Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 67 (2006) 1621–1628
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Magnesium ion regulation of in vitro rubber biosynthesis by *Parthenium argentatum* Gray

Bernardo M.T. da Costa [a], Jay D. Keasling [a,b,c], Colleen M. McMahan [d], Katrina Cornish [e,*]

[a] *Department of Chemical Engineering, University of California, Berkeley, CA 94720–3224, USA*
[b] *Department of Bioengineering, University of California, Berkeley, CA 94720–3224, USA*
[c] *Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA*
[d] *USDA-ARS, Western Regional Research Center, Albany, CA 94710, USA*
[e] *Yulex Corporation, 1945 Camino Vida Roble, Suite C, Carlsbad, CA 92008, USA*

Received 13 October 2005; received in revised form 23 February 2006
Available online 15 June 2006

This paper is dedicated to Professor R. Croteau on the occasion of his 60th birthday.

Abstract

Natural rubber is produced by a rubber transferase (a *cis*-prenyltransferase). Rubber transferase uses allylic pyrophosphate to initiate the rubber molecule and isopentenyl pyrophosphate (IPP) to form the polymer. Rubber biosynthesis also requires a divalent metal cation. Understanding how molecular weight is regulated is important because high molecular weight is required for high quality rubber. We characterized the in vitro effects of Mg^{2+} on the biosynthetic rate of rubber produced by an alternative natural rubber crop, *Parthenium argentatum* (guayule). The affinity of the rubber transferase from *P. argentatum* for IPP · Mg was shown to depend on the Mg^{2+} concentration in a similar fashion to the *H. brasiliensis* rubber transferase, although to a less extreme degree. Also, in vitro Mg^{2+} concentration significantly affects rubber molecular weight of both species, but molecular weight is less sensitive to Mg^{2+} concentration in *P. argentatum* than in *H. brasiliensis*.

Keywords: *Parthenium argentatum*; Compositae; *Hevea brasiliensis* Muell. Arg.; Euphorbiaceae; Regulation; Rubber transferase; *cis*-Prenyltransferase; Magnesium; FPP; IPP

1. Introduction

Although there are over 2500 plant species that produce natural rubber (Bealing, 1969; Bonner, 1991), there are only a few species that produce the high molecular weight rubber necessary for high product quality and performance (Swanson et al., 1979). Currently, the main commercial source of natural rubber is *Hevea brasiliensis* Muell. Arg. (Brazilian or Para rubber tree), which produces high molecular weight rubber obtained by tapping the laticifers in the tree bark (D'auzac et al., 1989). However, *H. brasiliensis* commercial production is limited to tropical regions of the world. Also, it is one of the most genetically uniform crops under cultivation, as it has a narrow breeding ancestry, and depends almost entirely on plantation-grown clonal trees (clonal scions grafted onto seedling root stocks), which makes it prone to pathogenic attack (Davis, 1997). *Parthenium argentatum* Gray (guayule), a native of the Chihuahuan desert of Mexico and Texas, is another plant species that produces high molecular weight rubber (Ray, 1993), and is an alternative source of natural rubber for commercial use. *P. argentatum* is being commercially developed as a source of latex for medical products because its rubber particle-associated proteins do not cross react with IgE (Type I latex allergy) and IgG antibodies to *H. brasiliensis* latex proteins (Siler and Cornish, 1994; Carey et al., 1995; Siler et al., 1996; Cornish et al., 2005).

* Corresponding author. Tel.: +1 760 476 0320; fax: +1 760 476 0321.
E-mail address: kcornish@yulex.com (K. Cornish).

0031-9422/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2006.04.010

Natural rubber is synthesized by rubber transferase (EC 2.5.1.20), a membrane bound *cis*-prenyltransferase (Archer and Audley, 1987; Madhavan et al., 1989; Cornish and Backhaus, 1990; Cornish, 1993; Cornish and Siler, 1995; Cornish et al., 1999), which requires an allylic pyrophosphate (APP) to initiate the rubber molecule, isopentenyl pyrophosphate (IPP) as the source of monomer used to elongate the polymer (Archer and Audley, 1987; Madhavan et al., 1989; Benedict et al., 1990; Cornish and Backhaus, 1990; Cornish, 1993; Tanaka et al., 1996; Scott et al., 2003; da Costa et al., 2005) and a divalent cation, such as Mg^{2+} or Mn^{2+}, as cofactor (Archer and Audley, 1987; Madhavan et al., 1989; Cornish and Backhaus, 1990; Cornish, 1993; Scott et al., 2003; da Costa et al., 2005).

