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MODIFICATION OF pH OF LATEX CYTOPLASM BY ETHYLENE

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Key Word Index—*Hevea brasiliensis*; Euphorbiaceae; rubber tree; latex; regulation of cytoplasmic pH; ethylene.

Abstract—In the absence of glycolytic activity the pH of isolated latex cytoplasm of *Hevea brasiliensis* increases progressively during the first hours of incubation. This increase is greatly enhanced after bark treatment with 2-chloroethylphosphonic acid. The process of alkalization is not enzymic and is oxygen dependent. This observation is discussed in relation to the increase in latex pH *in situ* under hormone action.

INTRODUCTION

The treatment of bark of the rubber tree with synthetic auxins or with 2-chloroethylphosphonic acid (CEPA) brings about an increase in latex pH which results in important changes in the activity of some key enzymes of carbohydrate catabolism in latex cytoplasm [1–4]. It enhances invertase activity and, consequently, glycolysis [1–3], and results in a decrease in the activity of pyruvate decarboxylase and an increase in the activity of phosphoenolpyruvate (PEP) carboxylase, which may modify the direction of the glycolysis-fed metabolic activities [4]. There is evidence that these pH-related changes in carbohydrate catabolism are of fundamental importance in the action of growth regulators on latex yield [1, 3]. The present paper is a contribution to the study of the mechanism of pH changes in latex produced by CEPA treatment.

RESULTS

During the initial time of incubation of isolated cytoplasmic latex serum, its pH decreases as a result of the absence of mitochondria and the accumulation of organic acids formed through glycolytic activity. In latex vessels the mitochondria are concentrated in parietal cytoplasm and few are expelled with latex on tapping [5].

As expected, the stimulation of glycolytic activity in the latex, by treatment of the bark with CEPA [1, 2], enhances the acidification of the incubated serum (Figs. 1a, b; 2a). However, the inhibition of glycolysis by NaF did not lead to pH stabilization but, surprisingly, resulted in a progressive rise in serum pH, this rise being several times greater after CEPA treatment (Figs. 1a, b; 2a). The initial pH of latex serum varies, in general, between pH 6.5 and 7.2 and in stimulated trees it does not increase by more than 0.4 in most cases [2–4, 6, 7]. The differences in the initial pH have, however, no significant effect on the changes observed *in vitro* in the absence of glycolytic activity (Table 1). This also follows from the observation that even after 12 hr bark treatment, i. e. at the time when the increase of latex pH *in situ* due to the action of ethylene is not yet detectable [6], the shift of pH to alkalinity during serum incubation in the presence of NaF is significantly enhanced (Fig. 1a). This effect grows stronger with time and after an interval of 6 days the increase in serum pH in the present experiment reached one unit in 2 hr of incubation, which is four times more than in serum from the control tree (Fig. 1b).

The increase in serum pH during the first 30 min after addition of NaF is generally slower than later on (Fig. 1a, b), and in some cases it was not observed at all (Fig. 2). This could be explained by the possible presence of glycolysis-derived metabolites at the time of inhibitor addition, initially enabling some metabolic activity leading to acidification. The possibility that microbial activity is involved in the increase in serum pH is made unlikely by the presence of NaF and by the observation that this increase is not affected by addition of sodium azide (Fig. 1c).

The increase of pH in incubated serum of both CEPA-treated and control trees could not be significantly modified by addition of NAD or NADH (5 μmol/ml; results not given here), which indicates that this increase does not consist of oxidation–reduction reactions involving these coenzymes. Further research on the nature of pH changes induced by inhibition of glycolysis has shown their independence of enzymic activities. The elimination of proteins by ultrafiltration on Amicon membrane PM 10 (Fig. 1d) and also heat inactivation of enzymes (Fig. 2b) had the same effect

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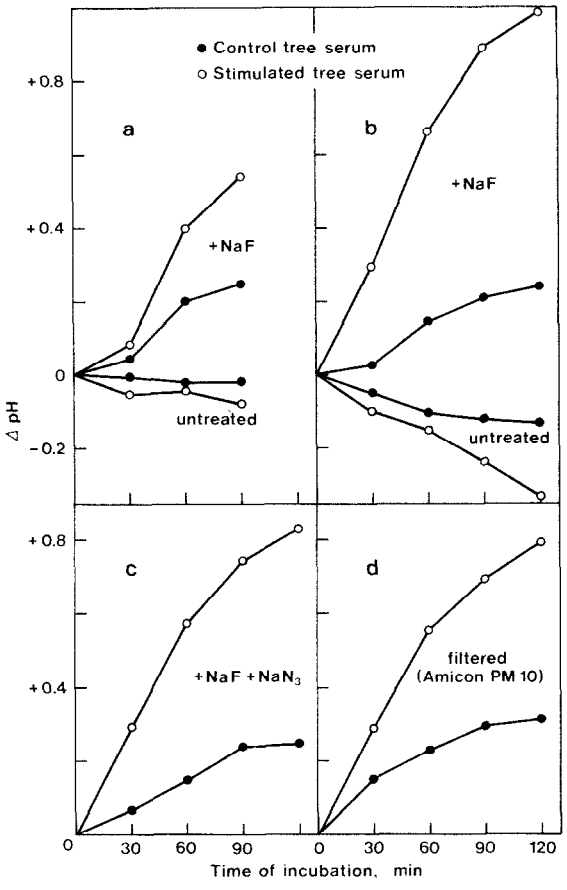

Fig. 1. Effect of CEPA on pH changes in incubated cytoplasmic latex serum 12 hr (a) and 6 days (b–d) after bark treatment. The serum of both control and stimulated trees was incubated without (untreated serum) and with NaF (a, b), in the presence of NaF and NaN_3 (c) and after elimination of high MW substances by ultrafiltration on Amicon membrane PM 10 (d). NaF and NaN_3 were added in dry state to 60 and 3 mM concentration, respectively. Before the experiment the trees were tapped on a full spiral cut once a week.

