Chapter 5

CONCLUSION

1. The partial *Hevea brasiliensis hmgs2* cDNA was isolated from the secondary screening of latex cDNA library by plaque hybridization using the *H. brasiliensis hmgs1* cDNA as a probe.
2. The full-length *H. brasiliensis hmgs2* cDNA was then completed by performing 5' Rapid Amplification of cDNA Ends with gene-specific primer for *H. brasiliensis hmgs2* cDNA.
3. The nucleotide sequence of *H. brasiliensis hmgs2* cDNA consists of 1,916 bp with the open reading frame of 1,392 bp. An open reading frame is flanked by 5' and 3' untranslated sequences of 316 bp and 213 bp, respectively. A putative polyadedenylation site (AAUAAA) was shown at position 1,861. (GenBank accession number AY534617).
4. The translated polypeptide of 464 amino acids with a predicted molecular mass of 51.27 kDa and an isoelectric point of 6.02 was deduced from the cDNA ORF between bases 317 and 1,708. Comparison of *hmgs1* with *hmgs2* showed 92% nucleotide sequence identity and 94% amino acid sequence identity. HMG-CoA synthase 2 differs in 28 amino acids from HMG-CoA synthase 1.
5. The expression of *H. brasisliensis hmgs2* mRNA is tissue specific, *hmgs2* is highly expressed in laticiferous cells.
6. Multiple sequence alignment of 30 HMG-CoA synthases in plants, mammals, amphibian, fish, insects, yeasts, worm, and bacterium showed they have 95%-23% amino acid identity. The multiple alignment of 22 HMG-CoA synthase sequences

and 8 ACP synthase III sequences show that these two enzymes are distantly related with about 18-55% identity between the two enzymes.

7. Proper multiple alignments showed that certain cysteine, histidine, and asparagine residues are completely conserved throughout all species of HMG-CoA synthase and ACP synthase III.
8. A phylogenetic tree constructed shows two major groups of HMG-CoA synthase and ACP synthase III. Gene duplications have a major role in the evolution of new biological functions and are selective by nature; this probably is how *hmgs2* occurs including HMG-CoA synthase and ACP synthase III diverged.
9. The recombinant HMG-CoA synthase was expressed in *E. coli* from a T7 promoter vector as a 6xHis-tagged fusion protein in soluble form. The recombinant protein in crude extracts was not stable for long term storage for the enzyme assay. The mutation at Cys^{117} and Asn^{326} conserved throughout all species affects the HMG-CoA synthase activity, indicating that Cys^{117} and Asn^{326} are important amino acids for the catalytic activity of HMG-CoA synthase. The functional role of Asn^{326} in HMG-CoA synthase activity is first reported in this study.
10. The possible secondary structure of HMG-CoA synthase was predicted based on the Protein Data Bank (PDB) information of *M. tuberculosis* ACP synthase III. The main topology displays a five-layered core structure, α-β-α-β-α, where each α comprises two α-helices and each β is made of a five-strand, mixed β-sheet.