

Composition of rubber particles of *Hevea brasiliensis*, *Parthenium argentatum*, *Ficus elastica*, and *Euphorbia lactiflua* indicates unconventional surface structure

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Abstract

Natural rubber (*cis*-1,4-polyisoprene) is formed inside plant cells from allylic diphosphate initiators and isopentenyl diphosphate, and packaged in subcellular rubber particles. In addition to rubber, rubber particles contain proteins, which vary in size in different species. Rubber transferase [EC 2.5.1.20], the enzyme that catalyzes the *cis*-1,4 polymerization of isoprene into rubber, is particle bound. Since membrane lipids have been identified in extracts of *Hevea brasiliensis* rubber particles, it has been suggested that rubber particles are covered by a half-unit membrane. We have examined the fatty acid and lipid composition of rubber particles from four rubber producing species, *H. brasiliensis*, *Parthenium argentatum*, *Ficus elastica*, and *Euphorbia lactiflua*. We demonstrate that although rubber particles from all four species contain significant amounts of phospholipids, glycolipids and sterols, there appears to be insufficient membrane lipid to constitute a half-unit membrane. Our results suggest that the rubber particle surface is a mosaic of protein, conventional membrane lipids and other components. The lipid covering may be a remnant of the rubber particle's origin in a unit membrane, with the addition of non-conventional components, including a large amount of non-phospholipid phosphate containing material, possibly polyisoprene mono- or diphosphate.

Key words

Cis-1,4-polyisoprene, natural rubber, oil bodies, rubber particles, *Parthenium argentatum*, *Hevea brasiliensis*.

Abbreviations

FAME, fatty acid methyl esters; GC/MS, gas chromatography/mass spectrometry; GLC, gas-liquid chromatography; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; TLC; thin-layer chromatography.

INTRODUCTION

Natural rubber (*cis*-1,4-polyisoprene) is formed inside plant cells from allylic diphosphate initiators and isopentenyl diphosphate, and packaged in subcellular rubber particles. Although over 2000 different plant species make natural rubber, currently, all commercial natural rubber comes from a single species, *Hevea brasiliensis*, the Brazilian rubber tree. *H. brasiliensis* produces rubber in a network of laticifer vessels, which can be tapped to obtain the latex, a complete cytoplasm consisting of rubber particles (25-45% of latex volume) as well as organelles and solu-

ble proteins (de Fay *et al.*, 1989). Electron microscopy reveals rubber particles as osmiophilic globules surrounded by a thin electron-dense layer (Backhaus and Walsh, 1983). Earlier workers classified rubber particles as spherosomes, along with oil bodies (Yatsu and Jacks, 1972). Whereas oil bodies have been extensively studied (Huang, 1992), less is known about rubber particle structure. Rubber particles range in size from about 10 μm to below 0.2 μm (Backhaus and Walsh, 1983; Cornish *et al.*, 1993). The surfaces of rubber particles are negatively charged, which helps keep them in suspension in latex (de Fay *et al.*, 1989). Rubber particles contain proteins, which vary in size

range in different species (Dennis *et al.*, 1989; Backhaus *et al.*, 1991; Cornish *et al.*, 1993). However, the structure of the rubber particle-cytoplasm interface, and the ontogeny of rubber particles, remain unclear.

A half-unit membrane structure for spherosomes was first suggested by Yatsu and Jacks (1972) with the inside of the membrane lipoidal and the outside polar. A half-unit membrane on rubber particles would permit the thermodynamically favorable compartmentalization of the hydrophobic rubber hydrocarbon chain inside the particle, and a polar surface to interact with the aqueous cytoplasm. This would provide a suitable environment for the rubber particle-bound enzyme rubber transferase [EC 2.5.1.20], which catalyzes the *cis*-1,4 polymerization of isoprene into rubber, using substrates synthesized by soluble enzymes (Archer *et al.*, 1963; Siler and Cornish, 1993). The amount of polar lipid and protein in oil bodies from maize, linseed, and safflower is enough to cover the particles with a half-unit membrane (Slack *et al.*, 1980; Stymne and Stobart, 1987; Tzen and Huang, 1992), although Kleinig *et al.* (1978) reported that oil bodies from carrot suspension cells do not have enough phospholipid and protein to constitute a half-unit membrane. Since membrane lipids (phospholipids, glycolipids, and sterols) have been identified in extracts of *H. brasiliensis* rubber particles (Ho *et al.*, 1975; Hasma, 1991), it has been assumed that rubber particles also are covered by a membrane (Dupont *et al.*, 1976; Hasma, 1991), more specifically, a half-unit membrane (Attanyka *et al.*, 1991). However, the necessary quantitative studies to confirm the presence of a true membrane surrounding rubber particles have not been reported. We have undertaken such a study to test the hypothesis of a half-unit membrane at the surface of rubber particles.

