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The Occurrence of 3-Hydroxy-3-methylglutaryl CoA Reductase (NADPH) in the latex of regularly-tapped *Hevea brasiliensis.*

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Key words: **Hydroxymethylglutaryl CoA reductase; *Hevea brasiliensis;* latex; Arrhenius plot; preincubation effect.**

RINGKASAN

Kajian telah dilakukan mengenai enzim 3-hydroksi-3-metil glutaril KoA reduktase (NADPH) dalam lateks yang diperolehi daripada pohon Hevea brasiliensis. *Kebanyakan aktiviti enzim ini berada dalam bahagian 'pecahan bawah' yang didapati apabila lateks digempar pada 42,000 g. Sebahagian kecil aktiviti juga didapati dalam bahagian zon Frey-Wyssling. Enzim dalam 'pecahan bawah' memerlukan NADPH secara khusus sebagai kofaktor. Optima pH nya ialah 6.6 – 6.9 dalam penimbal 0.1 M fosfat. Plot Arrhenius enzim ini didapati linear dalam lingkungan suhu 12 – 40° C dan anggaran tenaga pengaktifan Arrhenius ialah 57.3 kJ/mol (13.7 kcal/mol). Aktiviti enzim adalah tidak stabil jika lateks diperolehi dan digempar pada suhu ambien. Kehilangan aktiviti sekadar 30% juga berlaku apabila 'pecahan bawah' disimpan pada suhu – 15° C selama 24 jam. Tindakan pra-pengeraman enzim pada suhu 30° C selama 1 jam mengakibatkan kehilangan aktiviti sekadar 90% dan kesan ini tidak dapat dihentikan dengan membasuh 'pecahan bawah' atau dengan menambahkan bovine serum albumin (1%, w/v) atau NADPH (2 mM) atau dithiothreitol (10 mM) dalam campuran tindakbalas enzim. Aktiviti enzim dalam enapan 'pecahan bawah' didapati tepu dengan kepekatan 300 μM RS-HMG KoA dan anggaran K_m ialah 56 μM manakala V_{max} pula ialah 6.10 pkat/mg protin.*

SUMMARY

The enzyme 3-hydroxy-3-methylglutaryl CoA reductase (NADPH) from the latex of mature trees of Hevea brasiliensis *was studied. It was found to be mainly associated with the bottom fraction of centrifuged latex (42,000 g), although appreciable activity was also detected in the Frey-Wyssling zone. The bottom fraction enzyme has a specific requirement for NADPH as the cofactor and its pH optimum was 6.6 – 6.9 in 0.1 M phosphate buffer. The Arrhenius plot of the enzyme was linear within the temperature range of 12 – 40° C and the Arrhenius activation energy was estimated to be 57.3 kJ/mol (13.7 kcal/mol). The enzyme was very unstable when the latex was collected and centrifuged at ambient temperature. A 30% loss of activity also occurred when the bottom fraction was stored at –15° C for 24 hr. Pre-incubation of the enzyme at 30° C for up to 1 hr resulted in a 90% loss of activity and this was not prevented by washing the bottom fraction or by the addition of either bovine serum albumin (1%, w/v) or NADPH (2 mM) or dithiothreitol (10 mM) to the assay mixture. Enzyme activity in the washed bottom fraction was saturated at 300 μM RS-HMG CoA and the K_m and V_{max} were 56 μM and 6.10 pkat/mg protein respectively.*

INTRODUCTION

The reactions in the biosynthesis of natural rubber from acetate are well understood although the control mechanism operating over the pathway still remains to be elucidated (Lynen, 1969). One of the steps in this pathway is the reduction of HMG CoA[1] to mevalonate, catalysed by HMG CoA reductase (mevalonate: NADP oxidoreductase; EC 1.1.1.34). Mevalonate is further converted to isopentenyl pyrophosphate, which is the common building block of all isoprenoid compounds (Nes and McKean, 1977). Current interest in the reductase arises primarily from the observation that it is the rate-limiting enzyme in the synthesis of the isoprenoid cholesterol from acetate (Rodwell *et al.* 1976). There is evidence that the reductase may also be rate-limiting in rubber biosynthesis in *Hevea brasiliensis.* Lynen (1969) reported that the activity of the reductase was lowest in comparison to those of the other enzymes in the pathway of biosynthesis from acetate.

