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POSSIBLE MECHANISMS CONTROLLING MOLECULAR WEIGHT OF RUBBERS IN *HEVEA BRASILIENSIS*

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Key Word Index—*Hevea brasiliensis*; Euphorbiaceae; seedlings; rubber; M_r distribution; transesterification.

Abstract—The M_r and M_r distribution were determined by gel-permeation chromatography and osmometry for four rubbers from one-month- to three-year-old *Hevea brasiliensis* seedlings. A bimodal distribution was usually observed with M_r peaks at 2.0×10^6 and 1.2×10^5, although the high M_r peak was very small. The number-average M_r ($\bar{M}_n$) was one-tenth to one-sixth of that from a mature tree. There was a clear tendency for $\bar{M}_n$ to increase with the age of the tree. Transesterification reduced $\bar{M}_n$ by *ca* 40%. Part of the rubber from the *Hevea* seedlings was presumed to consist of molecules having the same ester branch-points as in the case of rubber from mature trees.

INTRODUCTION

Rubber from *Hevea brasiliensis* has a bimodal M_r distribution based on solvent fractionation [1, 2] and gel-permeation chromatography (GPC) measurements [3]. It has been inferred that this distribution results from branching, with the high M_r fraction presumed to have tri- or tetra-functional branch-points [4, 5]. The extrapolation of the plot of the number of branch points against M_r for fractionated rubbers suggested that the low M_r fraction consist of linear polymers, while the high M_r one comprises branched molecules [4].

Long-chain fatty acid groups esterified to rubber have been identified and transesterification reduced the number-average M_r ($\bar{M}_n$) to one-third in the case of rubber from commercial high-ammonia latex [6]. It was presumed that the branch-points are decomposed to provide linear chains, although the detailed structure of these points has not been determined. However, the transesterified rubber also showed a bimodal distribution, with a marked increase in the intensity of the low M_r peak. This finding suggests that this distribution is inherent in the mechanism of rubber biosynthesis in *Hevea* latex; the fact that the ratio of the peaks in the distribution is characteristic of each *Hevea* clone supports this idea. However, at present, there is no direct evidence on the mechanism of formation of the two types of rubber molecule.

Previously we carried out a series of structural studies on *Hevea* rubber to elucidate its biosynthesis [7] and to investigate the origin of its characteristic properties [8]. The present work, which is a study of the M_r distribution of rubber from *Hevea* seedlings, is an attempt to uncover the mechanism(s) controlling this distribution.

RESULTS AND DISCUSSION

Figure 1 shows the M_r distribution curves for four rubbers from *Hevea* seedlings of different ages, together with that for rubber from a mature tree. The bimodal distribution, with peaks at *ca* 2.0×10^6 and 1.2×10^5, was clearly observed for all the rubbers, except in the one from the youngest seedlings. The peak height of the high M_r fraction in the seedling rubbers is far less than that of the low M_r fraction, contrary to the distribution for rubber obtained from the mature tree. Hager *et al.* have previously analysed the rubber from six-month-old seedlings; it showed a $\bar{M}_n$ of 6×10^4, with a very broad distribution [9]. Analysis of 19 clonal rubbers from fresh latex, demonstrated that the low M_r fraction varied from 18 to 40% [10]. It is remarkable that the ratio of the peak height of the high and low M_r fractions decreases with increasing age of the tree (Table 1).

The average M_rs of the rubbers examined are compared in Table 2. There is a clear tendency for weight-average M_r ($\bar{M}_w$), determined by GPC, to increase as the age of the tree increases. A similar tendency was

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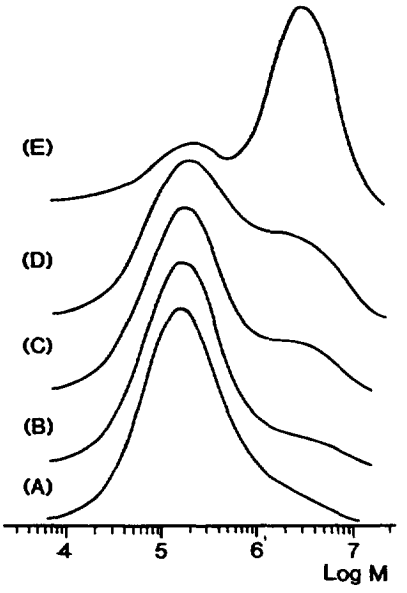

Fig. 1. M_r distribution of rubbers from (A) 1-month-old, (B) 3-month-old, (C) 7-month-old, (D) 3-year-old seedlings and (E) 17-year-old (mature) trees.

