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PHYSIOLOGICAL ACTIVATORS OF INVERTASE FROM *HEVEA BRASILIENSIS* LATEX

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Key Word Index—*Hevea brasiliensis*; Euphorbiaceae; latex; invertase activators; β-fructofuranosidase.

Abstract—Potassium or sodium nitrates or phosphates, and thiols such as reduced glutathione or cysteine, stimulate the activity of invertase from *Hevea brasiliensis* latex. These activators raise the V_{max} but do not affect the K_m of the enzyme for sucrose. The action of these effectors is additive. Their efficiency is pH dependent, being higher below pH 7.0 and markedly decreasing above it.

INTRODUCTION

The correlations established between activity of latex invertase from *Hevea brasiliensis* and the rubber yield of the tree [1–3] indicate that factors modulating *in situ* invertase activity may be of great importance to *cis*-polyisoprene regeneration between tappings of *Hevea*. Among other factors, slight physiological changes of the latex pH bring about large variations in enzyme activity which exhibits a narrow optimal pH range [2]. Hormonal treatments (auxinomimetics and Ethrel) used in rubber culture to improve production, have been shown to raise both the pH [2, 4–7] and invertase activity in the latex [2]. The influence of numerous natural components of latex on invertase activity has been tested, and the present work shows that this enzyme, in the cytosol [1, 8], is in contact with activators such as phosphates, nitrates and thiols, all of which are present in concentrations liable to influence invertase activity.

RESULTS AND DISCUSSION

Preliminary experiments indicated that, under the experimental conditions specified below, the invertase reaction was stoichiometric, linear with enzyme concentration and, for at least 30 min, linear with time. In 50 mM sodium cacodylate buffer (pH 6.8), sodium or potassium phosphate, sodium or potassium nitrate and cysteine activated latex invertase. Figure 1(A) shows the activation curves vs effector concentrations. The amount of compound necessary to obtain maximum activation, depended on the effector. The apparent activation coefficient (K_a) was estimated as 0.15 ± 0.05 mM for cysteine, 6 ± 1 mM for nitrates and 8 ± 1.5 mM for phosphates. In 50 mM phosphate buffer, at the same pH of 6.8, nitrates and cysteine also enhanced invertase activity.

Since neither cystine nor methionine significantly affected invertase activity, it seems that sulphydryl groups are involved in the activating mechanism. Nevertheless, all sulphydryl compounds were not equally effective. If, under given conditions, cysteine was taken as the reference for 100% activation, dithiothreitol had an estimated effect of 113%, dithioerythritol of 118%, reduced glutathione of 56% and 2-mercaptoethanol of 16%.

As Fig. 1(B) shows for sodium nitrate, none of the activators tested had any effect on the affinity of invertase for sucrose. Under these conditions, the K_m for sucrose, estimated at 6 ± 1 mM, was unchanged; only the V_{max} of the reaction was enhanced.

When the three types of activators were simultaneously added to the medium at saturation or near saturation an additive effect was observed. Table 1 shows relatively good agreement between experimental data and calculated data obtained by addition of the activation induced by each effector.

Table 2 shows that pH plays an important part in the effect of the activators on invertase activity. For all activators tested, this effect was considerable at pH 6.8, but greatly decreased at pH 7.2.

Although many invertase inhibitors of low MW as well as proteinaceous inhibitors have been identified [9–16], invertase activators are seldom mentioned in the literature. Matsushita and Uritani showed a positive effect of potassium nitrate, potassium fluoride and lithium chloride on alkaline invertase activity from sweet potato [17]. West *et al.* [18] noted that a low concentration of copper nitrate accelerated the activity of invertase from *Phytophtora megasperma* mycelium, and Sund and Linder [19] found that inorganic phosphate activated β-fructofuranosidase from *Streptococcus mitis*.

With regard to the invertase of the latex of *H. brasiliensis*, it is important to observe that although the activators tested had no effect on enzyme K_m, they did alter the reaction velocity. These effectors therefore act on the catalytic reaction, but not through enzyme–substrate binding. The fact that the activity of the enzyme might be regulated by several mechanisms, could be a reflection of its high MW [20].

