P14 & FP – **Targeting bacterial adhesion: synthesis and on-cell NMR-binding studies of new FimH multivalent ligands**

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Background: Bacterial adhesion is the first step in pathogen infection and bacterial adhesins are prime candidates as targets for antibacterial therapeutics such as specific-ligand-like inhibitors and vaccines [1].
Type 1 fimbriae of *Escherichia coli* are filamentous appendages that confer bacterial binding to glycoproteins with terminally exposed mannose [2]. Mannose-specific binding is mediated by a 30-kDa adhesive protein, FimH.
FimH is a virulence factor and an attractive therapeutic target for urinary tract infection (UTI) and Crohn's Disease (CD) [3].
Objective: Aim of this work was the development of new multivalent FimH ligands able to target this adhesin thus preventing the D-mannose sensitive adhesion of pathogenic bacteria to mammalian cells.
Moreover, we wanted to set up an NMR-based assay allowing a very rapid screening of FimH ligands and the structural characterization of their binding mode to bacterial cells expressing the protein on their surface.
Methodology: Monodispersed glycodendrimer based on pentaerythritol core and bearing a different number a FimH natural ligand, i.e. D-mannose, were synthesised through a convergent modular strategy exploiting Cu(I)-catalyzed azide-alkyne cycloaddition conjugation reaction (CuAAC, “click chemistry”).
Compound ability to inhibit D-mannose sensitive adhesion was tested by yeast agglutination inhibition assay [4].
The STD-NMR experiment acquired on samples containing bacterial living cells [5] was exploited to develop a rapid and sensitive experiment allowing verifying compound ability to bind FimH on *E. coli* cell surface.
Results: We synthesised a small library of potential FimH ligands constituted by glycodendrimers functionalized with a different number of D-mannose units (Man4, Man6, Man18).
STD-NMR experiments on living cells (*E. coli* CFT073, an uropathogenic strain expressing a high level of FimH) were set up to test compound ability to bind FimH on cell surface. A *E. coli* CFT073 ΔFimH strain was employed as negative control.
As expected, we found a strong correlation between the number of D-mannose units and compound affinity /inhibition potency. In particular, Man18 showed a MIC of 56 μM.
Conclusions / Implications for practice: The STD-NMR-based methodology and FimH ligands and inhibitors here described represent new tools towards effective therapeutic strategies against FimH-mediated bacterial adhesion, in particular for the treatment of UTI and CD.
Moreover, our study demonstrates the versatility of the STD-NMR experiment, also exploitable for the characterization of recognition events involving other proteins expressed on bacterial cell surface.

References

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