Character of Long-Chain Branching in Highly Purified Natural Rubber

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ABSTRACT: The nature of long-chain branching in natural rubber (NR) from *Hevea brasiliensis* was analyzed for NR purified by enzymatic deproteinization in the latex state followed by acetone extraction in the solid state to remove the proteins and neutral lipids, respectively. The treatment of purified NR in a toluene solution with a polar solvent, such as methanol or acetic acid, resulted in a clear decrease in the molecular weight, gel content, and Huggins' constant; this was caused by the decomposition of branch points in the purified rubber. This finding clearly showed that long-chain branching in the purified NR was mainly derived from the association of phospholipids linked with both terminal groups in the rubber chain via hydrogen bonds. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 115: 3645–3650, 2010

Key words: networks; rubber; structure

INTRODUCTION

It has been recognized that natural rubber (NR) from *Hevea brasiliensis* is composed of long-chain branched molecules.[1,2] In our structural studies, we proposed that the initiating terminal group or ω-terminal is a modified dimethylallyl group[3] that is able to form branch points by hydrogen bonding with proteins. As proof, the purification of NR latex by washing with centrifugation in the presence of sodium dodecyl sulfate as a surfactant can remove almost all of the proteins and results in a decrease in the gel content to the same level as that of deproteinized natural rubber (DPNR) obtained by the deproteinization of latex with a protease.[4] In the previous study,[5] the storage of DPNR latex in the presence of an ammonia solution showed an increase in the gel content, which indicated that the functional group at the ω-terminal formed branch points without the proteins. This finding suggests that the polar lipids (i.e., phospholipids) may interact with the ω-terminal to form the branch points in DPNR.

Another terminal group at the chain end, called the α-*terminal*, is presumed to be composed of a monophosphate or diphosphate group linked with phospholipids by hydrogen bonding between phosphate groups or ionic linkages with divalent metal ions.[6,7] The formation of crosslinking by ionic linkages between negative charges of phospholipids with divalent cations is plausible because the process has been implicated in many membrane-associated events.[8] The presence of Mg^{2+} ions is expected to form ionic linkages between rubber molecules. The decrease in the gel content of NR after the addition of ammonium sulfate [$(NH_4)_2SO_4$] to NR latex indicated that Mg^{2+} ions play a role in linking rubber molecules together by ionic bonding.[9] However, the formation of branch points through the linkage of phosphate terminals with phospholipids by hydrogen bonding cannot be neglected. As reported in our previous article, the addition of 1% (w/v) ethanol to a toluene solution of DPNR reduced the gel fraction.[10] This was supporting evidence to show the formation of branch points through hydrogen bonding, although it was difficult to determine the type of hydrogen bonding on the basis of the previous findings.

An attempt was made in this study to clarify the characteristics of the hydrogen bonding or ionic linkages of phosphate groups with divalent metal ions that predominantly contribute to the branched structure of NR chains. A polar solvent treatment was applied to decompose the branch points, which were formed by the hydrogen bonding of phospholipids and phosphate groups. If the branching formation was derived from mainly ionic linkages, the polar solvent would not decompose the branch points. As a model system to investigate the characteristics of the hydrogen bonding at the α-terminal, we used

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DPNR to prevent the effect of proteins in NR. Furthermore, the rubber was purified by acetone extraction to remove neutral lipids, which are not believed to affect the formation of branch points.

