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Molecular structure and storage hardening of natural rubber: Insight into the reactions between hydroxylamine and phospholipids linked to natural rubber molecule

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ABSTRACT: The effect of hydroxylamine on the molecular structure and storage hardening of natural rubber (NR) was investigated by the treatment of deproteinized NR (DPNR) latex with hydroxylamine. The hydroxylamine treatment decreased the content of long-chain fatty acid ester groups in DPNR from about 2–0.7 mol per rubber molecule. The molecular weight and molecular weight distribution changed apparently after treatment with hydroxylamine. The relative intensity of the ^{1}H NMR signals corresponding to phospholipids at the α-terminal group decreased after the hydroxylamine treatment. The Huggins '*k*' constant of hydroxylamine-treated DPNR showed the liberation of linear rubber molecules caused by decomposition of branch points derived from phospholipids. The absence of storage hardening in hydroxylamine-treated DPNR was observed to be caused by not only the reaction of hydroxylamine and aldehyde groups but also the removal of phospholipids as well as the breakdown of phospholipid aggregations as a result of hydroxylamime, contributing to the establishment of a newly proposed mechanism of hydroxylamine on the inhibition of storage hardening in NR. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43753.

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INTRODUCTION

Natural rubber (NR) is usually referred to as the rubber from *Hevea brasiliensis*. NR comprises not only long-chain branched *cis*−1,4-polyisoprene but also the biomolecules such as proteins, lipids and carbohydrates.[1] The progressive increase in hardness of a solid NR during storage under ambient conditions is known as storage hardening phenomenon. The storage hardening phenomenon in NR has long been recognized to be a factor affecting the processability such as an increase in Mooney viscosity that means a change in processing behavior and the requirement of more power consumption as well as longer time for mixing the rubber with other chemicals. This is one serious problem to restrict the use of NR. In spite of extensive research for many years, the actual mechanism of storage hardening has not been fully clarified.

A number of researchers have postulated that the storage hardening of NR is caused by the crosslinking reactions of so-called abnormal groups and nonrubbers assumed to exist in NR such as epoxide,[2,3] aldehyde[4,5] lactone,[6] and proteins or amino acids.[7] However, some hypotheses cannot be accounted due to the fact that the reaction of epoxidized synthetic polyisoprene and glycine cannot proceed the hardening.[8] Moreover, the removal of proteins and amino acids using enzymatic deproteinization for preparing deproteinized NR (DPNR) cannot inhibit the storage hardening.[9,10] The crosslinking reaction through lactone is also denied because no direct evidence to support the presence of lactone on NR molecules.[11,12]

Fortunately, the storage hardening can be suppressed by the addition of aldehyde-reactive chemicals such as hydroxylamine, semicarbazide, and hydrazine to produce a constant viscosity (CV) grade NR,[4,5,13–15] leading to the establishment of the most accepted postulation that aldehyde groups on the main chain molecule of NR are responsible for the progressive hardening process via aldol condensation reaction.[15] However, a study on fractionated and transesterified NR disclosed that aldehyde groups are not present on the main chain molecule of NR but are likely derived from auto-oxidation of unsaturated fatty acid of lipids bonded to NR molecules.[16] This could be supported by the observation that the removal of fatty acid bonded with NR also decrease aldehyde content to a same level as synthetic polyisoprene.[16]

Structural characterization of NR based upon a combination of spectroscopic techniques and a selective enzymatic scission including a chemical decomposition revealed the presence of proteins and phospholipids at the initiating ω-terminal and terminating α-terminal groups of NR molecules, respectively.[17–20] The both active functional groups possibly form certain networks, including branch points and gel.[12,21,22] Recently, our previous work proposed that proteins are not essential component for the occurring of storage hardening, whereas fatty acid ester groups in phospholipids are responsible for this phenomenon under low humidity.[9,10] Based on these findings, the storage hardening of NR might take place through the formation of phospholipids at the chain ends and/or aldol condensation reaction between aldehyde groups on the fatty acid ester groups of phospholipids at the chain end of the rubber molecules.

Therefore, the suppression of storage hardening of NR using hydroxylamine may involve with aldehyde groups and phospholipids in NR. In the present work, the reaction between hydroxylamine and phospholipids at terminating end of the rubber molecules was elucidated. In an attempt to acquire better understanding of the roles of hydroxylamine involved with phospholipids at the rubber chain end, the model compound of L-α-phosphatidylcholine (PC) as a representative model of the phospholipids in NR was firstly investigated to prove the effect of hydroxylamine on phospholipids. DPNR, wherein contains only the phospholipids as the active functional group, was treated with hydroxylamine to provide the indepth details in structural changes and storage hardening of NR.

EXPERIMENTAL

Sample Preparation

Deproteinized NR (DPNR) was prepared by deproteinization with urea. Freshly tapped NR latex, containing 0.6% ammonium hydroxide in the latex, was diluted to 30% dry rubber content (DRC) and then incubated with 0.1% w/v urea in the presence of 1% w/v sodium dodecyl sulfate (SDS, BDH Chemicals, England) at room temperature for 1 h, followed by centrifugation at 13,000 rpm for 30 min twice. The cream phase was dispersed in the water to make 30% DRC. DPNR latex (30% DRC) was treated with various concentrations of hydroxylamine hydrochloride (Sigma-Aldrich) [0–2 parts per hundred of rubber (phr)] in the basic condition by adjusting to pH 8–9 using ammonium hydroxide and incubated at room temperature for 1 h, followed by gradual coagulation with acetone. The hydroxylamine-treated DPNR was dried in an oven at 70 °C. The sample code for the hydroxylamine-treated DPNR with 1 phr hydroxylamine was designated as H-DPNR.