[1] Abbreviations used : HMG CoA, 3-hydroxy-3-methylglutaryl Coenzyme A; HMG, 3-hydroxy-3-methyl glutarate; PPO, 2, 5 – diphenyloxazole

Hepper and Audley (1969) also found that the rate of incorporation of HMG CoA into rubber exhibited a much larger seasonal variation compared to that for mevalonate under the same experimental conditions. In spite of the importance of these observations to our understanding of the regulation of rubber biosynthesis, there have been few follow-up studies on this enzyme. This report presents the results of an investigation of the reductase in the latex obtained from regularly tapped trees of *H. brasiliensis*.

MATERIALS AND METHODS

Chemicals

CoA (the Lithium salt), NADPH, NADH, HMG acid was purchased from New England Sigma Chemical Co., St. Louis, U.S.A. [3 – ^{14}C] HMG acid was purchased from New England Nuclear, U.S.A. All other reagents were of analytical grade.

Collection of latex

The latex was obtained from nine regularly tapped (S.2/d.2) trees of *H. brasiliensis* clone RRIM 600 (approx. 15 yr. old) grown in the University Farm. The latex from each tree was allowed to run to waste for 3 min after tapping and then collected for the next 30 min into a glass conical flask surrounded by crushed ice. The contents of all flasks were pooled to give a yield of 300 – 400 ml latex.

Fractionation of latex

The latex was centrifuged using a Beckman L5-65 Ultracentrifuge (Rotor 65) at 42,000 x g for 40 min exclusive of acceleration and deceleration time. This procedure separates out four main fractions of the latex, viz., the uppermost layer of rubber, the Frey-Wyssling zone, the C-serum and the bottom fraction consisting largely of lutoid particles (Gomez and Moir, 1979). The Frey-Wyssling zone from each tube was carefully removed using a syringe and the fractions obtained from several tubes were pooled and recentrifuged at 4000 x g for 3 min. The yellowish-orange sediment was resuspended in a buffer (pH7.1) of 0.1 M triethanolamine-HCl containing 2 mM dithiothreitol and 20 mM EDTA (Buffer A) and the reductase activity in this preparation was determined as described below. The C-serum was removed by puncturing the cellulose nitrate centrifuge tube and draining carefully into a beaker so as to exclude much of the rubber particles. Part of the centrifuge tube containing the bottom fraction was cut off and rinsed with ice-cold distilled water to remove the rubber. The bottom fraction was then scraped into a Potter-Elvehjem homogeniser and resuspended in Buffer A. This suspension was the enzyme source. In some experiments, bottom fraction pellets which were stored frozen (at approx. -15°C) were used.

All operations were performed at 0 – 4° C.

HMG CoA reductase assay

The enzyme activity was assayed essentially as described by Shapiro *et al.* (1974). The ^{14}C-labelled substrate was prepared by reacting CoA and [^{14}C] HMG anhydride (synthesised as described by Goldfarb and Pitot (1971), and with a specific activity of 200 μCi/mmol) and was used without further purification (Louw *et al.* 1969). This preparation contained about 86% of the total radioactivity as *RS*-[^{14}C] HMG CoA. Each enzyme reaction mixture contained: enzyme, 0.7 – 0.9 mg protein; NADPH, 0.4 μmol; *RS*-[^{14}C] HMG CoA, 120 nmol (specific activity was 444 dpm/nmol) and Buffer A in a final volume of 0.2 ml. Boiled enzyme was used as the control. The incubation was carried out at 30° C for times ranging up to 1hr and the reaction was terminated with 25 μl of 10 N HCl. The product of the enzyme reaction was purified as mevalonolactone by thin layer chromatography (TLC) using benzene: acetone (1:1, v/v). Unlabelled mevalonolactone was also spotted on to the TLC plate as a marker and this was visualised using iodine vapour. After removal of the iodine by evaporation, the zone of R_f 0.46–0.73 was scraped into a scintillation vial and the gel resuspended in 1 ml distilled water. Radioactivity was measured in 10 ml scintillatio cocktail (0.5%, w/v) PPO in a solvent of toluene: Triton X-100 (2:1, v/v). Samples were allowed to stabilise overnight before counting in a Packard 460C spectrometer. The counting efficiency was 92 – 94% and the recovery of mevalonate was about 95%.

