Study of a NADH-Quinone-Reductase Producing Toxic Oxygen from *Hevea* Latex

J. D'AUZAC

Université des Sciences et Techniques du Languedoc, Montpellier, France

C. SANIER

IRCA-CIRAD, Montpellier, France

AND

H. CHRESTIN

ORSTOM, Abidjan, Ivory Coast

Abstract in Bahasa Malaysia

Adalah ditunjukkan bahawa pokok-pokok getah tertentu yang kurang berhasil dan/ atau pokok-pokok yang berkulit separa kering menunjukkan adanya NAD(P)H-O_2 reduktase lutoid. Eksploitasi yang intensif menambahkan keaktifan enzim ini. Enzim ini yang berasal dari pengeringan sejuk-beku mudah terlarut dan menghasilkan ion-ion superoksida bila dicampurkan dengan NAD(P)H.

Enzim ini boleh diukur dengan beberapa cara, iaitu dengan mentitratkan keaktifan diaforasenya dengan menggunakan diklorofenol-indofenol, menghabiskan NADH pada 340 nm, menghabiskan oksigen dengan oksigraf dan pembentukan formazan dengan nitro biru tetrazolium.

Enzim ini telah diceriakan empat puluh tujuh kali secara pemeringkatan dengan amonium sulfat, pemisahan secara kromatografi melalui DEAE-Sefaros dan Ultrogel AcA 44. Keaktifan spasifiknya adalah tinggi. Berat molekulnya adalah lebih kurang 100 000 daton. Elektroforesis menunjukkan satu enzim sahaja. Sebatian kuinon yang hadir dalam lutoid adalah sangat perlu untuk keaktifannya. Enzim ini, sebenarnya adalah satu NAD(P)H-kuinon reduktase yang sanggup menerima banyak sebatian-sebatian kuinon dan juga sebatian-sebatian kuinon yang dihasilkan dari pengoksidaan sebatian fenol secara *in vivo* atau *in vitro* oleh peroksidase dan fenoloksidase yang wujud di dalam lateks.

Penurunan kuinon kepada hidrokuinon selalunya tidak lengkap dan disertai dengan pengeluaran separa kuinon dalam kuantiti yang tak tentu. Sebatian ini boleh mengauto-oksida dengan pengeluaran ion-ion superoksida dan menghasilkan hidroksil radikal (OH) dan oksigen (O^1_2) singlet dengan cara tidak langsung.

Berbagai perencat NAD(P)H-kuinon reduktase telah diuji. Perencat-perencat ini menghalang enzim tersebut ataupun membersihkan oksigen toksik yang dihasilkan dari tindak balasnya. Kemungkinan untuk menggunakan perencat-perencat ini secara *in situ* pada tapak torehan telah dicadangkan.

Abstract

It was previously shown that certain low yielding trees and/or trees with partial dry bark display a lutoidic NAD(P)H-O_2-reductase. Intensive exploitation increases the activity of this enzyme which is easily soluble from freeze-dried lutoids and which produces superoxide ions when NAD(P)H is added.

The enzyme may be titrated by its diaphorase activity with dichlorophenol-indophenol, by NADH consumption measured at 340 nm, by oxygen consumption measured with an oxygraph or by formazan formation with nitro blue tetrazolium.

The enzyme was purified forty-seven times by fractionation with ammonium sulphate, chromatographic separation on DEAE-Sepharose and Ultrogel AcA44. Its specific activity was high. The molecular weight was about 100 000 D. Electrophoresis revealed only one enzyme. Quinone compounds naturally present in lutoids are indispensable to its activity. The enzyme is, as a matter of fact, a NAD(P)H-quinone reductase which is able to accept many quinonic compounds and also those which are produced in vivo *or* in vitro *from phenolic compounds oxidised by endogenous latex peroxidase and phenoloxidase.*

The reduction of quinones to hydroquinones was always incomplete and was accompanied by production of varying amounts of semi-quinones which are auto-oxidisable, production superoxide ions and indirectly the hydroxyl radical (OH) and oxygen singlet (O^1_2).