Transesterified DPNR (TE-DPNR) was conducted by dissolving small pieces of DPNR in toluene and then treated with freshly prepared sodium methoxide ($NaOCH_3$) under a nitrogen atmosphere at room temperature for 3 h. The resulting rubber solution was neutralized by methanolic–HCl and subsequently purified by reprecipitation in methanol twice, followed by drying in a vacuum oven at 40 °C for 24 h.

Accelerated Storage Hardening[9]

A test portion of approximately 50 g from the homogenized sample was placed in a preheated (to 60 °C) desiccator over phosphorus pentoxide (P_2O_5, Kanto, Japan) for 30 min and then evacuated to 0.1 mmHg for 1 h. The evacuated desiccator was then placed in an oven at 60 °C for 0, 6, 12, 24, 48, and 72 h.

Model Study on Cleavage of L-α-Phosphatidylcholine (PC) Using Hydroxylamine

The reaction was performed at 1:2 mole ratios of PC from soybean (Sigma-Aldrich) and hydroxylamine hydrochloride. PC was dissolved in chloroform and subsequently treated with hydroxylamine in ethanol at room temperature for 1 h in the basic condition by adjusting to pH 8 using sodium hydroxide. After that, the mixture was evaporated to dry under nitrogen and finally analyzed using FTIR.

Characterizations and Physical Property Measurements

Prior to all characterizations, the rubber samples were purified by precipitation from toluene/acetone solution. The content of long-chain fatty acid ester group was determined using FTIR measurement based on calibration by a mixture of methyl stearate and synthetic *cis*−1,4-polyisoprene (Kuraprene IR10). The content of fatty acid ester group per weight of rubber was determined by the intensity ratio of peaks of carbonyl group at 1739 cm^{-1} (C═O) and unsaturated carbon (C═C) absorbance at 1664 cm^{-1}.

The gel content in rubber sample was determined by dissolving accurately weighed rubber in dried toluene at concentration of 0.1% (w/v) and then was kept in the dark without stirring for a week at room temperature. The insoluble fraction was separated from sol fraction by centrifugation at 10,000 rpm (14,900*g*) for 30 min. The gel fraction was coagulated using methanol and dried under vacuum at 40 °C. The weight of the gel fraction against the total sample weight was determined as a percentage of gel.

Number-average molecular weight ($\overline{M}_n$), weight-average molecular weight ($\overline{M}_w$) and molecular weight distribution (MWD) of NR samples were determined by size exclusion chromatography (JASCO-Borwin) using refractive index detector. Commercially available *cis*−1,4 polyisoprene (Polymer Standard Service GmbH, Germany) was used as standard for calibrating the molecular weight.

The intrinsic viscosity and Huggins' k' constant were measured with a single bulb Ubbelohde viscometer (SCHOTT, CT52) at 30 ± 0.01 °C in toluene. All of the solvents and rubber solutions were filtered through a glass filter prior to measurement. The measurement was repeated until three consecutive readings differed by ±0.01 s. The intrinsic viscosity and Huggins' k' constant were calculated using the below equation:

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

where η_{sp}/c, $[\eta]$, c, and k' represent the reduced viscosity, intrinsic viscosity, concentration expressed as g/dL, and Huggins' constant, respectively.

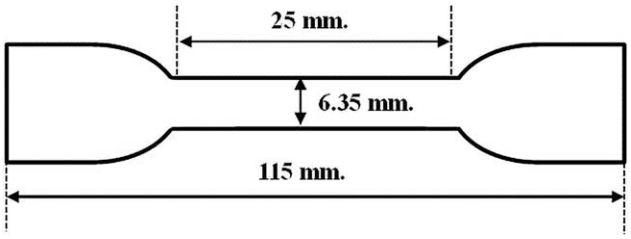

Figure 1. Shape of dumbbell test piece.

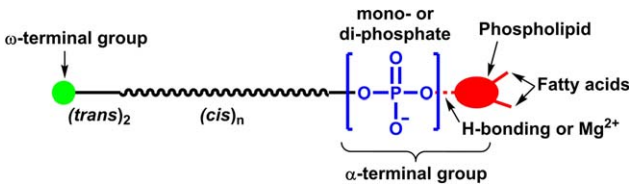

Figure 2. Proposed structure of NR linked to a phospholipid at the α-terminal end. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The ^{1}H NMR measurement was performed using a Bruker UltraShield™ spectrometer at 500 MHz. All spectra were recorded at 50 °C of samples in C_6D_6 solution at a concentration of 1% (w/v) with tetramethylsilane as an internal standard. All measurements were based on 512 scans obtained at a pulse repetition time of 10 s.

Mooney viscosity (MS_{1+4}) was determined using a TECHPRO Mooney Viscometer based on ISO 289-1. The temperature of testing was 100 °C using a small rotor. The rubber sample was preheated at 100 °C for 1 min, followed by a 4 min period of continuous shear at a strain rate of about 1 s^{-1} to measure the Mooney viscosity.

Determination of green strength was carried out using an Instron Model 5569 based on ISO 9026:2007. The testing crosshead speed of 500 mm/min was applied with load cell of 100 N. The test pieces were stamped out using a dumbbell die. The shape and length of dumbbell test piece are represented in Figure 1. The thickness of sample was 1 ± 0.5 mm. The measurement was repeated five times for each sample. The stress was calculated from the following formula:

$$\sigma = F/A$$

where σ = stress (MPa, N/mm^2), F = observed force (N), A = cross-sectional area of unstretched specimen (mm^2) and green strength = stress at rupture of specimen.

The strain was calculated from the following equation:

$$\alpha = (l - l_o)/l_o$$

where α = strain, l = observed distance between the grips of extensometer on the stretched specimen (mm), and l_o = original distance between the extensometer (mm).