Enzyme assays were carried out in triplicate and the measurements expressed as the mean ∓ S.D. in units of pkat (pmol mevalonate formed/sec).

Protein measurements

Protein was determined by the method of Lowry *et al.* (1951) after precipitating with trichloracetic acid (10%, v/v).

RESULTS

Identification of mevalonate as the product of enzyme reaction

Previous studies of the reductase in latex employed the indirect coupled enzyme assay (Lynen, 1969) or the method of solvent extraction followed by separation on TLC (Hepper and Audley,

1969). These methods are laborious and time-consuming, and the latter in particular has a low recovery of mevalonate (about 60%). In the present work, the rapid single-step TLC method of Shapiro *et al.* (1974) was used for the first time on the latex enzyme. Fig. 1 shows the result of a typical separation. The radioactive zone with an R_f of 0.56 at the peak of radioactivity cochromatographed with unlabelled mevalonolactone marker and was presumed to be the product of the enzyme reaction. This zone was absent when boiled enzyme preparation was used. The R_f value obtained compares favourably with those reported in the literature (Shapiro *et al.* 1974); Ito *et al.* 1979). The bulk of the radioactivity remaining at or near the origin was unreacted substrate mainly, and HMG acid.

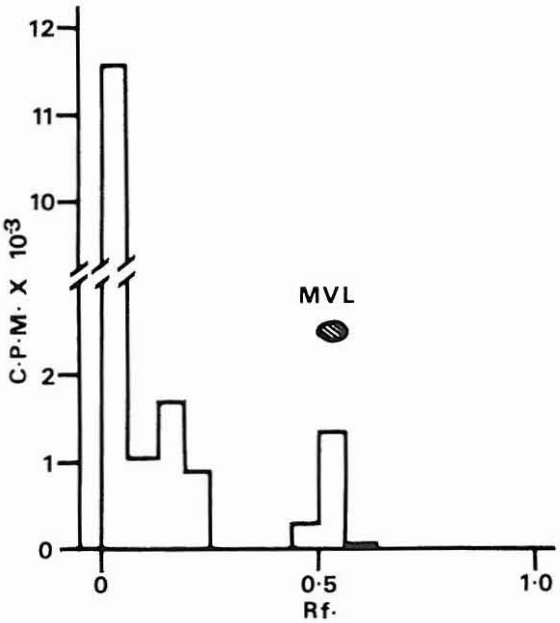

Fig. 1. Purification of mevalonate using TLC. *HMG CoA reductase activity in the bottom fraction was assayed as described in Materials and Methods. The above figure shows the radioactivity profile along the TLC plate. Unlabelled mevalonate (MVL) marker was visualised using iodine vapour. Under the same experimental conditions, both ^{14}C-labelled HMG CoA and HMG acid remained at or near the origin.*

Conditions for enzyme assay

The reductase in the bottom fraction appears to have a specific requirement for NADPH as the cofactor (Table 1). NADH was only 6% as effective. The rate of mevalonate formation was linear for up to 30 min incubation, and on using this incubation period, was also linear with up to 1.25 mg protein concentration. Under these conditions, the membrane-bound enzyme in the washed bottom fraction (see the legend to *Fig. 2*) was saturated at about 300 μM RS-HMG CoA. From a double reciprocal plot of the substrate saturation curve, an apparent K_m of 56 μM (*RS*-HMG CoA) and a V_{max} of 6.10 pkat/mg protein were obtained *(Fig. 2).* The K_m value obtained is similar to those reported for the rat liver enzyme (Langdon and Counsell, 1976). The pH optimum of the reductase in the bottom fraction was about 6.6 – 6.9 in 0.1M phosphate buffer (*Fig. 3*). This pH optimum is similar to that for the incorporation of HMG CoA into rubber (Hepper and Audley, 1969), as well as that of the reductase from various other sources (Brown *et. al.* 1973; Brooker and Russell, 1975a).