EXPERIMENTAL

Freshly tapped NR latex from the *H. brasiliensis* tree, provided by Thai Rubber Latex Co. (Chonburi, Thailand) was preserved in a 0.6% (w/v) ammonia solution for 1 month at room temperature. The NR latex was diluted to a 30% (w/v) dry rubber content and subjected to deproteinization by incubation with 0.04% (w/v) protease (KP 3939, KAO Co.) in the presence of 0.5% (w/v) Triton X-100 [Fluka, analytical reagent (AR) grade] at 37°C for 12 h. The latex was centrifuged at 13,000 rpm (25,100 g) for 30 min. The rubber cream fraction was redispersed in distilled water containing 0.5% (w/v) Triton X-100 and adjusted to a 30% dry rubber content. The process was repeated twice. The solid DPNR was obtained by the coagulation of the double-centrifuged DPNR latex with methanol (Lab Scan, AR grade) and drying at 50°C. Acetone-extracted deproteinized natural rubber (AE-DPNR) was prepared by the extraction of DPNR with acetone (Fluka, AR grade) in a Soxhlet extractor under a nitrogen atmosphere for 24 h to remove free fatty acids and fatty acid esters. This acetone extraction was proven not to cause the degradation of DPNR because the treatment did not result in a significant change in the molecular weight.

The polar solvent treatment was carried out by the addition of a 1–3% (v/v) polar solvent, such as methanol (Lab Scan, AR grade) or acetic acid (Fluka, AR grade), into a 1% (w/v) AE-DPNR in toluene solution. The mixed solution was stirred and incubated at room temperature for 9–72 h, followed by precipitation of the rubber by the pouring of the rubber solution into an excess amount of methanol. The precipitated rubber was recovered by drying in a vacuum oven at 40°C for 12 h.

The content of long-chain fatty acid ester groups was determined by Fourier transform infrared spectroscopy with a Jasco FT/IR 460 (Jasco International Co., Ltd., Tokyo, Japan). A calibration curve was obtained for a mixture of methyl stearate and synthetic *cis*-1,4-polyisoprene (Kuraprene IR10; Kuraray Isoprene Chemical Co., Tokyo, Japan). The content of fatty acid ester group per weight of rubber was determined by the intensity ratio of the peaks at 1739 cm^{-1} (C=O) to 1664 cm^{-1} (C=C).[11]

The molecular weight of the NR samples was determined by size exclusion chromatography (Jasco-Borwin) with two columns in series packed with crosslinked polystyrene gel with exclusion limits of 2.0×10^7 and 4×10^5. The rubber solution was prepared by the dissolution of rubber into tetrahydrofuran (LabScan, high performance liquid chromatography (HPLC) grade) at a concentration of 0.05% w/v and filtering through a Millipore prefilter and 0.45-μm membrane filter (Alltech). Tetrahydrofuran was used as an eluent with a flow rate of 0.5 mL/min at 35 ± 0.01°C. The effluent was monitored by its refractive index. Commercially available *cis*-1,4-polyisoprene (Polymer Standard Service GmbH, Germany) was used as standard sample for the calibration of the molecular weight.

The measurement of the intrinsic viscosity ([η]) was made by the dilution method with a single-bulb Ubbelohde viscometer (Schott Gerate GmbH, Mainz, Germany) at 30 ± 0.02°C. The viscosity-average molecular weight (M_v) was calculated according to the Mark–Houwink equation. The Huggins' constant (k') was calculated with the following equation:[12]

$$[\eta] = 33.1 \times 10^{-5} M_v^{0.71} \quad (1)$$

$$\eta_{sp}/c = [\eta] + k'[\eta]^2 c \quad (2)$$

where η_{sp}/c and c represent the reduced viscosity and concentration (g/dL), respectively.

RESULTS AND DISCUSSION

It was postulated that the formation of branch points at the α-terminal in NR is derived from the association of phospholipids linked with the phosphate terminal via hydrogen bonding.[6,7] This postulation can be proven by the decomposition of the gel fraction by the addition of small amounts of polar solvent to a solution of rubber in toluene. If this hydrogen bonding is responsible for branch formation, the addition of a polar solvent should lead to the decomposition of branch points and result in the liberation of free phospholipids. It was reported that the lipids in NR consist of 54% neutral lipids, 33% glycolipids, and 14% phospholipids.[13] However, only phospholipids were presumed to be associated with the formation of branch points.[6,7] It has been confirmed that neutral lipids in NR have no direct role in the formation of branch points because acetone extraction has no significant effect on the gel content.[14] The measurement of long-chain fatty acid ester content in this study provided information about the amounts of phospholipids, glycolipids, and neutral lipids. The effect of polar lipids on the formation of branch points was studied after the removal of neutral lipids by acetone extraction.