RESULTS AND DISCUSSION

Our previous work revealed the presence of phospholipids at the α-terminal group of NR, based on structural characterization of NR after treatment with lipase and phosphatase as demonstrated in Figure 2.[17] Phospholipids at the rubber chain ends are responsible for the branching formation and storage hardening of NR.[18] A recent study showed that the major component of phospholipids was phosphatidylcholine.[23,24] Therefore, the possible chemical reaction between hydroxylamine and phospholipids was first investigated by the use of L-α-phosphatidylcholine (PC) as a model compound. Figure 3 shows the FTIR spectra of PC before and after hydroxylamine treatment. The C═O stretching band at wavenumber of 1740 cm^{-1} corresponding to fatty acid ester in PC disappeared after the reaction with hydroxylamine. Moreover, the peak at wavenumber of 1560 cm^{-1}, assigned to N═H bending of amide, can be observed in the spectrum of the hydroxylamine-treated PC. This finding indicates that hydroxylamine decomposed fatty acid ester of PC and then liberated free fatty acids in the form of fatty hydroxamic acid (R—CO—NHOH), which is known as the product of the cleavage of esters with hydroxylamine.[25,26] The cleavage of fatty acid ester of PC with hydroxylamine is demonstrated in Figure 4. Hydroxylamine completely cleaves the ester linkages of PC at C1 and C2 position to give fatty hydroxamic acid and glycerophosphocoline, which is a water soluble compound.

Proteins and phospholipids at the terminating ends of the rubber chains have been proposed to originate the branch points and gel in NR.[12,21,22] In order to gain the understanding of the role of hydroxylamine on branch points and storage hardening of NR, based on the interaction of phospholipids at the chain ends, it is necessary to remove the branch points and gel containing proteins. Consequently, the effect of hydroxylamine was analyzed for the DPNR latex. Figure 5 exhibits the relationship between the long-chain fatty acid ester content in the DPNR and the concentration of hydroxylamine. Prior to the measurement of long-chain fatty acid ester content, the rubber samples were purified by precipitation from toluene/acetone to remove the free fatty acids. The content of long-chain fatty acid ester in the DPNR remarkably decreased to one-half after treatment with 1 phr of hydroxylamine and then attained the constant value of about 7–8 mmol/kg rubber. The hydroxylamine

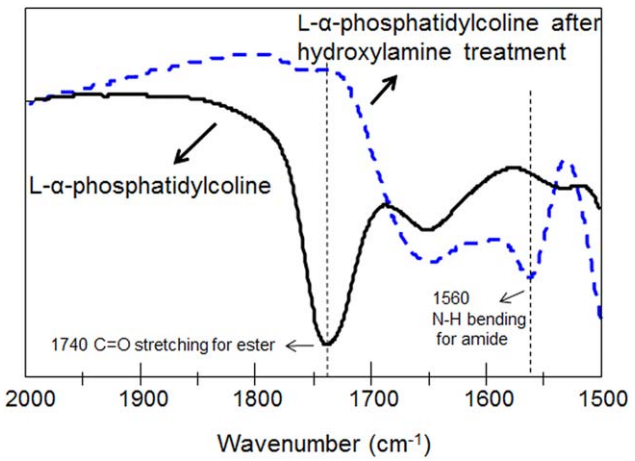

Figure 3. FTIR spectra of L-α-phosphatidylcholine before and after hydroxylamine treatment. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

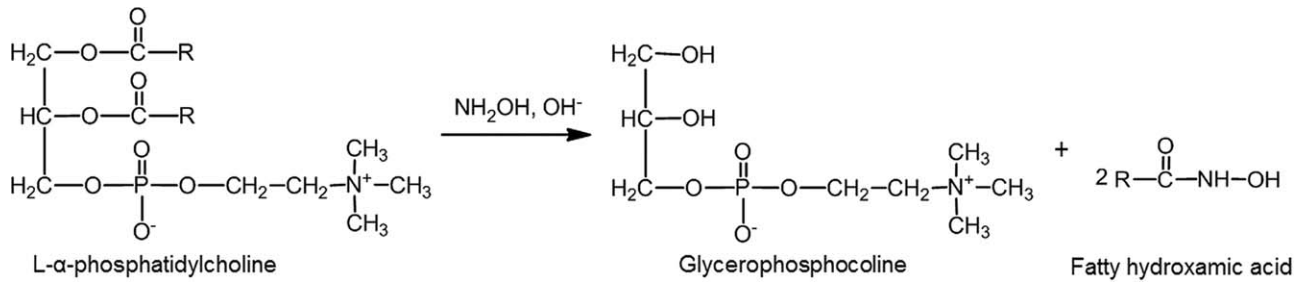

Figure 4. The cleavage of fatty acid ester of L-α-phosphatidylcholine with hydroxylamine.

