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Short communication

The low temperature induced rate of 3-hydroxy-3-methylglutaryl-CoA reductase in *Parthenium argentatum* Gray limits the theoretical rate of rubber formation

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ABSTRACT

The accumulative low temperature of 5–7 in the fall and winter of the Chihuahuan Desert induces rubber biosynthesis in *Parthenium argentatum* Gray (guayule) and the enzyme activities of 3-hydroxy-3-methylglutaryl-CoA reductase and rubber transferase. The conclusion that the induced rates of 3-hydroxy-3-methylglutaryl-CoA reductase and rubber transferase account, in part, for the induced rate of rubber biosynthesis is incomplete. The objective of this Short Communication was to determine if the induced rate of 3-hydroxy-3-methylglutaryl-CoA reductase supported the rate of rubber biosynthesis as determined by the rate isopentenyl pyrophosphate polymerization to rubber polymers catalyzed by rubber transferase. The rates of these 2 enzymes were measured in the bark of lower stems of guayule from July to December in plants transplanted to the field in the Chihuahuan Desert in May. At the peak of the low temperature induced rubber formation the induced rate of 3-hydroxy-3-methylglutaryl-CoA reductase of 29.9 nmol MVA h^{-1} g fr wt^{-1} was significantly lower than the induced rate of rubber transferase of 357.5 nmol IPP h^{-1} g fr wt^{-1}. The rate of 3-hydroxy-3-methylglutaryl-CoA reductase was 8.3% of the theoretical rate of rubber formation based on the rate of rubber transferase at saturating concentrations of isopentenyl pyrophosphate Mg^{2+} and dimethylallyl pyrophosphate initiator *in vivo*. A new interpretation of the induced developmental rate curves of 3-hydroxy-3-methylglutaryl-CoA reductase and rubber transferase is that the low temperature induced rate of 3-hydroxy-3-methylglutaryl-CoA reductase limits rubber biosynthesis in guayule. The new calculations support the conclusion that isopentenyl pyrophosphate from an alternate source, other than the mevalonic acid pathway may additionally supply isopentenyl pyrophosphate to rubber transerase for polymerization into natural rubber.
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1. Introduction

Bealing (1976) reported the rate of several enzymes of the Mevalonic Acid (MVA) Pathway in the latex of *Heveabrasiliensis*. The rate of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) of 0.078 nmol MVA ml^{-1} latex min^{-1} was only 0.35% of the theoretical rate of rubber formation as measured by the rate of rubber transferase (RT).

In *Partheniumar gentatum* Gray (guayule) rubber biosynthesis is stimulated by low temperature (Appleton and van Staden, 1989; Benedict et al., 2013, 2008, 1986; Bonner, 1943; Cornish and Backhaus, 2003; Downes and Tonnet, 1985; Gilliland et al., 1985; Goss et al., 1984; Ji et al., 1993; Sundar and Reddy, 2000; Veatch-Blohm et al., 2007). Guayule is indigenous to the Chihuahuan Desert of Northeastern Mexico and Southwestern Texas and Benedict et al. (1986) determined that the accumulative temperatures of 13 °C and below in the fall and winter of the Chihuahuan Desert stimulated RT and rubber biosynthesis. Ji et al. (1993) reported that the accumulative temperatures of 5–7 °C during the fall and winter of this desert stimulated HMGR-1 mRNA, HMGR, RT activities and rubber biosynthesis. It was concluded that the stimulated activities of HMGR and RT accounted for, in part, rubber biosynthesis but no determinations were made on the relation of the rate of HMGR to the rate of rubber formation.

Abbreviations: HMGS, 3-hydroxy-3-methylglutaryl-CoA synthase; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; RT, rubber transferase; MVA, mevalonic acid; MEP, 2-*C*-methyl-D-erythritol-4-phosphate; qRT–PCR, quantitative reverse transcriptase–polymerase chain reaction; IPP, isopentenyl pyrophosphate; DMAPP, dimethylallyl pyrophosphate.

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This communication is a new interpretation of the work published by Ji et al. (1993). We calculate that the rates of HMGR in guayule induced by the low temperatures of the fall and winter of the Chihuahuan Desert limit the theoretical formation of rubber.

2. Materials and methods

Section 2 is a brief review of the methods in the previous paper by Ji et al. (1993) important to this short communication.

2.1. Plant material

Seed of guayule were germinated in the greenhouse in small pots of vermiculite. After 2 mo of growth the seedlings were transplanted to field plots in the Chihuahuan Desert at the Texas Agrilife Experiment Station at Ft. Stockton, TX in May, 1992. The seedlings were planted in 30 ft. rows randomly throughout the field plots. Samples for enzyme, DNA and RNA analyses were taken randomly from plants in these replicate rows from June, 1992 to December, 1992.

2.2. Temperature

In the Chihuahuan Desert field plots the accumulative hours of 5–7 °C from May to December were calculated from continuous daily temperature recordings. The developmental plots of rubber biosynthesis and the activities of HMGR and RT in the guayule plants were from September–October to December.

