

METHYLBUTENOL SYNTHASE: BIOCHEMICAL CHARACTERIZATION, HOMOLOGY MODELING, AND IMPLICATIONS FOR UNDERSTANDING HEMITERPENE SYNTHASE EVOLUTION IN PLANTS

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Supplementary material

Table S1
PCR Primers used to clones and express MBO synthase

Primer ID	Sequence 5'-3'	Usage
Oligo(dt) ₁₇	TTTTTTTTTTTTTTTTTT	cDNA synthesis
Oligo(dt) ₁₇ .adapt1	GACTCGAGTCGACATCGATTTTTTTTTTTTTTTTTTT	cDNA synthesis
Adapt1	GACTCGAGTCGACATCG	3'RACE
PsabMBO 1F	TTCAGACTTGGATTTGCCAAAATCTTT	3'RACE
Pon3.1R	CTCTCCCTCTGTTTGAATGGCCGAACATGC	5'RACE
Pon4.1R	ATTGGAGGGGGTGATTAATTCTCCATCTTT	5'RACE
Pon 4.11F	GAATTCAGTTCATTATTTTGAGCTGC	Full length amplicon
Pon4.9R	ACCGTCATTGAGCCTGTGCCTTTATAA	Full length amplicon
Ppon4.14aF Nco1	c <u>catgg</u> <u>GT</u> AGACGCATAGCAAATCATCATTCCAAC	Expression constructs
Ppon4.10R Sac1	gagctcAAAGGCACAGGCTCAATGACGGTTGT	Expression constructs

All primers shown in a 5'-3' orientation. Lowercase letters indicate restriction sites, Underlined nucleotides were added to achieve the correct reading frame.

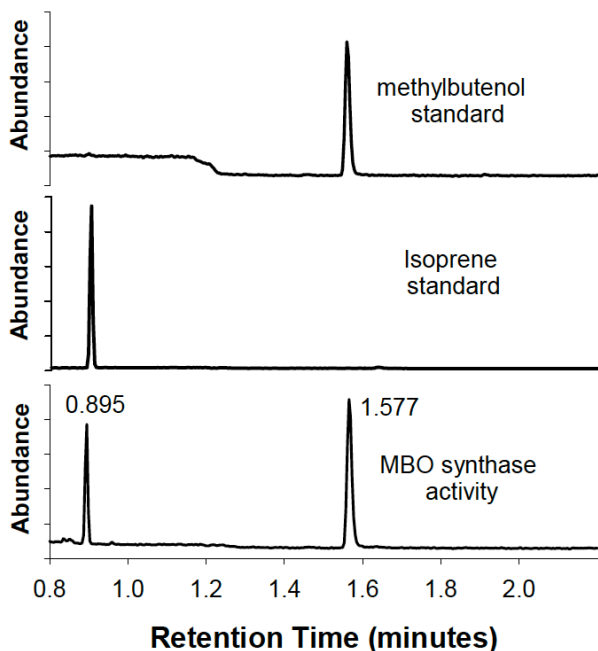


Fig. S1. GC/MS total ion trace showing retention times of authentic isoprene and MBO standards, and the corresponding isoprene and MBO peaks obtained from a typical MBO synthase enzyme assay. A 2-ml sample of gas phase standard or headspace from the MBO synthase assay was cryotrapped directly on the GC column (DB-5 column, Agilent; 10-m length, 0.1-mm I.D., and 0.34 μm film thickness) on a Agilent 6890N GC (Agilent) coupled to a 5975B mass selective detector) and desorbed at 40°C isothermally. MBO assays were incubated with 10 mM DMADP at 37°C for 60 min prior to injection.

