PHYSIOL. PLANT. 66: 108–114. Copenhagen 1986

Effect of nigericin on the H^+-translocating adenosine triphosphatase from tonoplast of *Hevea latex*

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Marin, B. 1986. Effect of nigericin on the H^+-translocating adenosine triphosphatase from tonoplast of *Hevea latex*. – Physiol. Plant. 66: 108–114.

Nigericin stimulated the ATPase activity of tightly-sealed membrane vesicles prepared from *Hevea brasiliensis* Müll.-Arg. lutoïds in the presence of K^+. This stimulation required a functioning membrane since it was membrane-bound and since it was not observed for the ATPase activity solubilized from the tonoplast by dichloromethane. The extent of nigericin-induced stimulation of tonoplast ATPase was proportional to the ΔpH collapsed by the ionophore in the presence of K^+.

Additional key words – Membrane vesicles, proton-motive force, tonoplast ATPase.

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Introduction

Vacuoles from *Hevea* latex are capable of hydrolyzing ATP (D'Auzac 1975, 1977, references in Marin 1985). This reaction is catalyzed by an enzyme bound to the tonoplast which also carries out an electrogenic influx of protons (Crétin 1982, Marin 1983, 1985, Marin et al. 1981a, b). The properties of this ATPase are well described (D'Auzac 1977; X. Gidrol 1984. Thesis, Univ. Aix-Marseille, France; Marin 1985). One important characteristic is its driving of the uptake of citrate (Marin 1982, cf. Marin 1985). In other less well characterized systems obtained from disrupted plant tissues the ionophore nigericin stimulates a microsomal anion-sensitive ATPase found to be a tonoplast-bound enzyme (Sze 1980, Churchill et al. 1983). It is not clear why this enzyme is sensitive to nigericin. The sensitivity could be related directly to the magnitude of pH gradient across the membrane of a tightly-sealed system, nigericin facilitating an electroneutral H^+/K^+ exchange across this membrane (Pressmann 1976). However, this nigericin stimulation of the membrane-bound enzyme could also be due to changes in the internal pH of such a vesicular system. Conditions required for maximal stimulation of the tonoplast H^+-translocating ATPase from *Hevea* latex are determined in the present paper. The results indicate that nigericin acts by dissipating the initial ΔpH rather than by a change in the internal pH.

Abbreviation – ΔpH, transmembrane pH gradient; Δψ, electrical transmembrane potential; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; MES, 2-(N-morpholino)-ethanesulphonic acid; $TPMP^+$, triphenylmethylphosphonium iodide; Trizma, Tris-base.

Materials and methods

Plant material. Latex was obtained from trees of *Hevea brasiliensis* Müll.-Arg. (clone Prang Besar 86) growing on the I.R.C.A. (Inst. de Recherches sur le Caoutchouc en Afrique) experimental plantation at Bimbresso, Abidjan, Ivory Coast. The fluid cytoplasm was harvested in ice-cold flasks as described previously (Marin 1982, Marin et al. 1981b). This latex is very rich in orga-

Received 2 April, 1985; revised 6 September, 1985

nelles called lutoïds which are equivalent to the vac-

described by Marin (1982, 1983). Bovine serum albumin

hydrochloride (1.5 TBq mol^{-1}) from Commissariat à l'Energie Atomique, Gif-sur-Yvette, France. All other reagents and chemicals were obtained from Labosi, France; Fluka Feinchemikalien GmbH, Ulm, West

these factors are taken into account, the extent of stimulation by nigericin varied between 25 and 120% above the control (in the absence of the ionophore). In addition, any treatment (physical and/or chemical) which

