APPENDIX

Sequenogram of nucleotide sequence by automate DNA sequencing

Nucleotide sequence was analyzed by an Applied Biosystems 377 sequencer (Perkin-Elmer, Norwalk, CT, USA).

Calculation of HMG-CoA synthase activity

The radioactivity of acid-stable [1-^{14}C] HMG-CoA was measured after heating at 90°C for 2 h, adding liquid scintillation fluid and counting decay events in a liquid scintillation counter. The radioactivity was shown in dpm

HMG-CoA synthase substrate stock (255 µl) containing, 250 µl of acetyl-CoA and 5 µl of [1-^{14}C] acetyl CoA (54.9 mCi/mmole). The 10 µl of stock HMG-CoA synthase substrate (50 nmole of acetyl-CoA plus ^{14}C-acetyl-CoA) was added into enzyme assay mixture (100 µl) as described in method 5.9, 40 µl of the assay mixture was determined the total radioactivity. Another 40 µl of remaining assay mixture was heated to remove the remaining ^{14}C-acetyl-CoA. The remaining radioactivity was from ^{14}C-HMG-CoA formed. The radioactivity of ^{14}C-acetyl-CoA was 3,256 dpm/40 µl. The assay mixture (100 µl) contained 50 nmole of acetyl-CoA plus ^{14}C-acetyl-CoA had a specific radioactivity value of 8,140 dpm (3,256 ×100/40). If the acetyl-CoA is completely converted to HMG-CoA, the ^{14}C-HMG-CoA radioactivity value should be 8,140 dpm. Therefore, 1 dpm of ^{14}C-HMG-CoA equaled 50 ×1/8,140 or 6.14 ×10^{-3} nmole. For example,

The radioactivity in the 40 µl reaction of sample is as follows

[1-^{14}C] HMG-CoA = 700 dpm and blank = 100 dpm

Total [1-^{14}C] HMG-CoA = 600 dpm

1 dpm = HMG-CoA 6.14x10^{-3} nmoles

Incubation time 2.5 min in the total of 100 µl reaction

The activity of HMG-CoA synthase = 3.7 nmoles/min.

If the reaction contains 120 ug of protein, the specific activity

= 30.83 nmoles/min/mg protein

IUB codes

A = adenine	S = G or C (Strong-3H bonds)
C = cytosine	W = A or T (Weak-2H bonds)
G = guanosine	Y = C or T (pYrimidine)
T = thymidine	B = C,G, or T
U = uracil	*D = A, G, or T*
K = G or T (Keto)	H = A, C, or T
M = A or C (aMino)	V = A, C, or G
R = A or G (puRine)	N = aNy base

Amino acids Classifications

Physicochemical properties	Amino acids
Hydrophobic aliphatic R groups	G A V L I M* C* P
Hydrophobic aromatic R groups	F Y W
Polar charged R groups	R** K** H D*** E***
Polar uncharged R groups	S T N Q

* = Sulphur R group, ** = Acidic R groups, and *** = Basidic R groups

Abbrevations and molecular weights for Amino acids

Amino acid	Three-letter abbrevation	One-letter symbol	Molecular weight (Da)
Alanine	Ala	A	89
Arginine	Arg	R	174
Asparagine	Asn	N	132
Aspatic acid	Asp	D	133
Asparagine or Aspatic acid	Asx	B	-
Cysteine	Cys	C	121
Glutamine	Gln	Q	146
Glutamic acid	Glu	E	147
Glutamine or Glutamic acid	Glx	Z	-
Glycine	Gly	G	75
Histidine	His	H	155
Isoleucine	Iso	I	131
Leucine	Leu	L	131
Lysine	Lys	K	146
Methionine	Met	M	149
Phenylalanine	Phe	F	165
Proline	Pro	P	115
Serine	Ser	S	105
Threonine	Thr	T	119
Tryptophan	Try	W	204
Tyrosine	Tyr	Y	181
Valine	Val	V	117

PUBLICATIONS

Sirinupong, N., Suwanmanee, P., Doolittle R.F. and Suvachittanont, W. (2004) Molecular cloning of a new cDNA and expression of 3-hydroxy-3-methylglutaryl-CoA synthase gene from *Hevea brasiliensis.* GenBank accession number AY534617.

Suwanmanee, P., **Sirinupong, N.** and Suvachittanont, W. (2004) Regulation of the expression of 3-hydroxy 3-methylglutaryl CoA synthase gene in *Hevea brasiliensis* (B.H.K) Mull. Arg. *Plant Science* 166: 531-537.

Sirinupong, N., Suwanmanee, P., Doolittle, R.F. and Suvachittanont, W. (2004) Molecular cloning of a new cDNA and expression of 3-hydroxy-3-methylglutaryl CoA synthase gene from *Hevea brasiliensis.* (Submitted).

PROCEEDINGS

Sirinupong, N., Suwanmanee, P. and Suvachittanont, W. (2000) Regulation of the expression of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) synthase gene in *Hevea brasiliensis* (Oral presentation). 18th International Congress of Biochemistry and Molecular Biology. Birmingham, UK. 16-20 July.

Sirinupong, N. and Suvachittanont, W. (2001) The latex cDNA library screening of a partial new *hmgs2* in *Hevea brasiliensis* (Poster presentation). Ph.D.Congress II: The Thailand Research Fund, Chonburi, Thailand.

Sirinupong, N., Doolittle R.F. and Suvachittanont, W. (2003) The putative catalytic residues in HMG-CoA synthase from *Hevea brasiliensis* (Poster presentation). Ph.D.Congress IV: The Thailand Research Fund, Chonburi, Thailand.