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Purification of Natural Rubber

JITLADDA TANGPAKDEE* AND YASUYUKI TANAKA*#

About two long-chain fatty acid ester groups per rubber chain are retained in Hevea *rubber, even after treatment of the rubber with proteolytic enzyme and reprecipitation or acetone extraction. The fatty acid esters and phosphorus compounds were perfectly removed by transesterification of rubber solution with sodium methoxide or saponification with KOH. Almost all the proteins were removed by saponification or deproteinisation, while they remained even after transesterification. The gel content of rubber was reduced to almost zero by transesterification or saponification. Huggins' k' constant of the soluble fraction was reduced apparently by these treatments. The branch-points comprising phospholipid esters in* Hevea *rubber were presumed to be decomposed to form linear molecules.*

The basic structure of natural rubber (NR) from *Hevea brasiliensis* has been confirmed to consist of an initiating terminal group, two or three *trans*-isopene units, a long sequence of *cis*-isoprene units and a terminated group aligned in that order[1], although detailed structure of both terminal groups has not been identified. In addition to the isoprene units, small amounts of various abnormal groups such as aldehyde[2], epoxide[3] and lactone[4] have been postulated to be responsible for the occurrence of branching and crosslinking reactions in NR.

Proteins in NR have been regarded as a reactive substance to produce branching by reaction with the abnormal groups[5] and act as an essential component to lead the outstanding properties of NR. However, Ichikawa, *et al.* have shown that the proteins do not participate in the fundamental properties of NR[6]. We have presumed that the long-chain fatty acid groups linked to rubber molecule as phospholipids are one of the major abnormal groups to form branch-points and gels in NR[7]. These findings prompted much attention on the prominent role of proteins, fatty acid groups and phospholipids present in NR. However, the definite structure of the ester linkages in NR even now has not been identified yet.

In this paper, we report about the component and structural changes in NR after different treatments, *i.e.* enzymatic deproteinisation, transesterification with sodium methoxide and saponification with potassium hydroxide. This work provides fundamental information on the whole structure of NR including the branching and gel formation.

EXPERIMENTAL

Fresh field latex (FL-latex) of RRIM 600 clone was preserved in 1% (w/v) sodium dodecyl sulphate for one day before use. The enzymatic deproteinisation was carried out by treatment

* Division of Applied Chemistry, Faculty of Technology, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184, Japan
# Corresponding author

of 10% DRC latex with 0.04% (w/v) *Alcalase 2.0T* and 1% (w/v) *Triton X-100* at 37°C for 24 h followed by centrifugation. The cream rubber was redispersed in 1% (w/v) *Triton X-100* to make 10% DRC and recentrifuged twice. Transesterification was carried out by treatment 1% (w/v) solution of rubber in toluene, with freshly prepared 1 *M* $NaOCH_3$ under N_2 atmosphere in the dark at room temperature for 2.5 h, followed by concentration with a rotary evaporator at 45°C and precipitation in methanol. Saponification was performed by reaction of 1% (w/v) of rubber in hexane/toluene (5:3, v/v) with 1.5 *M* KOH solution in 2-propanol/water (5:1, v/v), in the presence of 0.1% (w/v) methanolic pyrogallol as an antioxidant. The reaction mixture was refluxed at 70°C for 2 h under N_2 atmosphere. The hot saponified mixture was then filtered and washed several times with hot distilled water until the solution became clear-white, then concentrated by evaporation and precipitated in methanol. All the rubbers was further purified by extraction with acetone under N_2 atmosphere for 30 h. The purified rubber was dried *in vacuo* at room temperature and subjected to molecular weight and structural analyses.

All the rubbers were fractionated to several fractions, by solvent fractionation in the usual way[8]. The fractionated rubbers were subjected to analyse the Huggins' *k'* constant, using viscometric measurement[9].

The molecular-weight distribution (MWD) was determined by gel-permeation chromatography (GPC) using two columns in series, packed with styrene-divinylbenzene copolymers having exclusion limits of 2.0 x 10^7 and 5.0 x 10^4. Measurements were made using THF as an eluent, with a flow rate of 0.5 ml/min at 35°C, monitoring with RI and low-angle laser-light scattering (LALLS) detectors. Commercially obtained standard polystyrenes were used for calibration. The purified rubber at concentration of 0.01% (w/v) in THF was filtered through a Millipore LS prefilter and a 0.2 μm before injection.