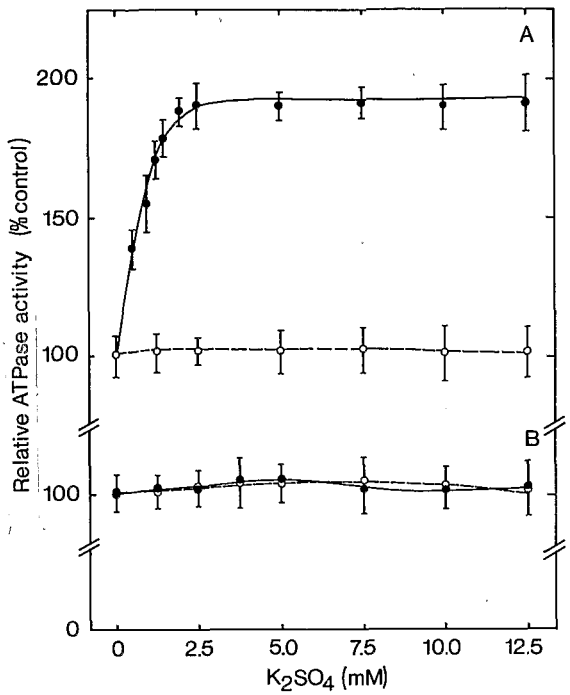

Fig. 2. Dependence of the nigericin stimulation of tonoplast ATPase on K^+ concentration. The ATPase activity of tonoplast ATPase activity (A) and the solubilized tonoplast ATPase (B) were measured in a medium containing 5 m*M* $MgSO_4$, 5 m*M* ATP and K_2SO_4 ranging between 0 and 12.5 m*M*, in the presence (●——●) or absence (○- - -○) of 20 n*M* nigericin. The ATPase activity was expressed as percentage of the activity assayed in the absence of K^+-salt and nigericin. The results are from one experiment representative of three. Control values are as followed: native ATPase, 1.75 ± 0.13 units (mg protein)$^{-1}$, solubilized ATPase, 109.3 ± 0.4 units (mg protein)$^{-1}$.

Tab. 1. Effect of different anions on the *Hevea* tonoplast ATPase. The ATPase activity of tonoplast vesicles (native) and the solubilized tonoplast ATPase (solubilized) was assayed at pH 7.0 as described in the experimental part. The final concentration of each salt was 50 m*M*. Nigericin concentration was 20 n*M*. The ATPase activity of tightly sealed tonoplast vesicles was 1.71 ± 0.11 units (mg protein)$^{-1}$. When the enzyme was solubilized from tonoplast membranes, the activity was 118.2 ± 0.4 units (mg protein)$^{-1}$. These values were set to 100%. All values represent the means of two to five experiments.

Treatment	ATPase activity			
	Native		Solubilized	
	− Nig.	+ Nig.	− Nig.	+ Nig.
No addition ($MgSO_4$ only)	100	103	100	102
KCl	171	340	143	141
KBr	161	280	131	134
KNO_3	42	55	39	40
K_2SO_4	103	210	102	98
K-imino diacetate	100	210	102	101
K-benzene sulphonate	98	200	95	96

cept NO_3^- which must be considered as a typical strong inhibitor of this ATPase (cf. Marin 1985). In contrast to the intact enzyme, nigericin had no effect on the activity of the solubilized ATPase (Tab. 1).

Effect of nigericin on the proton-motive force in tonoplast vesicles

In the absence of MgATP, but in the presence of K_2SO_4, the transmembrane ΔpH was reduced when the nigericin concentration increased (Fig. 3). Thus, at a concentration which stimulates the ATPase activity (20 n*M*), the ionophore decreased the ΔpH component by 0.8–

