Diurnal variation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in latex of *Hevea brasiliensis* and its relation to rubber content

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Summary. Temporal variation of HMG-CoA reductase activity in latex of *Hevea brasiliensis* was investigated. The latex enzyme showed diurnal variation of activity during a 24-h cycle. Peak activity occurred around sunset (18.00 h) and was approximately 2-fold above basal level during the daylight period. Rubber content in the latex also varied diurnally in a pattern similar to the variation of HMG-CoA reductase activity. Two clonal types used in the study (RRIM 600 and KRS 21) showed the same variation patterns. The results suggested a possible correlation between rubber biosynthesis and the regulation of HMG-CoA reductase activity.
Key words. Hevea brasiliensis; Euphorbiaceae; latex; hydroxymethylglutaryl CoA reductase; rubber biosynthesis.

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (mevalonate: $NADP^+$ oxidoreductase; EC 1.1.1.34) catalyzes the reduction of HMG-CoA to mevalonate. Mevalonate is then further converted to isopentanyl pyrophosphate, the building unit of isoprenoid compounds such as cholesterol and dolichol as well as natural rubber. HMG-CoA reductase has frequently been shown to be the rate limiting enzyme for cholesterol biosynthesis in mammalian system[2]. In rubber latex, this enzyme was found to have the lowest activity among the enzymes in pathways of rubber biosynthesis from acetate[3]. The physiological significance of the low activity of HMG-CoA reductase in rubber latex is unknown at present. However, it is likely to be one of the rate-limiting enzymes in rubber biosynthesis.

When rubber latex was centrifuged at 49,000 × g for 40 min, the HMG-CoA reductase was found mainly in the bottom fraction which consists of lutoid particles and a small amount of Frey-Wyssling complex[4]. HMG-CoA reductase was also reported to be a membrane-bound enzyme[5] as was the case for the mammalian enzyme. HMG-CoA reductase has shown to exhibit a circadian variabity of activity in mouse[6], rat[7,8] hamster[9], and swine livers and ileum[10]. The same variation pattern of sterol synthesis was also reported in rat liver[8]. The present study was designed to examine the temporal variation in *Hevea* latex HMG-CoA reductase activity and its relation to rubber content.

Materials and Methods. Chemicals. All solvents and chemicals used were of analytical reagent grade. DTT, NADPH, unlabeled HMG-CoA, and unlabeled mevalonolactone were purchased from Sigma. [3-^{14}C] HMG-CoA was purchased from Amersham, England.

Collection and fractionation of latex. The latex was obtained from three regularly tapped (S. 2/D. 2) trees of *Hevea brasiliensis*, clones RRIM 600 and KRS 21 (each approximately 15 years old). The two clones, grown at the rubber research center, Hat-Yai, show similar yield capacity. A half spiral tapping was performed every other day on the same set of trees at the times indicated in figures 1 and 2. After tapping, the latex was collected into a beaker surrounded by crushed ice for 30 min. Latex from each clone was separately pooled and fractionated by centrifugation at 49,000 × g for 40 min, exclusive of acceleration and deceleration times. The pellet at the bottom of centrifuge tube, 'bottom fraction', was pooled and assayed for HMG-CoA reductase activity. The top (floated) rubber layer was pooled and oven dried at 60 °C for 48 h before the determination of the percentage of rubber content (w/v).

Assay of HMG-CoA reductase activity. The HMG-CoA reductase activity was assayed in 0.2 ml of buffer A (0.15 M sucrose, 15 mM DTT, 37 mM EDTA, 75 mM Tris-HCl pH 7.0) containing bottom fraction protein, 1.0–1.8 mg; NADPH, 0.4 µmoles; DL-[3-^{14}C] HMG-CoA 120 nmoles (1060 dpm/nmole). Incubation was conducted at 37 °C for 30 min. The reaction was stopped by the addition of 25 µl of 10 N HCl; unlabeled mevalonolactone was then added, and the mixture was allowed to incubate for 30 min for complete lactonization of the incubation product, mevalonic acid. The precipitated protein was removed by centrifugation and the product of the enzyme was copurified with the unlabeled mevalonolactone by TLC using benzene:acetone (1:1 v/v) and visualized under iodine vapor according to the method of Iijima et al.[11]. The zone of mevalonolactone was scraped into a scintillation vial containing 10 ml of dioxane fluor. Samples were allowed to stabilize overnight before counting in a Packard liquid scintillation counter.