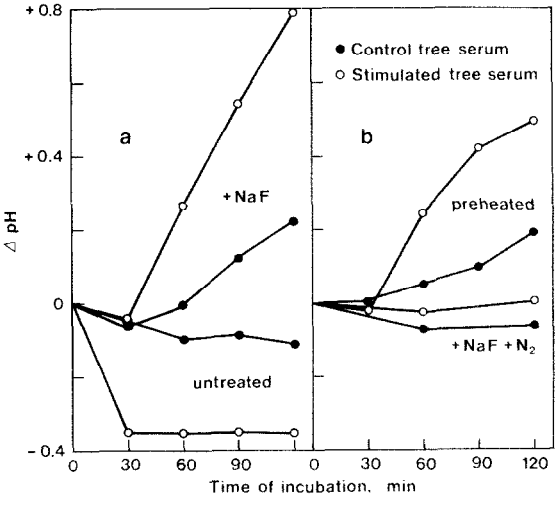

Fig. 2. pH changes during incubation of cytoplasmic latex serum of control and CEPA-stimulated trees; effect of NaF (60 mM) (a) of 10 min heating at 70° (b) and of serum saturation with nitrogen in the presence of NaF (b). Results on the latex taken 3 days after CEPA application on trees under regular tapping on a half spiral cut twice a week.

as inhibition of glycolysis by NaF. In both cases a rise in pH was observed without any modification of proportions between the serum of treated and control trees. The total increase in pH during the 2 hr of incubation was smaller than in the serum with NaF. This can be explained by the fact that heating itself caused an increase in pH in the serum of control and treated trees by 0.2 and 0.6 pH, respectively, and thus some diminution in the substances involved in the rise in pH. Also, some increase in pH occurred in the glycolytically inactive serum during ultrafiltration even at low temperature.

The changes in serum pH occurring in the absence of glycolytic activity can be partially inhibited by a reducing substance such as sodium metabisulphite (results not given here), and are completely stopped when the serum is incubated under nitrogen (Fig. 2b). Taken together, these results indicate that the increase in pH is the result of non-enzymic oxidation reactions.

Table 1. pH changes during incubation with NaF (60 mM) at 30° in cytoplasmic serum from control and CEPA-treated trees; examination of the dependence on initial pH

Serum of control tree pH			Serum of stimulated tree pH		
Initial	After 2 hr	Δ	Initial	After 2 hr	Δ
5.95	6.30	0.35	5.94	6.51	0.58
6.60	6.80	0.21	6.58	7.44	0.87
6.97	7.24	0.28	6.85	7.71	0.86
7.33	7.54	0.21	7.19	8.05	0.86

The initial pH of the serum was modified by microamounts of HCl or NaOH. The serum was separated from the first 30 ml latex fraction taken at the first tapping (6 days) after treatment.

DISCUSSION

There is some evidence that the control of pH in plant cytoplasm is based on regulation of the organic acid level through a metabolic pH-stat involving PEP carboxylase and malic enzyme [8, 9]. The pH activity curve of latex PEP carboxylase is consistent with this pH-stat scheme, as it exhibits a rapid increase in activity with the rise in pH [4] and the results described here show an enhancement of acidification of isolated latex cytoplasm at higher initial pH induced by CEPA treatment, this enhancement being also the result of an increase in glycolytic activity. However, in the latex *in situ*, the level of organic acids was not observed to rise at higher pH in CEPA-treated trees [10]. This indicates that the catabolism of organic acids in latex vessels rises parallel with the increase in their formation and suggests the existence of an additional mechanism of pH control operating especially under the effect of ethylene.

Such a mechanism may be related to the observation reported here, that the pH of isolated cytoplasmic serum increases after stopping glycolysis and that this increase is enhanced by bark application of CEPA. It is further shown that this effect is revealed before the rise in latex pH caused by the treatment. In other work, it was found that the primary known event in CEPA action is an increase in carbohydrate catabolism resulting from the fall in synthetic activity of sucrose synthetase [11], which precedes the described effect on pH *in vitro*. It seems probable that these events are related and that the stimulation of carbohydrate breakdown is the reason for increased formation, or entry of compounds, the transformation of which leads to the rise in pH *in vitro*, and to the later alkalization of latex *in situ*. This transformation is shown to be non-enzymic and oxygen dependent. The nature of these compounds is, however, not known and their identification is necessary to obtain a clearer concept of their suggested role in pH control in the cytoplasm.

EXPERIMENTAL

Plant material and treatment with CEPA. Regularly tapped adult rubber trees (*Hevea brasiliensis* Muell. Arg.) of the clone GT 1 were used for expts. The commercial prepn, Ethrel, was diluted with palm oil to a CEPA concn of 5% and thinly applied below the tapping cut to a 2 cm strip of scraped bark. Latex samples were collected under cooling with an ice bath and only the first 40 ml fraction was taken after normal opening of the tapping cut.

Isolation and incubation of latex cytoplasmic serum. The clear cytoplasmic serum was isolated from the latex by two refrigerated centrifugations. The first 15 min centrifugation at 10 000 rpm sedimented lutoid particles and the supernatant was recentrifuged 30 min at 30 000 rpm (Beckman rotor Ti 50) to separate the cytoplasmic serum from rubber particles. Serum samples (1 ml) were incubated in test tubes 16 mm (dia) shaken at 30°. pH of the serum was measured at 30 min intervals at the same temp.

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