Characterization of a β-glucosidase Aggregating Factor (BGAF) Responsible for the 'Null' β-glucosidase Phenotype in Maize (*Zea mays L.*)

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APPROVED:

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ABSTRACT

β-Glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21) catalyzes the hydrolysis of aryl and alkyl β-D-glucosides as well as glucosides with a carbohydrate moiety such as cellobiose and other ß-linked oligosaccharides. In maize (*Zea mays L.*), β-glucosidase exists as 120 kD homodimers, but also forms high-molecular-weight (HMW) aggregates in certain maize inbreds (nulls). In this study we show that the null β-glucosidase phenotype is caused by the formation of HMW enzyme aggregates (>1.5 X 10^6 Daltons), mediated by a β-glucosidase aggregating factor (BGAF). BGAF is a 32 kD protein that binds specifically to β-glucosidase and renders it insoluble during extraction. The data unequivocally demonstrate that BGAF is solely responsible for β-glucosidase aggregation and insolubility, and thus, the apparent null phenotype. Additionally, I have isolated the cDNA encoding BGAF and have identified BGAF as a member of the small heat-shock protein (sHsp) family.

Interestingly, BGAF binds to both maize β-glucosidase isozymes (Glu1 and Glu2), but does not bind to their sorghum homolog Dhurrinase-1 (Dhr1; Sorghum β-glucosidase), that shares 70% sequence identity with Glu1 and Glu2. Therefore, these proteins provide an excellent system to study functional differences at nonconserved residues and elucidate the mechanism of enzyme aggregation and insolubility. By examining the behavior of β-glucosidase chimeras in binding assays, I demonstrate that BGAF binding is conformation dependent, highly specific, and reminiscent of antigen-antibody interactions. Additionally, I have identified two disparate polypeptide segments in the primary structure of the maize β-glucosidase isozyme Glu1 that form a BGAF binding site in the tertiary structure of the enzyme.

Dedication

I would like to dedicate this thesis to my wife Keta for her love and support.

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