The gel content was determined by dissolving the purified rubber in dried toluene, which was kept in activated Molecular Sieves 4A, to give a concentration of 0.2% (w/v) and kept in the dark without shaking or stirring for 1 week at room temperature. The solution was centrifuged at 10 000 g for 40 min to separate the gel from sol fraction. The separated gel fraction was dried *in vacuo* and weighed to estimate the gel content.

FTIR measurements were made with a JASCO 5300 FTIR spectrometer. The quantity of the ester groups was determined by FTIR using a calibration curve obtained from a mixture of synthetic *cis*-1,4-polyisoprene and methyl stearate[10]. The nitrogen content was analysed using a Kjeldahl method[11]. The phosphorus content was analysed by digesting rubber with nitric acid according to the method of Moris *et al.*[12] The ^{13}C-NMR spectra were taken with a JEOL λ-500 spectrometer at 50°C in deuterated chloroform.

RESULTS AND DISCUSSION

The gel content in the rubber from FL-latex was reduced from 5% to 3% by deproteinisation and further decreased to about 1% by transesterification or saponification, as shown in *Table 1*. It is generally accepted that the gel fraction of NR is branched molecules which originated from crosslinking reaction due to abnormal groups present in the rubber matrix,

TABLE 1. ANALYSIS OF RUBBERS AFTER DEPROTEINISATION, TRANSESTERIFICATION AND SAPONIFICATION

Sample	N content (%, w/w)	Ester content (mmol/kg rubber)	P content (%, w/w)	Gel content (%, w/w)
Control (FL)	0.231	10.4	0.069	5.1
DP-NR	0.016	11.8	0.013	3.2
TE-NR	0.210	~0	~0	0.8
SAP-NR	0.011	~0	~0	1.2

which lead to storage hardening of rubber[5]. The decrease in gel content after these treatments implies that a part of the branch-points was decomposed by deproteinisation and almost all of them were disintegrated by transesterification.

Table 2 shows the change in molecular weight of rubbers after enzymatic deproteinisation, transesterification and saponification, compared with the control rubber. Deproteinisation resulted in an insignificant change in $\overline{M}_n$ and $\overline{M}_w$, while transesterification and saponification reduced the $\overline{M}_w$ and $\overline{M}_n$ to about two-third of the control sample. This indicates that proteins are not concerned with the branch-points observed here, although it was confirmed that proteins are involved in the gel formation[4].

Synthetic *cis*-1,4-polyisoprene was treated in a similar way as the transesterified NR. As shown in *Table 2*, there was practically no change in the molecular weight, suggesting that transesterification is a reaction which decomposes only the ester linkages, which compose the branch-points to form linear molecules.

The Huggins' *k'* constant of the fractionated rubbers from high to low M_w fractions was given in *Table 3*. It is clear that the *k'* values of the TE-NR and SAP-NR were in narrow ranges and smaller than those of the control rubber and DP-NR. It is well-known that the Huggins' *k'* constant of a linear polymer is smaller than that of the branched polymer[13,14]. This indicates that the control rubber and DP-NR contain branched molecules, while the TE-NR and SAP-NR are composed of linear molecules. This result supports the idea that transesterication and saponification breaks down the branch-points which are stable to the enzymatic deproteinisation.

Figure 1 shows FTIR spectra of the rubbers obtained after deproteinisation (DP-NR), transesterification (TE-NR) and saponification (SAP-NR) together with the control (FL-NR). It is clear that the intensity of the infrared band at 3280 cm^{-1}, which is assignable to ν_{NH}, markedly reduced in intensity and shifted to 3316–3320 cm^{-1} as the nitrogen content of the DP-NR and SAP-NR decreased. The characteristic bands of the amide and amine bondings at 1628 cm^{-1} and 1540 cm^{-1} also disappeared in the DP-NR and SAP-NR, while those bands clearly resided in TE-NR and the control rubber. As shown in *Table 1*, the nitrogen content of these rubbers was reduced to about 0.01% by deproteinisation or saponification, but transesterification gave an

TABLE 2 MOLECULAR WEIGHT OF RUBBERS AFTER DEPROTEINISATION, TRANSESTERIFICATION AND SAPONIFICATION

Sample	$\overline{M}_w \times 10^{-6}$ (LALLS)	$\overline{M}_w \times 10^{-6}$ (RI)	$\overline{M}_n \times 10^{-5}$ (RI)	$\overline{M}_w/\overline{M}_n$
Control (FL)	2.1	2 4	3.5	6 8
DP-NR[a]	1.9	2 3	3.2	7 1
TE-NR[b]	1.6	1 6	1 9	8 3
SAP-NR[c]	1.7	1 8	2.3	8 0
Cariflex-305[d]	2.2	2.0	5.7	3.3
Cariflex-305-TE[e]	2.0	2.0	5.9	3.4

