Phytochemistry, 1972 Vol 11, pp 167 to 169 Pergamon Press Printed in England

SHORT COMMUNICATION

RHODANESE OF TAPIOCA LEAF

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(*Received* 11 *May* 1971)

Abstract—Rhodanese activity was detected in a crude extract of tapioca (*Manihot utilissima*) leaves Optimal activity was found at a high pH (10 2–11 0) and temperature (57–59°) Under these conditions, rhodanese from 0 5 ml of the crude extract (75 mg leaf fr wt) catalysed the formation of 10 2 μmoles thiocyanate per 15 min

INTRODUCTION

RHODANESE (thiosulphate cyanide sulphur transferase, E.C 2 8 1 1) catalyses the formation of thiocyanate from free cyanide and a sulphur donor [1] The sulphur donor *in vivo* is believed to be thiosulphate[2] ($S_2O_3^{2-}$), although other –S–S⁻ compounds can be effective sulphur donors [3] Rhodanese is present in animal tissues where it is thought to be involved in cyanide detoxification, the highest levels being found in the liver.[4] The enzyme has been crystallized from ox liver by Sorbo,[5] and from ox kidney by Westley and Green [6] Rhodanese has also been found in a variety of bacterial species [7] The enzymatic formation of thiocyanate in several plants has been reported previously by Gemeinhardt [8] However, whether the formation of thiocyanate in this case is due to the presence of a plant rhodanese as claimed by Gemeinhardt,[8] or to the enzymatic degradation of the mustard oil glucosides,[9] now known to exist in several of the plants examined, is not known This communication describes some of the properties of crude tapioca (*Manihot utilissima*) leaf rhodanese

Tapioca is a cyanophoric plant widely cultivated in the tropics.[10] The cyanogenic glucoside, linamarin, is distributed in all parts of this plant[2,4,11,12] which, on treatment with dilute acid or the appropriate hydrolytic enzymes, yields equimolar amounts of hydrogen cyanide (HCN), glucose, and acetone [12] Previous studies on the metabolism of cyanogenic glucosides and related compounds have revealed the assimilation of HCN by both cyanophoric and non-cyanophoric plants into asparagine [12,13] From the work described here it would seem that the conversion of cyanide to thiocyanate could be equally important in cyanide detoxification This will be discussed

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RESULTS AND DISCUSSION

The effect of temperature on tapioca rhodanese activity was studied between 20 and 70° A high temperature optimum of 57–59° was observed for this enzyme A temperature optimum of 50° has been reported for ox liver rhodanese [14] *Ferrobacillus ferrooxidans* has a temperature optimum of only 25° [7] The pH optimum of tapioca rhodanese was determined to be between 10·2–11·0 and the enzyme was almost inactive below pH 8 Optima pH of 8–9 and 7 5–9 0 have been reported for the ox liver[14] and *F ferrooxidans*[7] rhodanese, respectively.

Rhodanese in tapioca leaf crude extract appeared to be extremely stable No appreciable loss of activity was found after storing the crude extract at 4–10° for more than a month Fresh crude extract incubated at 57° for nearly 10 hr showed only about 30% loss of activity in assay

Rhodanese in 0·5 ml of tapioca leaf crude extract (75 mg leaf fr wt) catalysed the formation of 10·2 μmoles thiocyanate in 15 min. At the high temperature used in the rhodanese assay, some 1·1 μmoles thiocyanate was found to be formed non-enzymatically However, non-enzymatic thiocyanate formation was negligible at room temperature (25–29°) From the absorption spectra of thiocyanate formed under optimal conditions, an absorption maximum of 460 nm for the thiocyanate-ferric nitrate colour complex was determined. (Fig 1)

It would appear that the activity of tapioca rhodanese at normal growth temperature and original pH of the crude extract would only be a minute percentage of that determined here