Characterization of NR after purification by deproteinization and acetone extraction

The characteristics of NR after purification by deproteinization and acetone extraction are shown in

TABLE I
Characteristics of the Rubber Samples After Purification by Deproteinization and Acetone Extraction

Sample	Nitrogen content (% w/w)	Ester content (mmol/kg of rubber)	Gel content (% w/w)
NR	0.812	30.1	42.9
DPNR	0.026	30.5	12.3
AE-DPNR	0.022	19.8	11.9

Table I. The nitrogen content of NR decreased to almost 0% (w/w) after deproteinization; this confirmed the efficiency of removing protein in NR by this process. Because the proteins were postulated to be associated with the branch points at the ω-terminal of NR,[3,4] the removal of proteins led to the decomposition of branch points. This was evidenced by the reduction of the gel content after deproteinization. However, DPNR contained 12% (w/w) of the gel fraction even after the removal of almost all of the protein through deproteinization. This finding demonstrated that some parts of the branch points at the ω-terminal were composed of a compound other than the proteins removed in DPNR. The acetone extraction of DPNR resulted in a reduction in the ester content from 30.5 to 19.8 mmol/kg of rubber, which indicated the removal of neutral lipids. There was no remarkable change in the gel content observed in DPNR after acetone extraction; this implied that the neutral lipids in NR had no direct role in the formation of the branch points.

Change in the gel content of the purified rubber after the polar solvent treatment

It is remarkable that the gel content of AE-DPNR drastically decreased after the addition of 2% (w/v) methanol and acetic acid to the rubber solution, as shown in Figure 1. The gel content of AE-DPNR decreased slightly from 12 to 9% after the methanol treatment, whereas it decreased to less than 5% after acetic acid treatment, more effectively with increasing acetic acid concentration. The effect of incubation time on the gel content of AE-DPNR is illustrated in Figure 2. In the case of methanol treatment, the gel content of AE-DPNR substantially decreased with increasing methanol concentration and incubation time. These findings clearly indicate that the polar solvent acted to decompose the branch points. Interestingly, the treatment by acetic acid was more effective in reducing the gel content than that of methanol. This indicates that acetic acid had a higher efficiency in decomposing branch points formed by hydrogen bonding between phospholipids than methanol because the polarity of acetic acid was higher than methanol. The minimum gel content

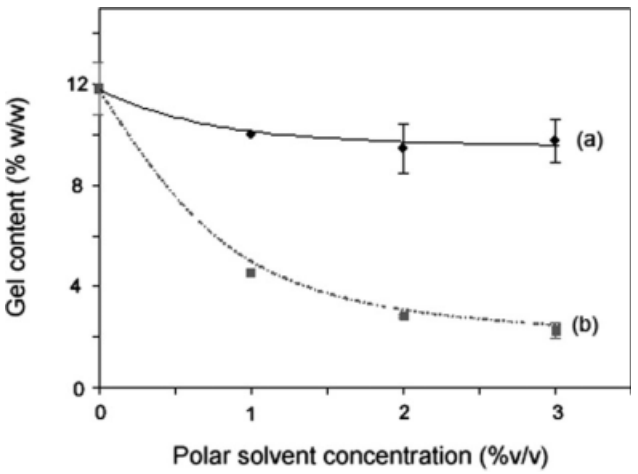

Figure 1 Changes in the gel contents of AE-DPNR during 9 h of incubation in 1–3% (v/v) of (a) methanol treatment and (b) acetic acid treatment.

was obtained when 2 and 3% (v/v) acetic acid was added to the rubber solution after an incubation time of 24 h.