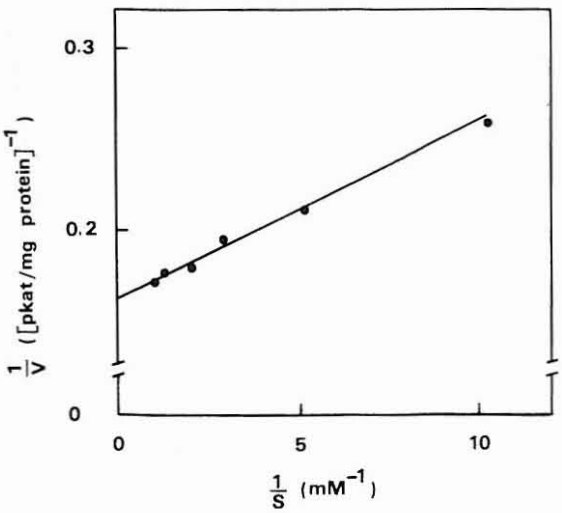

Fig. 2. A Lineweaver-Burke plot of the reductase activity in washed bottom fraction. *A fresh bottom fraction was resuspended in 5 mM triethanolamine-HCl buffer (pH 7.1) containing 2 mM dithiothreitol and then sonicated for 30 sec (Sonic 300 Dismembrator, Artek, U.S.A.). The suspension obtained was centrifuged at 103,000 g for 1 hr. The 103,000 g pellet was resuspended in Buffer A (washed bottom fraction) and enzyme activity assayed as described in Materials and Methods in the presence of varying amounts of RS-HMG CoA. Each point on the plot is the mean of triplicate measurements and the coefficiencts of variation for all points are less than 8%. The estimate for the apparent K_m and V_{max} is 56 μM and 6.10 pkat/mg protein respectively.*

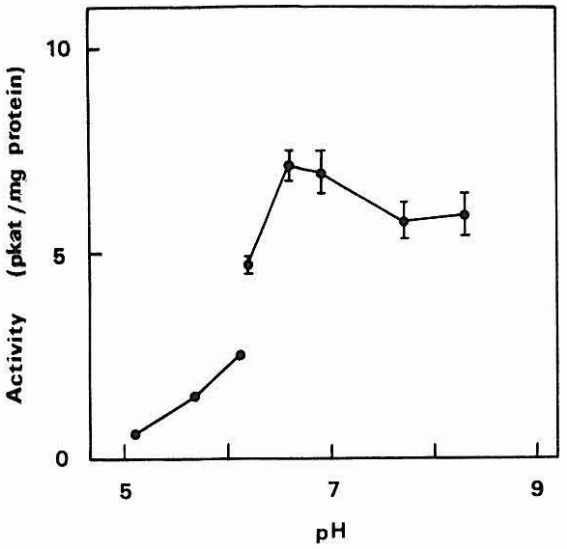

Fig. 3. pH profile of the reductase. *A fresh bottom fraction was resuspended in a buffer of a known pH and enzyme activity then assayed in this same buffer as described in Materials and Methods. A buffer of 0.1 M sodium citrate containing 20 mM EDTA and 2 mM dithiothreitol was used for the pH range of 5.0 – 6.5, while the buffer for the pH range 6.5 – 8.5 was 0.1 M sodium phosphate containing 20 mM EDTA and 2 mM dithiothreitol. Each point on the curve represents the mean,* ± S.D., of *triplicate measurements.*

TABLE 1
Cofactor Requirement of the reductase

Cofactor	Activity (pkat/mg protein)	%
NADPH (2 mM)	4.71 ± 0.83	100
NADPH omitted	0.13 ± 0.10	3
NADH (2 mM)	0.29 ± 0.19	6

A bottom fraction pellet stored at −15° C for 6 days was used in this experiment. Enzyme activity was measured as described in Materials and Methods in the absence and presence of the above nucleotides. Each value in the Table is the mean, ± S.D., of triplicate measurement.