Various inhibitors of NAD(P)H-quinone-reductase were tested; either they inhibited the enzyme itself, or they scavenged toxic oxygen produced by the reaction. The possiblity of using these inhibitors in situ *on the tapping panel is suggested.*

Chrestin[1,2], d'Auzac[3] and Chrestin *et al*[4] have shown that latex from low-yielding trees or trees with more or less dry bark is particularly characterised by the presence of an enzyme which consumes oxygen in the presence of NAD(P)H.

This NAD(P)H-O_2-reductase produces superoxides ions, $O_2^{\bullet-}$, which by non-enzymatic or superoxide dismutase (SOD) catalysed reactions lead to H_2O_2. Reaction between $O_2^{\bullet-}$ and H_2O_2 gives, non-enzymatically, the hydroxyl radical ($OH^{\bullet}$) which is able to attack double bonds of ethylenic fatty acids which are constituent elements of membrane phospholipids. Lutoidic membrane degradation occurs *in vivo* or *in vitro* and finally latex coagulation follows.

After repeated ethephon stimulation of *Hevea* it is sometimes possible to observe the acceleration of NADH-O_2-reductase activity[5].

The biochemistry of the cessation of latex flow is described elsewhere[6]. The object of the present paper is the biochemical study of the lutoidic enzyme responsible for the production of superoxide ions.

MATERIALS AND METHODS

Freeze-dried lutoids from latex produced by low yielding trees and/or trees with partial dry bark were obtained from the IRCA station in the Ivory Coast.

Tapped latex was collected in tubes on ice and rapidly ultracentrifuged in the laboratory (40 000 g × 30 min); the lutoid fraction was then collected and washed three times in an isotonic buffered medium (mannitol : 0.3*M*, triethanolamine-HCl (TEA) : 50 mM, pH 7.5). After that, lutoids were immediately freeze-dried and sent to France by air.

The NAD(P)H-O_2-reductase activity was measured on a freeze-dried lutoid suspension (1 g per 4 ml TEA, 50 mM, pH 7.5), which was homogenised and then, centrifuged (40 000 g × 30 min) or used without centrifugation. Oxygen consumption was measured polarographically after addition of NADH (0.35 mM final concentration).

The diaphorase [NADH-dichlorophenol-indophenol (DCPIP)-reductase] activity was measured spectrophotometrically at 620 nm with NADH (0.35 mM) and DCPIP (0.125 mM).

The NAD(P)H-quinone-reductase activity was measured either by the disappearance of NADH at 340 nm for quinone and duroquinone, or by the appearance of formazan after addition of nitro blue tetrazolium (NBT) (0.15 mM) at 540 nm for other quinones (menadione, 1-4 naphthoquinone, 1-4 chloranil : 5 μM). The last method determined superoxide ion ($O_2^{\bullet-}$) produced by auto-oxidisable semiquinones.

Electrophoresis was performed with disc polyacrylamide gel according to classical methods. Enzyme visualisation was carried out by use of DCPIP discolouration, or the appearance of formazan red-violet band with NBT and the different quinones.

RESULTS

Functioning of the Enzyme.

It was shown that a suspension of freeze-dried lutoids (2 g of, lyophilised in 8 ml of a TEA buffer, 25 mM, pH 7.5; 5 mM of EDTA; 5 mM of mercaptoethanol) or a clear supernatant obtained by ultracentrifugation of this suspension (40 000 g × 30 min) had practically the same NADH-O_2-reductase activity. It follows that the enzyme, probably of membraneous origin, was solubilised by freeze-drying and homogenisation. The following study was therefore performed on a clear lutoidic supernatant.