treatment in latex state was able to cleave the ester linkage of fatty acid ester groups in phospholipids at the chain end of NR, but not completely. On the other hand, all fatty acid ester groups in NR are completely decomposed by the transesterification and saponification in toluene solution.[12] This can be explained by the complicated aggregation forms of phospholipids (mixed micelles, multibilayers, vesicles) in aqueous medium, which are more difficult to attack using chemicals than the monomeric form in organic solvents.[27] Figure 6 displays the content of long-chain fatty acid ester and intrinsic viscosity of DPNR against the concentration of hydroxylamine. Since the rubber chain is terminated by phospholipids, the number of ester group per a rubber chain is regarded as an index of linked phospholipids on the rubber chain.[17] Here, the ester content per rubber chain was calculated based on the $\overline{M}_n$ values of rubber. The ester content decreased from 2.2 to 0.7 mol of ester groups per rubber molecule when the concentration of hydroxylamine increased to 1 phr. In proportion to the decrease in the ester content per rubber chain, the intrinsic viscosity, [η], decreased from 4.8 to 2.7 dL/g. The decrease in the [η] relates to the decrease in molecular weight of the rubber. It has been noted that high molecular weight fraction of NR is derived from not only the inherent mechanism in the biosynthesis, but also the branching formation.[21,22] Therefore, the decomposition of branch points can result in the decrease in molecular weight. The decrease in ester content per rubber chain together with the [η] after hydroxylamine treatment signifies that hydroxylamine could decompose phospholipids at the chain ends of the rubber, resulting in the decomposition of branch points in NR. The hydroxylamine-treated DPNR with 1 phr of hydroxylamine, designated as H-DPNR, was selected to use for the further characterizations. The decomposition of phospholipids at the α-terminal group of NR after hydroxylamine treatment was confirmed by the ^{1}H NMR spectra of DPNR and H-DPNR as shown in Figure 7. The spectrum of DPNR displayed triplet signals at 4.09 and 4.22 ppm assigned to methylene protons of a *cis*-isoprene unit next to monophosphate ($C\underline{H}_2OP$) and diphosphate ($C\underline{H}_2OPP$) groups, respectively.[17] The presence of phospholipid in DPNR was revealed by the clear signal at 3.49 corresponding to the methyl proton next to the nitrogen atom of the choline headgroup of a phospholipid ($N^+(C\underline{H}_3)_3$).[17] Furthermore, two multiplet signals resonating at 3.92 and 4.04 ppm attributed to nonequivalent methylene protons linked to a phosphate group ($C\underline{H}_2OP$) in the glyceride structure of phospholipids were found.[17] The relative intensity of phospholipids signal at 3.49, 3.92, and 4.04 ppm obviously decreased after hydroxylamine treatment as can be seen in the H^1 NMR spectrum of the H-DPNR. This clearly indicates that

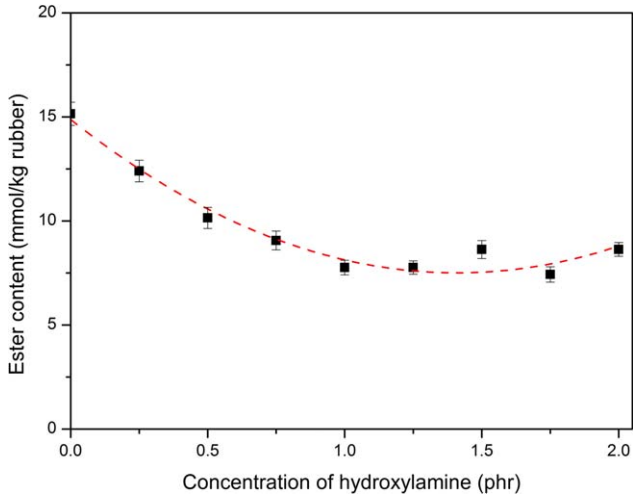

Figure 5. Content of long-chain fatty acid ester in DPNR after treatment with different hydroxylamine concentrations. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

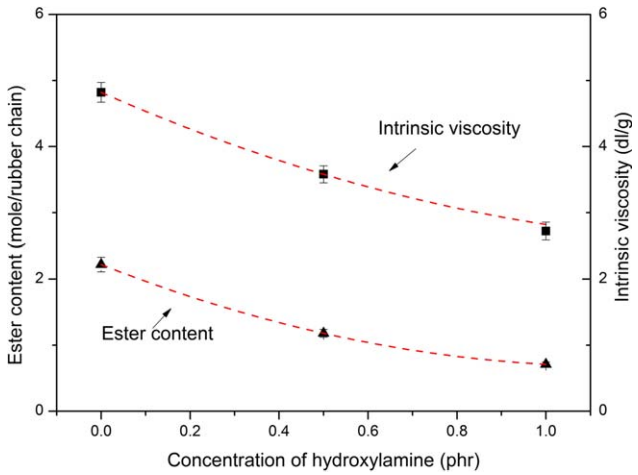

Figure 6. Intrinsic viscosity and content of long-chain fatty acid ester of DPNR after hydroxylamine treatment. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

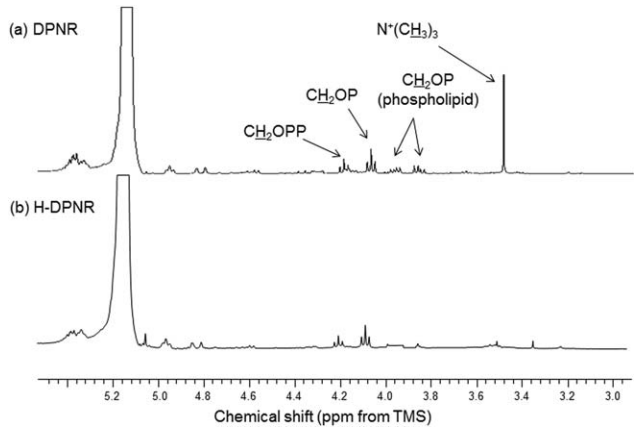

Figure 7. ^{1}H NMR spectra of DPNR and H-DPNR.