[a]Deproteinised natural rubber

[b]Transesterified natural rubber

[c]Saponified natural rubber

[d]Synthetic *cis*-1,4-polyisoprene

[e]*Transesterified synthetic cis-1,4-polyisoprene*

TABLE 3 HUGGINS' *K'* CONSTANT OF FRACTIONATED RUBBER AFTER DEPROTEINISATION, TRANSESTERIFICATION AND SAPONIFICATION

Rubber fraction	Huggins' *k'* constant			
	Control (FL)	DP-NR	TE-NR	SAP-NR
1	0 65 (6 08)[a]	0.59 (6.11)	0 45 (6 24)	0.4 (6.18)
2	0.55 (4.60)	0.57 (5 36)	0.43 (4.77)	0.43 (5.20)
3	0.57 (3.66)	0 59 (4.19)	0.44 (3.23)	0.40 (4.30)
4	0.50 (2 50)	0.46 (2.19)	0.40 (2.98)	0 42 (2 10)
5	0 45 (1 27)	0.43 (0.68)	0.35 (1 20)	0.36 (0.83)

[a]Values in the parentheses are intrinsic viscosities [η] of each fraction

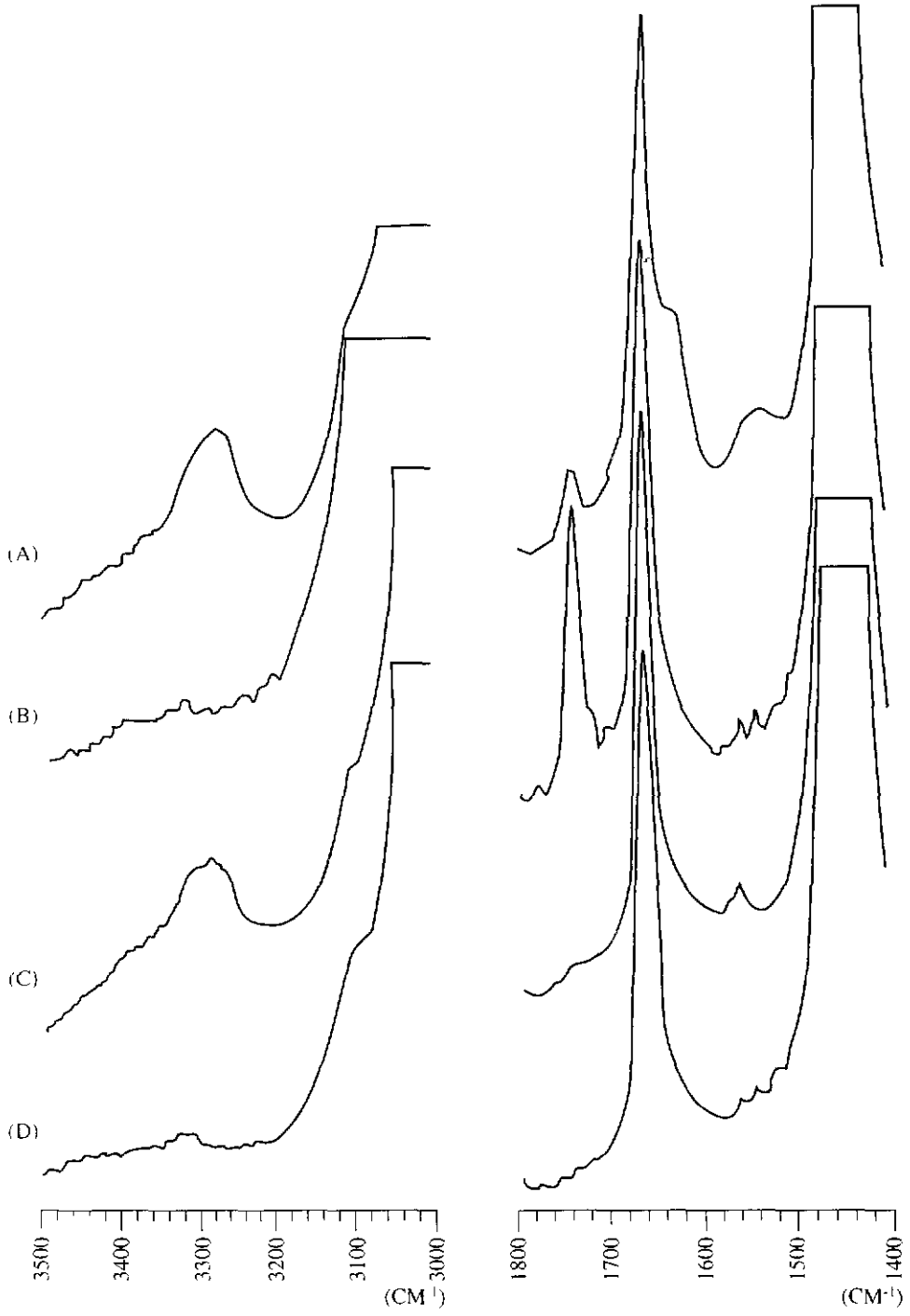

Figure 1 FTIR spectra of the rubbers from (A) control (FL-NR), (B) deproteinised NR (DP-NR), (C) transesterified NR (TE-NR) and (D) saponified NR (SAP-NR)

insignificant change in the nitrogen content. This indicates that the saponification with KOH hydrolyses amide linkages of rubber in hexane/ toluene solution as proteases do in the rubber latex. The residual nitrogen content in the DP-NR and SAP-NR was estimated to be about two to three mol-atom/rubber chain, based on the degree of polymerisation of 5000 for one rubber chain. This quantity of amide groups per rubber chain agrees well with our previous finding that the infrared band at 3316 cm^{-1} to 3320 cm^{-1} corresponds to the N-H band in oligopeptides[15]. Our ^{1}H-NMR study on the structure of NR suggests the presence of a special linkage at the initiating terminal[10]. Although there is no direct evidence to prove the linkage between oligopeptides and this terminal group at present, this finding implies that the amide groups remained in the rubber molecule even after high deproteinisation.

We have shown that the free fatty acid components were removed in the preliminary stage from NR by acetone extraction[7]. However, some fatty acids linked to the rubber chain remained and these could be detected by FTIR and ^{13}C-NMR measurements. Both the control rubber and DP-NR showed a clear infrared band at 1738 cm^{-1}, which is a characteristic of ester group in fatty acid esters. This ester bond was confirmed at