A lutoidic supernatant from trees with partial dry bark is able, after addition of NAD(P)H alone, in the cell of a 'Gilson Oxygraph', to consume considerable amount of O_2. Under these conditions the addition of Fe^{3+}/EDTA complex[1] is useless. The addition of exogenous and commercial SOD and catalase neutralised O_2 consumption completely. We therefore have:

$$NAD(P)H + H^+ + O_2 \xrightarrow{NAD(P)H\text{-}O_2\text{-reductase}} NADP^+ + 2O_2^{\cdot -} + 2H^+$$

$$O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 (SOD)$$

$$H_2O_2 \rightarrow H_2O + 1/2O_2 \text{ (catalase)}.$$

In order to study the functioning of NAD(P)H-O_2-reductase in a more elaborate way it was necessary to purify the enzyme. This differs from the studies of Chrestin[1,2] who worked with fresh and total lutoids.

Purification of the Enzyme

At first, the enzyme present in a clear supernatant B-serum was separated from low molecular weight compounds present. This contains the possible substrate of this and other enzymes able to interfere with the functioning of NADH-O_2-reductase.

Desalting of the crude B-serum was performed on an Ultrogel AcA 202 column (22 × 2.5 cm). Thus, a first peak containing the high molecular weight proteins was separated from two peaks corresponding respectively to low molecular weight proteins and solutes. The first protein peak obtained in this manner is totally devoid of enzymatic activity measured with an 'Oxygraph' in the presence of NAD(P)H. In contrast, an addition of an aliquot of the third peak (low molecular weight solutes) or of Fe^{3+}/EDTA[1] showed enzymatic activity. Nevertheless, the desalted enzyme functions easily with NAD(P)H (0.35 mM) and DCPIP (0.125 mM) alone, like a diaphorase, (EC. 1.66.99.2) with constitutive flavoprotein. The last method was used to follow the different steps of enzymatic purification.

The clear lutoidic supernatant may also be precipitated by ammonium sulfate between 35% and 65% of saturation. The active protein fraction obtained in this way was dissolved in a minimal volume of the same buffer.

This fraction was placed on a DEAE-Sepharose CL6B column (K 15/40 sephadex) equilibrated with TEA buffer (25 mM, pH 7.5). The enzyme retained on the column was eluted by a linear gradient of the same buffer with NaCl 0–0.4 *M* at a concentration of 0.135 ± 0.02 *M* of NaCl.

The active fraction determined again with NADH and DCPIP was concentrated by an Amicon filtration cell with a PM 15 membrane. The concentrate was placed on an Ultrogel AcA 44 (K 15/90) column equilibrated with TEA buffer (25 mM, pH 7.5 plus NaCl, 0.1 *M*). Markers with known molecular weights were also used (blue dextran 2×10^6, catalase 24×10^4, lactate dehydrogenase 14×10^4, malate dehydrogenase 7×10^4, peroxidase 4×10^4 and cytochrome C 1.25×10^4). The peak corresponding to the enzyme had a molecular weight of 101 000 ± 3 000 D.

Table 1 and *Figure 1* contain all the results of this purification.

TABLE 1. DIFFERENT STEPS OF THE PURIFICATION OF NAD(P)H-O_2-REDUCTASE

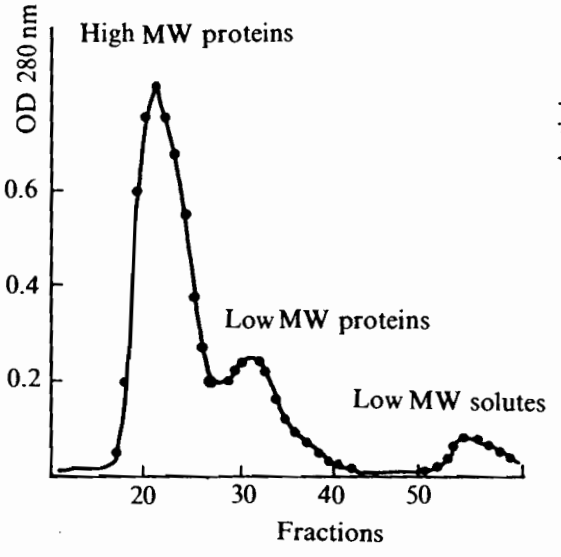
Step	Total units (n. kat DCPIP)	Proteins (mg)	Specific activity (n. kat)	urification X	Yield (%)
Lutoidic Supernatant (12)	7683	77.4	99	–	–
$(NH_4)_2SO_4$ 35% – 65%	6450	61.0	106	1.1	83.8
Desalting: Ultrogel AcA 202	5117	19.8	258	2.6	66.5
DEAE-Sepharose	4083	5.4	756	7.6	53.2
Concentration: Amicon PM 15	3783	2.1	1801	18.2	49.3
Molecular Sieving: Ultrogel AcA 44	3262	0.7	4667	47.0	42.4