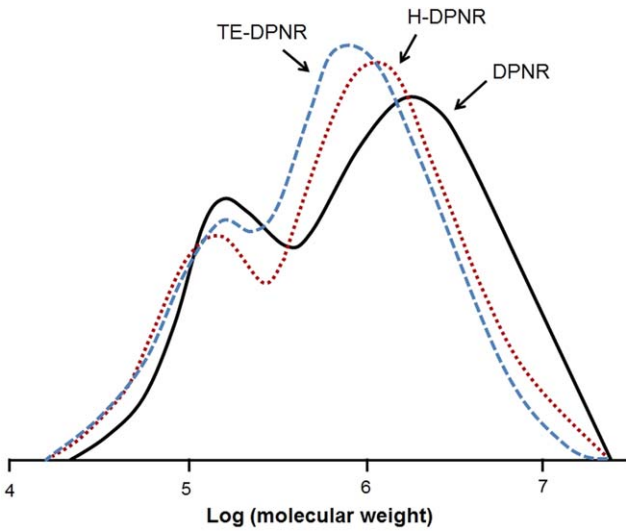

Figure 8. Molecular weight distribution of DPNR, H-DPNR, and TE-DPNR. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

hydroxylamine decomposes phospholipids at the α-terminal group of NR. As mentioned above, hydroxylamine can cleave the ester linkages of phospholipid at C1 and C2 positions to give the glycerophosphocoline. After the hydroxylamine treatment in the DPNR latex, the glycerophosphocoline, which might show the same H^1 NMR signals as phospholipids, was removed from NR molecules because it was dissolved in water.

Table I shows the content of long-chain fatty acid ester, gel content, molecular weight, and k' of the H-DPNR compared with the DPNR and TE-DPNR. The decrease in ester content after transesterification (TE-DPNR) and hydroxylamine (H-DPNR) treatments was accompanied by the decrease in both of the gel content and molecular weight. Taking into account the decrease in molecular weight, it can be seen clearly from the MWD curve of the DPNR, TE-DPNR, and H-DPNR in Figure 8. The transesterification as well as hydroxylamine treatments resulted in the significant shift of the bimodal peak from the high to low molecular weight. The decrease in the gel content and molecular weight might be due to the decomposition of branch points. However, if the decrease in the molecular weight is due to the decomposition of branch points, it should be accompanied with the occurrence of linear rubber molecules, which are provable using the Huggins' k' constant. It is well-known that the k' is a qualitative indicator of the presence of long-chain branching. The k' is almost independent of molecular weight and MWD. Moreover, it increases in proportion to the quantity of branching in the polymer chains.[28,29] It has been reported that k' values of about 0.45–0.65 refer to branched molecules, whereas values around 0.3 correspond to linear polymer.[28] It was found that the k' value of DPNR was ~0.6, whereas that of the TE-DPNR and H-DPNR was ~0.3. This finding is strong supporting evidence that branch points were entirely decomposed to form linear molecules after transesterification and hydroxylamine treatments. Consequently, it can be deduced that the removal of long-chain fatty acid ester group results in the decomposition of branch points. However, it is noteworthy that although the hydroxylamine treatment is not an effective method to remove phospholipids completely, unlike transesterification, the decomposition of branch points and the formation of linear molecules can be observed in the case of the H-DPNR. This is because some branch points in DPNR are derived from phospholipid aggregations via hydrogen bond, as confirmed by the decrease in the branch points and gel content after polar chemical treatments.[18,21,30] Since hydroxylamine is a strongly polar chemical, it can decompose branch points formed by phospholipid aggregations in DPNR. Therefore, it is reasonable to postulate that hydroxylamine plays a vital role in the decomposition of branch point of NR, resulting in the change in molecular structure of DPNR from branched to linear molecules as a result of the removal of phospholipids and the breakdown of phospholipid aggregations at the chain ends of NR as illustrated in Figure 9. It should be noted that the determination of branch points in NR was affected by the analysis techniques. The measurement of the Huggins' k' constant using viscometric analyses, the combination of ^{13}C-NMR measurement and

Table I. Structural Characteristics of DPNR, TE-DPNR, and H-DPNR

Characteristics	DPNR	TE-DPNR	H-DPNR
Ester content (mol/rubber chain)	2.22 ± 0.13	~0	0.71 ± 0.15
Gel content (% w/w)	16.7 ± 1.4	~0	~0
$\overline{M}_n\left(\times 10^5\right)$	1.47	1.18	1.08
$\overline{M}_w\left(\times 10^6\right)$	1.86	0.75	1.58
k'	0.64 ± 0.03	0.30 ± 0.02	0.35 ± 0.02

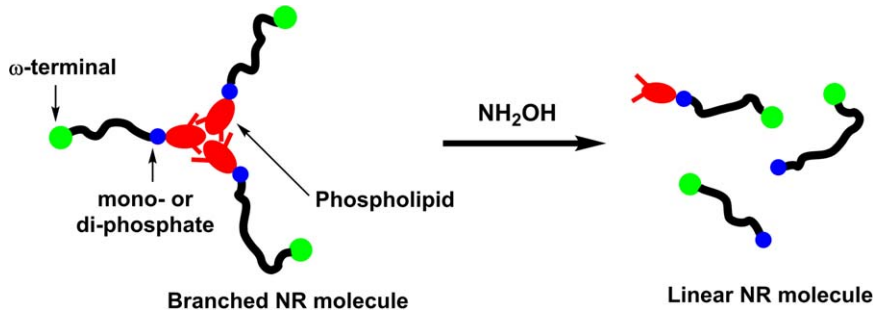

Figure 9. Scheme of proposed molecular structure NR before and after hydroxylamine treatment. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

osmometry as well as the combination of low-angle laser light scattering (LALLS) gel permeation chromatography (GPC) and viscometry provided the information that NR is composed of branched molecules, whereas a recent study using GPC coupled with a multiangle light scattering detector (SEC-MALS) proposed that NR is composed of almost linear molecules and nanoaggregates with rather compact structures.[12,22,31,32]

The decomposition of branch points should affect the stress–strain properties of the unvulcanized rubber that are important to certain processing operations in the rubber industry. These unvulcanized rubber properties are frequently referred to as "green strength".