We examined the composition of purified rubber particles from four plant species. Three of the plants produce rubber as latex in laticifer cells. *H. brasiliensis* produces long chain rubber, with average molecular mass 1.31×10^6 (Swanson *et al.*, 1979). *E. lactiflua*, a desert plant native to Chile, produces intermediate length rubber, and *F. elastica*, Indian rubber tree, produces mostly short chain rubber. Unlike laticifers, the desert shrub *P. argentatum* (guayule) accumulates rubber in the cytoplasm and vacuoles of parenchyma cells, predominantly in the bark. *P. argentatum* produces long chain rubber comparable in chain length and commercial quality to *Hevea* rubber.

RESULTS

Fatty acid composition of rubber particles

The compositions of fatty acids, identified as FAME, from rubber particles of *H. brasiliensis*, *P. argentatum*, *F. elastica* and *E. lactiflua* rubber particles are given in table 1. The rubber particle FAME extracts contained some unidentified material in addition to the identified FAME. More of this material was extracted with longer exposure to solvents, and the extracts became more viscous, suggesting extraction of rubber, short-chain polyprenols, or other isoprenoids: *H. brasiliensis* latex contains many different isoprenoid compounds such as isoprenoic acids (Hasma and Subramaniam, 1986), and tocotrienols have been identified in extracts of *H. brasiliensis* rubber particles (Hasma, 1991). *P. argentatum* plants contain up to 10% resins, including guayulins (sesquiterpene cinnamic acid esters) (Sidu *et al.*, 1995), and molecular mass analysis of rubber extracted from *F. elastica* rubber particles revealed large amounts of substances with molecular mass of 1000 or less (Cornish *et al.*, 1993). A molecular mass of 1000 would reflect an average chain length of 15 isoprene units.

Rubber particle phospholipids

In order to reduce the interference from unidentified material in the FAME mixture, and to examine the membrane lipid fatty acids of most interest, the lipid extracts were separated into lipid classes. The phospholipid fatty acid profiles of the four plant species are different, but all have fatty acids typical of plant membranes (tab. 1). The amounts of phospholipid were determined from the amounts of FAME assuming two fatty acids per molecule (tab. 2). Phospholipids identified by TLC were phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphatidic acid (PA) (fig. 1). Two of the major phospholipids in *F. elastica* (indicated by arrows) are unconventional (fig. 1). The relative abundance of the two bands varies in different preparations, and generally exceeded the amount of PC and PE. Although unidentified, these lipids do contain fatty acids and phosphorus. Similar bands were observed as minor components in phospholipid extracts from the other species. The phospholipid profile of *E. lactiflua* was similar to that of *P. argentatum* (TLC plate not shown). Hasma (1991) detected phospholipid in *H. brasiliensis* rubber

Table 1. Distribution of fatty acids in lipids of *H. brasiliensis*, *P. argentatum*, *F. elastica* and *E. lactiflua* rubber particles. Percentage based on total of all fatty acids identified.

	Fatty acid													Total ($\mu\text{g mg}^{-1}$)
	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0	24:0	26:0	28:0	Hydroxy	FA	Furanoid
<i>H. brasiliensis</i>														
Total	4		10	4	11									71
Neutral lipids	6		12	4	13	2	1							63
Phospholipid	8	2	26	13	43	5	2							
Glycolipid	8		20	9	17	2	3					2		39
<i>P. argentum</i>														
Total	9		2	8	53	28								10
Neutral lipids	8		1	5	53	33								
Phospholipid	33		8	10	40	4			4					
Glycolipid	10		3	4	14	7			2			60		
<i>F. elastica</i>														
Total	10				16		4	21	18	13	18			8
Neutral lipids	26		2	2	42	3	5	12	8					
Phospholipid	39		11	14	37									
Glycolipid	18	2	12	12	14	1	1		1			39		
<i>E. lactiflua</i>														
Total	64		9	18	9									1
Neutral lipids	52	22	3	4	8	3	4	3						
Phospholipid	29	18	11	12	26	4								
Glycolipid	6		4	5	10	2						73		

particle extracts, and Dupont *et al.* (1976) reported that the major component of *H. brasiliensis* rubber particle phospholipid extracts was PC, with small amounts of PE and phosphatidylglycerol, and no detectable PA. Our results show large amounts of both PC and PE in *H. brasiliensis* rubber particles (fig. 1).