In vitro, a number of APPs can be used by rubber transferases from *P. argentatum*, *H. brasiliensis* and *Ficus elastica* Roxb. (Indian rubber tree) to initiate the rubber molecule (Archer and Audley, 1987; Madhavan et al., 1989; Cornish and Siler, 1995). In vivo, farnesyl pyrophosphate (FPP) is thought to be the main initiator of rubber molecules as it has a lower binding constant than other APPs (Cornish, 2001), is produced in the cytosol of laticifers, which is the same compartment as rubber transferase, and NMR data indicates that at least in *H. brasiliensis* the rubber molecule has a *trans* double bond at the initiation site (Tanaka et al., 1996). In vitro, changes in IPP and APP concentrations affect the rubber molecular weight of the rubber produced by *H. brasiliensis*, *P. argentatum* and *F. elastica* (Castillón and Cornish, 1999; Cornish et al., 2000). Under identical [IPP] and [FPP] (IPP and FPP concentration, respectively) in vitro conditions, *H. brasiliensis* and *P. argentatum* produce rubber with half the molecular weight of that made by *F. elastica* (Cornish et al., 2000), while, in vivo, *F. elastica* produces rubber with a lower molecular weight than *H. brasiliensis* or *P. argentatum*. This discrepancy suggests that the rubber transferases themselves are not the primary determinators of rubber molecular weight and was attributed to the higher affinity of *F. elastica* rubber transferase for IPP in the presence of FPP (Cornish et al., 2000). This result also suggests that plants regulate their rubber molecular weight in vivo by a mechanism other than, or in addition to, regulation of [IPP] and [FPP]. Possible mechanisms could involve regulation of polymer termination or regulation of cofactor availability.

In vitro, the metal ion cofactor concentration was shown to affect the IPP incorporation rate by the rubber transferases from *F. benghalensis* (Kang et al., 2000b) and *F. carica* (Kang et al., 2000a), *F. elastica* (Scott et al., 2003), *H. brasiliensis* (Kang et al., 2000a,b; Scott et al., 2003; da Costa et al., 2005) and *P. argentatum* (Scott et al., 2003). The rubber transferase from *F. elastica*, *H. brasiliensis* and *P. argentatum* requires the metal ion as a cofactor and as an activator, such that, at low metal ion concentrations, the metal ion deinhibits the rubber transferase activity, whereas at high concentrations, it inhibits the rubber transferase activity (Scott et al., 2003). Therefore, there is a metal ion concentration, $[A]_{max}$, that gives a maximal IPP incorporation rate, V_{max} (Scott et al., 2003). Throughout the remainder of this paper we shall refer to the metal ion as a cofactor even though it also acts as an activator. It also has been shown that the rubber transferase from *F. elastica*, *H. brasiliensis* and *P. argentatum* can bind FPP, FPP · metal or metal ion alone, whereas it can bind IPP · metal or metal ion alone, but not IPP alone, and that Mg^{2+} is the in vivo cofactor (Scott et al., 2003).

In vitro, the concentration of Mg^{2+} radically affects the affinity of the *H. brasiliensis* rubber transferase for IPP · Mg, which suggests that the Mg^{2+} concentration may have a regulatory role in rubber biosynthesis (da Costa et al., 2005). The metal ion cofactor concentration also affects the molecular weight of the rubber produced by *H. brasiliensis* (da Costa et al., 2005).

Here, we characterize the role of Mg^{2+} concentration in rubber biosynthesis by enzymatically active rubber particles purified from *P. argentatum*. The effect of $[Mg^{2+}]$ on initiation, biosynthetic rate, molecular weight and substrate affinity was determined and then compared to rubber biosynthesis in *H. brasiliensis*.

2. Results and discussion

2.1. IPP incorporation rate dependence on $[Mg^{2+}]$

In vitro, the IPP incorporation rate by *P. argentatum* was $[Mg^{2+}]$ dependent (Fig. 1). At low levels of $[Mg^{2+}]$,

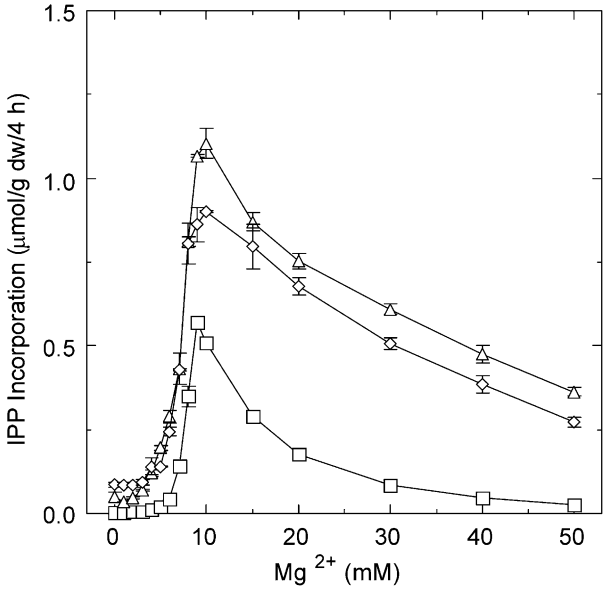

Fig. 1. Dependence of [^{14}C]-IPP incorporation on magnesium ion concentration by rubber transferases of *Parthenium argentatum* purified rubber particles. Incorporation rates of [^{14}C]-IPP were measured in 15 µM FPP, 7 mM EDTA, varying $[Mg^{2+}]$ and different [IPP]: 37.5 µM, □; 375 µM, △; 1000 µM, ◇. The error bars in the figure represent the standard deviation of triplicates.