DESOXYHEMIGOSSYPOL O-METHYLTRANSFERASE FROM COTTON STEMS

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Abstract

Terpenoid aldehydes and naphthofuran precursors are synthesized in diseased tissues as part of an active defense mechanism. The terpenoid aldehyde hemigossypol (HG) is derived by oxidation of the terpenoid naphthofuran desoxyhemigossypol (dHG). The enzyme dHG O-methytransferase (dHG OMT) catalyses the transfer of a methyl group from S-adenosyl-L-methionine to dHG to form desoxyhemigossypol-6-methyl ether (dMHG). dMHG can undergo the same oxidation as dHG to yield the methylated derivative of HG (i.e., hemigossypol-6-methyl ether). The methylated terpenoids are only about one-half as toxic as the unmethylated parents to microbial pathogens. Antisense constructs of dHG OMT gene may prevent the conversion of dHG to dMHG by blocking the synthesis of the enzyme, and thereby increase resistance of the cotton plant to pathogens. To facilitate cloning of the dHG OMT gene, the dHG OMT was purified for sequence information.

dHG OMT was purified to homogeneity from the cotton stele tissue inoculated with *Verticillium dahliae* by a Q-Sepharose anion exchange column, an Ultrogel AcA34 gel filtration column, a 3-hydroxy-4-methoxyphenethylamine-Sepharose affinity column, a 3,4-dimethoxyphenethylamine-Sepharose affinity column, and a 2',5'-ADP-Sepharose 4B affinity column. An overall 851 fold purification has been achieved. The purified enzyme showed a single band at 41.2 kDa. on SDS-PAGE. It had a native molecular weight of 81.4 kDa. and consisted of two identical subunits. The enzyme exhibited high substrate specificity. For instance, hemigossypol, 2,7-dihydroxycadalene, caffeic acid, 4-methylcatechol, and 2,3-dihydroxynaphthalene were not active as substrates for the enzyme. Mg^{2+} ion is not required for the enzyme activity. Thiol group blocking reagent, p-chloromercuribenzoate, inhibited the enzyme activity by 30% at 1 mM and 98% at 10 mM concentrations, indicating possible involvement of thiol groups in the enzyme's active center. Substrate-saturation kinetic data were obtained with the dHG OMT preparations purified to homogeneity, and were typical Michaelis-Menten type. A k_m of 4.6 mM and a k_m/k_{cat} of 5.08×10^4 $s^{-1}(mol/L)^{-1}$ were determined for dHG and a k_m of 81.4 mM and a k_m/k_{cat} of 1.83×10^3 s^{-1} $(mol/L)^{-1}$ were determined for SAM. The enzyme showed strong affinity toward dHG.

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