Title:	Mechanistic Insights into the Stabilisation of Gram Negative Bacterial Biofilms by HU And Cloning, Expression and Purification of IHF
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Abstract:	Like histone and many other DNA binding proteins in eukaryotes, bacteria have NAPs (nucleoid associated proteins) which confine their chromosome in a small space called a nucleoid. NAPs (nucleoid associated proteins) help in organising the bacterial chromosome into domains for effective compaction and gene expression. Outside bacterial cells, NAPs have been implicated in biofilm formation, maintaining the structural integrity of the biofilm (being one of the major components of EPS, or extracellular polymeric substance). Despite intensive studies on their role in biofilms their architectural role has not been fully understood. Our study exposes the role of HU- the most abundant DNA binding protein in biofilms, as a “glue” between DNA and LPS, helping bacteria to be embedded in the EPS matrix, as a component of a larger ongoing study in this area. Interaction of HU with LPS was validated through flow cytometry, microscale thermophoresis, and glutaraldehyde crosslinking experiments. We further explored whether the results were applicable to other DNABII proteins as well, and this got us interested in IHF (Integration –Host Factor), a DNA binding protein in biofilms highly similar to HU sequentially and structurally. Purification of this protein in substantial quantities had been difficult. Individual proteins were unable to fold hence we co-transformed plasmids encoding both IHF proteins (A and B) into the same cell. In the study we showed a time-dependent expression of the protein(s) with maximum overexpression occurring at fifth and sixth hours. Despite being soluble and available in the supernatant, the proteins’ hexahistidine tags appeared to be unavailable to bind with Ni-NTA. Subsequently, we purified the protein through cation exchange chromatography. From the studies we hypothesised the existence of a balance between the IHF proteins for their substantial expression.
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