Table II shows the green strength of DPNR, H-DPNR, and TE-DPNR. DPNR showed the high green strength (5.70 MPa), indicating that protein has no direct effect on the green strength. On the contrary, a very low green strength (<1 MPa) can be observed in the case of the H-DPNR and TE-DPNR. This might be due to the decomposition of branch points derived from phospholipids. Another factor affecting green strength of NR is strain-induced crystallization (SIC) of NR.[33] The SIC of DPNR, TE-DPNR and H-DPNR could be represented by their stress–strain curves as shown in Figure 10. It can be seen that the stress upturn of DPNR occurred at smaller strains (~460% strain) compared with that of H-DPNR (~700% strain). In the case of TE-DPNR, it could not be observed any increase in stress during stretching. This indicates that the decrease in phospholipids after the hydroxylamine and transesterification treatments affects the SIC of NR. This finding can be supported by our previous study using *In situ* wide-angle X-ray diffraction (WAXD) during tensile deformation. The WAXD pattern at strain of 6.0 of DPNR exhibited crystalline reflections, while that of transesterified sample only showed an isotropic scattering halo from random amorphous chains.[34] This can be implied that removal of phospholipids play a significant role on the SIC, contributing to the decrease in green strength of NR. However, quite different green strength and stress–strain behaviors between TE-DPNR and H-DPNR could be observed. This is attributed to the better efficacy of transesterification for the removal of phospholipids, leading to the efficient suppression of branching formation and SIC. The decomposition of some branch points derived from phospholipids might be the reason why CV grade NR (hydroxylamine-treated NR) shows the excellent processabilty, which premastication process can be almost completely eliminated.

Table II. Green Strength of DPNR, TE-DPNR, and H-DPNR

Sample	Green strength (MPa)
DPNR	5.70 ± 0.67
TE-DPNR	0.11 ± 0.06
H-DPNR	0.66 ± 0.04

The storage hardening of NR can be monitored by an increase in the gel content and Mooney viscosity of the rubber, which is accelerated the storage hardening by keeping the rubber under low humidity condition using P_2O_5.[9] Figure 11 shows the change in the gel content and Mooney viscosity of the DPNR, H-DPNR, and TE-DPNR during accelerated the storage hardening. It can be seen clearly that the gel content and the Mooney viscosity of the DPNR significantly decreased after the hydroxylamine and transesterification treatments. This is due to the decomposition of branch points originated from phospholipids. After storage, the apparent increase in the gel content and

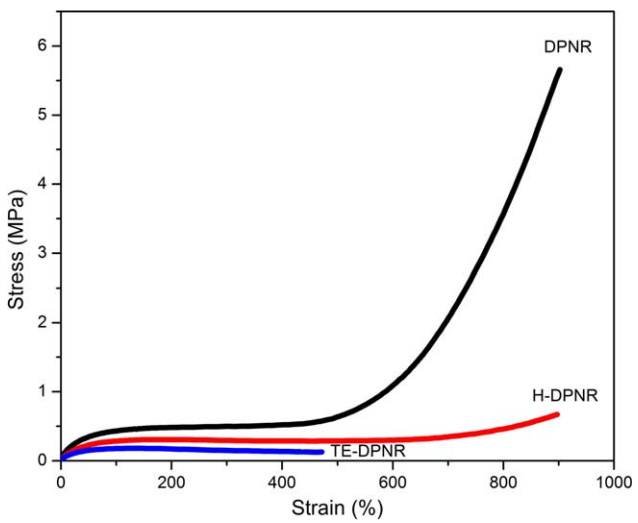

Figure 10. Stress–strain curves of DPNR, H-DPNR, and TE-DPNR. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