The enzymatic activities are expressed in katals. The reaction used was the discolouration of DCPIP by reaction with NADH.

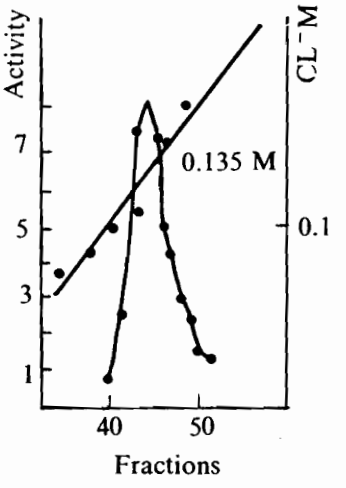
Study of the Purified Enzyme

The pH-curve determined by Chrestin[2] on the crude enzyme gave a value of 7.5 for the optimum pH. Apparent K_m for NADH was $45 \pm 11 \mu$M and between 22μM and 30 μM for oxygen. These values were not determined again on the purified enzyme.

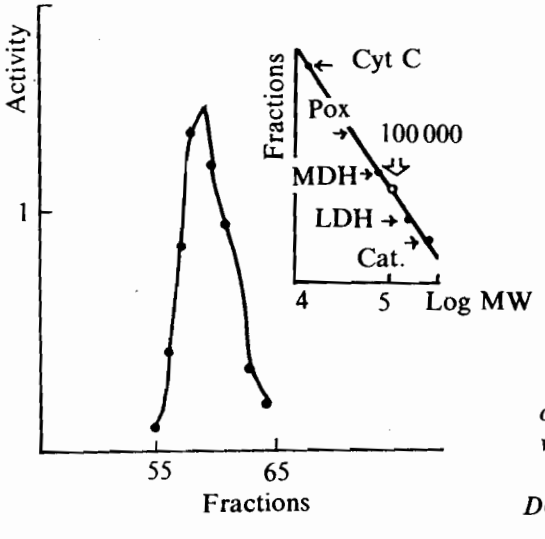
Chrestin[2] had also shown that naphthoquinones greatly increased the O_2 consumption with crude lutoids and this leads to the hypothesis according to which the



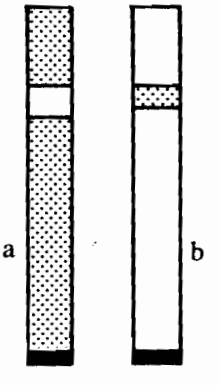
Desalting on an ultrogel AcA 202 column



chromatography on a DEAE-Sepharose CL6B column



Molecular sieving on an ultrogel AcA 44 chromatographic column



Electrophoresis of the purified enzyme. a: Enzymatic visualisation of the purified enzyme; incubation with NADH (0.35 mM) DCPIP (0.125 mM) in a TEA 50 mM, pH 7.5 buffer. b : Enzymatic visualisation with NADH (0.35 mM), menadione (0.1 mM) NBT (0.15 mM) to obtain a red-violet precipitate band of formazan.

Figure 1. Different steps of the purification of NADH-quinone-reductase.

NAD(P)H-O_2-reductase was, as a matter of fact, a NAD(P)H-quinone reductase. Our study of the purified enzyme took this direction.

