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CHARACTERIZATION OF TONOPLAST PYROPHOSPHATASE FROM *HEVEA BRASILIENSIS* LATEX

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A pyrophosphatase (PPase) is evidenced on the lutoidic membrane in *Hevea brasiliensis* latex. Presence of Mg^{2+} is needed during the membrane washing, to preserve the tonoplastic enzyme activity. PPase functioning is Mg^{2+}-dependent. Mg^{2+} and pyrophosphate (PPi) K_m are measured. The substrate affinity is high (50 µM). It is relatively thermosensitive. Many products were tested on the PPase functioning. K^+ is a strong activator. It increases the reaction activity but does not modify the enzyme affinity. Among the monovalent cations, only Rb^+ has a similar effect. No divalent cation can take the place of Mg^{2+}, but some of them such as Ca^{2+} or Mn^{2+} are inhibitors. Inhibition induced by cysteine, pCMB and NEM indicates the importance of SH groups in the enzyme active site. DIDS which breaks anion transport, inhibits the enzyme. DCCD and $NaNO_3$, inhibitors of lutoidic ATPase, have no effect on the lutoidic PPase. Taking the results into account, it is possible to compare latex tonoplastic and cytosolic PPases. The physiological role of this tonoplastic PPase is discussed.

Key words : *Hevea brasiliensis*, Pyrophosphatase, Lutoid, Tonoplast, Latex, Rubber synthesis.

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INTRODUCTION

Intracellular regeneration of latex between two tappings results in strong metabolic activity in the laticifers, especially intense synthesis of isoprene, and accounts for 30 to 50 per cent of the fresh weight of latex and 90 per cent of dry weight of latex (Compagnon, 1986). During isoprenic synthesis, pyrophosphate (PPi) is released into the medium whenever rubber transferase transfers an isopentenyl pyrophosphate (IPP) lined to the elongating chain of the polymer (Archer and Audley, 1969). PPi accumulation can inhibit anabolic processes (Kornberg, 1962; Amir and Cherry, 1971) and enzymatic systems prevent this from taking place. The process involves either transferase enzymes capable of using PPi as Pi posphate donor, or hydrolase enzymes cutting it in 2 Pi. Concerning the first ones, fructose-6-phosphate 1-phosphotransferase (PP-PFK:EC 2.7.1.90) was found in rubber tree latex (Prevot *et al.*, 1987). Concerning the second, acid phosphatases in the lutoid (Pase : EC 3.23.2.) (Jacob and Sontag, 1974), and a specific cytosolic inorganic pyrophosphatase (PPase : EC 3.6.1.1) (Jacob *et al.*, 1989) are present in the cytosol. The presence of a PPase has been reported in tonoplasts of many plant cells (Walker and Leigh, 1981; Rea and Poole, 1985; Schumaker and Sze, 1987). The present work shows the presence of this enzyme on the lutoidic membrane which is a latex cell tonoplast (d'Auzac *et al.*, 1989) and confirms recent results (Prevot *et al.*, 1991). A number of characteristics of the enzyme are determined and its physiological role in latex is discussed.