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SHORT COMMUNICATION

Proteolipids of Natural Rubber Particles

H. HASMA*

About 40% of the membrane proteins of the rubber particles were found to be proteolipids. They were hydrophobic proteins containing 70% non-polar amino acids and were closely associated with phospholipids and glycolipids. SDS-gel electrophoresis revealed one main protein band at a molecular weight of 14 300 with two minor ones in between 14 300 and 24 000.

Proteolipids, first discovered by Folch and Lees[1], are a type of protein-lipid complex which differ from lipoproteins in being soluble in chloroform/methanol mixture but insoluble in water. They have been found characteristically as membrane components of many plant, animal and bacterial cells[2] especially in brain white matter (BWM) where they constitute about 50% of the central nervous system myelin proteins[3]. Generally, they are hydrophobic and non-covalently associated with lipids[1,4,5]. However, proteolipids containing covalently bound long-chain fatty acids linked to specific amino acids have also been found[6,7].

Proteolipids have not previously been reported to be present in *Hevea brasiliensis* latex. This communication describes the isolation and characterisation of proteolipids associated with natural rubber (NR) particles.

EXPERIMENTAL

Isolation of Proteolipids

Fresh latex from clone RRIM 600 was centrifuged on a Beckman L8-70 ultracentrifuge at 19 500 r.p.m. using rotor 21 for about 1 hour. The rubber phase and bottom fraction were collected.

The rubber phase was redispersed in the minimum of water, filtered and added dropwise to five volumes of a continuously stirred chloroform/methanol (2:1, volume/volume) mixture. The extracts separated from the rubber coagulum were washed with salt solutions [0.6% aqueous NaCl and 0.1*M* NaCl in 0.023*M* *tris*-HCl (pH 8.8)[4]]. A lower chloroform fraction and a thin whitish interfacial layer were isolated. The chloroform layer was concentrated on a rotatory evaporator. Further addition of a chloroform/methanol (2:1, volume/volume) mixture left an insoluble portion. This insoluble portion containing proteolipids was collected. The interfacial layer was precipitated with acetone, centrifuged and dried.

The bottom fraction was freeze-thawed three times and then centrifuged. The sedimented membrane components, washed several times with water to remove the remaining B-serum, were extracted with a chloroform/methanol (2:1, volume/volume) mixture and the proteolipids isolated as described in the rubber phase.

Determination of Proteins, Phospholipids, Glycolipids and Amino Acids

Protein content was determined by multiplying the amount of nitrogen[8] with its conversion factor 6.25. Similarly, multiplying phosphorous content[9] by 25.9[10] and the sugar content[11] by 4.7[10] gave the amount of phospholipids and glycolipids, respectively.

*Rubber Research Institute of Malaysia, P.O. Box 10150, 50908 Kuala Lumpur, Malaysia

Amino acid analyses were done on a Technicon automatic amino acid analyser. The amino acids were obtained by hydrolysing the proteolipids in 6*N* HCl at 110°C for 24 hours.

SDS-urea Polyacrylamide Gel Electrophoresis

Electrophoresis on SDS-urea polyacrylamide gel was carried out according to the method of Chans and Lees[12] at a constant current of 99mA for 2½ hours.

RESULTS AND DISCUSSIONS

By extracting the rubber particles with chloroform/methanol, there was a loss of about 40% nitrogen content in the rubber phase. As this nitrogen is attributed mainly to the proteins associated with the rubber particles it implied that the chloroform/methanol mixture extracted some proteins. Concentrating the water washed chloroform/methanol extracts to almost dryness left a portion of the extracts insoluble in the same solvent mixture. Nitrogen determination showed the residue to contain about 7%–10% nitrogen which was considered too high for any nitrogen-containing lipids. Acid hydrolysis of the residue showed a high concentration of neutral amino acids. All these observations seem to fit well with the description of proteolipids by Folch and Lees[1]. Further analysis described below confirmed the presence of proteolipids in the membrane components of rubber particles.

As observed by other workers[1,4,5], the proteolipids from the rubber particles also showed a variation in lipid components, depending on the method of isolation. Lipid contents decreased from 30% in water washed chloroform/methanol extracts to 7%–13% in proteolipids obtained from the interfacial layer on washing the extracts with sodium chloride solutions of different pH *(Table 1)*. The decrease in lipid content could be due to the 'splitting' of the protein-lipid linkages by the salt[4] or the proteolipid apoprotein (the protein moiety of the proteolipid) itself undergoing changes at alkaline pH leading to its precipitation with parallel release of associated lipids[5]. The level of lipids reflected the purity of proteolipid apoprotein as *Table 1* shows that the protein content increased from 62% to 81% with the level of lipids decreasing from 30% to 7%. A crystalline proteolipid containing 86% proteins and 2% lipids was obtained from the interfacial layer of the chilled unwashed chloroform/methanol extract of the rubber phase which had been redispersed in excess water.

Characteristic of membrane proteolipid, the lipids highly bound to the apoprotein were mainly phospholipids and glycolipids. Some neutral lipids were also detected. Preliminary investigations showed that the phospholipid, glycolipid and neutral lipid composition of the rubber particle proteolipid was similar to that present in the total lipid extracts of the whole *H. brasiliensis* latex[13].