In accordance with the work of Wosilait *et al.*[7], de Viljoen *et al.*[8], and Lind *et al.*[9] we have proposed the reaction hypothesis in *Figure 2*. The quinones might be intermediates in the reaction catalysed by the NAD(P)H-O_2-reductase enzyme. The formation of $O_2^{\cdot-}$ detected by colorimetric analysis after NBT reaction as described by Chrestin[2] was used and it was confirmed that the addition of exogenous SOD and catalase was effective in the reduction and neutralisation of the appearance of formazan. We therefore sought the efficiency of different quinones as regards production of $O_2^{\cdot-}$. the K_m and V_{max} of these different quinones in the production of formazan were determined. It was shown that duroquinone, menadione, 1-4 naphthoquinone and 1-4 chloranil had considerable affinity for the purified enzyme and that naphthoquinone and menadione had a high reaction as for the production of $O_2^{\cdot-}$ *(Table 1)*.

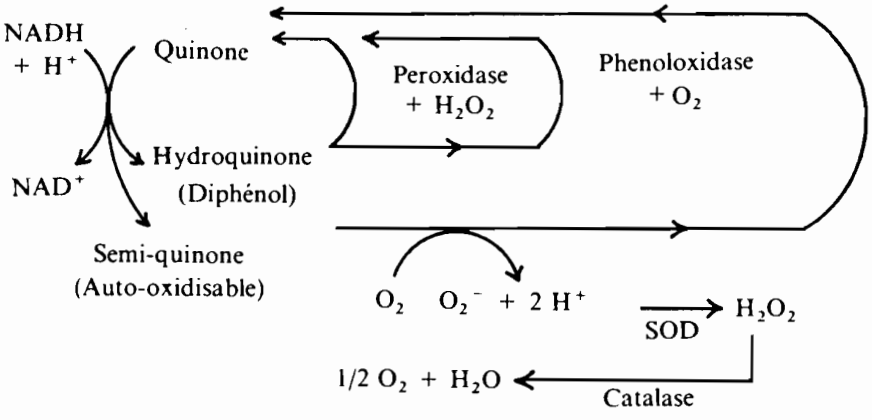


Figure 2. Proposed representation of the reactions catalysed by NADH-quinone-reductase. Certain endogenic oxidases of the lutoidic fraction can transform di- or polyphenols into quinonic compounds which are reduced by NADH partially into hydroquinones or into auto-oxidisable semi-quinones which initiate the production of toxic oxygen forms ($O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$, $O^1{}_2$).

Inhibitors of this reaction were then sought knowing that 2,4 dinitrophenol (2,4 DNP) and dicumarol were usually considered as powerful inhibitors of NADH-quinone-reductase[8,9]. 2,4 DNP (1mM) and dicumarol (1mM) had practically no effect on $O_2^{\cdot-}$ production as detected by formazan production.

In contrast, if the same $O_2^{\cdot-}$ production is considered it is seen that different molecules are effective to inhibitors *(Table 3)*. Examination of the results show that propyl-gallate (PG), which is indicated as a scavenger of $O_2^{\cdot-}$ and SOD-like compounds are very efficient. It is likely that they do not inhibit NADH-quinone-reductase; it is more

TABLE 2. KINETIC CONSTANTS OF NAD(P)H-QUIONONE-REDUCTASE TO VARIOUS QUINONES

Substrate	$K_m(\mu M)$	V_{max} (m. kat. ml^{-1} formazan)
Quinone	130	0.006
Duroquinone	70	0.029
Menadione	15	0.044
Naphthoquinone 1-4	7	0.047
Chloranil	13	0.013