Rubber particle glycolipids

The fatty acid compositions of the glycolipids of the four species also are conventional (tab. 1). High levels

of hydroxy fatty acids were detected in three of the species, ranging in size from 16:0 to 24:0. Tentative identification by mass spectrometry included 16:0 and 24:0 in *P. argentatum* and *E. lactiflua*, and 16:0 in *F. elastica*. Hydroxy fatty acids are common constituents of cell surface glycosphingolipids in many organisms, including plants (Lynch and Steponkus, 1987). Unidentified compounds, possibly isoprenoids, were present with FAME in the total glycolipid fraction. These compounds did not contain fatty acid or sterol, and were not characterized further.

Table 2. Composition of rubber particles from *H. brasiliensis*, *P. argentatum*, *F. elastica* and *E. lactiflua*.

	(nmol mg ⁻¹)							Protein (mg mg ⁻¹)
	Glycolipid fatty acid	Phospholipid fatty acid	Sterol glucosides	Sterols	Hexose	Phosphate	Free sterol composition	
<i>H. brasiliensis</i>	3.7	2.6	0.06	2.7	8.2	9.8	61% β -sitosterol 15% stigmasterol 24% fucosterol	0.002
<i>P. argentatum</i>	2.2	0.5	nd	0.4	17.6	9.6	81% β -sitosterol 9% stigmasterol	0.001
<i>F. elastica</i>	2.4	0.6	nd	0.5	3.8	3.1	sitosterol only	0.0015
<i>E. lactiflua</i>	6.2	0.23	0.6	0.6	19.5	1.3	sitosterol only	0.048

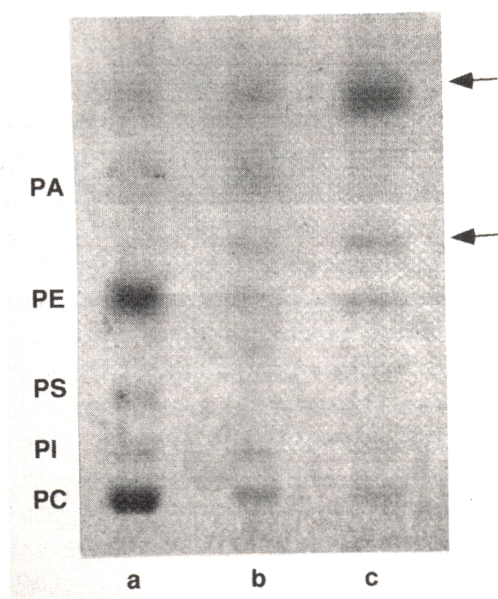


Figure. 1. Rubber particle phospholipids. Lipids were extracted from rubber particles of *H. brasiliensis* (a), *P. argentatum* (b), and *F. elastica* (c), and the phospholipid fraction separated by thin layer chromatography and compared to phospholipid standards. The phospholipids identified were phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphatidic acid (PA). Two of the major phospholipids in *F. elastica* (indicated by arrows) are unconventional.

The major glycolipid bands in each sample were steryl glycoside or glycosphingolipid (which comigrated on TLC). Except for *H. brasiliensis*, the samples contained no detectable galactosyl diglycerides, and acylated steryl glycosides were minor components. Hasma (1991) also detected free and esterified steryl glycosides in *H. brasiliensis* rubber particle extracts, as well as monogalactosyl and digalactosyl diglycerides. Glycosphingolipids contain one fatty acid and one long-chain base. Therefore, for quantitation, we assumed a single fatty acid or sterol per molecule (corrected for the galactosyl diglycerides in the case of *H. brasiliensis*) (tab. 2).

