Pertanika 5(1), 123–125 (1982)

COMMUNICATION (IV)

Enzymes of *Hevea brasiliensis* latex. Adenylate Kinase, Sulphate Adenylyltransferase (ATP-sulphurylase) and Thiosulphate Sulphurtransferase (Rhodanese)

RINGKASAN

Penyiasatan fasa serum dari lateks Hevea brasiliensis *mendalilkan kewujudan ketiga enzim yang berikut: adenilat kinase EC 2.7.4.3; sulfat adenililtransferase (ATP-sulfurilase) EC 2.7.7.4; tiosulfat sulfurtransferase (rodanese) EC 2.8.1.1.*

INTRODUCTION

The latex within the vessels of *Hevea brasiliensis* is cytoplasmic and the material obtained by tapping the tree is an exceptionally attractive object for biochemical study. It contains numerous organelles of several kinds, the main ones being rubber particles, lutoids and Frey-Wyssling complexes. These are all suspended in a fluid known conventionally as latex serum and corresponding with the cytosol in the latex vessels (see review by Gomez and Moir, 1979). Many enzymes have been studied in *Hevea* latex, for example those involved in glycolysis (d'Auzac and Jacob, 1969; Bealing, 1969) and rubber biosynthesis (Archer and Audley, 1967; Lynen, 1969) and the acid hydrolases occurring in the lutoids, which identify these organelles as a kind of lysosome (Pujarniscle, 1968, 1971). Recent advances include the finding that two basic proteins characteristic of lutoids are lysozymes (Tata *et al.*, 1976) and the purification of a magnesium-dependent phosphatase from the serum phase (Souciet *et al.*, 1980).

An unpublished survey by Dr B.G. Audley and one of us (G.F.J.M.) shows that seventy enzymes have been described in *Hevea brasiliensis* latex. This communication reports the detection of the three further enzymes listed in the title.

MATERIALS AND METHODS

Latex was collected from *Hevea brasiliensis* trees of clone RRIM 600 growing in 'Ladang 8' of the farm of Universiti Pertanian Malaysia. These trees had been planted in about 1967; at the time of the work described here they were being tapped on the second half-spiral panel (Panel B), three times a week (Tuesday, Thursday, Saturday). After each tree was tapped the latex was run to waste for 3 min, then collected for 30 min into a flask in an ice bath. The latex from nine trees was pooled and a portion centrifuged at 2° for 40 min at 25,000 r.p.m. (58,000 g_{max}) in rotor No 65 of a Beckman L5-65 ultracentrifuge. The latex separated into the rubber cream, a sediment ('bottom fraction') and the serum phase ('C serum') essentially as described by Moir (1959). The tubes were punctured to recover the C serum: the lower, less turbid portion was used.

Adenylate kinase, EC 2.7.4.3, was assayed by the method of Aminuddin (1974). The reaction mixture (1 ml) contained TRIS-HCl (35 μmol; pH 7.5), $MgCl_2$ (0.5 μmol), ADP (0.4 μmol) and C serum (0.05 ml). It was incubated for one min at 30° after starting the reaction by adding ADP. 1.0 ml 5% (v/v) perchloric acid was then added; after centrifugation for 5 min at about 3000 g 0.1 ml of the supernatant was mixed with 1.9 ml of ice-cold water and ATP determined in an aliquot of the diluted sample using the continuous bioluminescence assay method of Balharry and Nicholas (1971) as modified by Aminuddin and Kooi (1980).

Sulphate adenylyltransferase (ATP-sulphurylase), EC 2.7.7.4, was assayed by the continuous bioluminescence method of Balharry and Nicholas (1971) as modified by Aminuddin and Kooi (1980).

Thiosulphate sulphurtransferase (rhodanese), EC 2.8.1.1, was assayed by measuring the thiocyanate formed from cyanide and thiosulphate according to the method of Sörbo (1955) with some modifications. The substrates were buffered in 0.1 M borate, pH 10.5; substrates and C serum were equilibrated at 47° before being mixed and incubated at the same temperature for 10 min. The reaction was stopped by the addition of 35% (w/w) formaldehyde, followed by the ferric

nitrate reagent for colour development; in the control, formaldehyde was added to the substrates before C serum.

Protein was estimated in C serum directly, without prior precipitation, using the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Brzozowska *et al.* (1974) have reported the presence of some free aromatic amino acids in C serum, while Tata (1980) has shown that the use of bovine serum albumin as a standard when the Lowry method is applied to C serum proteins results in values considerably above the true level. On both counts, therefore, the procedure we used overestimates the protein content of C serum. Nevertheless it was considered adequate for the purposes of this preliminary study.

RESULTS AND DISCUSSION

All three enzymes were found in the C serum from latex. Table 1 shows typical figures for their activities.

We have not yet investigated any differences in levels of activity that may occur between clones or with season. We are mainly concerned here with the occurrence of these enzymes in latex. However, the ATP-sulphurylase has been studied further: the results will be published separately.

The thiosulphate sulphurtransferase is notably stable. At 57° the enzyme in C serum had almost twice the activity shown in the assays at 47°. There also seemed to be no loss of activity in C serum kept frozen for a week. In these properties the enzyme resembles the thiosulphate sulphurtransferase reported by Chew and Boey (1972) in the leaves of tapioca *(Manihot utilissima). H. brasiliensis* and *M. utilissima* both belong to Euphorbiaceae and both contain the same cyanogenic glycoside, linamarin (Gorter, 1912).

ACKNOWLEDGEMENTS

We thank Dr Idris Abdol, Director of the University Farm, and his staff for supplying the rubber trees and for help in tapping them. We also thank Cik Chew Mee Li, Encik Arifen Kamarudzaman and Encik Mior Ahmad Alang Mior for their skilled technical assistance and Cik Hafizah bte Abdul Kadir for typing the manuscript.

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TABLE 1

Enzyme activities and protein content of C serum of latex.

All measurements on each sample were made on the day the latex was collected. Dates of collection are shown. Values are means of duplicate or triplicate determinations except for ATP-sulphurylase where four and five determinations were averaged for samples 1 and 2 respectively.

Enzymes detected	C serum samples 1 (28/10/80)	2 (4/11/80)	3 (11/11/80)
		Activity in pkat/mg/protein	
Sulphate adenylyltransferase (ATP-sulphurylase)	39	41	
Adenylate kinase	196	187	
Thiosulphate sulphurtransferase (Rhodanese)		55	64
		Protein content in mg/ml C serum	
	16.8	15.5	16.3

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(Received 25 March 1981)