CHARACTERIZATION OF DLP-1 AND DLP-2 FROM *B anthracis*: IRON IN AND IRON OUT, DNA BINDING AND PROTECTION

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Bacterial Dps (DNA Binding Proteins during Starvation) proteins are mini-ferritins that accumulate during bacterial stationary phase. Dps genes respond to stress signals. The proteins provide multiple layers of physical and chemical protections to host cells[1].

Molecular mechanisms have been partly studied in mini-ferritins. Sequence identity among Dps isoforms, and between mini-ferritins and maxi-ferritins is very low. However, they share similar structure and functions, suggesting that functional similarities are preserved in the secondary, tertiary and quaternary structures. Here, we report biochemical properties of two Dps isoforms from *B. anthracis*, Dlp-1 and Dlp-2, as studied *in vitro*.

Dlp-1 and Dlp-2 oxidize Fe in a di-iron coupled mechanism that mimics maxi-ferritin[2], with slower rates but apparently similar mechanism. Dlp-2 utilizes H_2O_2 to oxidize Fe^{2+} with faster rates than O_2, while in Dlp-1, addition of H_2O_2 triggered protein-independent oxidation of Fe^2, indicating that the two proteins may have different functions *in vivo*. Rates of reductant/chelator removal of mineral iron inside mini-ferritins is controlled by the protein cage as in maxi-ferritins, and the temperature dependence rate change and subdomain melting (CD spectroscopy) are similar to maxi-feriritn[3] for Dlp-2.

Many mini-ferritins bind DNA and protect from Fe^{2+}/H_2O_2 degradation. The behavior of Dlp-1 and Dlp-2 differ and coincide with Fe^{2+} oxidation properties: 1) Dlp-1 binds DNA but does not protect; 2) Dlp-2 does not bind but protects DNA; 3) Together Dlp-1 and DLp-2 bind and protect DNA more completely than does either protein alone. The protection of DNA from oxidative cleavage by Dlp-1 and Dlp-2 mini-ferritins may contribute to the ability of *B anthracis* to resist oxidative burst and H_2O_2 host killing macrophages and neutrophils.

In summary, Dlp-1 and Dlp-2 mini-ferritins have distinct properties in solution, including DNA binding, DNA protection, and Fe^{2+} oxidation activity, that likely relate to different cellular functions. The two genes may be distinctively regulated by different stress challenge. The relative ratios between Dlp-1 and Dlp-2 homopolymers, or heterogonous co-assembly of Dlp-1 and Dlp-2 subunits could be differentially regulated by changes in iron/oxygen level and nutrient status as are the catalytically active (H) and inactive (L) maxi-ferritins. Supported by Part Support: Cooley's anemia Foundation and NIH-DK20251.

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2. Liu, X., Theil, E. C. (2004) *Proc Natl Acad Sci USA*. **101** 8557-8562.
3. Liu, X., Jin, W., Theil, E. C. (2003) *Proc Natl Acad Sci USA*. **100** 3653-3658.