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Biochemical Characterization and Crystal Structures of *aedes aegypti* Alanine Glyoxylate Aminotransferase

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Aedes aegypti alanine glyoxylate aminotransferase (Ae-AGT) is a homodimeric protein, which catalyzes the irreversible transamination (detoxification) of the intermediary two-carbon metabolite glyoxylate to glycine, and also transfers the amino group of alanine to pyruvate. Here, we report the recombinant protein production, expression profile, biochemical characterization and crystal structure of Ae-AGT in its PMP and PLP forms at 1.90 and 1.55 A, respectively. Using an insect-baculovirus expression system, Ae-AGT was expressed, purified and biochemically characterized for the substrate specificity. The enzyme shows high substrate specificity, is active towards only alanine, serine and glycine of 24 tested amino acids. Aedes mosquitoes have an isoenzyme of AGT sharing 48% amino acid sequence identity to a previous reported mosquito 3-hydoxykynurenine aminotransferase/AGT. This AGT is mainly detected in pupae and adults. The structure was solved by a combination of single-wavelength anomalous dispersion and molecular replacement approaches. The solved structure shows that the enzyme is a homodimer, and that the two subunits are stabilized by a number of hydrogen bonds, salts bridges, and hydrophobic interactions. Each subunit is divided into an N-terminal arm and small and large domains. Based on it's folding, the enzyme belongs to the prototypical fold type, aminotransferase subgroup I. The three-dimensional structure shows a strictly conserved 'PLP-phosphate binding cup' featuring PLP-dependent enzymes. The interaction between Cys284 (A) and Cys284 (B) is unique in Ae-AGT, which might explain the cysteine effect of Ae-AGT activity.