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Impact of Mutations on the Higher Order Structure and Activity of a Recombinant Uricase

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Abbreviations used: HOS, Higher Order Structure; NUV-CD, Near UV Circular Dichroism; FUV-CD, Far UV Circular Dichroism; DSC, Differential Scanning Calorimetry; DSF, Differential Scanning Fluorimetry; SEC-MALS, size exclusion chromatography with multi angle light scattering; DLS, Dynamic Light Scattering; PBS, phosphate-buffered saline; UA, uric acid; NRMSD, normalized root mean square deviation.

ABSTRACT:
This study explores the structural and functional changes associated with a low temperature thermal transition of two engineered bacterial uricase mutants. Uricase has a non-covalent homo-tetrameric structure, with four active sites located at the interface of subunits. Using differential scanning calorimetry, a low temperature transition was identified at 42ºC for mutant A and at 33°C for mutant B. This transition was stabilized by the uricase inhibitor, oxonic acid, suggesting a strong structural relationship to the active site. For mutant B, there was a reversible loss of enzymatic activity above the low temperature transition. Spectroscopic measurements demonstrated that there was also a reversible loss of secondary and tertiary structures and an increase in surface hydrophobicity. However, the hydrophobic core environment and the tetrameric structure were not altered over the low temperature transition suggesting that the changes occurred primarily at the surface of the enzyme. The protein became aggregation-prone at temperatures approaching the cluster of higher temperature melting transitions at 84°C, indicating these transitions represent a global unfolding of the protein. Our findings shed light on the structural changes that affect the uricase mechanism of action and provide new insights into how enzyme therapeutic development may be approached.