BIOSYNTHESIS OF GOSSYPOL: PURIFICATION AND PROPERTIES OF DESOXYHEMIGOSSYPOL 6-O-METHYLTRANSFERASE

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Abstract

Desoxyhemigossypol 6-O-methyltransferase (dHG 6-OMT) was partially purified from the cotton stele tissue inoculated with *Verticillium dahliae* by ultracentrifugation, anion exchange chromatography, and gel filtration chromatography on a FPLC column. The enzyme which was purified 267-fold catalyzed the transfer of the methyl group of S-adenosyl-L-methionine (SAM) to the 6-hydroxy group of desoxyhemigossypol (dHG), one of the several sesquiterpenoid phytoalexins induced in response to the fungal attack.

The enzymatic methylation of dHG was established with radioactive [methyl-^{14}C]-SAM. The biosynthetic products were separated by HPLC and desoxyhemigossypol 6-methyl ether (dMHG) was identified as the methylated product. This finding was also directly confirmed by the feeding experiment with [methyl-$^{2}H_3$]-SAM using a purified enzyme preparation. The enzymatically labeled deuterated phytoalexins were separated by HPLC. The fraction corresponding to dMHG had an identical UV spectrum with the authentic dMHG. This fraction was analyzed by GC-MS and the only significant major GC peak was identified as deuterated dMHG.

The purified dHG 6-OMT had a native molecular mass of 81 kD. The enzyme did not require a divalent cation for activity and several divalent cations such as Cu^{2+}, Co^{2+}, Zn^{2+}, and Mn^{2+} appreciably inhibited the enzyme activity. Thiol blocking reagents also strongly inhibited the enzyme activity, indicating possible involvement of thiol groups in the active center of the enzyme. The enzyme exhibited relatively high substrate specificity. For instance, hemigossypol, another sesquiterpenoid phytoalexin induced by *V. dahliae,* was not active as a substrate for the enzyme. Other phytoalexins, such as 2,7-dihydroxycadalene and 7-hydroxycadalene, as well as caffeic acid, which was involved in the lignin synthesis , were also found not active as substrates for the enzyme. Substrate-saturation kinetics of the purified enzyme for dHG and SAM were typical Michaelis-Menten type with K_m values of 4.5 and 20.8 μM, respectively.

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