Rubber particle sterols

Sterols were detected in rubber particle extracts from the four plant species (tab. 2). The amounts of sterols were likely underestimated because sterol esters were not analyzed, since they were incompletely resolved by TLC from interfering compounds, possibly isoprenoids. However the amount of sterol esters present appeared to be less than that of free sterols. Steryl glycosides (acylated or non-acylated) were only

detectable in *H. brasiliensis* and *E. lactiflua*, and contained only β -sitosterol. Amounts of free sterols comparable to those detected in purified rubber particles (tab. 2) were reported in *H. brasiliensis* latex (Hasma and Subramaniam, 1986), and free sterols were identified, but not quantified, in *H. brasiliensis* rubber particle extracts (Hasma, 1991). Unlike our results from purified rubber particles (tab. 2), sterol glycosides from latex contained all three sterols (Hasma and Subramaniam, 1986). Acylated sterols also were reported in *H. brasiliensis* latex and rubber particles, but amounts were not determined (Hasma and Subramaniam, 1986; Hasma, 1991).

Rubber particle neutral lipids

Much of the total fatty acid is in triglycerides. *H. brasiliensis* triglyceride has an unusual furanoid fatty acid, 10,13-epoxy-11-methyloctadeca-10,12-dienoic acid, which was first identified in *H. brasiliensis* latex where it constituted about 86% of the total esterified acids (Hasma and Subramaniam, 1978), and also was reported in the neutral lipid fraction of *H. brasiliensis* rubber particles (Hasma, 1991). Our results confirm that it is the major esterified fatty acid in the neutral lipid fraction of purified *H. brasiliensis* rubber particles, but it was not detected in rubber particle extracts from *P. argentatum*, *F. elastica* or *E. lactiflua* (tab. 1), and was not present in *H. brasiliensis* phospholipids. A peak with the same retention time as a furanoid fatty acid was detected in extracts of total glycolipids from *H. brasiliensis*, but not in any individual glycolipids analyzed. Since the neutral lipids, including triglycerides, are thermodynamically unlikely to be on the rubber particle surface, they were excluded from calculations of surface coverage. The neutral lipids would partition with the rubber, which makes up over 96% of rubber particle mass.

Rubber particle protein, hexose and phosphate

The amounts of protein, hexose, and phosphate derived from rubber particles of the four species are given in table 2. The amounts of hexose and phosphate present exceed the amounts which can be readily attributed to glycolipids and phospholipids, respectively. Surface staining of *H. brasiliensis* rubber particles indicated the presence of complex poly-saccharides (Hébrant, 1980), and the most abundant proteins in *F. elastica* and *P. argentatum* rubber particles are

glycoproteins (Backhaus *et al.*, 1991; Siler and Cornish, 1993).

Previous attempts to quantify glycolipids and phospholipids in latex and rubber particle extracts were obtained by multiplying the experimentally determined amounts of sugar and phosphorus by the conversion factors 4.7 and 25.9 respectively (Hasma and Subramaniam, 1986; Hasma, 1991). This method assumes that all of the sugar and phosphorus are attributable to conventional glycolipids and phospholipids, with a single sugar per glycolipid. Our data indicate that this method overestimates the amounts of these lipids present.

DISCUSSION

Rubber is an end product of metabolism, which cannot be reutilized by the plant (Archer and Audley, 1973). Therefore, packaging of rubber within rubber particles is a one-way process, requiring no mechanism for release. In contrast, oil bodies serve a dual purpose in storage and release of triacylglycerols for energy. More than 90% of the proteins on oil bodies are oleosins, a family of small (15-26 kDa) alkaline proteins unique to the oil bodies, and all oleosins contain specific conserved structural domains which determine the positioning of the protein within the oil body membrane and may bind lipases during germination (Huang, 1992). However, rubber particle-bound proteins vary widely among species (Cornish *et al.*, 1993), suggesting most are non-conserved. Rubber particles from *H. brasiliensis* have more than 30 proteins, while rubber particles from *P. argentatum* and *F. elastica* have only a few detectable proteins (Cornish *et al.*, 1993). The most abundant *H. brasiliensis* and *P. argentatum* rubber particle proteins are 14.6 kDa, and 52 kDa, respectively (Dennis *et al.*, 1989; Backhaus *et al.*, 1991), but neither protein is rubber transferase (Dennis *et al.*, 1989; Pan *et al.*, 1995), which has yet to be conclusively identified in any species. Furthermore, sequence comparisons show no homology between the 14.6 kDa and 52 kDa proteins (Attanyaka *et al.*, 1991; Pan *et al.*, 1995), and between either of these rubber particle proteins and maize oleosin (Qu and Huang, 1990). Antibodies raised to intact rubber particles of *H. brasiliensis* and *P. argentatum* recognized almost exclusively the 14.6 kDa and 52 kDa proteins, respectively (Siler *et al.*, 1996). Antibodies raised against solubilized

H. brasiliensis rubber particle proteins recognized additional epitopes not exposed in intact rubber particles (Siler and Cornish, 1994). Thus, both proteins likely are localized at the rubber particle surface.

