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RELATIONSHIP OF ENDOGENOUS SUBSTRATE TO SPECIFICITY OF *S*-ALKYL CYSTEINE LYASES OF DIFFERENT SPECIES

MENDEL MAZELIS* and LESLIE FOWDEN
Department of Botany and Microbiology, University College, London WC1E 6BT

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Key Word Index—*Acacia georginae*; *Albizzia julibrissin*; Leguminosae; *S*-alkyl cysteine lyase.

Abstract—The presence of *S*-alkyl cysteine lyases was established in germinating seedlings of *Acacia georginae* and *Albizzia julibrissin*. The enzymes were present in both the cotyledons and the radicle (hypocotyl and root). The specific activity of enzyme in the latter organ was much higher than in the cotyledon. The lyase of each species showed greater affinity for those alkyl cysteine derivatives peculiar to the particulal species.

INTRODUCTION

THE SEEDS of many species in the Leguminosae contain a variety of *S*-alkyl cysteine derivatives among their non-protein amino acids.[1] Compounds of this type form prominent seed constituents in the closely-related genera *Acacia*[2] and *Albizzia*.[3] Germination of these plants is frequently associated with the production of strong odours suggestive of volatile sulphur compounds, and a C–S lyase cleaving *S*-alkyl cysteines and their sulphoxides has been partially purified from *Albizzia lophanta* seeds.[4] The cleavage products of an *S*-alkyl cysteine were identified as the related alkyl mercaptan, pyruvate and ammonia, and the reaction may be summarized as:

$$R\text{–}S\text{–}CH_2CH(NH_2)COOH + H_2O \rightarrow RSH + MeCOCOOH + NH_3.$$

The *Albizzia lophanta* enzyme utilized L-djenkolic acid, a natural constituent of the non-protein nitrogen pool of the species, most rapidly. However, the enzyme split *S*-carboxy-ethyl-L-cysteine far less readily, although this compound has been found in a number of *Acacia* spp.[2] and in *Albizzia julibrissin*.[3]

No information is available on the presence of this enzyme in other species of *Albizzia* or *Acacia*. The present report shows that *Acacia georginae* and *Albizzia julibrissin* contain C–S lyase activity and compares the levels of enzyme present in cotyledons and radicles [hypocotyl + root] after germination. The activity of the lyase from each species was determined in relation to the species' own sulphur amino acids and towards substrates characteristic of other species.

* Present address: Department of Food Science and Technology, University of California, Davis, California, CA 95616, U.S.A.

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RESULTS AND DISCUSSION

The distribution of the *S*-alkyl cysteine lyase among the various organs of *Acacia georginae* and *Albizzia julibrissin* seedlings is shown in Table 1; the enzyme shows a much higher specific activity in the hypocotyl and root than in the cotyledon. However, the cotyledon contains so much more protein than the other tissues that, in the case of *Albizzia ulibrissin*, the total lyase activity present in cotyledons is double that in the rest of the seedling.

TABLE 1. DISTRIBUTION OF *S*-ALKYL CYSTEINE LYASE IN FOUR-DAY-OLD SEEDLINGS OF *Acacia georginae* AND *Albizzia julibrissin*

Organ	Specific activity (milliunits per mg) *Acacia georginae*	*Albizzia julibrissin*
Cotyledon	67	57
Hypocotyl	400	443*
Root	583	

The substrate was L-djenkolic acid sulphoxide.
* This result was obtained using enzyme isolated from hypocotyl + root.

A new sulphur-containing amino acid, *S*-(2-hydroxy-2-carboxyethylthiomethyl)-L-cysteine ($C_7H_{13}O_5NS_2$) has recently been isolated from *Acacia georginae* seed.[5] Structurally, this new compound is intermediate between djenkolic acid and dichrostachinic acid. The latter is also a prominent component of the seed extracts of this species. *Albizzia julibrissin* does not contain detectable amounts of dichrostachinic acid or the new amino acid but does have considerable *S*-carboxyethylcysteine and *S*-carboxyisopropylcysteine. The activity of the lyase of *Acacia georginae* with various cysteine derivatives was tested. All of these compounds are found in the non-protein nitrogen fraction of *Acacia georginae* seed extracts except for *S*-carboxyethylcysteine. The latter compound was only utilized by this enzyme

TABLE 2. K_m VALUES AND SPECIFIC ACTIVITIES FOR VARIOUS SUBSTRATES WITH CYSTEINE LYASES

Compound	K_m (mM)	*Acacia georginae* Specific activity* (milliunits/mg)	*Albizzia julibrissin* K_m (mM)
L-Djenkolic sulphoxide	9·1	1780	12
L-Dichrostachinic acid	4·8	960	4·8
S-(2-Hydroxy-2-carboxyethylthiomethyl)-L-cysteine	1·6	1180	3·5
S-Carboxyethyl-L-cysteine	50	150	1·0
L-Djenkolic acid		1380	

at 8% of the rate measured for djenkolic acid sulphoxide. In contrast the lyase from *Albizzia julibrissin* exhibited the same activity with both compounds.

The Michaelis constants of various substrates with the lyase of both species were deter-

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mined (Table 2). The K_m values for djenkolic acid and its sulphoxide were similar for both enzymes, whilst the K_m for *S*-(2-hydroxy-2-carboxyethylthiomethyl)-L-cysteine, found in *Acacia georginae*, was somewhat lower with the *Acacia* enzyme than with the *Albizzia* lyase. However, the most striking finding was the large difference in the Michaelis constants for *S*-carboxyethylcysteine determined with the two enzymes. The K_m for the *Albizzia julibrissin* lyase is many times lower than that for the *Acacia georginae*. It appears that each lyase has a greater affinity for the substrate found endogenously in the species from which the enzyme was extracted.

The physiological role of the enzyme is uncertain. It is known that volatile sulphur compounds are toxic to many fungi: for instance, mercaptans and sulphides are toxic to *Colletotrichum circinans*[6] and *Botrytis allii*.[6] Mercaptans also prevent the germination of sclerotia of *Sclerotium cepivorum*.[7] During the early germination period while the cotyledon, hypocotyl and radicle are in the soil, they are particularly susceptible to invasion by soil pathogens. The breakdown of the *S*-substituted cysteines by lyase action might release compounds having fungistatic or fungicidal activity and so contribute to the successful establishment of the seedling.

EXPERIMENTAL

Enzyme preparation. Seeds from *Acacia georginae* and *Albizzia julibrissin* were germinated in the dark at 30°. After 4–5 days the plants were harvested and the cotyledons separated from the hypocotyl and root. Each organ was ground in the cold with 0·05 M Tris buffer pH 7·5. The ground material was filtered through muslin and the filtrate centrifuged for 15 min at 17000 *g*. The supernatant solution was then decanted and either dialyzed against 0·05 M Tris pH 7·5 for 4 hr or passed through a Sephadex G25 column to remove endogenous substrates.

Reaction mixture. The usual reaction mixture consisted of the following components: Tris pH 8·0, 60 mM; pyridoxal-5′-phosphate, 25 mM; *S*-alkyl cysteine, 100 mM; enzyme solution. The final volume was 1·0 ml. Incubation was carried out at 25° and the reaction terminated at the desired time by the addition of 3 ml 10% trichloroacetic acid.

Assays. Pyruvate was assayed by the total keto acid method of Friedemann and Haugen.[8] A unit of enzyme activity produces 1 μmol of pyruvate per min under the conditions of assay. Protein was determined by either a biuret assay[9] or spectrophotometrically.[9]

Chemicals. The substrates used were natural isolates. Other chemicals were obtained from commercial suppliers.

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