2.3. Enzyme extraction and assay

2.3.1. HMGR

The procedure for the assay of HMGR in the paper of Ji et al. (1993) followed the procedure described for the assay of HMGR in corn leaves (Ji et al., 1992). This extraction and differential centrifugation of the bark homogenate resulted in the isolation of a 105,000 × *g* pelleted membrane fraction that was suspended in phosphate buffer and assayed for HMGR activity at 30 °C for 60 min. The mean of the activity of the membrane bound HMGR from 3 individual plants selected randomly from the guayule field plots was plotted against the time of year to establish the developmental curve in the previous paper Ji et al. (1993). Lines representing the range of HMGR activity in the 3 plants were drawn on the developmental curve. The exposure of the guayule plants to the low temperatures of 5–7 °C from September–October to December 20, 1992 induced HMGR activity by 6-fold.

2.3.2. MVA kinase, IPP isomerase and RT

Aliquots of the bark homogenate obtained by grinding the bark samples in liquid N_2 and suspending the ground samples in Tris–HCl buffer were used for the assays of MVA kinase, IPP isomerase and RTas described in the previous paper Ji et al. (1993). The temperature for these assays was 30 °C for 30 min. The MVA kinase and IPP isomerase activities were determined by analyzing 2 plants throughout the year.

The activities of RT bound to rubber particles present in the bark homogenates were determined by the procedure of Madhaven et al. (1988). The mean of the activity from 3 plants was plotted against the time of year to establish the developmental curve. Lines representing the range of RT activity in the 3 plants were drawn on the developmental curve. The assays of RT were run at 30 °C for 60 min. The temperature for determining the optimal activity of 30 °C had previously been established by determining the RT activities from 5 to 40 °C (Benedict, 1991). These results demonstrated that the low temperature did not induce a cold-sensitive RT enzyme. The exposure of the guayule plants to the accumulative low temperatures of 5–7 °C from September–October to December 20, 1992 induced RT activity by 9-fold.

2.4. Northern blots

Examination of RNA from the bark of the lower stems of guayule plants from June to November with a tomato HMGR-1 cDNA probe demonstrated higher levels of HMGR mRNA in June during seedling growth and in November during induced rubber biosynthesis (Ji et al., 1993). The higher levels of HMGR mRNA also corresponded to high levels of HMGR activity in June for the synthesis of cellular membranes and in November for rubber biosynthesis. The higher HMGR mRNA throughout the low temperature of the fall and winter of the Chihuahuan Desert demonstrated that the low temperatures induced HMGR gene transcription and accounted for, in part, the higher levels of HMGR resulting in higher enzymatic rates of HMGR.

2.5. Rate of rubber biosynthesis

The *in vitro* rate of RT in nmol IPP h^{-1} g fr wt^{-1} of the homogenized stem bark at saturating concentrations of IPP, Mg^{2+} and DMAPP is equal to the *in vivo* rate of rubber biosynthesis nmol rubber h^{-1} g fr wt^{-1} if the *in vivo* concentrations of IPP, Mg^{2+} and DMAPP are sufficient to saturate the cellular rubber particle bound RT. The *in vivo* concentration of IPP, Mg^{2+} and DMAPP in guayule stem bark has not been measured therefore the *in vitro* rate of RT is equal to the theoretical rate of rubber biosynthesis. The *in vitro* rates of HMGR, MVA kinase or IPP isomerase limit the theoretical rate of rubber biosynthesis if the rate of one of these enzymes is below the theoretical rate of rubber biosynthesis.

3. Results

The data in Table 1 is from the developmental curves of HMGR, RT and rubber formation were determined from stem samples from August 20, 1992 to December 20, 1992 published by Ji et al. (1993). The rubber content of the plants increased from 500 to 4400 mg $plant^{-1}$ from September 29 to December 20. During the biosynthesis of rubber, in the low temperature period of 5-7 °C, the HMGR activity increased from 5.0 to 29.9 nmol MVA h^{-1} g fr wt^{-1} and RT activity increased from 56.3 to 357.5 nmol IPP h^{-1} g fr wt^{-1}. The statement that these findings demonstrate that the development of activities of HMGR and RT may, in part, account for rubber biosynthesis may be correct, but is incomplete.

Table 1
The development of the rates of HMGR and RT in guayule in field plots in the Chihuahuan Desert presented as developmental curves in previous paper (Ji et al., 1993).

Date	HMGR (nmol MVA h^{-1} g fr wt^{-1})	RT (nmol IPP h^{-1} g fr wt^{-1})	Rubber (mg $plant^{-1}$)
August 20	3.0	28.0	0.0
September 8	2.1	47.0	25.0
September 29	5.0	56.3	500.0
October 14	12.1	50.0	700.0
November 15	21.7	143.2	2730.0
December 20	29.9	357.5	4400.0

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