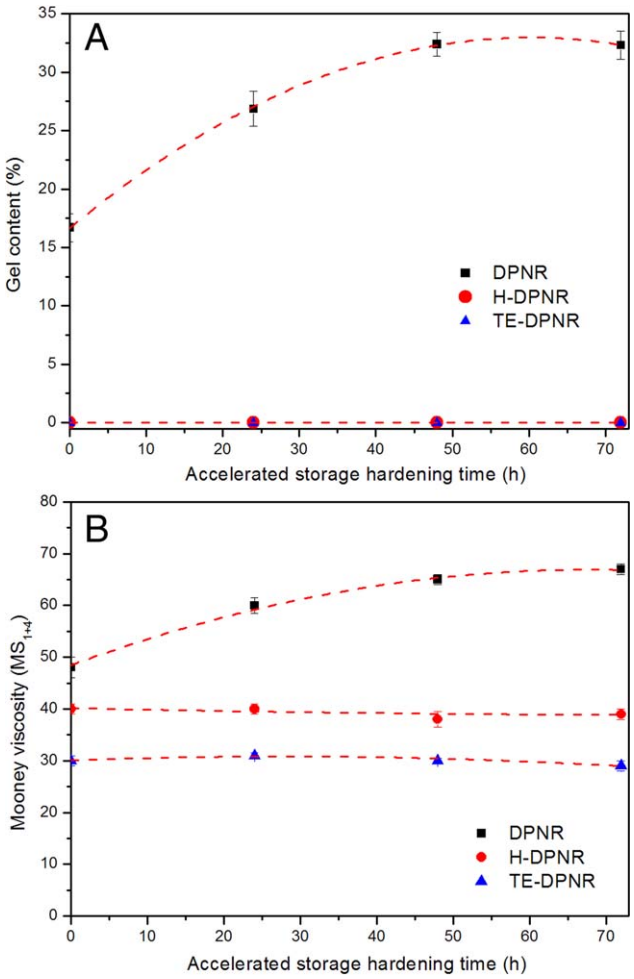

Figure 11. Change in gel content (A) and Mooney viscosity (B) of DPNR, H-DPNR, and TE-DPNR during accelerated storage hardening. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Mooney viscosity of DPNR was observed, indicating that protein is not responsible for the storage hardening phenomenon, as reported in the previous studies.[9,10] On the other hand, the constant gel content and Mooney viscosity of TE-DPNR during the accelerated storage hardening signifies that the lack of phospholipids in TE-DPNR might be the main reason for the absence of the storage hardening. It was very clear that there was no change in the gel content and Mooney viscosity of the H-DPNR during accelerated storage hardening. As mentioned above, the hydroxylamine treatment could remove phospholipids from the rubber and was able to obstruct the formation of phospholipid aggregations at the chain ends of rubber molecules. Consequently, the new possible mechanism of hydroxylamine on the inhibition of the storage hardening in NR can be proposed that hydroxylamine decomposes phospholipids and prevents phospholipid aggregations, contributing to an inability to form networks. Nevertheless, the former postulation that hydroxylamine inhibits storage hardening by the reaction with aldehyde should not be neglected due to the fact that other viscosity-stabilizing agents such as semicarbazide and hydrazine, which can react with aldehyde groups, also suppress the storage hardening in NR. It has been reported that the viscosity of hydroxylamine-treated rubber was obviously lower than that of semicarbazide and hydrazine-treated rubbers.[35] Here, our present work suggests that hydroxylamine can decompose branch points derived from phospholipids. This is likely to be the cause of lower viscosity of hydroxylamine-treated CV rubber in comparison with semicarbazide and hydrazine-treated rubbers.

A study on fractionated NR revealed that aldehyde content increased in line with molecular weight of the rubber,[16] indicating that aldehyde groups are not auto-oxidative degradation products of NR because if aldehyde groups originate from the degradation of the rubber chain, the low molecular weight fraction should have a high aldehyde content. Since the removal of long-chain fatty acid ester bonded with NR decreased aldehyde content to a same level as synthetic polyisoprene, aldehyde groups are likely derived from auto-oxidation of unsaturated fatty acid of phospholipid bonded to NR molecules.[16] Therefore, aldehyde-reactive chemicals such as hydroxylamine, semicarbazide and hydrazine possibly prevent storage hardening by the reaction with aldehyde groups present on phospholipids bonded to NR molecules. Accordingly, the storage hardening of NR should be due to the chemical crosslinking reaction that is probably the aldol condensation between aldehyde groups on the long-chain fatty acid of phospholipid molecules at the α-terminal group of rubber molecules.

Table III. Gel Content and Ester Content of Stored DPNR (72 h under Accelerated Condition) Precipitated from Toluene Solution after Treatment with Transesterification, Polar Solvent, Hydroxylamine, and Hydrazine

Rubber sample	Gel content (% w/w)	Ester content (mmol/kg rubber)
Stored DPNR (control)	32.3 ± 1.1	15.15 ± 0.45
Stored DPNR treated with transesterification	~0	~0
Stored DPNR treated with polar solvent[a]	28.7 ± 1.5	14.57 ± 0.21
Stored DPNR treated with hydroxylamine[b]	3.1 ± 0.5	4.34 ± 0.25
Stored DPNR treated with hydrazine[c]	26.6 ± 1.2	14.76 ± 0.36

[a] 2% (v/v) ethanol in toluene solution.
[b] 1% (w/v) hydroxylamine/ethanol in toluene solution at pH 8.
[c] 1% (v/v) hydrazine in toluene solution.

In order to verify this assumption, the DPNR after accelerated storage hardening for 72 h was subjected to treatment with transesterification, polar solvent, hydroxylamine as well as hydrazine. The transesterification treatment was done by the same procedure as the preparation of TE-DPNR, while the polar solvent, hydroxylamine, and hydrazine treatments were carried out by the addition of small amounts of ethanol, hydroxylamine, and hydrazine, respectively into the rubber solution. Table III exhibits the gel content and ester content of the stored DPNR (72 h under accelerated condition) after the transesterification, polar solvent, hydroxylamine, and hydrazine treatments. The gel content and ester content decreased to ~0% after the transesterification treatment. It is evident that the network formation during storage hardening is composed of phospholipids. The type of phospholipid interactions caused by storage hardening was proven by the observation of gel content which slightly decreased after the polar solvent treatment. This infers that in the case of storage hardening, the interaction of phospholipids derived from hydrogen bonds is a minority, but the vital interaction of phospholipids is the chemical interaction. This finding supports the assumption that network formation during storage hardening is derived from the chemical crosslinks via aldehyde groups on the long-chain fatty acid of phospholipids. However, our previous work reported that the gel content of the rubber from high ammonia preserved latex and deproteinized NR latex decreased to almost 0% after treatment with 1–2% ethanol, suggesting that the network formation of NR is ascribed to the hydrogen bond of proteins and phospholipids.[21] Consequently, it can be deduced that the inherent network and the network caused by storage hardening are likely dissimilar. It was noteworthy that the obvious decrease in gel content was accompanied by the decrease in ester content after the hydroxylamine treatment, whereas the insignificant decrease in gel content and ester content was observed after the hydrazine treatment. This supporting evidence confirms the ability of hydroxylamine to decompose phospholipids, which could not be found in hydrazine. This makes hydroxylamine an excellent chemical for controlling viscosity of NR.

CONCLUSIONS

The hydroxylamine treatment of DPNR showed the decomposition of branch points derived from phospholipids at the α-terminal group, resulting in the change in molecular structure of DPNR from branched to linear molecules. The decomposition of branch points was due to the cleavage of ester linkages of phospholipids and the breakdown of phospholipid aggregations as a result of hydroxylamine. The storage hardening of NR was proposed to be caused by the chemical crosslinking reaction between aldehyde groups on the long-chain fatty acid of phospholipid molecules at the α-terminal group of NR. Apart from the reaction with aldehyde groups, hydroxylamine can inhibit storage hardening in NR effectively, since it can decompose phospholipids and prevent phospholipid aggregations.