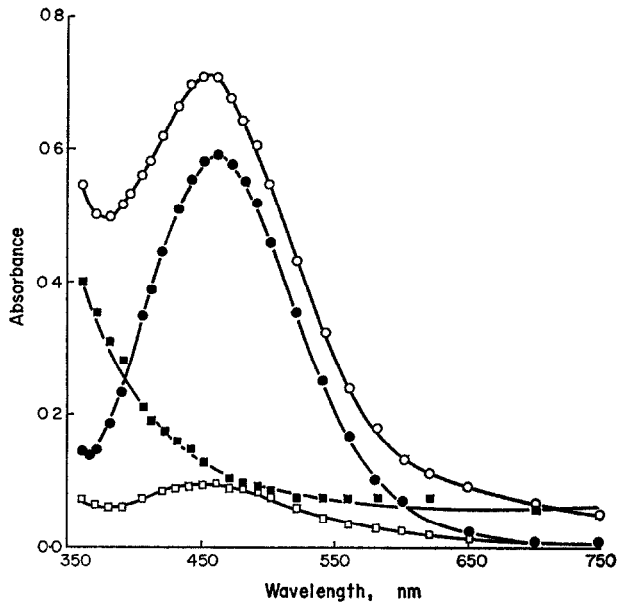

FIG 1 ABSORPTION SPECTRA OF THIOCYANATE–FERRIC NITRATE COMPLEX
Reaction mixtures, incubated at 57° and pH 11 0, contained the following reagents 0 125 M KCN (1 0 ml), 0 125 M $Na_2S_2O_3$ (1 0 ml) and 0 2 M arginate buffer (1 0 ml) together with (1) □—□ water (0 5 ml), (2) ○—○ enzyme (0 5 ml crude extract) and (3) ■—■ denatured enzymes (0 5 ml crude extract in 0 5 ml 35% w/w formaldehyde) (4) ●—● is curve (2) minus (3)

[14] B H SORBO, *Acta Chem Scand* **7**, 1137 (1953)

at high temperature and pH It can be calculated, however, that there would be sufficient rhodanese activity in tapioca leaves to assimilate any free cyanide released by cyanogen hydrolysis in the cellular microenvironment (the total cyanide content of young tapioca leaf ranging from 174 to 622 ppm fr. wt [15]) This would suggest a role for rhodanese in cyanide detoxification in this plant No free cyanide was detected in the freshly prepared crude extract Alternatively, it has been suggested that the HCN liberated from the hydrolysis of cyanogenic glucosides in plants is detoxified by conversion to the amide carbon and nitrogen of asparagine and thereby conserving the nitrogen atom [12,13] It would be of interest, therefore, to know whether rhodanese is widely distributed in both cyanophoric and non-cyanophoric plants and what is the relative physiological significance of these two mechanisms of cyanide detoxification *in vivo*

EXPERIMENTAL

Preparation of the crude enzyme extract Freshly harvested young tapioca leaves of the *kekabu* variety were washed with H_2O and blotted dry with filter paper Petioles and mid-ribs of the leaves were removed from the leaf blades and discarded Leaf blades were pooled, weighed, and ground in acid-washed sand in a minimum amount of H_2O using, a mortar and pestle The homogenate together with several washings was made up to 100 ml with H_2O for 15 g of fresh material Leaf debris in the homogenate was removed by passing the extract through cheesecloth The crude extract obtained was used for the enzyme studies The pH of this crude extract was 6 2

Enzyme assay Rhodanese activity of the crude extract was determined by the amounts of thiocyanate formed from cyanide and $S_2O_3^{2-}$, according to a modified method of Sorbo [5] Substrates and the crude extract were equilibrated at the reaction temperature before mixing Substrates were not buffered in the determination of the temperature optimum of rhodanese The pH of these reaction mixtures was 8 7 In the determinations of the pH optimum, substrates were buffered in 0 2 M Tris-HCl for pH range 7–10 and in 0 2 M arginate for pH range 10–12

Reaction mixture was incubated for 15 min and the reaction was stopped by the addition of 35% (w/w) HCHO (0 5 ml) Ferric nitrate reagent (2 5 ml per assay) (containing 50 g $Fe(NO_3)_3\,9H_2O$ and 525 ml HNO_3 S G 1 40, and H_2O to 2 l) was added to give the colour reaction The reddish brown solution was then diluted with 25 ml H_2O A fine suspension often appeared and was removed by filtration

Extinction was read at 460 nm against H_2O A value of $\epsilon^{1\,cm}_{mM}$ 55 8 was used to calculate thiocyanate concentration A control reaction was carried out by mixing the crude extract with formaldehyde before the addition of substrates Rhodanese activity is expressed in μmoles thiocyanate per 0 5 ml crude extract

Acknowledgements—Tapioca leaves were generously supplied by the Faculty of Agriculture We are grateful to Dr Eric E Conn for his valuable discussion This work was supported in part by a grant from the China Medical Board (70-135)

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Key Word Index—*Manihot utilissima*, Euphorbiaceae; tapioca, rhodanese, thiosulphato, cyanido sulphur transferase