Yatsu and Jacks (1972) proposed that spherosomes such as oil bodies could form as a result of oil being secreted into the lipoidal area between the layers of a unit (bilayer) membrane. Lipid-producing membranes in the endoplasmic reticulum apparently accumulate lipid between the phospholipid layers, eventually forming an oil body (Wanner *et al.*, 1981). Rubber particles could form in a similar way, starting with a membrane-bound rubber transferase. The elongating hydrocarbon chain of the first rubber molecule synthesized would likely segregate to the hydrophobic interior of the bilayer. As more rubber molecules are formed, the interior of the membrane would swell, eventually pinching off to form a particle of rubber surrounded by a membrane monolayer. Such a partitioning within a phospholipid bilayer has been suggested for dolichol (*cis*-1,4 polyisoprene), with the neutral dolichol in between the monolayers and the phosphate group of the dolichol phosphate located at the membrane-cytosol interface (McCloskey and Troy, 1980). Dolichol, found in most membranes of eukaryotic cells, differs from natural rubber only with respect to chain length. Dolichols contain from 17 to 24 isoprene residues, whereas natural rubber contains thousands.

As rubber polymerizes at the surface of the rubber particle, and the growing hydrocarbon chain diffuses into the interior of the rubber particle, the hydrophilic negatively charged diphosphate end-group likely remains at the surface, where it can react with isopentenyl diphosphate bound to the rubber transferase active site (Archer *et al.*, 1963). The smallest rubber particles that can be detected are less than 0.1 μm (Backhaus and Walsh, 1983), and likely contain only a few rubber molecules.

Electron spin resonance (EPR) studies using a series of stearic acid spin probes, intimated the presence of a membrane at the surface of rubber particles from *H. brasiliensis*, *P. argentatum*, *F. elastica*, and *E. lactiflua* (Siler *et al.*, 1995). The volume occupied by such a membrane was calculated for each species (tab. 3), assuming that a half-unit membrane on a rubber particle would have a thickness of 2.5 nm, and using previously determined mean rubber particle diameters (Cornish *et al.*, 1993). Estimates were made of the fraction of the membrane volume which would

be taken up by phospholipids, glycolipids, sterols and proteins, using the experimentally determined values for each given in table 2. While the amounts of rubber particle lipid and protein vary among the four species, all have significant amounts of conventional membrane lipids. The fraction of rubber particle surface shell occupied by protein is much lower for *H. brasiliensis* and *P. argentatum* than for *F. elastica* and *E. lactiflua* (tab. 3). This is consistent with EPR studies, which indicate low lipid-protein ratios for the surfaces of *F. elastica* and *E. lactiflua* rubber particles, and higher lipid-protein ratios for *H. brasiliensis* and *P. argentatum* (J.J. Windle, personal communication).

Although the amounts of typical membrane components are significant, on average there appears to be insufficient phospholipid, glycolipid, sterol and protein to provide complete monolayer coverage of the rubber particles, with the exception of *E. lactiflua* which contains a high percentage of protein (tab. 3). Millichip *et al.* (1996) reported that sunflower oil bodies treated with 9 M urea to remove non-oleosin protein and the bulk of the phospholipid, were stable and of a similar diameter to those present *in vivo*, but had less than 0.1% of the phospholipid required to form a half-unit membrane surrounding the particles, and no phosphatidylserine or other negatively charged lipids. They suggest that the urea-washed oil bodies were covered by an oleosin protein coat rather than a conventional membrane, and that oleosin plays the major role in oil body stabilization (Millichip *et al.*, 1996). However, this report has been disputed by Ratnayake and Huang (1996), who contend that a calculation error in Millichip *et al.* (1996) underestimated the amount of phospholipid. Rubber particles from *E. lactiflua* appear to be covered mostly with protein, with a very small amount of membrane lipid (tab. 3). How-

ever, rubber particles from *H. brasiliensis*, *P. argentatum*, and *F. elastica* have considerably less protein than *E. lactiflua* or oil bodies.