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REFERENCES

1. Allen, P. W.; Bloomfield, G. F. Natural Rubber Hydrocarbon; Bateman, L., Eds.; MacLaren and Sons: London, **1963**; Chapter 1, p 1.
2. Burfield, D. R. *Nature* **1974**, *249*, 29.
3. Burfield, D. R.; Gan, S. N. J. *Polym. Sci. Polym. Chem. Ed.* **1975**, *13*, 2725.
4. Sekhar, B. C. Inhibition of hardening in natural rubber, In: Proceedings in the Natural Rubber Research Conference; The Rubber Research Institute of Malaysia: Kuala Lumpur, **1960**; p 512.
5. Sekhar, B. C. *Rubber Chem. Technol.* **1962**, *35*, 889.
6. Gregg, E. C.; Macey, J. R.; Macey, J. H. *Rubber Chem. Technol.* **1973**, *46*, 47.
7. Gregory, M. J.; Tan, A. S. Some Observations on Storage Hardening of Natural Rubber, In: Proceedings in International Rubber Conference: Kuala Lumpur; **1975**; p 28.
8. Subramaniam, A.; Wong, W. S. *J. Nat. Rubber Res.* **1986**, *1*, 58.
9. Yunyongwattanakorn, J.; Tanaka, Y.; Kawahara, S.; Klinklai, W.; Sakdapipanich, J. *Rubber Chem. Technol.* **2003**, *76*, 1228.
10. Amnuaypornsri, S.; Nimpaiboon, A.; Sakdapipanich, J. *Kautsch. Gummi Kunstst* **2009**, *62*, 88.
11. Eng, A. H.; Ejiri, S.; Kawahara, S.; Tanaka, Y. *J. Appl. Polym. Sci. Appl. Polym. Symp.* **1994**, *53*, 5.
12. Tangpakdee, J.; Tanaka, Y. *J. Rubber Res.* **1997**, *12*, 112.
13. Chin, P. S. *J. Rubber Res. Inst. Malaysia* **1968**, *22*, 56.
14. Bristow, G. M. *NR Technol.* **1977**, *8*, 21.
15. Gorton, A. D. T. *Rubber Indus.* **1974**, *8*, 142.
16. Eng, A. H.; Tangpakdee, J.; Kawahara, S.; Tanaka, Y. *J. Nat. Rubber Res.* **1997**, *12*, 11.
17. Tarachiwin, L.; Sakdapipanich, J. T.; Ute, K.; Kitayama, T.; Bamba, T.; Fukusaki, E.; Kobayashi, A.; Tanaka, Y. *Biomacromolecules* **2005**, *6*, 1851.
18. Tarachiwin, L.; Sakdapipanich, J. T.; Ute, K.; kitayama, T.; Tanaka, Y. *Biomacromolecules* **2005**, *6*, 1858.
19. Tanaka, Y.; Eng, A. H.; Ohya, N.; Nishiyama, N.; Tangpakdee, J.; Kawahara, S.; Wititsuwannakul, R. *Phytochemistry* **1996**, *41*, 1501.
20. Tangpakdee, J.; Tanaka, Y. *Phytochemistry* **1998**, *48*, 447.
21. Tangpakdee, J.; Tanaka, Y. *Rubber Chem. Technol.* **1997**, *70*, 707.
22. Tangpakdee, J.; Tanaka, Y. *J. Rubber Res.* **1998**, *1*, 14.
23. Liengprayoon, S.; Sriroth, K.; Dubreucq, E.; Vaysse, L. *Phytochemistry* **2011**, *72*, 1902.
24. Liengprayoon, S.; Bonfils, F.; Sainte-Beuve, J.; Sriroth, K.; Dubreucq, E.; Vaysse, L. *Eur. J. Lipid. Sci. Technol.* **2008**, *110*, 563.
25. Reddy, S. A.; Kumar, M. S.; Reddy, G. R. *Tetrahedron Lett.* **2000**, *41*, 6285.

26. Porcheddu, A.; Giacomelli, G. Synthesis of Oximes and Hydroxamic Acids; Rappoport, Z.; Liebman, J.F., Eds.; John Wiley & Sons: West Sussex, **2009**; Chapter 6, p 163.
27. Kepsil, C. R.; Dennis, E. A. *Biochemistry* **1981**, *20*, 6079.
28. Bristow, G. M. *J. Polym. Sci.* **1962**, *62*, S168.
29. Amber, M. R.; Mate, R. D.; Purdon, J. R. *J. Polym. Sci. Polym. Chem. Ed.* **1974**, *12*, 1759.
30. Amnuaypornsri, S.; Tarachiwin, L.; Sakdapipanich, J. T. *J. Appl. Polym. Sci.* **2010**, *115*, 3645.
31. Sakdapipanich, J. T.; Kowitteerawut, T.; Suchiva, K.; Tanaka, Y. *Rubber Chem. Technol.* **1999**, *72*, 712.
32. Kim, C.; Beuve, J. S.; Guilbert, S.; Bonfils, F. *Eur. Polym. J.* **2009**, *45*, 2249.
33. Grechanovskii, V. A.; Poddubnyi, I. Y.; Ivanova, L. S. *Rubber Chem. Technol.* **1974**, *47*, 342.
34. Amnuaypornsri, S.; Sakdapipanich, J.; Toki, S.; Hsiao, B. S.; Ichikawa, N.; Tanaka, Y. *Rubber Chem. Technol.* **2008**, *81*, 753.
35. Honghai, H.; Beilong, Z.; Weiyong, D.; Li, D.; Yongzhou, W.; Pingyue, W.; China, J. *Trop. Agric.* **2010**, *30*, 1.

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