Localisation of the reductase in latex

Fresh latex is a cytoplasmic suspension of numerous rubber particles and lutoids, and a smaller number of Frey-Wyssling complexes and other subcellular particles (Dickenson, 1969; Gomez and Moir, 1979). Centrifugation of the latex at about 42,000 g results in its separation into the rubber fraction, a small yellowish band containing mainly fragments of Frey-Wyssling particles (the Frey-Wyssling zone), the clear C-serum which is the cytosol and the sedimented bottom fraction made up largely of lutoids and some intact Frey-Wyssling complexes (Gomez and Moir, 1979).

The localisation of the reductase activity in the Frey-Wyssling zone, the C-serum and the bottom fraction is shown in Table 2. The bulk of the enzyme specific activity, as well as its activity/ml latex, was associated with the bottom fraction. The Frey-Wyssling zone also contained appreciable activity (about 23 – 32% of the specific activity in the bottom fraction) but in terms of volume of latex, the activity was low because this zone constituted only a small proportion of the centrifuged latex. The activity in the C-serum was quite neglible. Resuspending the bottom fraction in fresh C-serum resulted in a decrease in enzyme specific activity (Table 2) but on closer examination, the difference may not be significant in view of the large standard deviation in the measurement of activity. When boiled and deproteinated C-serum was used instead, there was a marked activation (about 50%) of the enzyme. Enzyme activity in the rubber fraction was not determined. Hepper and Audley (1969) however, reported that this fraction had neglible reductase activity.

Stability of the reductase

The effect of storing the bottom fraction at −15° C on enzyme activity was examined and the results in Table 3 show a decrease in activity of about 30% within 24 hr, and beyond this period for up to eight days, the activity remained at about 50% of that of the fresh enzyme. Dithiothreitol (2 mM) increased enzyme activity by up to 38% in the fresh bottom fraction (Sipat, 1982a) and up to 30% in the stored bottom fraction (Table 3). In the latter case however, the level of activity obtained still did not reach that of the fresh enzyme. Compared to the reductase obtained from seedling plants (Hepper and Audley, 1969), the enzyme in the present study appears to be more stable in spite of the absence of any added thiol compound. This difference may be due to the presence of endogenous thiols in the bottom fraction of latex from mature trees (Tan and Audley, 1968).

In view of reports that the rat liver enzyme is cold-labile, (Brown *et al.* 1973; Heller and Gould, 1974) the effect of collecting and centrifuging the latex under ambient temperature was also examined. Under this condition, the activity

TABLE 2
Distribution of reductase activity in latex

Latex fraction	Activity (pkat/mg protein)	(pkat/ml latex)
Expt. 1		
Frey-Wyssling zone	1.54 ± 0.06	0.14
C-serum	0.18 ± 0.05	0.51
Bottom fraction in Buffer A (1:1, pellet/v)	4.80 ± 1.03	8.69
Bottom fraction in C-serum (1:1, pellet/v)	3.54 ± 1.03	–
Expt. 2		
Frey-Wyssling zone	1.03 ± 0.31	0.08
C-serum	0.16 ± 0.01	0.44
Bottom fraction in Buffer A (1:1, pellet/v)	4.40 ± 0.28	9.68
Bottom fraction in boiled C-serum (1:1, pellet/v)	6.68 ± 0.11	–

The above results were obtained from two separate experiments. Fresh latex was fractionated into the various fractions and these were assayed for enzyme activity as described in the Materials and Methods. In Expt. 2, the C-serum was boiled for 10 min and the precipitated protein removed by centrifugation (3000 × g, 20 min). The supernatant obtained was then used to resuspend the bottom fraction. Each value in the Table is the mean, ± S.D. of triplicate measurements.

