MAGNESIUM AFFECTS RUBBER BIOSYNTHESIS IN *FICUS ELASTICA*

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ABSTRACT

Rubber biosynthetic rate and molecular weight are affected by the concentration of magnesium, a rubber biosynthesis activator, in *in vitro* assays of washed rubber particles (WRP). Maximum rubber transferase activity occurred at about 1 mM Mg^{2+} and was inhibited by concentrations above 10 mM Mg^{2+} for *Ficus elastica* WRP. The Mg^{2+} concentration in the latex cytosolic fraction was 231 mM suggesting that the living plants have inhibitory Mg^{2+} levels in their laticifer cytosols. Gel permeation chromatography was used to study the molecular weight and distribution of natural rubber from *F. elastica*. Latex containing rubber particles was collected from young and old *F. elastica* trees. Light (enzymatically-active) and heavy (enzymatically less active) WRP were isolated from fresh latex. All the *F. elastica* samples contained >92% low molecular weight materials. These lower molecular weight fractions had markedly different polymer sizes (Mw): 45,630 *g*/mol for heavy WRP and 2,860 *g*/mol for light WRP, but the significance of these differences is not yet understood.

INTRODUCTION

Natural rubber biosynthesis occurs in laticifers of *Ficus elastica* in microscopic rubber particles. *Ficus elastica* , the Indian rubber tree, is a potential multi-use plant, belongs to the Moraceae family, and produces latex in laticifers, like the commercial Brazilian rubber tree, *Hevea brasiliensis*[1](Fig.1). Natural rubber, *cis*-1,4-polyisoprene, is an important strategic industrial material. Rubber transferase, a membrane-bound protein complex on rubber particles, catalyzes rubber biosynthesis by using isopentenyl pyrophosphate (IPP) as the monomer, an allylic pyrophosphate (farnesyl pyrophosphate, FPP) as an initiator, and magnesium ions as cofactor/activator[2,6,8]. Previous studies demonstrated that the optimal concentrations of magnesium for the rubber transferase activity are different among the different natural rubber species[5,7-10]. The weight average molecular weight (*Mw*) of the rubber in the latex are smaller for *F. elastica* (401,000) when compared to those of *H. brasiliensis* and *Parthenium argentatum* (>1,000,000) [7,9,10].

MATERIALS & METHODS

F. elastica latex was collected and light (enzymatically-active) and heavy (enzymatically less active) washed rubber particles (WRP) were isolated by centrifugation and washing[4,6]. Proteins present in latex and WRP were extracted. Latex from individual *F. elastica* trees also was collected for rubber transferase activity analysis and for metal element determination by using inductively coupled plasma (ICP). Both latex and WRP were analyzed by using gel permeation chromatography, with refractive index detection, to determine rubber molecular weight and distribution.