the long-chain fatty acids linked to the rubber chain and was estimated to be about two molecules per rubber chain. It is clear that this band definitely diminished and the ester content became zero after transesterification or saponification. The removal of the ester groups was also confirmed by ^{13}C-NMR analysis. *Figure 2* (*A*, *B* and *C*) show the ^{13}C-NMR spectra of the DP-NR, SAP-NR and TE-NR rubbers, respectively. The former apparently showed signals at δ 14.0, 29.7 and 34.4, which were corresponding to the terminal methyl (-$\underline{C}H_3$), methylene sequence [-($\underline{C}H_2$)$_n$-] and terminal methylene (-$O_2C\underline{C}H_2$-) carbons of a long-chain fatty acid, respectively. These signals completely disappeared after transesterification or sanponfication. The long-chain fatty acids were confirmed to be liberated from rubber chain as methyl esters after transesterification[7]. These facts suggest that the abnormal groups having IR band at 1740 cm^{-1} are fatty acid esters and not as a lactone.

It is clear from *Table 1* that the amount of phosphorus in NR decreases significantly after deproteinisation and became zero after transesterification or saponification. A part of phosphorus constituents such as lipoproteins can be removed in the deproteinisation process. The residual phosphorus content in this stage is calculated about one mol-atom P/rubber chain. This may be regarded as a terminal group derived from the modification of diphosphate group in the termination step of the biosynthetic pathway, which probably is a phospholipid.

It is interesting that both the amounts of phosphorus and fatty acid ester groups decreased to zero after transesterification or saponification. This result supports the assumption that a phospholipid consisting of two molecules of long-chain fatty acids which probably originate the phosphorus and long-chain fatty acid components in NR. The reduction of gel content, molecular weight and Huggins' *k'* constant of rubbers after transesterification or saponification also implies that the phospholipid should be a principle component to produce the branch-points in NR. The detailed structure of the branch-points will be discussed in a subsequent paper.