Protein measurements. Protein was determined by the method of Lowry et al.[12] after trichloroacetic acid (10% v/v) precipitation.

Results and discussion. The latex HMG-CoA reductase obtained from the bottom fraction of 49,000 × g centrifugation exhibited

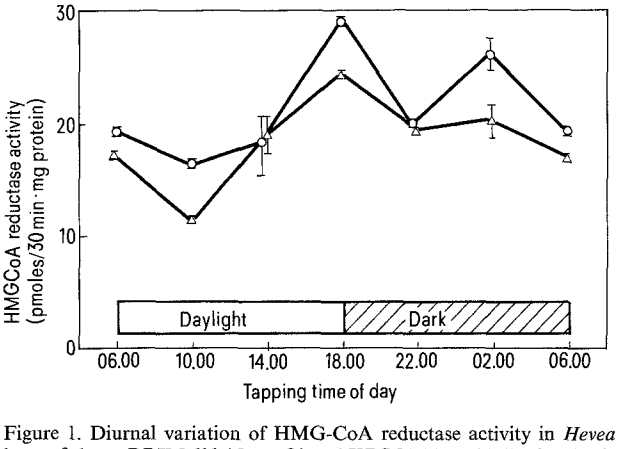

Figure 1. Diurnal variation of HMG-CoA reductase activity in *Hevea* latex of clones RRIM 600 (○—○) and KRS 21 (△—△). Each value is the mean ± SD of triplicate measurements. The unit of enzyme activity was defined as pmoles of mevalonate produced per 30 min/mg protein.

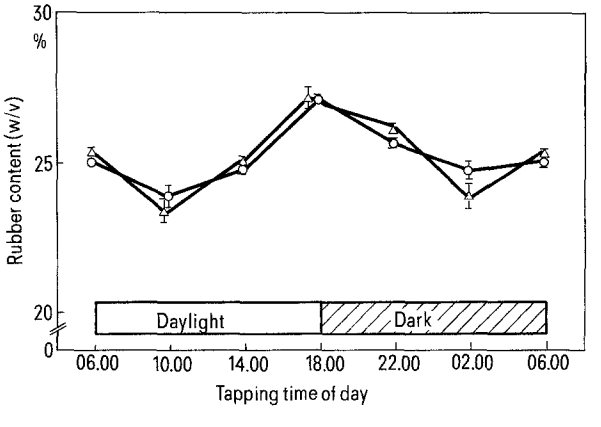

Figure 2. Diurnal variation of percentage of rubber content (W/V) in *Hevea* latex of clone RRIM 600 (○—○) and KRS 21 (△—△). Each value is the mean ± SD of triplicate measurements.

variation of enzyme activity during the cycle of 24-h study. Maximum activity was observed at the beginning of sunset (18.00 h), when it was 2.1-fold higher than the minimum activity during the early daylight period (10.00 h) in RRIM 600 and 1.7-fold in KRS 21 (fig. 1). Figure 1 shows similar patterns for both clonal types investigated. The apparent regulation of the light-dark phase is probably due to physiological processes associated with photosynthesis. The process of rubber biosynthesis requires three components: acetyl-CoA as building block, NADPH as reducing agent, and ATP as energy source. All three components are generated by degradation of carbohydrate which is an end-product of photosynthesis[3]. The carbohydrates are being synthesized and accumulated throughout the photosynthesis period, increasing in level by the end of the day[13,14]. During the dark period one or more of the three components become a limiting factor causing the decline in HMG-CoA reductase activity. The requirement of these components for rubber biosynthesis could be considered analogous to the study on diet fed to animals. The diurnal variation in *Hevea* HMG-CoA reductase is probably a natural daily cycle of fasting and feeding similar to that suggested earlier for the rat liver enzyme[8,15].