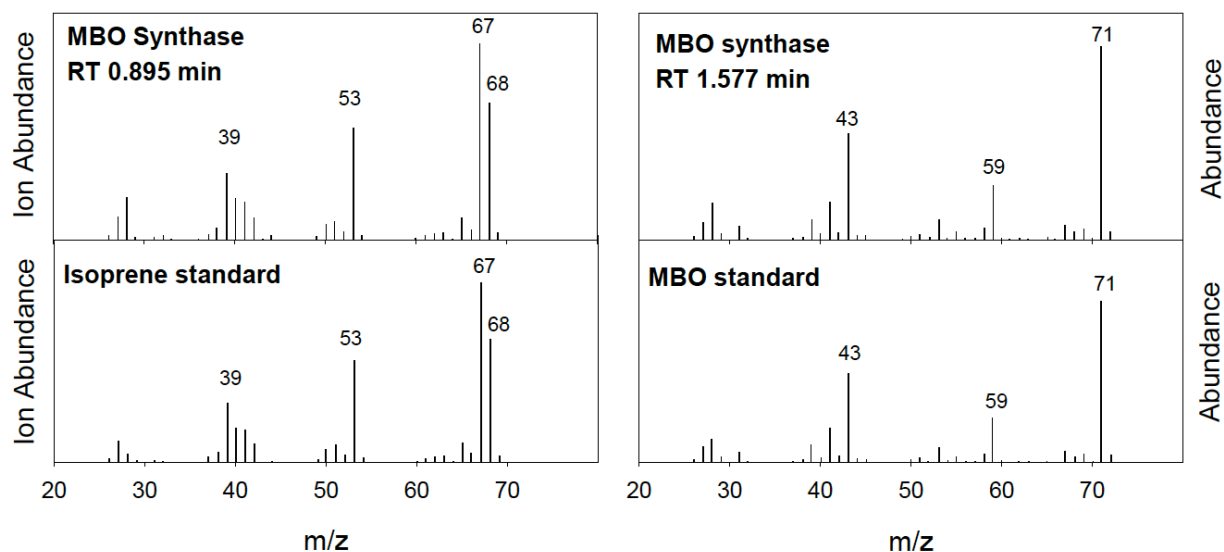


Fig. S2. Mass spectra of authentic isoprene and MBO standards and the corresponding isoprene and MBO peaks identified by GC retention time. A 2-ml sample of gas phase standard or headspace from the MBO synthase assay was cryotrapped directly on the GC column and desorbed at 40°C isothermally. No molecular ion ($m/z=86$) was detected in the MBO spectrum. Other details as for Fig. S1.

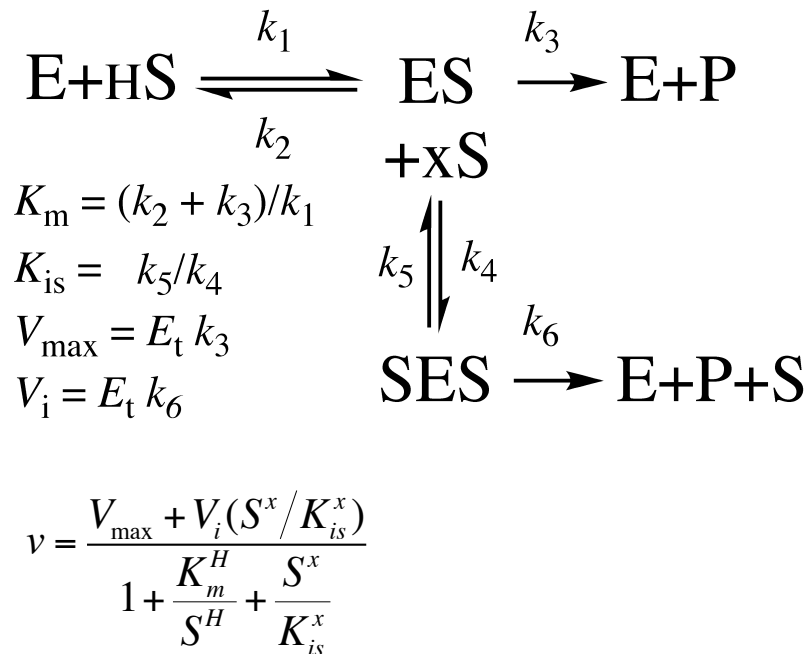


Fig. S3. Enzyme kinetic model allowing cooperativity and cooperative substrate inhibition adapted from (1,2). H = the Hill coefficient describing cooperativity, K_{is} is the binding affinity of the substrate to the enzyme-substrate complex, and x = the number of substrate molecules that could bind to and inactivate the enzyme substrate complex. This model allows the substrate-inhibited form of the enzyme to have some activity but fitting many data sets did not support this possibility for hemiterpene synthases and so was not used in this study. Unrealistic values of some parameters can give good fits so following the suggestion of LiCata and Allewell, K_{is} was constrained to be $\geq K_m$ and x was constrained to be ≤ 2 .

1. Pastra-Landis, S. C., Evans, D. R., and Lipscomb, W. N. (1978) *J. Biol. Chem.* **253**, 4624-4630
2. LiCata, V. J., and Allewell, N. M. (1997) *Biophys. Chem.* **64**, 225-234