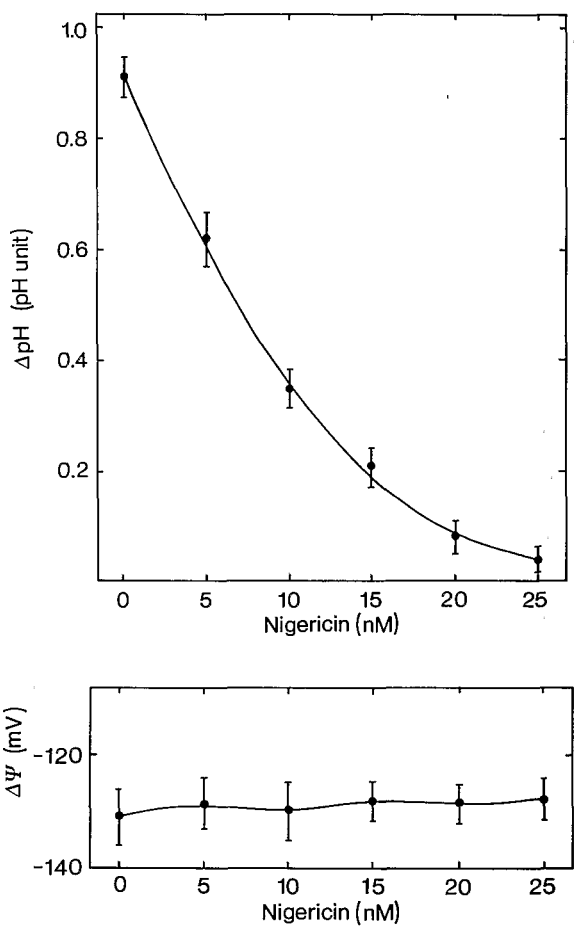

Fig. 3. Effect of nigericin on the magnitude of the electrochemical proton gradient in *Hevea* tonoplast vesicles. Tonoplast vesicles were added to an incubation medium adjusted at pH 7.0 by addition of Trizma, as described in Materials and methods. Data reported in this figure corresponds to a typical experiment where the magnitude of the electrochemical proton gradient was measured after the addition of the indicated nigericin concentration. The transmembrane pH gradient (ΔpH; top) and the electrical potential difference (Δψ; bottom) across the tonoplast were calculated from the transmembrane distribution at equilibrium of methylamine and $TPMP^+$, respectively. The results are from one experiment representative of three.

0.9 pH units. On the other hand, the membrane potential was not affected by the addition of nigericin, even at high concentrations (Fig. 3, bottom). Consequently, it can be postulated that nigericin stimulates the tonoplast ATPase activity because it decreases the pH gradient existing initially across the tonoplast membrane. Any constraint on the proton pump imposed is thus removed.

pH-dependence

The nigericin-induced stimulation was, on a percentage basis, relatively independent of the external pH value, with the greatest stimulation at the optimal value for the tonoplast ATPase (Fig. 4). In this experiment, as also demonstrated previously, the transmembrane pH gradient changes as the external pH varies (Marin 1982, 1983, Marin et al. 1982). However, this ΔpH also increases as the tonoplast-bound ATPase functions as a proton pump (Marin 1982, Marin et al. 1982). Consequently, when the tonoplast ATPase operates, the transmembrane ΔpH is maximal when external pH values are between 6.8–7.0 (Marin 1982, Marin et al. 1982). Under these conditions, when MgATP was added to the incubation mixture, the extent of the nigericin-stimulation of tonoplast ATPase was the greatest when the dissipated initial ΔpH was the greatest (Fig. 4). The collapsing effect induced by the ionophore was maximal under these conditions. The stimulation of the tonoplast ATPase was directly proportional to the magnitude of ΔpH across the membrane (Fig. 5).

