Title:	Role of the bacterial nucleoid associated protein “HU” in cell-cell and cell-DNA interactions through the binding of HU with eDNA and Lipopolysaccharide
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Abstract:	Bacteria in biofilms are embedded within a matrix of extracellular DNA (e-DNA) derived from the lysis of bacterial cells, or cellular secretions. In biofilms, negative charges decorate surfaces of both bacteria and DNA, creating scope for repulsive interactions. An abundant and non- sequence-specific DNA-binding protein such as HU, which is decorated with positive charges could potentially bind to both DNA and to bacteria, to function as a charge-neutralizing glue. HU is already known to be present in bacterial biofilms (in association with e-DNA) and limiting for biofilm formation (with anti-HU antibodies disrupting biofilms). The work in this thesis demonstrates : (1) that HU binds to free lipopolysaccharide (fLPS) as well as to the surfaces of bacterial cells [i.e., to cellular LPS (cLPS) present in bacterial outer membranes]; (2) that binding of HU to fLPS or cLPS can involve either (a) HU’s canonical DNA-binding site, or (b) HU’s non-canonical DNA-binding site; (3) that addition of micellar fLPS to free HU (fHU) generates large molecular assemblies; (4) that addition of fHU to cells bearing cLPS generates large cellular assemblies (bacterial clumps); (5) that the charged head-group of the lipid A component of LPS contains two hexose-linked sugar-phosphate moieties that bind to lysine/arginine residues on fHU’s DNA-binding sites in specific geometries. Further, the thesis (6) examines the stability of HU’s dimeric interface, and (7) constructs a protein-engineered (HU simulacrum) construct containing both types of DNA-binding sites, with other regions removed.
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