Rubber particles, unlike oil bodies (Tzen and Huang, 1992), can coalesce when pressed against each other. Electron micrographs of rubber-containing cells in guayule show spherical rubber particles, as well as some larger irregular-shaped rubber particles, which may have been formed by the coalescence of smaller particles which are pushed together by cell contents (Backhaus and Walsh, 1983), indicating that rubber particles have form-retaining as well as fluid properties. Lee *et al.* (1994) reported that guayule rubber particles can aggregate during preparation under conditions that do not cause aggregation of oil bodies. Once such aggregation has occurred, the rubber coagulates to form insoluble clumps. However, the rubber particles used in the studies reported here were prepared using centrifugation speeds much lower than those used by Lee *et al.* (1994), and did not result in aggregation. Rubber particle size distributions for a given species were consistent among different rubber particle preparations (data not shown). Therefore it is reasonable to assume that loss of surface area during preparation is unlikely, and that the sizes used in our determinations of surface coverage are representative of those found *in vivo*. Pendle and Swinyard (1991) found no significant change in rubber particle size distributions in *H. brasiliensis* latex during storage up to one year, and concluded that there was negligible, if any, spontaneous agglomeration of the latex particles.

There may be other membrane components which were not examined in this study. There is more lipid-associated sugar than can be accounted for by a single

Table 3. Surface coverage of rubber particles by lipid and protein. The values for mean rubber particle diameter are from Cornish *et al.* (1993).

	<i>H. brasiliensis</i>	<i>P. argentatum</i>	<i>F. elastica</i>	<i>E. lactiflua</i>
Mean rubber particle diameter (μm)	0.96	1.41	3.8	0.42
Rubber particle (mg prot. mg^{-1})	0.002	0.001	0.002	0.048
% of 2.5 nm shell filled				
Phospholipid	4.5	1.3	4.1	0.2
Glycolipid	12.9	11.2	33.0	9.5
Sterol	9.8	2.0	6.9	1.8
Protein	9.2	6.8	27.3	97.7
Total lipid and protein	36.4	21.3	71.3	109.3
Non-phospholipid phosphate-containing compounds	29.6	47.8	38.5	1.8
Grand total	66.0	69.1	109.8	111.1

hexose per glycolipid (*tab. 2*). This may indicate multiple sugar residues per molecule, as well as the presence of other sugar esters, perhaps glycosylated isoprenoids structurally related to dolichol or saponins. Furthermore, phosphate analyses showed that there is more phosphate than can be accounted for by phospholipid (*tab. 2*), indicating the presence of unidentified phosphate containing compounds. The extra phosphate unaccounted for by phospholipid may include isoprenoid mono- or diphosphates, or polyisoprene diphosphate at the particle surface, as hypothesized by Archer *et al.* (1963). We calculated the maximum volume of such material by assuming that all of the non-phospholipid phosphate was on isoprenoid monophosphates, with a diameter similar to that of a membrane lipid (0.7 nm) (*tab. 3*). Such material could make up a significant part of the surface of rubber particles from *H. brasiliensis*, *P. argentatum*, and *F. elastica*, but not *E. lactiflua* rubber particles, which, as discussed earlier, appear to be coated with much more protein than the other species (*tab. 3*).

Calculations of rubber particle surface coverage are dependent on estimates of rubber particle size. Cornish *et al.* (1993) used two different statistical analysis methods for their estimations of mean rubber particle size, which gave slightly different results. The mean rubber particle sizes reported for *H. brasiliensis* using the two methods were 0.96 μm used in our calculations for *table 3*, and 1.23 μm (Cornish *et al.*, 1993). Using the larger value, the calculation of total surface area covered by phospholipid, glycolipid, sterol and protein increases from 36.4% (*tab. 3*) to 46.6%, still insufficient to provide complete coverage.

Thus, our results suggest that the rubber particle surface is a mosaic of protein and conventional membrane lipids and non-standard components, including a large amount of phosphate containing material, possibly polyprenyl phosphate. It is not, however, a conventional half-unit membrane.

METHODS

Plant materials. *Hevea brasiliensis* latex was generously provided by J. R. Bugansky, Senior Botanist, Plantations Operations, The Goodyear Tire & Rubber Co., from *H. brasiliensis* trees (clone PB260) at the Goodyear Plantations in Sumatra. *Euphorbia lactiflua* latex was generously supplied by A. Pooley, Universidad de Concepcion, Chile. *Ficus elastica* plants were obtained from a local nursery and grown in a

greenhouse at Albany, CA. *F. elastica* latex was obtained by excising the stems and petioles and collecting the exudate. *Parthenium argentatum* plants (line 11591) were field-grown at the U.S. Water Conservation Laboratory, Phoenix, AZ. Bark from mature plants was air-transported on ice to Albany, CA.