Complying with the hydrophobic nature of the proteins, the amino acids of *H. brasiliensis* proteolipids comprised about 70% neutral amino acids *(Table 2)*. Out of the eighteen common protein amino acids only fourteen were detectable. Tryptophan is usually destroyed by 6*N* HCl hydrolysis of proteins[14]. The presence of carbohydrates in the glycolipid

TABLE 1. PROTEIN AND LIPID CONTENT OF THE PROTEOLIPIDS ASSOCIATED WITH THE RUBBER PARTICLES

Washing reagent	Composition (%)		
	Proteins	Phospholipids	Glycolipids
Water	62.5	23.0	6.9
Sodium chloride	75.0	10.2	2.6
Tris-HCl (NaCl)	81.3	6.0	1.5

TABLE 2. AMINO ACID COMPOSITION OF PROTEOLIPIDS ASSOCIATED WITH THE RUBBER PARTICLES

Amino acid	Proteolipids at the interphase	Proteolipids from the $CHCl_3$ faction
Lysine	8.0	6.9
Histidine	—	—
Arginine	2.5	2.6
Aspartic acid	10.1	10.0
Threonine	3.7	4.2
Serine	7.0	6.8
Glutamic acid	9.6	9.5
Proline	3.9	6.4
Glycine	7.5	7.6
Alanine	12.9	11.8
Cysteine	—	—
Valine	12.4	11.5
Methionine	—	—
Isoleucine	5.5	6.1
Leucine	8.2	8.0
Tyrosine	3.8	3.9
Phenylalanine	4.5	4.4
Polar (%)	30.2	29.0
Neutral (%)	69.8	71.0

component of the rubber proteolipids enhanced the destruction of methionine and cysteine during acid hydrolysis[15]. Histidine was not present in the proteolipids of NR particles as simultaneous hydrolysis of cow BWM proteolipids showed that the amino acid was not destroyed by the acid hydrolysis process. *Table 2* shows that there was no major difference in the amino acid composition of the proteolipids from the chloroform layer and from the interphase. Both proteolipids had high content of alanine, valine, glutamic acid and aspartic acid, each constituting about 9%–13% of the total amino acids. The relatively high concentration of the latter two polar amino acids in rubber proteolipids was not unusual as it has been reported that such exceptions to the general abundance of non-polar amino acids do occur in the proteolipids of sarcoplasmic reticulum Ca^{2+}-ATPase[2] and defatted soybean meals[16].

SDS-urea polyacrylamide gel electrophoresis of the rubber particle proteolipids showed a prominent band at a molecular weight of 14 300 with a second and a faint third one between 14 300 and 24 000 *(Figure 1)*. A similar pattern was observed in proteolipids obtained at the interphase, regardless of their state of delipidation. The proteolipids from the chloroform layer, however, contained mainly the lower molecular weight proteolipids. The other plant proteolipids which showed a similar protein pattern to the NR particle proteolipids were the soybean proteolipids[16]. They too gave two protein bands on SDS-urea polyacrylamide gel electrophoresis; a denser band at the

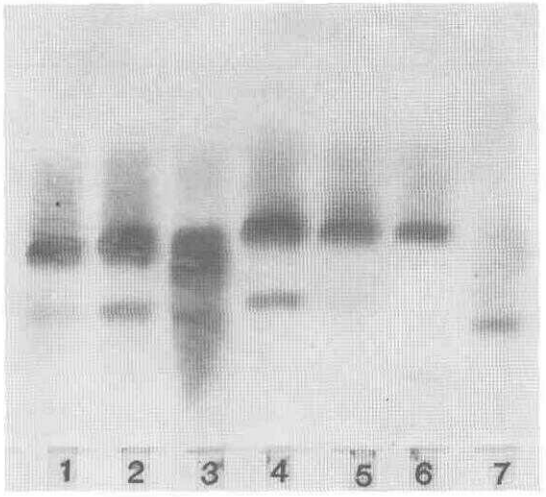

Figure 1. SDS-urea polyacrylamide gel patterns of proteolipids from NR particles. 1 = proteolipids obtained after washing the chloroform-methanol extracts with water, (2) with sodium chloride, (3) with tris-*HCl (NaCl), 4 = proteolipids at the interphase, (5) in the chloroform fraction, 6 = lysozyme, and 7 = trypsinogen.*

molecular weight of 15 000 and a less dense one at 13 000.

The triple proteolipid apoprotein bands on SDS-urea polyacrylamide gel electrophoresis might not indicate three different kinds of proteolipids but merely represent specific states of aggregation of a single protein as shown in the case of BWM proteolipids[12]. Differences in molecular shape would also result in differences in SDS binding capacity.

Although proteolipid is a membrane protein, it is not characteristic to all membranes[1]. In *H. brasiliensis* latex the concentration of the proteolipids in the membrane surrounding the non-rubber particles was low, being only 3%–6% of the total proteins, compared to 40%–50% in the rubber particles. Moreover, a certain portion of the proteolipids of the non-rubber particles must be derived from the rubber particles which were sedimented together with the former in the bottom fraction of the ultracentrifuged latex. Thus, the hydrophobic proteolipids of *H. brasiliensis* seem to show preference for the membrane surrounding the equally hydrophobic rubber molecules rather than the plasma membrane of the non-rubber particles. This perhaps suggests a specific function of the proteins in the membrane of the rubber particles; for example in the stability of NR latex.

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