The activators studied here are present in the

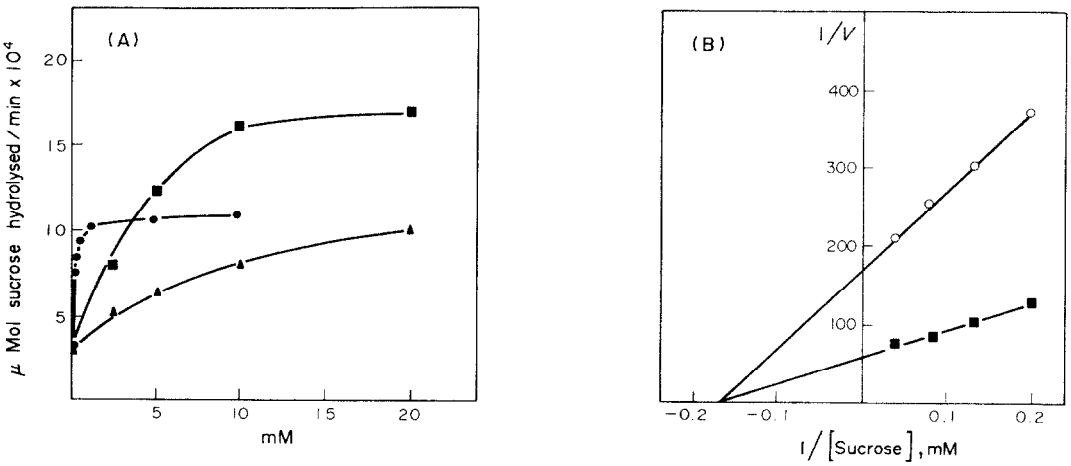

Fig. 1. Effect of activators on the activity of latex invertase from *H. brasiliensis* in 50 mM sodium cacodylate buffer, pH 6.8. (A) Enzyme activity related to different concentrations of activators: sucrose, 50 mM ■——■, sodium nitrate; ▲——▲, sodium phosphate; ●——●, cysteine. (B) Double reciprocal plot for sucrose hydrolysis by latex invertase with and without sodium nitrate: ○——○, control; ■——■, sodium nitrate, 2.5 mM.

Table 1. Additive effect of the latex invertase activators

	Activity (μmol/min)	
	Experimental data	Calculated data
Control	0.23	—
Sodium nitrate (10 mM)	1.08	—
Sodium phosphate (10 mM)	0.53	—
Cysteine (0.5 mM)	0.63	—
Sodium nitrate (10 mM) + sodium phosphate (10 mM)	1.44	1.38
Sodium phosphate (10 mM) + cysteine (0.5 mM)	1.06	0.93
Sodium nitrate (10 mM) + cysteine (0.5 mM)	1.60	1.48
Sodium nitrate (10 mM) +sodium phosphate (10 mM) +cysteine (0.5 mM)	1.83	1.78

Calculated data are obtained by the addition of the activator effect of each effector. Sodium cacodylate buffer, pH 6.8, was used.

Table 2. Efficiency of latex invertase activators at two different pHs using sodium cacodylate buffer, 50 mM

	pH 6.8		pH 7.2	
	Activity (μmol/min)	Activation (%)	Activity (μmol/min)	Activation (%)
Control	0.30	—	1.09	—
Sodium phosphate (2.5 mM)	0.48	+60	1.39	+27
Sodium nitrate (5.0 mM)	0.89	+197	1.53	+41
Cysteine (0.5 mM)	0.75	+150	1.66	+49.5

cytoplasmic serum at such concentrations [21–24] (Jacob, J. L., unpublished results) that they may have a stimulatory effect on invertase. In addition the variation in the latex pH from 6.3 to 7.1 [2], and even more and the variation from pH 6.4 to 7.4 in the cytoplasmic serum [6, 7], imply that activator effects can fluctuate considerably in this medium. Usually, physiological concentrations of these effectors are not saturating in relation to invertase activation, but some external factors might alter this situation. Thus hormonal treatments used to stimulate rubber yield modify the thiol and inorganic phosphate concentration of the latex [24, 25]. Consequently, it is possible that these treatments influence β-fructofuranosidase activity in this way and so help to regulate isoprenoid metabolism.

These results were obtained with freeze-dried serum submitted to molecular filtration to eliminate compounds which might have interfered with the assay of enzyme activity. When unfiltered serum was used, the same kind of activation was also obtained.

EXPERIMENTAL

Plant material. Latex from the Ivory Coast was collected in ice-cooled flasks and immediately centrifuged at 40 000 *g* for 60 min. The aq. phase, or cytoplasmic serum, which contained the invertase was separated, freeze-dried, and stored at −20°. This procedure did not alter the enzyme activity.

Pretreatments of enzyme soln. Two procedures were used to eliminate substances which might have interfered with the invertase assay: (1) 500 mg of freeze-dried serum, dissolved in 1 ml 0.1 M sodium cacodylate buffer, pH 7.0, was passed down a Sephadex G-25 column equilibrated with the same buffer; (2) 500 mg freeze-dried serum was dissolved in 10 ml of distilled H_2O and submitted to ultrafiltration through an XM 300 Amicon membrane until a ten-fold concn of the enzyme soln was obtained. The ultrafiltration was repeated ×3 and the invertase soln adjusted to the initial vol. No activity was detected in the filtrate, confirming the high MW of the latex enzyme (> 300 000). In addition, no enzymatic activity capable of perturbing the invertase assay, e.g., hexokinase or glucose-6-phosphate dehydrogenase, was found in the β-fructofuranosidase soln remaining on the filter.

Latex invertase assay. The reaction medium consisted of 50 mM sodium cacodylate or NaPi buffer of the desired pH, and 50 mM sucrose. Effector(s) and sucrose had been previously prepared in the same buffer and adjusted to the selected pH. The addition of 10 or 15 μl of invertase soln started the reaction. The vol. incubated was 1 ml, reaction time 15 min and temp. 30°. The reaction was stopped by dipping the tube into a boiling water bath for 2 min. After cooling in iced water, 2 ml 0.3 M triethanolamine buffer, pH 7.4, containing 3 mM $MgSO_4$ were added, and glucose and fructose determined enzymatically [26].

Enzyme unit. One unit of enzyme was defined as the amount which hydrolysed 1 μmol sucrose/min at 30°.

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