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RHODANESE IN HIGHER PLANTS

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Key Word Index—Cyanophoric and non-cyanophoric species; rhodanese; high pH and temperature optima.

Abstract—Rhodanese activity was detected in crude leaf extracts of 12 randomly selected plant species consisting of 9 non-cyanophoric and 3 cyanophoric species. In each case, the enzyme exhibited high activity at pH 10·4 and 55°. There appeared to be no correlation between rhodanese activity and the cyanophoric nature of the plant.

INTRODUCTION

IN A previous paper, we reported rhodanese (thiosulphate: cyanide sulphur transferase, E.C. 2.8.1.1) activity in crude leaf extract of tapioca (*Manihot utilissima*); the enzyme had high pH (10·2–11·0) and temperature (57–59°) optima.[1] A role for rhodanese in cyanide detoxification has been suggested since the cyanogens of tapioca on hydrolysis provide a readily available endogenous source of HCN.[1,2] However, the physiological significance of plant rhodanese is still unclear.

The work reported here was undertaken to find out whether rhodanese was present in both cyanophoric and non-cyanophoric species. With one exception, the plant species were selected randomly for investigation, and were obtained from the surrounding compounds of the Faculty of Medicine. Crude leaf extracts of 12 different plant species were assayed for rhodanese activity. *Hevea brasiliensis* leaves were kindly supplied by the Rubber Research Institute of Malaysia.

RESULTS AND DISCUSSION

Of the 12 species examined, 3 were found to contain cyanogens by the picrate paper test,[3] namely *M. utilissima*, *H. brasiliensis* and *Elasteriospermum* sp.

Rhodanese activity was detected in all 12 species, as shown in Table 1. Enzyme activity at pH 7·9 was generally low and the average difference in activity between 37 and 55° was not more than 2-fold. High activity was recorded for all the plant species at the more alkaline pH 10·4. At this pH, enzyme activity was also markedly raised by increasing the incubation temperature from 37 to 55°.

The highest rhodanese activity was observed in extracts of *Elasteriospermum* sp., while *Thevetia* sp. exhibited the lowest (see Table 1). The former species is cyanophoric but the latter is not. However, another cyanophoric species *H. brasiliensis*, under similar conditions, showed relatively low activity. It would seem, therefore, that there is no correlation between rhodanese activity and the cyanophoric nature of individual plant species.

Our results imply an ubiquitous distribution of rhodanese in higher plants. Since the

[1] CHEW, M. Y. and BOEY, C. G. (1972) *Phytochemistry* **11**, 167.
[2] NARTY, F. (1970) *Z. Pflanzenphysiol.* **62**, 398.
[3] BUTLER, G. W. and BUTLER, B. G. (1960) *Nature* **187**, 780.

enzyme is present in both cyanophoric and non-cyanophoric plant species, it is quite likely that it has a functional role other than, or in addition to, its role in cyanide detoxification. The possibility that rhodanese has a more important natural role in plants, such as in sulphur metabolism, merits attention.

TABLE 1. RHODANESE ACTIVITY OF SOME HIGHER PLANTS

Plant species	μmol thiocyanate per 15 min/g leaf fr. wt pH 7·9 37°	pH 7·9 55°	pH 10·4 37°	pH 10·4 55°
Cyanophoric				
Elasteriospermum sp.	11·3	5·6	31·8	74·3
Manihot utilissima (*kekabu variety*)	2·0	0·5	22·0	50·2
Hevea brasiliensis	3·6	0·5	3·1	24·1
Non-cyanophoric				
Cassia spectabilis	10·8	12·3	14·9	42·0
Duranta sp.	1·3	2·0	7·7	24·1
Artocarpus lanceifolius	5·1	6·7	38·9	62·5
Mesua ferrea	10·8	6·7	4·1	39·5
Pentaspadon officinale	7·2	2·6	22·5	46·1
Allamanda sp.	1·5	1·5	8·7	25·1
Canangium odoratum	0·5	1·0	7·7	21·0
Acacia sp.	1·0	1·0	35·9	51·8
Thevetia sp.	0·5	1·0	3·6	8·7

Results are the average of duplicate assays.

EXPERIMENTAL

Picrate paper test. Alkaline picrate paper was used to detect the presence of cyanogens in leaves.[3] Only young leaves were used. A folded leaf, or portion of a leaf, was inserted into a small vial (5 × 1·5 cm) and 2 drops of toluene were added. A piece of picrate paper strip (5 × 0·5 cm) was then placed in the vial. The vial was closed with a screw cap such that the paper strip was held marginally above the leaf. Samples in the vials were incubated at 35° for 24 hr. In a positive reaction, the yellow paper strip turned orange to brick-red in colour.

Preparation of crude leaf extract. Freshly harvested young leaves were used to prepare the extracts for rhodanese assay. Leaves were washed with H_2O and blotted dry between filter papers. The petioles and mid-ribs of leaves were removed from leaf blades and discarded. Leaf blades were pooled, weighed and ground to powder in liq. N_2 with a mortar and pestle. For every 5 g leaf material, 10 ml of 0·1 M mannitol in 0·1 M borate buffer at pH 7·2 were used for enzyme extraction. This extraction medium was used to preserve the cell organelles intact.[4] Cell debris and the intact cell organelles in the leaf homogenate were removed by centrifugation at 30 000 *g* for 1 hr, since plant rhodanese is located in the cytosol.[5] The clear supernatant solution so obtained was the crude leaf extract and was pale yellow in colour in all cases except *Canangium odoratum*, in which it was purplish.

Enzyme assay. Rhodanese activity of crude leaf extract was determined by measuring the amounts of thiocyanate formed from CN^- and $S_2O_3^{2-}$ according to a modified method of Sörbo.[1,6] Enzyme activity was assayed at pH 7·9 and 10·4, each at two different incubation temps, 37° and 55°. Substrates were buffered in 0·1 M Tris-HCl for pH 7·9, and in 0·1 M arginate for pH 10·4. Reaction mixtures contained the following reagents: 0·5 ml leaf extract, 1·0 ml buffered 0·125 M KCN and 1·0 ml 0·125 M $Na_2S_2O_3$.

[4] BONNER, JR., W. D., (1965) in *Plant Biochemistry* (BONNER, J. and VARNER, J. E., eds.), p. 89, Academic Press, New York.
[5] BOEY, C. G. (1972) M.Sc. Thesis, University of Malaya.
[6] SÖRBO, B. H. (1953) *Acta Chem. Scand.* 7, 1129.

After incubation (15 min), the reaction was stopped by the addition of 35% (w/w) HCHO (0·5 ml). Ferric nitrate reagent (2·5 ml per assay) was added to give the colour reaction.[1] The reddish brown solution was then diluted with 25 ml H_2O. Extinction was read at 460 nm against H_2O. A value of ϵ^{1cm}_{mM} 55·8 was used to calculate thiocyanate concentration. A control reaction was carried out by mixing the crude extract with HCHO before the addition of substrates. Rhodanese activity is expressed in μmol thiocyanate per 15 min/g leaf fr. wt.

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