TABLE 3
Storage stability of the reductase

Period (days at −15°C)	Dithiothreitol (mM)	Activity (pkat/mg protein)
Fresh	0	4.19 ± 0.10
	2	4.33 ± 0.26
1	2	2.94 ± 0.46
2	0	1.72 ± 0.06
	2	2.24 ± 0.07
8	0	2.14 ± 0.22
	2	2.49 ± 0.18

Several bottom fraction pellets were prepared as described in Materials and Methods. One pellet was used fresh while the remaining were used after storage at −15°C for the stated duration. The enzyme activity was determined as described in Materials and Methods and where indicated, dithiothreitol was also omitted from Buffer A. Each value in the Table is the mean, ± S.D., of triplicate measurements.

obtained was 0.36 ± 0.03 pkat/mg protein as compared to an activity of 5.63 ± 0.42 pkat/mg protein under refrigeration (0–4° C).

Effect of temperature

The effect of varying the incubation temperature on the activity of the reductase in the bottom fraction is represented as the Arrhenius plot of enzyme activity vs the reciprocal of the absolute temperature *(Fig. 4)*. The plot is linear within the temperature range of 12 – 40°C and the Arrhenius activation energy (E_a) was estimated to be 57.3 kJ/mol/(13.7 kcal/mol). Washing the bottom fraction in hypotonic buffer did not significantly alter the Arrhenius plot of the membrane-bound enzyme (Sipat 1982b). The Arrhenius plot characteristics of the latex enzyme differ from those of normal-fed rat liver microsomal enzyme where at least one distinct break in the plot is observed at 28°C (Sipat and Sabine, 1982: Venkatesen and Mitropoulos, 1982). The differences in the Arrhenius plot characteristics may be related to the physical state of the membranes with which the respective enzymes are associated. In contract to the rat liver endoplasmic reticulum,

the membranes of the major organeller in the bottom fraction, ie. the lutoids, are known to be relatively non-fluid due to the high content phosphatidic acid and to the high degree of unsaturation of the fatty acids (Dupont *et al.* 1976).

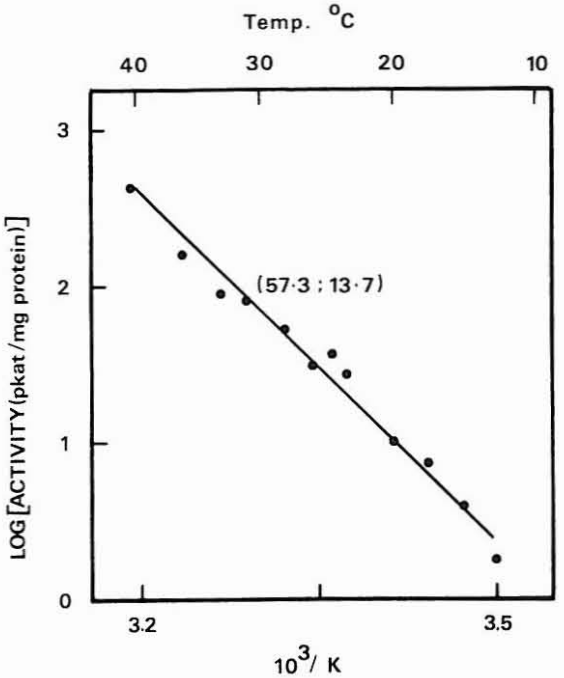

Fig. 4. Arrhenius plot of the reductase. *The assay for reductase activity, using fresh bottom fraction, was as described in Materials and Methods except that the incubation temperature was varied from 12 – 40° C via a series of waterbaths, each controlled to within ± 0.2° C by a Haake Model E52 heater (Berlin, Germany). Each point on the plot represents the mean of triplicate measurements and the coefficients of variation for all points were less than 10%. The activation energy is given in the bracket, first in kJ/mol and then in kcal/mol.*