TABLE 3. INHIBITORS OF SUPEROXIDE FORMATION BY NAD(P)H-QUINONE-REDUCTASE

Compound	Concentration for 50% inhibition (μM) $O_2^{\cdot -}$ measured by formazan)
Monophenols	
Hydroxybenzoic acid	700
Polyphenols	
Quercetin	300
Dicumarol	> 1000
Catechine	> 1000
Propyl-gallate	30
SOD-like compounds	
Cu^{2+} (3-5-di-isopropyl-salicylic acid)$_2$	40
Cu^{2+} (Acetyl-salicylic-acid)$_4$	10
Antioxidants	
β-Carotene	> 1000
α-Tocopherols	> 1000
Butyl-hydroxytoluene (BHT)	> 1000
Butyl-hydroxyanisol (BHA)	> 1000

likely that they are effective in scavenging the product of this reaction : $O_2^{\cdot -}$. This was particularly clear for copper-complexes, well known as SOD-like compounds, having an action very close to that of the SOD enzyme and which are widely used in pharmacology for this reason[10].

If the criterion used was no longer the production of $O_2^{\cdot -}$ but NADH consumption, with duroquinone as an intermediate substrate, dicumarol inhibited the enzyme by 50% for 275 μM, quercetin for 60 μM, 4-hydroxybenzonic acid for 300 μM, copper-acetyl salicylic complex for 100μM. It appears likely in this case that there is inhibition of the NADH-quinone reductase itself.

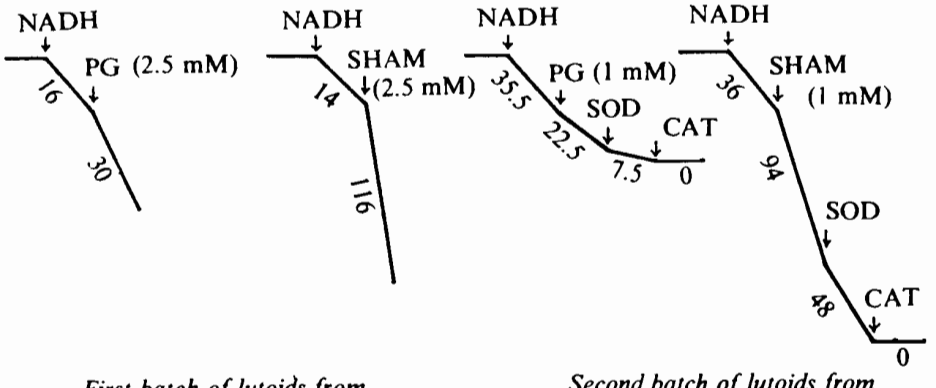
All these results were obtained with purified enzyme. It should be pointed out that if a crude lutoidic supernatant (B-serum) is used some of these molecules will act in a completely different way, because they may have been transformed by the enzymes of

this crude B-serum. Thus, in an experiment carried out with a batch of lutoids from trees with partial dry bark, PG, a classical scavenger of O_2^- and salicylhydroxamic acid (SHAM), an inhibitor of alternative respiration, acted at a concentration of 2.5 mM, as activators of NADH and O_2 consumption and O_2^- production, the latter being detected by formazan *(Table 4)*. It can be assumed that these phenolic compounds are oxidised by the enzyme of crude B-serum into quinoid compounds.

TABLE 4. ACTIVATION OF NADH-O_2-REDUCTASE IN LUTOIDIC SERUM

Compound	NADH consumed	O_2 consumed (m. kat. ml^{-1})	Formazan formed
Control + NADH	5.3	3.2	1.2
NADH + PG (2.5 mM)	15.0	6.8	–
NADH + SHAM (2.5 mM)	36.3	26.3	19.2

In another experiment carried out with another batch of lutoids also from trees with more or less partial dry bark, the results obtained with a 1 mM concentration of the same molecules were quite different *(Figure 3)*. SHAM increased O_2 consumption and formazan production while PG was ineffective. SOD and catalase led us to confirm that these molecules really produce $O_2^{\cdot-}$, probably by their transformation into quinoid compounds by endogenic peroxidase and polyphenoloxidases which are present in the 'sedimentable fraction'.



First batch of lutoids from partially dry bark trees

Second batch of lutoids from partially dry bark trees

Figure 3. Oxygen consumption measured by polarograph. The arrows indicate different additions during the course of polarographic O_2 consumption.