It is generally observed that rubber trees tapped after sunrise give less latex than those tapped during night time[16]. Supporting data were obtained in this study. The percentage of rubber content in latex in either clone RRIM 600 or KRS 21 was highest at sunset, 18.00 h, and remained relatively high throughout the night. During the daylight period the rubber content was lower, and it was lowest at 10.00 (fig. 2). The percentage of rubber content varied diurnally, closely parallel to the pattern exhibited by HMG-CoA reductase activity. This relationship showed that the level of HMG-CoA reductase activities was closely associated with the rubber content in the latex. The correlation suggested the importance of this enzyme in the regulation of rubber biosynthesis.

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High resolution isoelectric focusing of juvenile hormone esterase activity from the hemolymph of *Trichoplusia ni* (Hübner)

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Summary. Juvenile hormone esterase (JHE) activity from the hemolymph of larval *Trichoplusia ni* was analyzed by two different isoelectric focusing (IEF) methodologies. Use of techniques capable of progressively higher resolution split ultimately what appeared at lower resolution to be a single peak into two discrete peaks of JHE activity (pI 5.5 and 5.3). Neither peak was a degradation artifact of the other caused by conditions of IEF.
Key words. Juvenile hormone esterase; *Trichoplusia ni;* isoelectric focusing.

Juvenile hormone (JH), an endocrine of central importance in insect metamorphosis, is thought to be regulated, in part, by ester hydrolysis. JH esterase (JHE) activity is present in many insect orders[2], and has been shown to peak in the hemolymph twice during the last larval stadium of many Lepidoptera[3]. Recent extensive studies and reviews have centered around *Trichoplusia ni* (Hübner) as a model for the study of lepidopteran JHE[4,5]. Their results have led to the conclusion that there are not multiple forms of JHE in the *T. ni* model system, rather JH ester hydrolysis is due to a single enzyme or polypeptide chain.

In the present study we have re-examined the results of isoelectric focusing techniques with respect to the number of *T. ni* JH esterases which are identifiable on polyacrylamide IEF gels.

Materials and Methods. Insects. The laboratory colony of *T. ni* was maintained at 28 °C, 14:10 light:dark[6]. Last day, feeding stage larvae were bled as described previously[3]. Chemicals. Radiolabeled JH I, II and III were obtained from NEN while unlabeled homologs were obtained from Sigma or Calbiochem-Behring. Preliminary tests showed profiles of JHE activity obtained from IEF gels are the same using all three homologs.

Isoelectric focusing. Polyacrylamide gels (5% with 3% cross linking) were cast and held overnight prior to use. LKB Ampholine® or Pharmacia Pharmalyte® was used to create pH gradients of either 3.5–9.5 or 4–6.5. Alternatively, LKB Immobiline® compounds (which are not as salt sensitive) were used to create a pH gradient of 5–6. The gels were run on a LKB Multiphor apparatus, in either the 9-cm or 23-cm direction. 20 µl of hemolymph diluted 1:1 with double distilled water (dHOH) were added to an 0.5 × 1.0 cm paper wick placed at the edge of a lane. On some Immobiline gels 60 µl of such diluted hemolymph were added in 3 portions. After electrofocusing at power settings and a time duration described by the supplier (see figure legend), the gel was sliced into sections. Each section was placed into 400 µl of dHOH, for pH determination or phosphate buffer (pH 7.4, I = 0.2 M) for assay of enzyme activity, and eluted overnight at 4 °C. Each sample of eluate was appropriately diluted and assayed in duplicate for JHE activity.

JHE assay. A partition assay previously described[7] was used to measure JHE activity, except that samples in which the enzymatic activity was terminated with the methanolic stopping solu-