RESULTS

Light and heavy WRP were separated by centrifugation and rubber transferase activity was determined. Most rubber transferase activity was associated with the light WRP. The Heavy WRP had about 1/3 of rubber transferase activity compared to that of light WRP (data not shown). WRP proteins were extracted and analyzed along with latex and its cytosolic fraction (Fig.2). Few proteins were associated with the light WRP- one major protein is about 12 kDa and a few minor proteins were present between 30-45 kDa. There are several dominant proteins associated with heavy WRP with molecular sizes of about 30 kDa (two proteins), and between 10-21 kDa (3-4 proteins). Several WRP proteins stained only with silver, including a protein larger than 200 kDa, which is likely the 376 kDa protein observed in earlier research[3,4]. This protein was absent in the cytosolic fraction and, in contrast to early work, also missing from the light WRP[3,11]. Rubber transferase activity in *F. elastica* WRP is dependent upon Mg^{2+} concentration (Fig.3). The maximum rubber transferase activity occurred at about 1 mM Mg^{2+} which is similar to that of *P. argentatum* and *H. brasiliensis*. The rubber transferase activity was inhibited approximately 50% when Mg^{2+} concentrations increased to 20 mM and was completely inhibited at 100 mM Mg^{2+}. The dependence of IPP incorporation on chelating EDTA concentrations was examined using WRP isolated without Mg^{2+} in the wash buffer (data not shown). There was very little rubber transferase activity in *F. elastica* WRP compared to the minimal EDTA concentrations for maximum inhibition of rubber transferase activity of 0.01 mM EDTA for *H. brasiliensis* and 0.25 mM EDTA for *P. argentatum.* The rubber transferase activity in latex tapped from eight individual *F. elastica* plants varied among plants but was at least eight times below the maximum activity of WRP determined *in vitro* (IPP incorporation at 0.04-0.32 vs. 2.6 µmol/g dry rubber), suggesting the presence of inhibitors in the cytosol that are effectively removed by washing. The rubber transferase activity of latex from a single *F. elastica* tree was decreased when exogenous magnesium increased, and was completely inhibited at 50 mM Mg^{2+} (Fig.4). Metal element analysis of latex cytosolic fractions revealed the endogenous magnesium level is in the range of 212 to 247 mM, suggesting that the living plants have inhibitory Mg^{2+} levels in their laticifer cytosol.

Figure 1. *Ficus elastica* plant

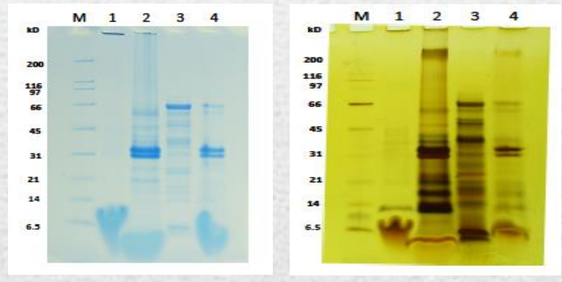

Figure 2. SDS-PAGE analysis of *Ficus elastica* proteins from latex, cytosol, light and heavy WRP. Left panel: Coomassie blue stained; Right panel: Silver stained. M: MW markers; 1-4 are proteins samples extracted from 1: Light WRP; 2: Heavy WRP; 3: Cytosolic fraction; 4: Latex.

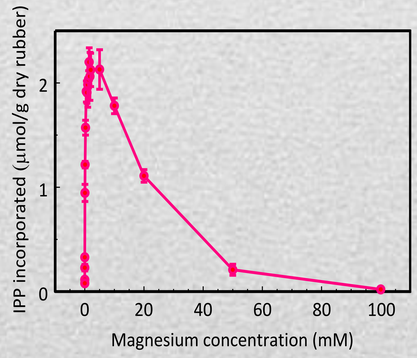

Figure 3. Dependence of IPP incorporation on Mg^{2+} concentration in *Ficus elastica* WRP.

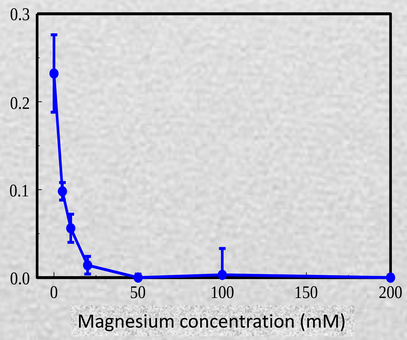

Figure 4. Effect of Mg^{2+} concentration on rubber transferase activity in *Ficus elastica* latex.