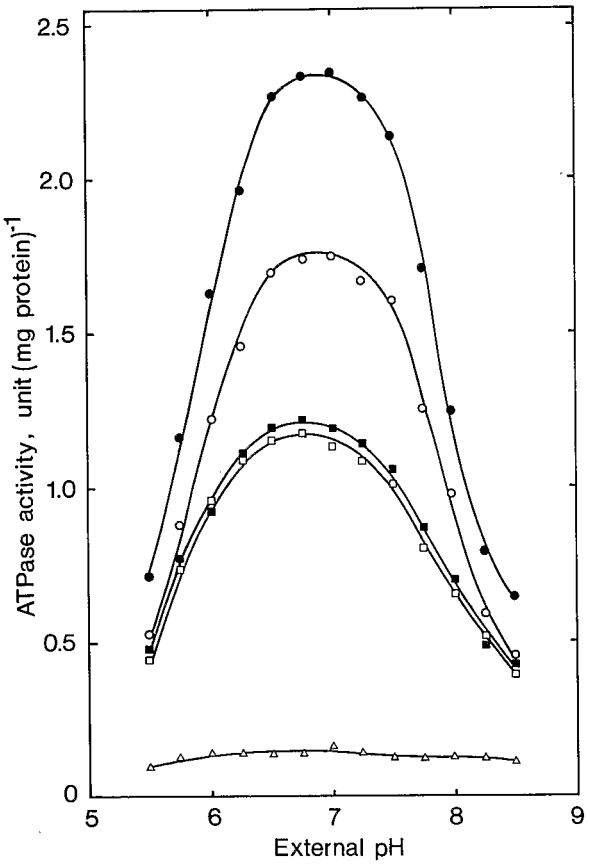

Fig. 4. Effect of external pH on the stimulation of *Hevea* tonoplast ATPase by nigericin. Tonoplast vesicles were incubated at different pH values from pH 5.0 to pH 8.0. Sufficient Trizma or H_2SO_4 was added to bring the pH to the desired value. In each case, 5 m*M* ATP and 5 m*M* $MgSO_4$, buffered at the tested pH value, were added. The activity was measured in the presence (●) or in the absence (○) of 20 n*M* nigericin in a medium containing 50 m*M* K_2SO_4. For control, K^+ was omitted, and 20 n*M* nigericin was either absent (□) or present (■). When ionophore was omitted, 0.5% ethanol was added. △, without Mg^{2+} in the medium. The results are from one experiment representative of three.

Discussion

The data reported in the present paper describe the conditions required for maximal stimulation of tonoplast ATPase from rubber tree (*Hevea brasiliensis*) by nigericin. This stimulation is due to the preexisting ΔpH across the tonoplast membrane and not to the value of the internal pH of the tonoplast vesicles. The tonoplast ATPase activity is directly related to the transmembrane

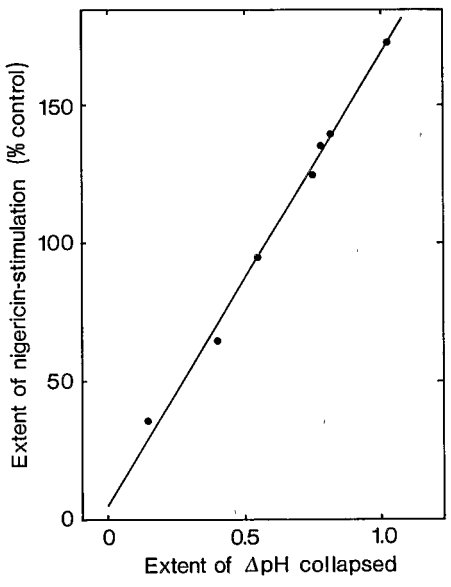

Fig. 5. Dependence of the extent of the nigericin-stimulation of tonoplast ATPase on the initial magnitude of ΔpH collapsed by the ionophore. These data come from different experiments conducted on tonoplast vesicles exhibiting different initial ΔpH gradient. The same amount of lyophilized tonoplast fractions was vesiculated at different pH values but the resulting tonoplast vesicles were incubated at the same pH value (7.0). The extent of nigericin-stimulation of tonoplast ATPase was measured after the addition of 20 n*M* nigericin. The results are presented as percentage of the rate of ATP hydrolysis and correspond to the means ± SE. The value of the control is 2.05 ± 0.15 units (mg protein)$^{-1}$.

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