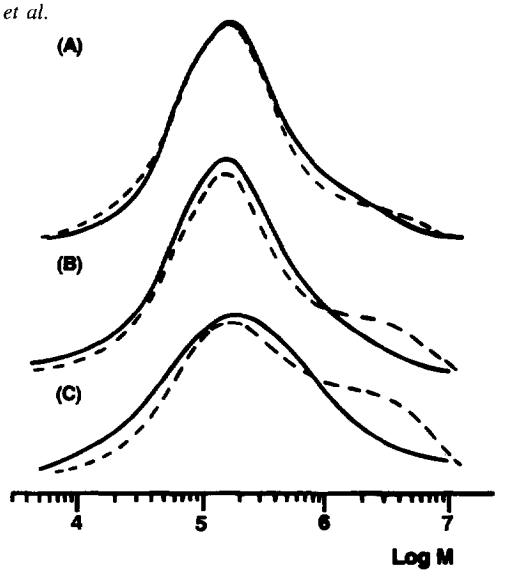

Fig. 2. Changes in M_r distribution after transesterification. Broken lines show the M_r distribution before transesterification of rubbers from (A) 3-month-old, (B) 7-month-old and (C) 3-year-old seedlings.

Table 1. Ratios of high and low M_r rubber obtained from *Hevea* of different ages*

Age of plant	Low M_r	High M_r
1 month	0.93	0.07
3 months	0.90	0.10
7 months	0.83	0.17
3 years	0.72	0.28
17 years (mature)	0.23	0.77

*Values calculated from ratio of peak height of high and low M_r peaks (cf. Fig. 1).

observed for $\bar{M}_n$ measured by GPC and osmometry. It is noteworthy that the polydispersity ($\bar{M}_w/\bar{M}_n$) of these seedling rubbers is narrow and increases with tree age.

The fact that all the samples showed M_r peaks at *ca* 1.2×10^5 and 2.0×10^6 suggests that there are certain controlling mechanisms for the biosynthesis of high and low M_r rubbers. The relationship between M_r distribution and tree age (Fig. 1 and Table 1) is not in accordance with that reported for rubbers from a five-year-old and a mature tree [2]. The difference may be ascribed, in part, to the location where the trees were grown; the former in England, the latter in Malaysia. Seasonal variation and regional differences in our samples can be ignored, because they were collected in the same season and in a limited locality.

The origin of the bimodal M_r distribution of rubber has been ascribed to the formation of branched chains. This implies that the low M_r fraction is composed of the fundamental biogenic macromolecular units, which link up to form the branched molecules of the high M_r fraction. Branch-points were found to be decomposed by transesterification when deproteinized rubber was treated with sodium methoxide [11]. However, the bimodal character of the distribution persists after such treatment [11, 12].

Figure 2 shows the M_r distributions of seedling rubbers before and after transesterification. The intensity of the high M_r peak decreases markedly, while the low M_r one remains unaltered in the transesterified three-month-, seven-month- and three-year-old seedling rubbers; this is confirmed by the data given in Table 1. These finding prove that a part of the high M_r fraction of the seedling rubbers is composed of branched molecules that are reduced in M_r by transesterification.

The gel content of the seedling rubbers obtained

Table 2. M_rs of rubber obtained from *Hevea* of different ages, determined by GPC and osmometry

	GPC			Osmometry
Age of plant	$\bar{M}_w \times 10^{-5}$	$\bar{M}_n \times 10^{-4}$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_n \times 10^{-5}$
1 month	2.7	6.6	4.1	1.1
3 months	2.9 (2.1)*	6.1 (6.0)	4.8 (3.5)	1.4 (0.87)
7 months	4.4 (3.0)	7.3 (7.1)	6.0 (4.2)	1.9 (0.94)
3 years	4.5 (3.5)	7.3 (7.2)	6.2 (4.9)	2.0 (1.2)
17 years (mature)	25 (19)	27 (18)	9.3 (10.6)	12 (10)
(Low M_r fraction)†	1.3	8.1	1.6	1.0

*Values in parentheses are M_rs after transesterification.
†Obtained from rubber of 17-year-old tree.

from fresh latex was *ca* 1%, similar to that of fresh rubber from mature trees (2–3%). However, it was observed that gelation occurred very easily in the case of seedling rubbers after purification and the gel fraction was solubilized by transesterification. This suggests that low M_r rubber from seedlings has reactive functional groups which can form a gel, as presumed for the microgel present in rubber from mature trees [13].

