Extractable Proteins in Natural Rubber Latex Films

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Introduction

Natural rubber latex (NRL) is a unique elastomeric polymer and can be made into thin surgical gloves and other products, of comparatively high strength, good flexibility and tactile sensitivity, with surprisingly good resistance to a wide range of liquid chemicals. Latex from the tree *Hevea brasiliensis* is the source material used in the manufacture of latex gloves. In the medical field especially, the rising concerns for protection from blood-borne pathogens and the inherent barrier characteristics of the latex products has precipitated a massive demand for latex examination and surgical gloves. There is evidence suggesting that the water-extractable proteins in latex are the cause of the allergic reactions. At the same time, much effort has been expended in devising ways to decrease the soluble proteins in NRL products (Yeang & Yusof, 1993). The aim of this study was extract and characterize water extractable proteins from films of NRL, vulcanized by conventional and by ionizing radiation methods.

Materials and Methods

Preparation of NRL films

Films were prepared with high ammonia (HA) type centrifuged NRL from Malaysia by casting method followed by leaching with water at 70°C for 15 minutes and drying at 70° C for 1 and a half hour. One sample consisted of unvulcanized NRL (NRL), two samples were irradiated with either 250kGy (RVNRL) for the sample without n-butyl acrylamide (n-BA) or 12 kGy for the sample with n-BA (RVNRLs), another sample consisted of vulcanized by the conventional process latex (VNRL) with sulfur.

Preparation and Electrophoretic Separation of Latex Proteins

Latex films extracts were prepared from 4 types of films: NRL; NVRL, RVNRL and RVNRLs . 45 g of each film were cut into pieces (1x1cm) and shaken for 24 hs in about 300 ml of distilled water at room temperature. The unextracted proteins were further extracted by the same procedure as above in pH 9.6, 50 mM carbonate-bicarbonate buffer. The eluates were freeze dried and the dissolution of proteins was in 3 ml of distilled water. 200 g of a HA NRL were coagulated with about 102 ml 1N acetic acid. The serum obtained was dialyzed against water for 6 hs, changing water each hour. The dialyzed serum was freeze dried and dissolved in 2 ml distilled water. Protein concentration was determined by Lowry method using serum albumin (BSA) as the calibration standard.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of eluates of 4 types of latex films, as well as serum, were carried out according to Laemmli,U.K.(1970). The gels were stained with Coomassie Blue and Silver.

High Pressure Liquid Chromatography

The water extracts above were submitted to high pressure molecular exclusion chromatography on a Toso-Haas TSK-2000 column (7.5x600 mm), eluted with 25 mM pH 7 ammonium bicarbonate with a 1ml/minute flow and detection at 220 nm.

Results and Discussion

As can be seen in table I, the extractability of the proteins in water or alcaline buffer was different, according to Beezhold (1993). The amount of water extractable protein is very small but this amount would be essentially the one that can cause hypersensitivity reactions as reported by several authors (Sunderasan and Yeang, 1993; Shamsul Bahri et al, 1993). The difference of vulcanization method also resulted in different extractability. The VRNL film presented the highest protein extractability of all the samples in water. The extraction in buffer, following a prime extraction in water suggests that a large amount of rubber bound proteins are not water-extractable.

TABLE I: EXTRACTABLE PROTEINS IN WATER AND PH 9,6 CARBONATE BUFFER (µG PROTEINS/G LATEX FILM).

	H_2O	Buffer
NRL	n.d.	125
VNRL	108	69
RVNRL	n.d.	20
RVNRLs	73	42

n.d.= not detectable

The SDS-PAGE and chromatographic data (figures 1 and 2) indicate that the predominant protein in all samples, except RVNRL was a protein of about 14 kDa, which we believe is Rubber Elongation Factor (REF) (Palosuo, T. 1995). The NRL water extracted presented very low amounts of proteins. On the other hand, on the buffer extracted NRL we could observe a higher protein concentration as well as tailing in the high molecular weight zone. VNRL extraction in both systems resulted in a very diffuse band in the 14 kDa zone, which could explain the 2 neoformed peaks in the chromatographic profile. We could also observe a diffuse high molecular weight tailing suggesting thermical degradation of the proteins. The high radiation dose applied on RVNRL resulted in a low extractability with a faint 14 kDa band,

confirmed by chromatography, and a little amount of aggregated material. RVNRLs presented a chromatographic profile very similar to RVNRL but with a slightly higher extractability. The predominant protein was the 14 kDa one, but peptides of about 7 kDa and smaller could also be observed. The tailing observed in SDS-PAGE was also observed on the cromatographic profile indicating formation of high molecular weight products.

