A Ligand Function of Glutathione *S*-Transferase

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Glutathione *S*-transferases (GSTs) are ubiquitous enzymes and abundant in plants. They are intimately involved in plant metabolism and stress defense related to reactive oxygen species. Our project assigned particular reactions including novel ones to certain GST-isoforms. Transformed *E. coli* was used to express recombinant GST-isoforms from maize. An *N*-terminal His tag allowed their purification by affinity chromatography. Three GST-monomers had a molecular weight of 26, 27, 29 kDa, and aggregated to dimers when assayed for their enzymic properties. Four dimeric isoforms were used to study how they interact with tetrapyrroles (of the chlorophyll biosynthesis pathway). It was found that protoporphyrin IX (Proto IX), Mg-protoporphyrin and other tetrapyrroles are bound non-covalently ("liganded") to GSTs but not conjugated with reduced glutathione. This binding is non-covalent, and results in inhibition of conjugation activity, the degree depends on type of the porphyrin and GST-isoform. I_{50}-values between 1–10 μM were measured for Proto IX, the inhibition by mesoporphyrin and Mg-protoporphyrin was 2- to 5-fold less. The ligand binding is non-competitive for the substrate 1-chloro-2,4-dinitrobenzene and competitive for glutathione. The dimer GST 26/26 prevents the (non-enzymic) autoxidation of protoporphyrinogen to Proto IX, which produces phytotoxic reactive oxygen species in the light. GST 27/27 protects hemin against degradation. Protoporphyrinogen is formed in the plastid and then exported into the cytosol. Apparently binding by a suitable GST-isoform ensures that the highly autoxidizable protoporphyrinogen can safely reach the mitochondrium where it is processed to cytochrome.

Key words: Ligand Function, Glutathione *S*-Transferase, Protection of Protoporphyrinogen