"Formation of protein aggregates and glycation products in *Escherichia coli and Klebsiella pneumoniae* exposed to desiccation-rehydration stress"

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In natural environments, bacteria often enter an anhydrobiotic state due to water loss. Desiccation results in molecular condensation and membrane disruption. Reduction of the hydration shell around proteins causes their inactivation, unfolding, and aggregation. The loss of protein stability and increased molecular crowding favor harmful reactions, including non-enzymatic glycosylation (glycation or the Maillard reaction), which leads to irreversible cross-linking of macromolecules and the accumulation of advanced glycation end-products (AGEs). Preliminary studies demonstrated that although glycation may induce protein aggregation *in vitro*, aggregates formed in *Escherichia coli* exposed to desiccation contained relatively low levels of AGEs compared to the soluble protein fraction.

Protein aggregation in bacteria caused by various intrinsic or environmental stresses disrupts proteostasis and exerts detrimental effects, including loss of protein function, nonspecific interactions with macromolecules, sequestration of functional proteins, and membrane damage. Recent studies have demonstrated that under specific conditions protein aggregates in bacteria play protective roles as functional compartments. Preliminary experiments suggested that glycation, rather than protein aggregation, is the main cause of *E. coli* death under desiccation stress.

The aim of this work was to verify this hypothesis and extend the study of stress-induced protein aggregation and glycation in *E. coli* and *Klebsiella pneumoniae*. The influence of osmolytes (carnosine, glycine-betaine, and trehalose) on bacterial viability, protein aggregation and glycation was investigated. It was found that protein aggregation was inhibited or enhanced depending on the osmolyte and its concentration used. Lower concentrations of carnosine, betaine, and trehalose inhibited the formation of protein aggregates and glycation products, thereby increasing *E. coli* viability under desiccation-rehydration stress. Although high concentrations of betaine and trehalose significantly enhanced protein aggregation, glycation was still inhibited and *E. coli* cells survived desiccation stress better than bacteria grown without osmolytes. Independent of concentration, all osmolytes caused enhanced protein aggregation and increased viability of *K. pneumonia* MKP103. Therefore, these results confirmed that glycation rather than protein aggregation is the main cause of cell death upon desiccation stress.

Further experiments showed that in *E. coli*, Nε-lysine acetylation may prevent the formation of carboxymethyl-lysine (CML), a non-fluorescent AGE product, thereby improving bacterial survival under desiccation-rehydration stress. The OmpC porin was identified as one of the most glycated proteins in *E. coli.*

The relationship between cell viability, protein aggregation, and glycation was also investigated using macrocolonies of the *K. pneumoniae* clinical isolate 577-BA. The macrocolonies formed two subpopulations: a mucoid/capsular center and a non-mucoid/non-capsular ring. The comparison of the center and ring genomes revealed that the appearance of the non-mucoid/non-capsular ring subpopulation resulted from the disruption of the *wbaP* gene by Insertion Sequence (IS)5 insertion. The *wbaP* gene encodes undecaprenyl-phosphate galactose phosphotransferase, an enzyme catalyzing the first step of capsule production. The loss of capsule was advantageous for the ring subpopulation, as it facilitated biofilm formation. The ring subpopulation was characterized by higher viability, lower levels of AGEs, and greater protein aggregation than the center subpopulation. Taken together, these results confirmed that the primary cause of bacterial viability loss during stress is glycation, not protein aggregation, suggesting that aggregates may have protective functions.