The decrease in the gel content after the polar solvent treatment of AE-DPNR in the toluene solution demonstrated that the branch points were formed by hydrogen bonding. However, it was remarkable that the gel fraction in AE-DPNR was not completely removed after the polar solvent treatment, even after treatment with a great amount of acetic acid. This finding clearly indicated the presence of another kind of branching involved in gel formation. One of the possibilities was formation of branches by ionic linkages by magnesium ions, which are always present in rubber latex as a cofactor for rubber biosynthesis. In general, at least two branch points per

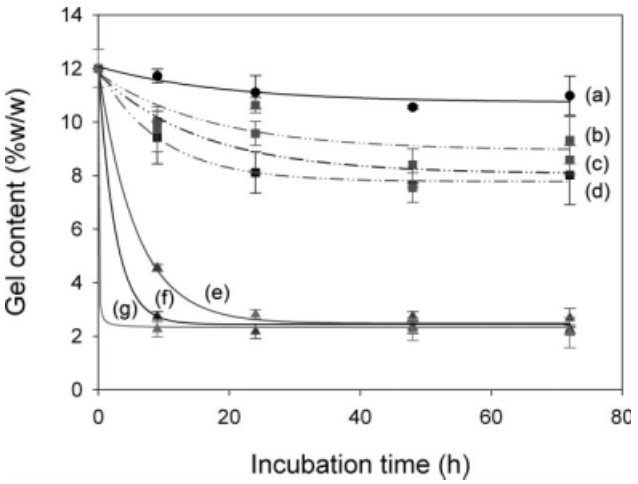

Figure 2 Gel contents of (a) AE-DPNR, (b) AE-DPNR after treatment with 1% (v/v) methanol, (c) 2% (v/v) methanol, (d) 3% (v/v) methanol, (e) 1% (v/v) acetic acid, (f) 2% (v/v) acetic acid, and (g) 3% (v/v) acetic acid after various times of incubation.

TABLE II
Molecular Weight Characteristics of AE-DPNR After Treatment with the Polar Solvent by Incubation for 24 h and After Transesterification

Sample	Gel content (% w/w)	M_n ($\times 10^5$)	M_w ($\times 10^6$)	Polydispersity index (M_w/M_n)
AE-DPNR	12.0	1.88	1.29	7.01
AE-DPNR + 2% methanol[a]	8.1	1.38	1.14	8.27
AE-DPNR + 3% methanol	9.6	1.33	1.12	8.44
AE-DPNR + 2% acetic acid	2.8	1.20	1.00	8.33
AE-DPNR + 3% acetic acid	2.8	1.22	0.98	8.08
Transesterified AE-DPNR	0	0.94	0.49	5.27

[a] AE-DPNR after treatment with 2% (v/v) methanol into rubber solution.

rubber chain are required to form the gel fraction. Consequently, the formation of branch points at both terminals, that is, the ω-terminal and the α-terminal, had to be taken into consideration. Another possibility was the formation of branch points by a radical reaction to form a so-called hard gel. This could be neglected on the basis of the finding that the residual gel in AE-DPNR could be solubilized by transesterification with $NaOCH_3$ in a toluene solution of rubber, which is shown later (cf. Table II).

Change in the ester content of the purified rubber after the polar solvent treatment

The ester contents of AE-DPNR after the methanol and acetic acid treatments in the toluene solution are shown in Figure 3. The polar solvent treatment of AE-DPNR in the toluene solution led to an insignificant decrease in the ester content for both methanol and acetic acid at different concentrations and incubation times. The inefficient removal of long-chain ester groups by the polar solvent treatment was possibly caused by the fact that the polar lipids could not be separated from AE-DPNR by ordinary precipitation because of their solubility in methanol, even if the hydrogen bonds were decomposed effectively. Therefore, the decrease in the gel content with the constant ester content of AE-DPNR inferred that the polar solvent had the ability to decompose the branch points formed by phospholipid molecules, whereas it was inefficient in removing dissociated phospholipids from the rubber by precipitation from the toluene solution into methanol.

