195f Biochemical and Biotechnological Studies of Hyperthermophilic *Themotoga* Xylose Isomerases for High Fructose Corn Syrup Production at Elevated Temperatures

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Enzymes from hyperthermophiles represent attractive biocatalysts for applications requiring high-temperature operations. One such application is the conversion of glucose to fructose by xylose (glucose) isomerase (XI) [D-xylose ketol isomerase EC 5.3.1.5] in the production of high fructose corn syrup (HFCS). Current HFCS production utilizes immobilized XIs from mesophilic sources, mainly *Streptomyces*, which limits operating temperatures to 60ºC. Operating at this temperature minimizes heat inactivation but limits conversion to 42-45% fructose. However 55% fructose syrups are desirable for use in food and soft drinks, thus an expensive chromatographic enrichment step is required to produce the 55% syrup. Higher temperatures favor fructose production; a more thermostable, thermoactive XI would allow the process to operate at higher temperature and, hence increase conversion rates. Operating at temperatures around 95ºC could theoretically produce 55 % fructose syrup. Because of XI's leading position in the immobilized enzyme market, abundant sequence, structural, and mechanistic information is available, which makes XI's an attractive model system for investigating protein stability and biocatalysis at elevated temperatures. Based on sequence analysis, XI's have been categorized into two classes, I and II. The Class II enzymes contain a ~35 amino acid insert at the N-terminus that is lacking in the Class I enzymes, although the function of the insert is unknown. While the stability and activity of the Class I enzymes are very similar, the Class II enzymes vary, with the *Thermotoga neapolitana* enzyme (TNXI) being one of the most thermostable XI currently known. Our work focuses on the examining the TNXI enzyme and its use for high temperature HFCS production. The structural stability of TNXI was investigated with respect to the different activating metals (Mg^{2+}, Mn^{2+}, and Co^{2+}) and compared with other Class II XIs from mesophilic (*Escherichia coli, Bacillus licheniformis*), and thermophilic (*Thermoanaerobacterium thermosulfurigenes*) sources using differential scanning calorimetry (DSC). Factors contributing to XI's thermostability and the potential for using a thermostable XI in HFCS production will be discussed.