DISCUSSION

It would thus appear that lutoid NAD(P)H-reductase reacting with DCPIP like a diaphorase (EC. 1.66.99.2) is in fact a NADH-quinone-reductase.

Latex is particularly rich in free or bound phenolic compounds[12]; these can be oxidised into quinones by the polyphenoloxidases and peroxidases in latex[11]. Very reactive quinoids can react with latex enzymes, and participate, in particular, in the coagulation of latex[13].

NADH-quinone-reductase can act as a detoxifying enzyme for quinones thus reduced to hydroquinones (diphenols). However, the experiment shows that a proportion of the quinones is partially reduced to semi-quinones that are self-oxidisable by O_2 with the production of O_2^- superoxide ions.

These, by a series of reactions that are well known[14] and that are enzymatic or not, will lead to various molecular forms of toxic oxygen such as hydrogen peroxide (H_2O_2) and especially the radical hydroxyl ($OH^\bullet$) and singlet oxygen (O^1_2).

The role of these toxic forms of oxygen with regards to ethylenic fatty acids and membrane phospholipids is well known in the animal kingdom[15,16]. It was demonstrated in *Hevea* latex by Chrestin [1,2,6].

It should be noted that the NADH-quinone-reductase revealed here can function with other electron acceptors such as potassium ferricyanide or oxidised cytochrome C. Such activity was revealed in lutoids by Moreau *et al.*[17] in 1975. In would seem unlikely that it is a NADPH-cytochrome P-450 reductase with flavoprotein, and the absence of inhibition by 2,4-DNP and dicumarol would appear to distinguish it from diaphorase[8,9] with flavoprotein.

All this would seem to give latex NADH-quinone reductase a certain originality.

In addition, the enzyme does not appear to be very specific since quinones as different as benzoquinone, menadione and chloranil, together with SHAM oxidation derivatives and propyl-gallate, can be used and lead to the formation of $O_2^{\bullet -}$.

This work also shows that various molecules can be used to scavenge the $O_2^{\bullet -}$ produced (PG, SOD-like compounds, quercetin) or for direct inhibition of NADH-quinone-reductase (inhibition of NADH consumption).

This therefore suggests the possibility of operating *in situ*, *i.e.* on the tapping panel either to inhibit NADH-quinone-reductase or to scavenge the various forms of toxic oxygen produced directly or indirectly as a result of over-exploitation. This would make it possible to prevent membrane destabilisation of latex organelles and particularly of the lutoids and the Frey-Wyssling particles, which have been shown to be responsible at best, to limiting the flow duration and at worst, to cause *in situ* coagulation and bark dryness[1-4,18].

REFERENCES

1. CHRESTIN, H. AND BANGRATZ, J. (1983) Une activité enzymatique endogéne NAD(P)H dépendante, responsable de la dégradation perodative des organites membranaires et de la coagulation précoce ou *in situ* du latex d'*Hevea brasiliensis*. *C. R. Acad. Sci.*, **296**, Série III, 101.

2. CHRESTIN, H. (1984) Le compartiment vacuo-lysosomal (les lutoides) du latex d'*Hevea brasiliensis*, son rôle dans le maintien de l'homéostasie et dans les processus de senescence des cellules laticiféres. Thése de Doctorat d'Etat, Université de Montpellier II (U.S.T.L.), France.

3. D'AUZAC, J. CHRESTIN, H. MARIN, B. AND LIORET, D. (1982) A Plant Vacuolar System : the Lutoids from *Hevea brasiliensis* Latex. *Physiol. Vég.*, **20(2)**, 311.

4. CHRESTIN, H. BANGRATZ, J. D'AUZAC, J. AND JACOB, J. L. (1984), Role of the Lutoidic Tonoplast in the Senescence and Degeneration of the Laticifers of *Hevea Brasiliensis*. *Z. Pflanzenphysiol. Bd 114 S*, 261.