Degree of branching and molecular weight of the purified rubber after the polar solvent treatment

The degree of branching in NR can be described qualitatively by k', that is, a higher k' value indicates a higher degree of branching. The k' value significantly decreased after polar solvent treatment, as shown in Figure 4. The k' value of AE-DPNR decreased from 0.65 about 0.35 after the addition of methanol and acetic acid to the rubber solution. The decrease in the k' values after polar solvent treatment indicated the decomposition of branch points and liberated linear rubber chains, which showed k' values of about 0.3–0.4.[15,16]

Other evidence showing the presence of hydrogen bonding in AE-DPNR was further provided by the

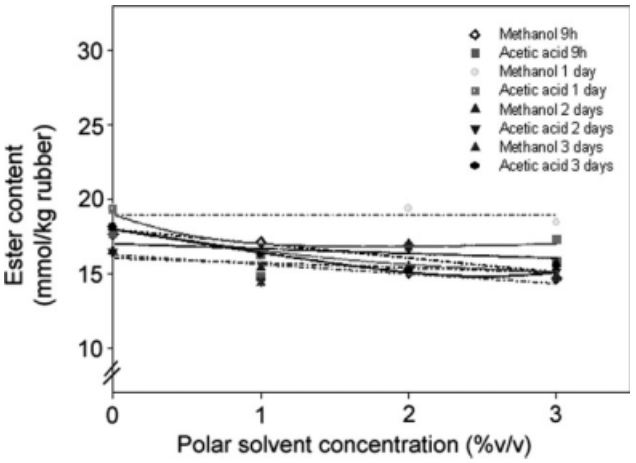

Figure 3 Changes in the ester contents of AE-DPNR after 1–3% (v/v) polar solvent treatment for different incubation times.

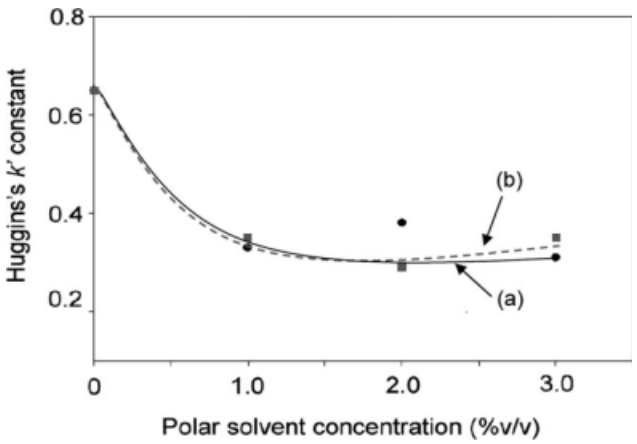

Figure 4 Dependence of k' of AE-DPNR on the concentrations of (a) methanol and (b) acetic acid with incubation for 24 h at 30°C.

changes in the molecular weight and molecular weight distribution after polar solvent treatment, as shown in Figure 5. We observed that the addition of the polar solvent resulted in a significant shift in the molecular weight. The decreases in the number-average molecular weight (M_n) and weight-average molecular weight (M_w) from the original values are tabulated in Table II. The M_n and M_w values of AE-DPNR decreased about 10–30% after treatment with methanol and acetic acid. These results demonstrated the decomposition of branch points by the polar solvent treatment; this confirmed the presence of hydrogen bonding mainly to form branch points in AE-DPNR. The molecular weight of acetic acid treated AE-DPNR was higher than those observed for transesterified AE-DPNR. Because of the formation of linear rubber molecules after the transesterification of DPNR,[17] the polar-solvent-treated AE-DPNR samples should have contained some branched molecules, although their k' values corresponded to the linear rubber molecules. This discrepancy could be explained reasonably by the assumption of the presence of some branch points partly composed of two linear rubber molecules, which were derived from the ionic linkage.