Effect of pre-incubation on reductase activity

HMG CoA reductase from *Ipomea batatas* was found to be inactivated by pre-incubation (Suzuki and Uritani, 1977) whereas the enzyme from the rat liver does not exhibit such a behaviour and in fact, is activated by this treatment (Heller and Gould, 1974). In the light of these findings, the response of the latex enzyme towards pre-incubation was studied and the results presented in *Fig. 5* show that as much as 90% of its activity was lost upon pre-incubation for 1 hr at 30° C. Washing the bottom fraction in a buffer containing Triton X-100 (0.1%, w/v) did not prevent this inactivation (Table 4). Various compounds were tested for their efficacy as a stabiliser. Of these, bovine serum albumin has been found to minimise the loss of activity upon pre-incubation of the reductase from *I. batatas,* presumably by acting as a competitive substrate for any endogenous proteinases, and also by binding any inhibitory free fatty acids released by the action of endogenous phospholipases (Suzuki and Uritani, 1977). NADPH has also been found to stabilise the solubilised enzyme from rat liver (Ackerman *et al.* 1974; Tormanen *et al.* 1976). As shown in Table 5, both these compounds did not prevent the inactivation due to pre-incubation. Dithiothreitol on the other hand increased the enzyme activity when added to the assay mixture during pre-incubation (Table 5). An examination of the time-course of preincubation in the presence of dithiothreitol however, showed that this compound merely activated the enzyme activity, but did not prevent the loss of activity of the activated enzyme during the preincubation (Sipat, 1981).

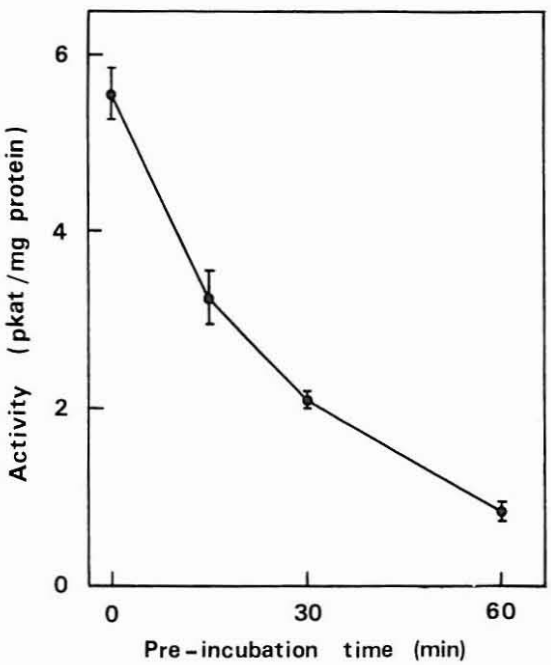

Fig. 5. Effect of pre-incubation on reductase activity. *A fresh bottom fraction was resuspended in Buffer A from which the dithiothreitol was omitted and enzyme activity was then assayed as described in Materials and Methods except that the enzyme was pre-incubated for the stated duration before starting the reaction by the addition of NADPH and the substrate. Each point on the curve represents the mean, ± S.D., of triplicate measurements.*

TABLE 4
Effect of pre-incubation on the activity of the reductase in washed bottom fraction

Enzyme source	Pre-incubation period (min)	Activity (pkat/mg protein)	%
Bottom fraction	0	5.31 ± 0.41	100
	30	2.85 ± 0.23	54
Washed bottom fraction	0	1.98 ± 0.05	100
	30	0.75 ± 0.04	38

A freshly prepared bottom fraction was resuspended in either Buffer A or Buffer A containing 0.1% (w/v) Triton X-100. The latter suspension was mixed vigorously, allowed to stand in ice for 15 min and then centrifuged at 103,000 × g for 1 hr. The 103,000 × g pellet (washed bottom fraction) was resuspended in Buffer A. Enzyme activity was measured as described in Materials and Methods except that the enzyme was pre-incubated at 30°C for 30 min before starting the reaction by the addition of the cofactor and substrate. Each value in the Table represents the mean, ∓ S.D., of triplicate measurements.