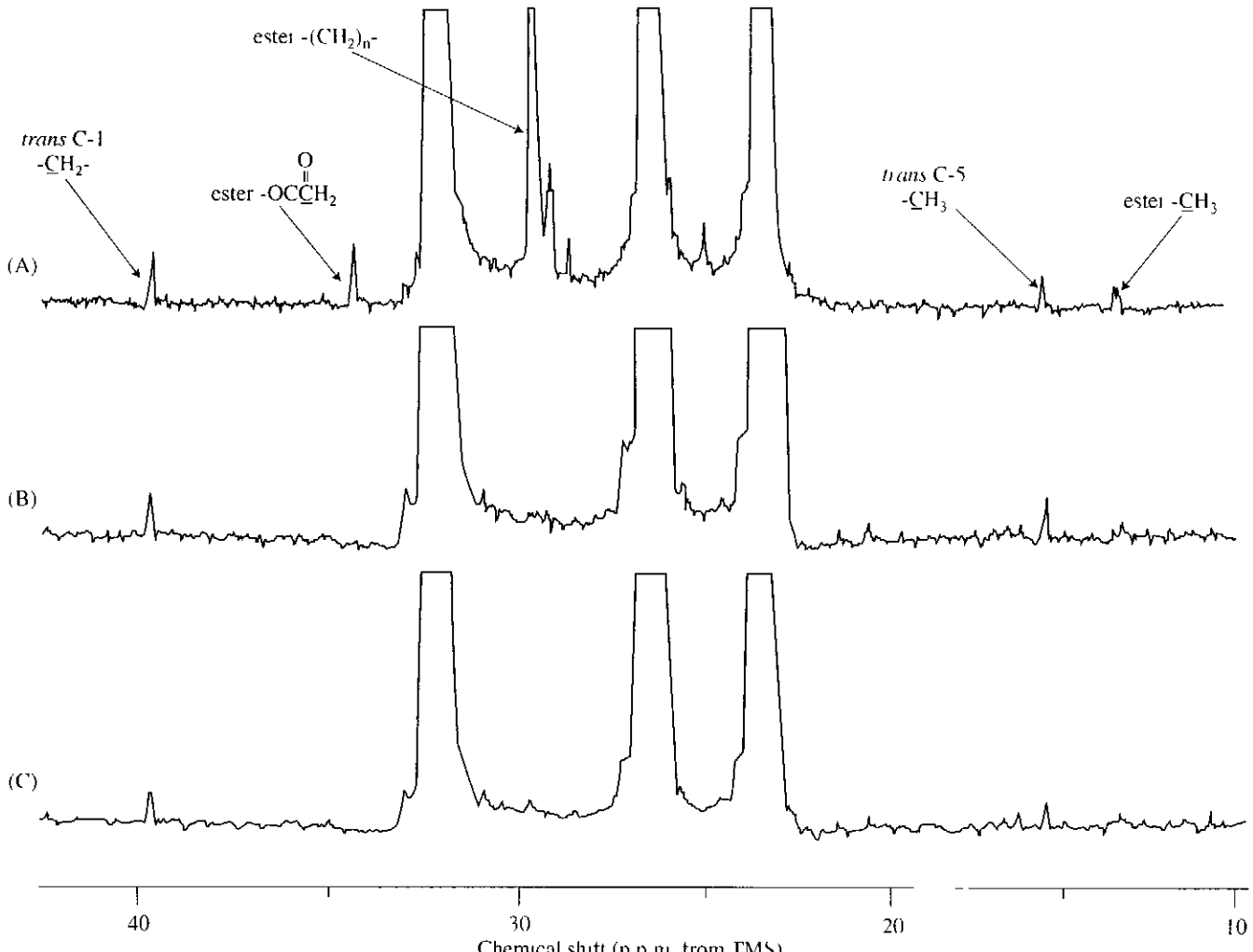

Figure 2 ^{13}C-NMR spectra of (A) deproteinised NR (DP NR), (B) transesterified NR (TE NR) and (C) saponified NR (SAP-NR)

CONCLUSION

Enzymatic deproteinisation breaks down the protein linkages selectively and remained as oligopeptide groups The phospholipid ester in NR was severed by transesterification, resulting in the linear rubbers as the products Saponification can remove both of the protein and phospholipid linkages to form the linear rubber molecules like those obtained from deproteinisation followed by transesterification Transesterification and saponification gave the reduction in gel content, k' and molecular weight, showing that the branch-points might be composed of phospholipid.

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