Switching first contact: photocontrol of \textit{E. coli} adhesion to human cells$^\dagger$

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We have shown previously that carbohydrate-specific bacterial adhesion to a non-physiological surface can be photocontrolled by reversible \textit{E/Z} isomerisation using azobenzene-functionalised sugars. Here, this approach is applied to the surface of human cells. We show not only that bacterial adhesion to the azobenzene glycoside-modified cell surface is higher in the \textit{E} than in the \textit{Z} state, but add data about the specific modulation of the effect.

Cell adhesion is a fundamental principle of biology. Many of its aspects have been elucidated,$^1,2$ but the molecular details of cell interactions are not fully understood. As the first contact of cells is mediated through their glycosylated surfaces, our research on cell adhesion is focused on carbohydrate recognition.

All organisms can recognise and distinguish between carbohydrates with the help of specialised proteins, the lectins.$^3$ Lectin function has been described in the context of, \textit{i.e.}, signalling, trafficking, and quality control, thus involving indispensable processes of life.$^4,5$ Also bacterial cells utilise their own lectins to mediate adhesion to glycosylated surfaces such as the membrane of the target cells.$^6,7$ Furthermore, bacterial adhesion enables bacterial colonization, biofilm formation, biofouling, or it precedes infection of cells.$^8,9$ Hence, investigation and control of bacterial adhesion is important, especially in a medical context, given the high incidence rates of infectious diseases worldwide.$^{10}$

In recent years we have studied the \textit{\textalpha}-D-mannoside-specific adhesion of \textit{Escherichia coli} bacteria,$^{11,12}$ which is mediated by the bacterial lectin FimH. FimH is located at the tips of adhesive organelles, called type 1 fimbriae,$^{13}$ which are projecting from the bacterial cell surface. Previously we have shown that type 1 fimbriae-mediated bacterial adhesion to a non-physiological surface can be photochemically controlled. In this specific case azobenzene-functionalised \textit{\textalpha}-D-mannoside derivatives were assembled on a gold surface in the form of a glyco-SAM (self-assembled monolayer).$^{14,15}$ Photochemical isomerisation of the azobenzene moiety between its \textit{E} and \textit{Z} form then allowed to reversibly switch the orientation of the attached carbohydrate ligands. In parallel with this \textit{E/Z} isomerisation, the adhesiveness of the surface was altered leading to reduction of bacterial adhesion in the \textit{Z} state of the SAM by $\sim 80\%$ in comparison to the \textit{E} state.

While the azobenzene photoswitch is long known$^{16}$ and has been used before for switching of the properties of designer surfaces,$^{17-20}$ our work on the control of bacterial adhesion through re-orientation of carbohydrate presentation is unprecedented. Here it has been our goal to challenge our system in the context of cell–cell adhesion, thus changing the artificial glyco-SAM surface against the plasma membrane of human cells. For this, we had to incorporate azobenzene mannoses in the cell surface of live cells, effect their \textit{E/Z} isomerisation to switch the orientation of the attached mannose moieties, and to finally test the influence of this isomerisation on \textit{E. coli} adhesion.

Our experimental approach is explained in Fig. 1: metabolic oligosaccharide engineering (MOE)$^{21,22}$ was employed to install bioorthogonal azido groups on the cell surface. This allows their subsequent modification with alkyne-functionalised azobenzene glycosides by well-known click chemistry.$^{23-26}$ In a final step, photochemical \textit{E/Z} isomerisation of the installed azobenzene moieties was effected by irradiation with light of the appropriate wavelength, and subsequently bacterial adhesion was measured via high-resolution live-cell fluorescence microscopy. For these experiments, human microvascular endothelial cells, variant 1 (HMEC-1) and GFP-fluorescent type 1 fimbriated \textit{E. coli} PKL1162$^{27}$ were used.

To install the required bioorthogonal azido functional groups on the cell surface, HMEC-1 were incubated with 50 $\mu$M tetra-acetylated \textit{N}-azidoacetyl-$\textit{\textalpha}$-mannosamine (Ac$_4$ManNAz, Fig. 1). Ac$_4$ManNAz is taken up by the cells and proceeded to result in azido labelling of terminal sialic acid units of membrane glycoproteins. When Ac$_4$GalNAz is employed on the other hand, the
azido label is incorporated into mucin-type glycoproteins. For the chemical functionalisation of the azido-modified cells, the azobenzene derivatives 5–7, carrying an alkyne functional group were required (Scheme 1). Synthesis started with the known \( p \)-aminophenyl glycosides 1 and 2, which were subjected to a classical azo coupling with phenol to furnish the respective hydroxyazobenzene derivatives 3 and 4. Then, Williamson etherification with propargyl bromide led to the desired alkyne-functionalised azobenzene glycosides 5 and 6. The azobenzene alkyne 7 was needed as control and was directly obtained from hydroxyazobenzene.