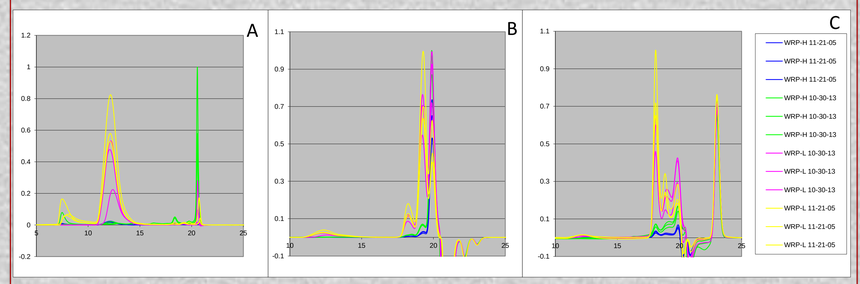

Figure.5. GPC analysis of *Ficus elastica* light and heavy WRP. Three runs on each sample were analyzed. WRP-H: heavy WRP; WRP-L: light WRP; WRPs were isolated on 11-21-2005 and 10-30-2013. Detector response vs. retention time (min) A: light scattering; B: refractive index; C: UV absorbance.

RESULTS

Samples	Mw (*g*/mol)	Mw/Mn	low Mw (RI) %	Low Mw area UV	Low Mw rubber (*g*/mol)
Ficus latex A	1,037,667	2.66	94.38	0.053	4,952
Ficus latex B	1,036,667	3.03	94.56	0.054	9,698
Ficus latex C	1,202,333	2.84	96.35	0.054	8,777
Ficus latex D	1,181,000	2.95	95.35	0.054	8,467
Ficus WRP light 1	1,199,033	2.36	95.18	0.015	2,041
Ficus WRP heavy 1	566,467	1.26	98.62	0.004	66,523
Ficus WRP light 2	1,429,000	2.21	92.48	0.014	3,671
Ficus WRP heavy 2	1,188,667	1.92	99.15	0.001	24,740

Table 1. Summary of GPC analysis. Rubber molecular weight and distribution determined by using gel permeation chromatography. *Ficus elastica* latex samples A&B were latex from 6-month old trees and C&D were latex from 2-year-old trees. *F. elastica* light and heavy WRP 1 (10-30-2013) were analyzed along with control WRP 2 (11-21-2005). Weight average molecular weight (Mw)(*g*/mol) were determined by light scattering detector and average % low Mw peaks were detected by using a differential refractive index.

F. elastica light, heavy WRP and latex samples collected from individual young and old *F. elastica* trees were analyzed by using gel permeation chromatography (GPC) for rubber molecular weight and distributions (Fig. 5 and Table 1). All the *F. elastica* samples contained a small proportion of high molecular weight natural rubber, approximately 1.0 x 10^6 *g*/mol except one heavy WRP sample (566,467 *g*/mol) but contained >92% low molecular weight materials by differential refractive index detection. These lower molecular weight fractions had markedly different polymer sizes, average 45,630 *g*/mol for heavy WRP and 2,860 *g*/mol for light WRP.

CONCLUSIONS & DISCUSSION

Firstly, our studies show that the optimal magnesium concentration for rubber transferase in *F. elastica* is about 1 mM, similar to those of other rubber-producing species, *H. brasiliensis* and *P. argentatum.* This is contrast to an earlier report, where the optimal concentration was considerably higher suggesting that different lines of *F. elastica* may differ or that growth conditions can have significant effects[5,9,10]. Secondly, rubber transferase activity was inhibited by magnesium concentrations above 10 mM particularly in *F. elastica* which declined more rapidly compared to the other two species. Magnesium concentrations in *H. brasiliensis* latex ranged from 1 to 5.6 mM (unpublished data) but were much higher in *F. elastica* latex (231 mM) which would be highly inhibitory to rubber transferase activity. Thirdly, as the molecular weight of rubber molecules from *H. brasiliensis* is very high, but low (most rubber molecules >92%) in *F. elastica,* the high magnesium concentrations in *F. elastica* might play a significant role in the synthesis of low molecular weight rubber molecules. It has previously been shown in *P. argentatum* and *H. brasiliensis* that excess Mg^{2+} will reduce molecular weight[7,8]. Fourthly, a protein larger than 200 kDa was associated with heavy WRP but not in light WRP. This is probably the 376 kDa protein reported in early studies which may plays an important role in rubber biosynthesis[2,3,11].

ACKNOWLEDGEMENTS & REFERENCES

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