Proposed structure of branching and gel in the purified rubber

Transesterification is known to be a method for decomposing the branch points formed by phospholipids[18] to form linear rubber molecules in the case of DPNR.[17] Consequently, the decomposition of the residual gel fraction of 12% (w/w) in DPNR by transesterification clearly indicated that the phospholipids were the major component of the branch

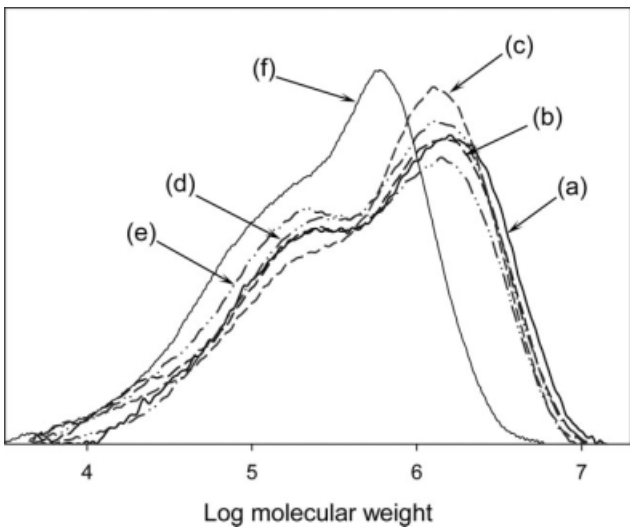

Figure 5 Molecular weight distributions of (a) AE-DPNR, (b) AE-DPNR after treatment with 2% (v/v) methanol, (c) 3% (v/v) methanol, (d) 2% (v/v) acetic acid, (e) 3% (v/v) acetic acid, and (f) AE-DPNR after transesterification.

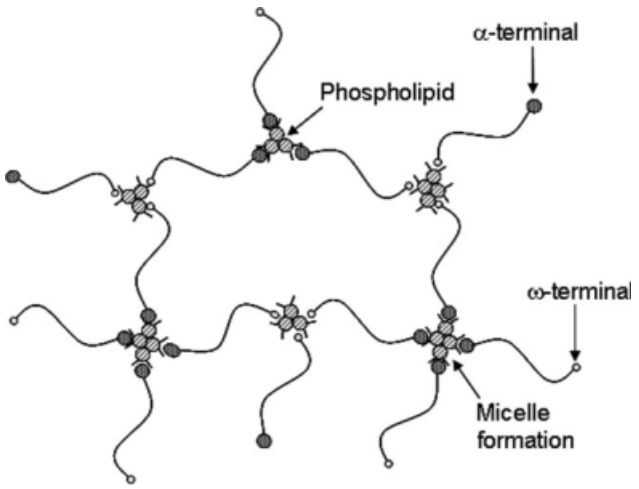

Figure 6 Proposed structure of branching and gel formation in DPNR.

points at both terminals, that is, the α- and ω-terminals in DPNR. This finding revealed that the branch points in AE-DPNR originated by the interaction of phospholipids to link the rubber chains together. That is, the proteins at the ω-terminal thus far postulated in NR should have been phospholipids in the case of DPNR, as illustrated schematically in Figure 6. Although the structure of the ω-terminal was not clarified yet, we assumed that the branch points at the ω-terminal derived from the interaction via ionic linkages between an unidentified functional group at the ω-terminal of the rubber molecule and phospholipids, in which the phospholipids associated together by the formation of a micelle structure.

CONCLUSIONS

Decreases in the molecular weight, gel content, and k' value were observed for AE-DPNR with the addition of 1–3% methanol and acetic acid in a toluene solution. These indicated the decomposition of branch points mainly formed by the association of phospholipids via hydrogen bonding. This assumption was supported by the fact that the polar nature of the solvent added to toluene strongly affected the ability to decompose the branch points; that is, acetic acid was more effective than methanol. However, the formation of branch point by ionic linkages with a divalent metal ion between rubber molecules and phospholipids was postulated because the gel fraction remained in AE-DPNR even after the treatment with the polar solvent. This was proven by the disappearance of the gel fraction by the transesterification of DPNR, which decomposed all of the lipids composed of phospholipids.

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