At first, the azobenzene \( \alpha \)-mannoside 5 was conjugated to azido-functionalised HMEC-1 after Ac\(_4\)ManNAz labelling. For this reaction, 200 \( \mu \)M azobenzene \( \alpha \)-mannoside, 50 \( \mu \)M CuSO\(_4\), 250 \( \mu \)M tris(3-hydroxypropyltriazolylmethyl)amine, 1 mM aminoguanidine, and 2.5 mM sodium ascorbate in buffered saline solution were added. 32 At first, the azobenzene (a) was exposed to UV light (365 nm) for 5 minutes. The azido groups were then reduced with a 200 \( \mu \)M solution of sodium ascorbate in buffered saline solution (b). Bioorthogonal click chemistry (c) then allows conjugation with azobenzene (AB) glycosides (such as AB-\( \alpha \)-mannosides, AB-Man) at the cell surface. Reversible E/Z isomerisation (d) employing UV or visible light, respectively, allows to change the orientation of the azobenzene-conjugated sugar and to manipulate sugar-specific bacterial adhesion in parallel.

Fig. 1 Our approach of switching the adhesivity of cells. Azido groups can be incorporated into cell surface glycoconjugates according to metabolic oligosaccharide engineering (MOE): (a) synthetic carbohydrates (Ac\(_4\)ManNAz or Ac\(_4\)GalNAz) are taken up by the cells (HMEC-1) and processed by their biosynthetic machinery (b). Bioorthogonal click chemistry (c) then allows conjugation with azobenzene (AB) glycosides (such as AB-\( \alpha \)-mannosides, AB-Man) at the cell surface. Reversible E/Z isomerisation (d) employing UV or visible light, respectively, allows to change the orientation of the azobenzene-conjugated sugar and to manipulate sugar-specific bacterial adhesion in parallel.

Scheme 1 Synthesis of alkyne-functionalised azobenzene glycosides and labelling of engineered human cells (HMEC-1). The \( \alpha \)-mannopyranoside 5 was used as specific ligand for bacterial adhesion and the \( \beta \)-o-glucopyranoside 6 and 7 were needed as control compounds.

Fig. 2 Switching of ManAB configuration allows to control adhesion of E. coli. After incubation of two sets of Ac\(_4\)ManNAz-engineered HMEC-1, conjugated with 5 in Z-configuration, bacterial adhesion is similar for both sets. Switching of ManAB configuration to E (365 nm) increases bacterial adhesion (second incubation). Error bars are standard error of the mean (SEM) of experiments with four independent sets of cells/condition. ***(p < 0.001 (cf. ESI,† Fig. S14)).
E and Z (Fig. 3). The development of the recorded bacterial GFP-fluorescence is then a direct measure for the adhesion of *E. coli*. The obtained curves clearly show that alternating irradiation using light of 365 nm and 488 nm has a strong effect on bacterial adhesion (Fig. 3a). After irradiation of the flow chamber with 488 nm light, the slopes of the bacterial GFP signal are significantly higher than after irradiation with 365 nm. This is also clearly seen after fitting of the curves, assuming a linear development of fluorescence (Fig. 3b).

Only after several switching events (starting at 1500 s), the measured slopes are no longer correlated with azobenzene configuration. This can be attributed to increasing coverage of HMEC-1 with bacterial cells (see also microscopic images, ESI† Fig. S19). When more and more bacteria adhere to bacterial cells and not to HMEC-1, the adhesion is naturally not sensitive to E/Z isomerisation.

As under static conditions, the results obtained in the flow experiments indicate that the orientation of mannoside 5 conjugated to the surface of HMEC-1 has a strong influence on the adhesion of bacteria, suggesting that the bent Z form is less accessible for bacteria than the E form. Thus, adhesion is reduced after $E \rightarrow Z$ isomerisation. Next, two important control experiments were performed in order to understand the observed effects in greater detail. First, the question of carbohydrate specificity of the observed effects was addressed by employing the azobenzene derivative 7 (AB in Fig. 4) devoid of a carbohydrate, and the β-D-glucoside 6 (GlcAB in Fig. 4) for HMEC-1 labelling. The glucoside 6 exhibits a similar structure and polarity as 5 but is no ligand for the bacterial lectin FimH. Labelling of HMEC-1 was done as before and bacterial adhesion measured after irradiation. All experiments were compared to azido-labelled HMEC-1, which had not been reacted with either of the azobenzene derivatives (Control in Fig. 4).

