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Aggregation properties as potential markers for classifying rubber particle proteins

Natural rubber is an important biopolymer that mainly consists of *cis*-1,4-polyisoprene. The rubber tree *Hevea brasiliensis* is the only commercially viable source of natural rubber because of its good yield and the product's high quality. Rubber elongation factor (REF) and small rubber particle protein (SRPP) are abundant proteins associated with *H. brasiliensis* (Hb) rubber particles. Their amino acid sequences show a high homology, and both proteins encode a common REF domain. The amount of HbREF in latex is proportional to the rubber content. In 1989, a model was proposed in which *trans*-prenyltransferase interacted with the protein named REF to form rubber transferase, a *cis*-prenyltransferase. However, the available data does not support this model. Therefore, REF has been supposed to play other roles. HbREF may have a role in stabilizing rubber particles and may be involved in natural rubber biosynthesis. HbSRPP (204 aa) is characterized by a longer protein sequence than HbREF (138 aa), and the C-terminal-proximal region is SRPP specific. Many SRPP homologs have been found in both rubber- and non-rubber-producing plants. In several rubber-producing plants, RNAi-mediated gene silencing has been performed to analyze the roles of SRPP homologs in rubber biosynthesis. However, the inhibition of SRPP homologs' expression levels affects natural rubber biosynthesis in various plant- and homolog-dependent ways. Although rubber particle proteins from many sources have been named a REF and SRPP, no sound basis for discriminating between the two has been established. None of the HbREF counterparts in other species are similar to it in size nor do they lack the SRPP-specific region. Thus, the amino acid sequence of HbREF is thought to be unique. This indicates that SRPP homologs might act as REFs in natural rubber biosynthesis and vice versa.

HbREF and HbSRPP auto-assemble differently in bulk solutions. HbREF forms amyloid fibrils and micrometer-sized amorphous aggregates that precipitate, while HbSRPP forms numerous small polymorphic particles that do not precipitate. We investigated whether some of the rubber particle proteins, regarded as SRPP homologs, have an aggregation property similar to that of HbREF. We cloned two cDNAs encoding REF/SRPP-family proteins (FcREF/SRPP-1 and -2) from *Ficus carica* latex. Their amino acid sequences were similar in size, highly homologous with each other and contained the SRPP-specific region, which are characteristics that classified both proteins as SRPPs. Recombinant FcREF/SRPP-1 and -2 were expressed in *Escherichia coli* and their aggregation properties were examined using a Congo red binding assay, agarose gel

electrophoresis and transmission electron microscopy. FcREF/SRPP-1 resembled HbREF in precipitation and amorphous aggregate-growth behaviors (Fig. 1).

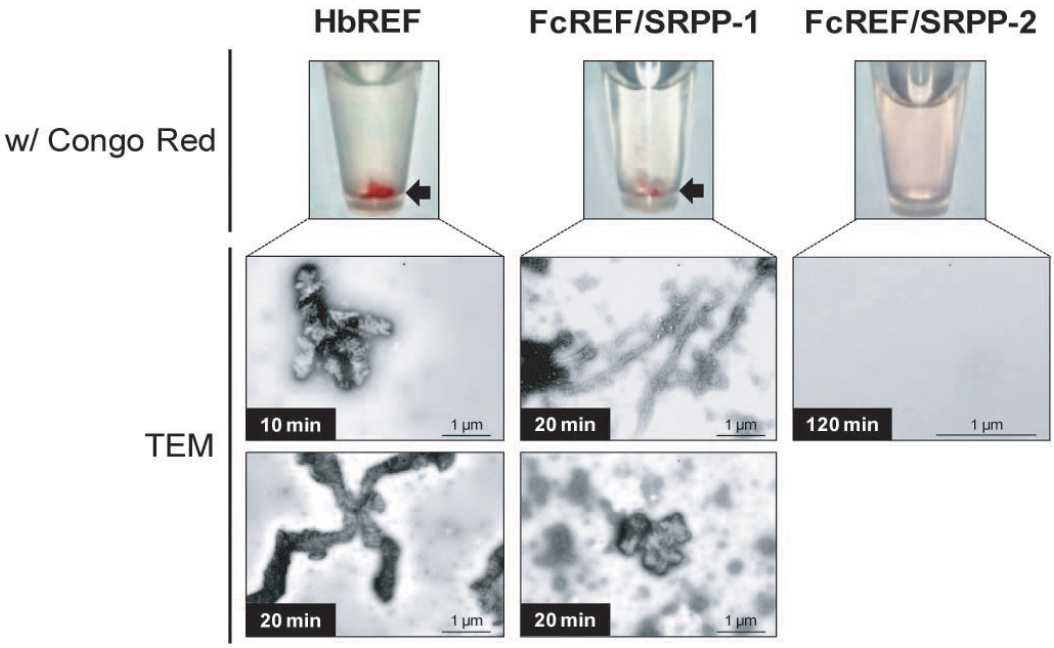

Fig. 1. Analysis of protein aggregation properties.

The aggregation properties of FcREF/SRPP-2 were distinctly different from those of FcREF/SRPP-1 but similar to those of HbSRPP. Therefore, FcREF/SRPP-1 and -2 should be classified and renamed as FcREF and FcSRPP, respectively. Determining the aggregation properties in this manner is a useful benchmark for classifying REF/SRPP-family proteins from plants other than *H. brasiliensis*. Correct classifications will help reveal the roles of REF and SRPP in whole latex for rubber production and latex coagulation.

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