Preparation of rubber particle suspensions. Rubber particles were purified from latex of *H. brasiliensis*, *F. elastica*, and *E. lactiflua* by centrifugation and resuspension as previously described (Cornish *et al.*, 1993). *P. argentatum* bark was homogenized and rubber particles purified as previously described (Backhaus *et al.*, 1991). Rubber particle dry weight was determined by pipetting purified rubber particles on tared 0.22 μm filters, allowing the filters to dry in a 37°C oven, and weighing.

Protein assay. Rubber particle proteins were solubilized with Triton X-100 and quantified using the 2,2'-bichinonic acid (BCA) protein assay (Pierce, Rockford, IL), as modified by Siler and Cornish (1995).

Lipid extraction and analyses. For determination of total fatty acid composition, aliquots of washed rubber particle suspensions were dried under a stream of nitrogen and transesterified in methanolic HCl. Methyl heptadecanoate was used as internal standard. Fatty acid methyl esters (FAME) were extracted with hexane and analyzed by GLC and GC/MS (Goodrich-Tanrikulu *et al.*, 1994). FAME were identified by retention times and mass spectra in comparison to standards. In analysis of individual lipid classes, aliquots of rubber particle suspensions were extracted as described in Goodrich-Tanrikulu *et al.* (1994) and the lipids fractionated into neutral lipids, glycolipids, and phospholipids on Sep-Pak silica columns (Lynch and Steponkus, 1987). FAME were prepared from aliquots of these fractions, or for some determinations, lipids in the three fractions were additionally separated by thin layer chromatography (TLC) using the solvent systems described in Goodrich-Tanrikulu *et al.* (1994). Bands corresponding to individual phospholipids, glycolipids, triacylglycerols and free sterols were removed for analysis, after brief iodine staining. TLC plates were run with standards and stained with the following detection reagents to aid in identification: iodine (general), molybdenum blue (phosphorus), α -naphthol/sulfuric acid (sugars). Free sterols were eluted in hexane/isopropyl alcohol (3/2, v/v), concentrated to dryness, and dissolved in chloroform for GC analysis. Other lipid-containing bands were transesterified directly, either as above for analysis of FAME or, for individual glycolipids, by the more rigorous procedure of Lynch and Steponkus (1987), followed by analysis of FAME and liberated sterols. Sterols were identified by GC/MS using standards (β -sitosterol, stigmasterol, fucosterol and campesterol) for mass spectra comparisons. Sterols were quantified by GLC using a non-polar capillary column method similar to that described in Goodrich-Tanrikulu and Travis (1995). Cholestane was used as external standard. The glycolipid

fatty acid composition was based on extracts from total glycolipids, with identity of the fatty acids confirmed using fatty acid profiles of individual glycolipids separated on TLC plates. Phosphorus in rubber particles was analyzed by the method of Bartlett (1959). Sugar was assayed by the phenol-sulfuric acid method (Kushwaha and Kates, 1981). All analyses were done at least in duplicate, except for GLC of sterols which were single analyses.

Calculations of rubber particle surface coverage by membrane components. The coverage of rubber particle surface by lipid and protein as reported in table 3 was estimated for each species as follows. Rubber particles were assumed to be spherical. Assuming that a half-unit membrane would have a thickness of 2.5 nm (Tzen and Huang, 1992), and using previously determined mean rubber particle diameters (Cornish *et al.*, 1993), the volume occupied by such an outer shell was calculated for each species. The average rubber particle weight was calculated assuming a density of 0.934, the density of rubber. The volume of a membrane lipid on rubber particles was calculated from the experimentally determined amounts of lipid (nmol mg⁻¹ rubber particle) and the particle weight, assuming a 2.5 nm long cylinder with diameter 0.7 nm (Tzen and Huang, 1992). The volume of rubber particle protein for each species was calculated from the experimentally determined amounts reported in table 2, assuming a protein density of 1.3 g cm⁻³. For calculation purposes, it was assumed that all of the rubber particle protein is embedded within a 2.5 nm shell on the outer surface of the particle.

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