5. CHRESTIN, H. (1985) La stimulation á l'Ethrel de l'Hevea ; jusqu' ou ne pas aller trop loin. *Caoutchs Plast.*, **62(647/648)**, 75.

6. CHRESTIN, H., JACOB, J. L. AND D'AUZAC, J. (1986) *Biochemical Basis of the Cessation of Latex Flow and Physiological Bark Dryness. Proc. Int. Rubb. Conf. Kuala Lumpur 1985*, **3**, 20.

7. WOSILAIT, W. D. AND NASON, A. (1954) Pyridine Nucleotide-quinone Reductase. I. Purification tion and Properties of the Enzyme from Pea-seeds. *J. biol. Chem.* **206**, 255.

8. VILJOEN, C. D. CLOETE, F., BOTES, D. P. AND KRUGER, H. (1983) Isolation and Characterisation of NAD(P)H-deshydrogenases from Seeds of the Castor Bean. *Phytochem.*, **22(2)**, 365.

9. LIND, C., HOCHSTEIN, P. AND ERNSTER, L. (1982) DT-Diaphorase as a Quinone-reductase : a Cellular Control Device against Semiquinone and Superoxide Radical Formation. *Archs Biochem. Biophys.*, **216(11)**, 175.

10. SORENSON, J. R. J., OBERLEY, L. W., CROUCH, R. K. AND KENSLER, T. W. (1984) Pharmacological Activities of SOD-like Copper Complexes. *Oxygen Radicals in Chemistry and Biology (Bors, W., Saran, and M. Tait, D. ed.)*, p. 821. Berlin, New York: De Gruyter.

11. COUPÉ, M., PUJARNISCLE, S. AND D'AUZAC, J. (1982) Compartimentation de diverses oxydoréductases (peroxydase, o. diphénol-oxydase et malate déshydrogénase) dans le latex d'*Hevea brasiliensis*. *Physiol. Vég.*, **10(3)**, 459.

12. HANOWER, P., BROZOZOWSKA-HANOWER, J., CHRESTIN, H., AND CHEZEAU, R. (1979) Composés phénoliques du latex d'*Hevea brasiliensis*. *Phytochem.*, **18**. 686.

13. BRZOZOWSKA, J., HANOWER, P., AND LIORET, C. (1978) Etude du mécanisme de la coagulation du latex d'*Hevea brasiliensis*. II. Systémes enzymatiques impliqés dans le processus : les Phenol-oxydases. *Physiol. Vég.*, **16(2)**, 231.

14. FRIDOVICH, I. (1978) The Biology of Oxygen Radicals. *Science*, **201**, 875.

15. SLATER, T. F. (1984) Free-radical Mechanisms in Tissue Injury. *Biochem. J.*, **222**, 1.

16. PORTER, N. A., LEHMAN, L. S. AND WUJEK, D. G. (1984) Oxidation Mechanisms of Polyinsaturated Fatty Acids : *Oxygen Radicas in Chemistry and Biology (Bors, W., Saran, M. and Tait, D. ed)*, p. 235. Berlin, New York : De Gruyter.

17. MOREAU, F., JACOB, J. L., DUPONT, J. AND LANCE, C. (1975) Electron Transport in the Membrane of Lutoids from the Latex of *Hevea brasiliensis*. *Biochem. biophys. Acta*, **396**, 116.

18. De FAY, E. (1980) Etude histologique des écorces d'*Hevea brasiliensis* atteint de la maladie des encoches séches. *C. R. Acad. Sci.*, **291**, Serie D, 865.

Auzac J. d', Sanier C., Chrestin Hervé. (1986).

Study of a NADH-Quinone-reductase producing toxic oxygen from Hevea latex.

In : Rajarao J.C. (ed.), Amin L.L. (ed.) International rubber conference 1985 : proceedings. Kuala Lumpur : Rubber Research Institute of Malaysia, 102-112.

International Rubber Conference, Kuala Lumpur (MYS), 1985/10/20-25.