yl cysteine sulfoxide content results in the different flavours and odours of the many Allium species. In onions it is particularly the presence or absence of PrenCysSO which is responsible for the lachrymatory effect of onions, and AllCysSO which produces the characteristic taste of garlic. Early work established that PrenCysSO when hydrolysed by alliinase is converted into propenyl sulphenic acid, which is chemically unstable and undergoes rearrangement to form the lachrymator compound propenal sulfoxide (Virtanen 1965).

Allium species also contain other non-volatile sulphur compounds, the γ-glutamyl peptides, which are not hydrolysed by alliinase and do not therefore contribute to flavour production. For example in the onion
bulb the majority of the PrenCysSO is bound as \( \gamma \)-glutamyl PrenCysSO (Lancaster & Shaw 1991), which effectively removes a large proportion of the PrenCysSO from contributing to lachrymator production (Whitaker 1976). In Allium species there are as many as 18 sulphur-containing \( \gamma \)-glutamyl peptides which have been identified by Virtanen and Suzuki and their respective co-workers (reported in Lancaster & Shaw 1989). The function of the \( \gamma \)-glutamyl peptides in the metabolism of the plant remains unclear. Originally they were generally considered to function as reserves of nitrogen and sulphur, and have since been implicated in the transport of amino-acids in cells (Kasai & Larson 1980). In 1989 however, Lancaster and Shaw proposed that some of the \( \gamma \)-glutamyl peptides (e.g. \( \gamma \)-glutamyl PrenCysSO) may be intermediates in the biosynthetic pathway of alk(en)yl cysteine sulfoxides.

A number of pathways for the biosynthesis of the alk(en)yl cysteine sulfoxides have been proposed. Early biosynthetic studies also showed that the uptake of labelled sulphur compounds resulted in many labelled \( \gamma \)-glutamyl peptides as well as alk(en)yl cysteine sulfoxides. These early studies gave rise to an important question concerning the biosynthetic relationship between \( \gamma \)-glutamyl peptides and the alk(en)yl cysteine sulfoxides. Lancaster & Shaw (1989) used a pulse chase experiment (\( ^{35} \)SO\(_4\)) to investigate the sequence of appearance of label in sulphur compounds in order to determine the biosynthetic relationship between flavour precursors and the \( \gamma \)-glutamyl peptides. Their work, performed on excised leaves, indicated that within the first 24h after the leaves were exposed to \( ^{35} \)S the label appeared predominantly in the fractions containing \( \gamma \)-glutamyl peptides. After 24h, as the amount of label declined in the \( \gamma \)-glutamyl peptides, the amount of label in the alk(en)yl cysteine sulfoxides fractions began to increase, thus indicating in this single experiment that the \( ^{35} \)S label was incorporated into the \( \gamma \)-glutamyl peptides before the alk(en)yl cysteine sulfoxides. This scheme was confirmed by Parry & Lii (1991) who showed that addition of \( \gamma \)-glutamyl cysteine to methacrylic acid gave rise to \( \gamma \)-glutamyl-S-2-carboxypropylcysteine which in onion undergoes sequential decarboxylation to \( \gamma \)-glutamyl-S-1-propenyl cysteine, oxidation to \( \gamma \)-glutamyl-S-1-propenylcysteine sulfoxide and finally cleavage by \( \gamma \)-glutamyl transeptidase to PrenCysSO.

More recently however, Ohsumi et al. (1993) have shown that in differentiating tissue cultures of garlic AlICysSO is formed by the oxidation of AlICys in agreement with the pathways proposed by Suzuki et al. (1961, 1962), Sugii et al. (1963), Granroth (1970) and Turnbull et al. (1980). Thus there are two main pathways proposed so far for the synthesis of PrenCysSO. The first involves no \( \gamma \)-glutamyl precursors (as reported by Suzuki et al. 1961, 1962 and Sugii et al. 1963) and Granroth (1970) while the second pathway is based on the presence of \( \gamma \)-glutamyl alkenyl cysteine compounds as the precursors (Lancaster & Shaw 1989). It is of course possible that both biosynthetic pathways are functional.

Much of the previous work that has been performed on the biosynthetic route has been done on excised plant organs and cell tissue culture systems. To our knowledge no one has yet made a detailed study of the dynamics of the complex interaction between the two chemical groups in the intact plant. Excised leaves are effectively senescent tissue and undifferentiated tissue cultures are meristematic tissue so that the results obtained from such material may be of limited relevance to the healthy intact plant. The work presented in this paper was obtained by pulse labelling (\( ^{35} \)SO\(_4\)) on intact growing plants in an attempt to resolve the relative importance of \( \gamma \)-glutamyl peptides in the biosynthesis of the sulfoxides. In addition we present data on the compartmentation of the flavour precursors and their intermediates in the cell.

Materials and methods

Plant material

Onion sets of variety Stuttgarter Riesen were obtained from Bridgemere Garden World, Cheshire, U.K. and stored at 4 °C in the dark until used. Sets were encouraged to shoot by supporting them above water for 1 week as described in Edwards et al. 1994. Water was replaced with Hoaglands and Arnon solution for 1 week then fresh culture solution was added which contained \( ^{35} \)SO\(_4\) (in the form Na\(_2^{35}\)SO\(_4\), obtained from Amersham International PLC, Buckinghamshire, U.K.). When shoots were labelled prior to fractionation the plants were exposed to the same radioactive source for 7 days before being excised and the shoot material was used for preparation of protoplasts. For the pulse labelling experiments, onion sets were exposed to the label for a total of 24h, then transferred to fresh nutrient solution. Two plants were harvested at 18h, 24h, 28h, 67h and 73h after the initial exposure to radioactive solution then tissue was taken from the shoots, roots, outer brown scale, middle fleshy scale and the inner.