The structure of rubbers obtained from seedlings was found to be fundamentally the same as that from mature trees, by ^1H and ^{13}C NMR measurements. The presence of a *trans–trans–cis*-sequence at the initiation terminal was clearly observed and small signals corresponding to both terminal groups were also seen in both spectra (data not shown). Detailed analysis of the structure of the terminal groups will be presented in a subsequent paper, in connection with the initiation and termination mechanisms of rubber formation in *Hevea* seedlings.

At least four possible reasons can be suggested why young *Hevea* plants produce mainly low M_r rubber, while mature trees produce both M_r types. (1) The probability of the termination reaction decreases rapidly with increase in the particle size of the rubber. (2) The activity of latex isopentenyl diphosphate isomerase decreases with the age of the tree; this would reduce the rate of initiation, which might lead to an increase in M_r. (3) Two types of rubber transferase are present, which produce high and low M_r rubbers; the activity of these is governed by the age of the tree. (4) *Hevea* seedlings produce linear molecules almost exclusively, but mature tree produce branched molecules.

Hager *et al.* have assumed that the mechanisms controlling M_r and a possible relation between M_r and particle size for the unimodal distribution, based on the M_r distribution, i.e. $\bar{M}_z:\bar{M}_w:\bar{M}_n = 3:2:1$, in mature guayule [9]. They have supposed that there is an intermediate building block in the formation of rubber molecules and that the low M_r fraction, having a $\bar{M}_w$ less than 10^5, is that block which represents the first stable droplet and can further polymerize on another enzyme until its large size hinders the geometrical positioning required for further polymerization. However, these assumptions do not appear to relate to the origin of the bimodal distribution in *Hevea* rubber. Subramaniam has shown that the M_r of rubber is not directly related to the particle size in latex [3].

EXPERIMENTAL

Latex was collected from branches and stems of *H. brasiliensis* seedlings, and from a mature tree (17-year-old), growing in Hat-Yai, Thailand. Latex was collected directly into hexane and the resulting hexane soln was washed with hot H_2O several times, in order to eliminate H_2O-soluble components, followed by centrifugation at 10 000 *g* for 30 min to break the emulsion which occurred at the above step. The supernatant was evapd to dryness under red. pres. and the resulting rubber purified by 6 reprecipitations with EtOH from toluene. The low M_r frs of the rubber form the mature tree were obtained in the usual way [9]. Transesterification was carried out by treatment of a soln of 1% (w/v) of rubber in toluene with 1 M NaOMe under N_2 in the dark at room temp. for 2.5 hr, followed by neutralizing with 1 M HCl in MeOH. The gel content was determined separately for the rubbers obtained from centrifuged fresh latex and coagulated rubber with EtOH and dried *in vacuo*. The resulting rubber was dissolved in toluene to give a concn of 0.2% (w/v) and kept in the dark without shaking or stirring for 1 week at room temp., then centrifuged at 10 000 *g* for 30 min to separate gel. The resulting gel was dried *in vacuo* and weighted for gel content measurements.

M_r distributions were determined by GPC using 2 columns in series, packed with styrene–divinylene copolymer, and having exclusion limits of 2.0×10^7 and 5.0×10^4. Measurements were made using THF as eluent with a flow rate of 0.5 ml min^{-1} at 35°, monitoring with RI and low-angle laser-light-scattering detectors. Commercially obtained standard polystyrenes were used for calibration. Samples for GPC were prepd from purified rubbers as described above at a concn of 0.01–0.02% (g/dl) in the THF used for GPC, at room temp. in the dark and were filtered through a Millipore LS prefilter and a 0.2 μm membrane filter. Absolute $\bar{M}_n$s were obtained with a membrane osmometer. Measurements were made at 35° in filtered toluene solns.

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