# GOSSYPOL PATHWAY IN COTTON: S-ADENOSYL-L-METHIONINE: DESOXYHEMIGOSSYPOL 6-O-METHYLTRANSFERASE

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## Abstract

Cotton produces four phytoalexins in response to infection by *Verticillium dahliae:* desoxyhemigossypol (dHG), desoxyhemigossypol-6-methyl ether (dMHG), hemigossypol (HG), and hemigossypol-6-methyl ether (MHG). These compounds are precursors of a binaphthyl pigment, gossypol (G) found in cotton. Bioassays show that the methylated phytoalexins are approximately one-half as toxic to several soilborne pathogens compared to their unmethylated counterparts. Using antisense technology we may prevent the conversion of unmethylated cotton phytoalexins to methylated ones by blocking the synthesis of the corresponding enzymes, and thereby increase the resistance of the cotton plant to pathogens.

It was found that the crude enzyme extract contained an O-methylation activity for dHG but not for HG, suggesting that MHG was produced by oxidation of dMHG in a reaction analogous to the conversion of dHG to HG in the biosynthesis of G, and an O-methyltransferase that converts dHG to dMHG leads to all the methylated compounds later in the pathway. The crude enzyme extract contained S-adenosyl-L-methionine (SAM) dependent O-methyltransferase activities of 3.44, 15.93, 20.71, and 27.56 pkat per mg protein with diverse cosubstrates such as: dHG, 4-methylcatechol, 2,3-dihydroxynaphthalene and ethyl 3,4-dihydroxyhydrocinnamate. The rate of the enzymatic methylation of the cosubstrates was lowest for dHG. Since it has been reported that *V. dahliae* induces lignin biosynthesis in cotton, it was conceivable that methylation to dHG by the cotton extract was the result of lignin OMT's utilization of o-diphenol or o-dinaphthol cosubstrates. However, we have purified the enzyme, dHG 6-O-methyltransferase (dHG-6-OMT) to homogeneity from infected cotton stem and this purified enzyme showed no methylating activity with a diverse array of o-diphenol and o-dinaphthol cosubstrates such as: HG, 2,7-dihydroxycadalene, caffeic acid, 4-methylcatechol, 2,3-dihydroxynaphthalene, or ethyl 3,4-dihydroxyhydrocinnamate. The specific utilization of dHG as a cosubstrates by the purified dHG-6-OMT demonstrates that the dHG-6-OMT induce by *V. dahliae* in cotton stele tissue requires a dihydroxy substituted naphthofuran ring for the methylation reaction and the probable function of the enzyme is to methylate dHG.

The purified enzyme had a native molecular weight of 81.4 kD and consisted of two subunits with mol. wts of 42.1 kD. The purified enzyme does not require $Mg^{2+}$ for methylation reaction and the reaction was unaffected by the addition of EDTA. The activity of dHG-6-OMT was inhibited by iodoacetoamide and p-CMB similar to other OMTs from plants, indicating the necessity for an -SH group for enzymatic activity. The $k_m$ values of dHG-6-OMT for dHG and SAM were 4.6 µM and 81.4 µM. The $k_m/k_{cat}$ values for dHG and SAM were $5.08 \times 10^4$ $s^{-1}(mol/L)^{-1}$ and $1.83 \times 10^3$ $s^{-1}(mol/L)^{-1}$.

The purified dHG-6-OMT was subjected to partial amino acid sequence analysis and yielded several peptide fragment sequences. Based on these sequence information, a pair of degenerate primers have been designed and used to clone a 326 bp cDNA fragment by RT-PCR. Research is currently underway to clone the full cDNA clone of dHG-6-OMT.

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