These experiments show that adhesion of *E. coli* to cells reacted with either 6 or 7, is significantly decreased compared to untreated cells. This observation suggests a shielding effect for both derivatives, prohibiting bacterial adhesion to the surface of HMEC-1 to some extent. On the other hand, this effect is not significantly sensitive to E/Z isomerisation. This finding indicates that the adhesion of bacteria to cells treated with 5 is indeed carbohydrate-specific. Control cells showed a slight response to irradiation with 365 nm light, but the effects caused by 6 or 7 are more significant.

How can the orientation of a rather small molecule (such as an azobenzene glycoside) within the complex environment of a cell’s surface exert such a pronounced effect on cell adhesion? To answer this question, the azobenzene mannoside ligand was positioned differently on the surface of HMEC-1. This is possible by labelling HMEC-1 with Ac$_4$GalNAz instead of Ac$_4$ManNAz. Whereas Ac$_4$ManNAz leads to labelling of sialic acids, which are almost exclusively found at the terminal positions of cell surface glycans, Ac$_4$GalNAz labels mucin-type glycoproteins. These are characterised by LacNAc moieties, which are localised deeper within the glycosylated cell surface.28

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**Fig. 3** (a) The azobenzene mannoside 5 was switched several times between Z and E while flowing a continuous stream of bacterial solution over the cells. The adhesion of bacteria was lower if 5 was in Z-configuration. Only after several switching cycles this tendency was no longer observed, caused by increasing coverage of the cells with bacteria. During irradiation with 365 nm (light grey bars) or 488 nm (dark grey bars), the flow was not stopped. (b) The slopes of linear curves that were fitted to the data of the middle panel clearly resembles the effect of reversible switching. Error bars are given as SEM of the fitting. For easier comparison, the slopes are set to start at a common origin by subtraction of the fluorescence intensity of the first frame from the subsequent frames.

**Fig. 4** Control experiments. Left & middle: Ac$_4$ManNAz labelling and conjugation with AB (no sugar residue) or GlcAB (β-Glc instead of α-Man) leads to reduced adhesion in comparison to control cells independent of AB configuration. Right: Ac$_4$GalNAz labelling and conjugation with ManAB leads to increased adhesion independent of AB configuration. Errors are given as SEM. Four independent sets of cells were tested for each condition. *p < 0.1, **p < 0.01, ***p < 0.001.
Indeed, bacterial adhesion to HMEC-1, labelled with Ac₄GalNAz and modified with mannose 5 is increased in comparison to control cells (Fig. 4). This observation can be rationalised by the increased concentration of mannoses present on the cell surface after modification. However, the observed increased adhesion is not photosensitive in this case. As LacNAc groups are typically not exposed at the cell surface, conjugated ManAB moieties might be buried under a layer of other glycoconjugates so that an orientational change of mannose ligands is not effective. On the other hand, we cannot measure the extent of azobenzene isomerisation, which might be different in both investigated cases (Ac₄ManNAz or Ac₄GalNAz labelling).

Taken together, we show that the azobenzene mannose 5 can be employed to influence adhesion of *E. coli* to HMEC-1. We postulate the following mechanism that underlies this photocontrol: (i) bacterial FimH binds to mannose, which serves as ligand for specific interaction between the bacterium and the modified cell surface (cf. Ac₄ManNAz + 5 vs. Ac₄ManNAz + 6). (ii) If no mannose ligand is present or if it adopts a disadvantageous orientation (Z-configuration), the azobenzene groups shield the cell, leading to reduced binding of *E. coli* (cf. Ac₄ManNAz + 5 vs. Ac₄ManNAz + AB). (iii) The FimH ligand mannose must be localised at the terminus of glycans to ensure that the change in configuration upon a Z/E transition is not small (Ac₄ManNAz + 5 vs. Ac₄GalNAz + 5).

It is surprising that this orientation effect can be found not only on an artificial, ordered glyco-SAM as we described before, but even in the complex, in comparison rather chaotic setting of the cell surface. Of course, this finding bears implications for the way how cells interact with their environment and offers options for prevention of bacterial adhesion with temporal and spatial resolution.

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Notes and references