Natural rubber (NR) is synthesized by the rubber transferase (RTase) on rubber particles (RPs) in latex. Due to the heterogeneity of the RPs in latex, it is difficult to precisely characterize the RTase activity. In this study, we separated the RPs of *Hevea brasiliensis* with different particle size distributions, via stepwise centrifugations. Analyses of protein compositions and size distributions of NR in the RPs suggest that RPs in *Hevea* latex can be categorized into two distinct subclasses, the larger RPs (termed 1kRP, 2kRP, and 8kRP) and the smaller RPs (termed 20kRP and 50kRP). Precise enzymatic assays using the RPs revealed that 50kRP showed the highest RTase activity, whereas the larger RPs, which had been regarded to have quite low activity, also exhibited a comparable activity to the smaller RPs. Immunological detections of *cis*-prenyltransferases in the RPs showed that the abundance of these enzymes correlates with the extent of RTase activity.

Purification of rubber particles from *Hevea brasiliensis* into 5 different fractions showed the existence of distinct border line to distinguish small and large rubber particles.