TABLE 5
Effect of pre-incubation in the presence of various compounds on reductase activity

Assay condition	Activity (pkat/mg protein)	%
No pre-incubation	2.57 ± 0.14	100
Pre-incubated 1 hr.	0.04 ± 0.02	2
Pre-incubated 1 hr. in the presence of BSA (1%, w/v)	0.05 ± 0.01	2
Pre-incubated 1 hr. in the presence of NADPH (2 mM)	0.12 ± 0.03	5
Pre-incubated 1 hr. in the presence of dithiothreitol (10 mM)	6.07 ± 0.47	236

A freshly prepared bottom fraction was resuspended in Buffer A from which dithiothreitol was omitted. Enzyme activity was measured as described in Materials and Methods except that for the pre-incubation step, the enzyme or the enzyme plus the stated compound, was pre-incubated at 30°C for 1 hr. before the reaction was started by the addition of the cofactor and the substrate. Each value in the Table is the mean, ± S.D. of the triplicate measurements.

DISCUSSION

The present work reports on the occurrence of the reductase in the latex of regularly tapped mature trees. Some of the properties of this enzyme are consistent with the earlier observations on the incorporation of HMG CoA into rubber (Hepper and Audley, 1969), e.g. the specificity for NADPH as the cofactor and the stimulatory effect of the heavy fraction (600 g) on HMG CoA incorporation. Although some activity was found in the Frey-Wyssling zone, the bulk of the reductase activity was particulate (42,000 g) and more recently, was shown to be membrane-bound (Sipat, 1982b). The practical problems encountered in the preparation of pure membranes of each of the organelle types present in the bottom fraction render it difficult to determine unequivocally the organelle localisation of the enzyme. Several lines of evidence, however, indicate that the reductase may be mainly associated with the lutoids. Firstly, the bottom fraction is made up predominantly of lutoids (Dickenson, 1969). Measurements of the enzyme activity in latex fractions enriched in either lutoid fragments or Frey-Wyssling complexes showed that the former had a specific activity about 5-fold higher than that of the latter (Sipat, 1982b). Secondly, the linear Arrhenius plot of enzyme activity indicates that the reductase is located in a relatively non-fluid membrane, which is characteristic of the membranes of the lutoids (Dupont *et al.* 1976). Thirdly, the lutoids appear to be derived from the vacuoles (Dickenson, 1964) while the latter in turn is thought to originate from the endoplasmic reticulum (Matile, 1969), which is the major site for the reductase in the rat liver (Rodwell *et al.* 1976) and in *P. sativum* (Brooker and Russell, 1975b). The endoplasmic reticulum incidently, has rarely been observed in the bottom fraction (Gomez and Moir, 1979) although it is seen in electron micrographs of the laticifer (Gomez, 1974).

Like the reductase from *I. batatas* (Suzuki and Uritani, 1977), the enzyme in the bottom

fraction is also sensitive to pre-incubation. This feature will have a negative effect on attempts to define the optimal assay conditions for the enzyme *in vitro*. The resulting loss of activity is not due to the action of endogenous proteases, since the addition of bovine serum albumin gave no protection. The action of various phospholipases, however, would alter the lipid composition of the membrane in which the enzyme is located and, if it is lipid-dependent, may result in the loss of activity. This apparently, is the explanation for the sensitivity of reductase from *I. batatas* (Suzuki and Uritani, 1977). In the case of the latex enzyme, phospholipase D, while present in the C-serum, has not been detected in the lutoid fraction (Dupont *et al.* 1976). Thus the observed behaviour of the latex enzyme is yet to be explained.

In view of the membrane-bound nature of the reductase, it is quite pertinent to examine the effect of the soluble cellular component (the C-serum) on enzyme activity. While the results presented earlier (Table 2) on this aspect may be ambiguous, more recent work has established beyond doubt that the C-serum has an activating effect (Isa, 1982; Isa and Sipat, 1982). The activator in the C-serum is heat-stable and appears to be a protein. Further work is being carried out to characterise the activator and to determine its mechanism of action. An understanding of the interaction between the reductase and the other components in latex would contribute to our knowledge of the function and role of this enzyme in the regulation of rubber biosynthesis.

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