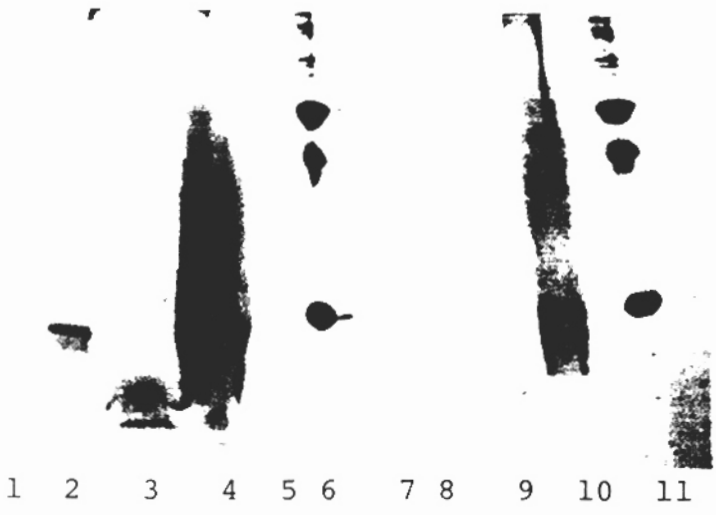

Figure 1. SDS-PAGE of latex films extracts. Lanes: 1-NRL; 2-NRL; 3-VNRL; 4-VNRL; 5-Serum 6-Molecular weight markers (66, 44 and 17 kDa); 7-RVNRL; 8-RVNRL; 9-RVNRLs; 10-RVNRLs; ; 11- Molecular weight markers..Even: buffer extracts. Odd: water extracts.

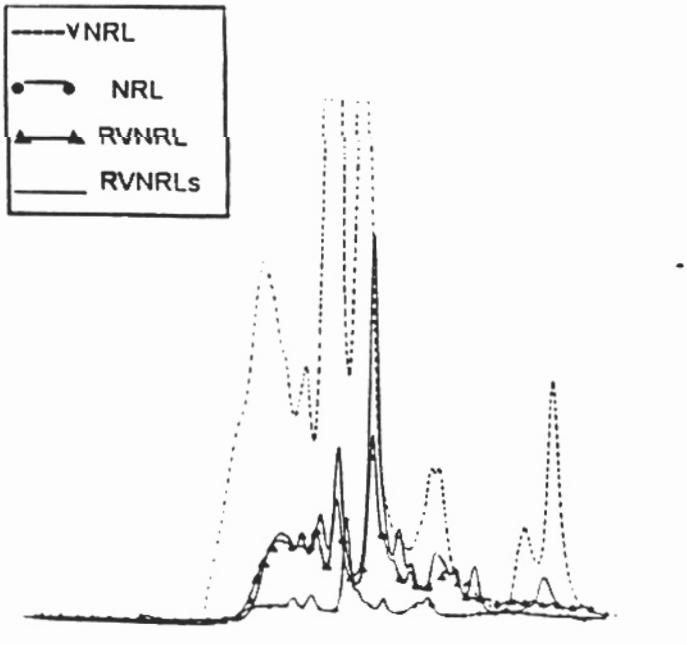

Figure 2: Chromatographic profile of the water extracts

Several groups have already observed that proteins irradiation leads to denaturation, and immunochemical modifications, mainly in immunoreactivity by destruction of protein domains responsable for the antibody recognition (epitopes), (Nascimento, 1991; Kume & Matsuda, 1995). According to Puig (1971), the proteins are present at a concentration of about 1% in latex. During the irradiation, these compounds are destroyed and the cross-linking is more efficient when the proteins are removed by this way. Our results showed that irradiation effectively modified protein structure and extractability. These data indicate irradiation as a promisefull tool for the production of hypoalergenic latex goods. Further experiments are being carried out by our group intending to characterize the immunological behaviour of irradiated latex proteins.

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References

Yeang, H.Y. and F. Yusof. J.nat.Rubb.Res., **8(2)**, 154-161 (1993).

Laemmli, U.K., Nature, **227**, 680-685. (1970)

Beezhold, D., International Conference:Latex Protein Allergy,19-28 (1995)

Sunderasan, E. and H.Y.Yeang. J.nat. Rubb.Res., **8(4)**, 293-298 (1993).

Samsul Bahri, A.R., S. Hamzah, H.M. Ghazaly and H.Y. Yeang. J.nat.Rubb.Res., **8(4)**, 299-307 (1993).

Palosuo, T.,International Conference:Latex Protein Allergy,11-15 (1995)

Nascimento,N., Master degree dissertation (1991)

Kume, T. and T. Matsuda, Radiat. Phys. Chem., **46(2)**, 225-231 (1995)

Puig, J.R., Atomic En. Rev., **9(2)**, 373-379 (1971).