Bacterial Adhesion

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Switching of Bacterial Adhesion to a Glycosylated Surface by Reversible Reorientation of the Carbohydrate Ligand**

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Abstract: *The surface recognition in many biological systems is guided by the interaction of carbohydrate-specific proteins (lectins) with carbohydrate epitopes (ligands) located within the unordered glycoconjugate layer (glycocalyx) of cells. Thus, for recognition, the respective ligand has to reorient for a successful matching event. Herein, we present for the first time a model system, in which only the orientation of the ligand is altered in a controlled manner without changing the recognition quality of the ligand itself. The key for this orientational control is the embedding into an interfacial system and the use of a photoswitchable mechanical joint, such as azobenzene.*

Adhesion of cells to surfaces is an essential process in life. This process is involved in the formation of tissues and organs, but also infection and the organization of biofilms. Thus, cellular adhesion is relevant in the context of many medical and technical applications.[1] The elucidation of the mechanisms of cell adhesion is crucial for our understanding of health and disease states of organisms, in particular in the area of bacterial infections. An important mechanism for the regulation of bacterial cell adhesion to their host cells depends on the specific binding to the carbohydrates presented at the cell surface, which is mediated by adhesive organelles of bacteria, called fimbriae.[2] Fimbriae comprise specialized proteins of the lectin type to recognize carbohydrate ligands.[3] Particular virulent bacteria possess so-called type 1 fimbriae, which are terminated by an α-D-mannoside-specific lectin named FimH.[4] These type 1 fimbriae are expressed in several hundred copies on the bacterial cell surface to achieve tight adhesion through multivalent protein-carbohydrate interactions. In case of uropathogenic *Escherichia coli* (UPEC), adhesion to the surface of urothelial cells is mediated by FimH binding to oligomannoside residues of the glycoprotein uroplakin Ia.[5] As a consequence of this process, bacterial invasion and severe infection of the host organism can occur.

The carbohydrate specificity of FimH has been elucidated in great detail.[2,6] As in all carbohydrate–protein interactions, carbohydrate binding is specific with respect to constitution and configuration of the carbohydrate ligand. However, carbohydrate recognition occurs within the context of the glycocalyx, a highly complex glycoconjugate layer surrounding the cells with a thickness of 100 nm and more. In contrast to many other biological entities, the glycocalyx has no apparent supramolecular structure or fold, respectively. Hence, there is no conclusive understanding to date, on how carbohydrate–protein interactions on the cell surface are orchestrated in this seemingly disordered environment.

As cell–cell adhesion is mediated and regulated by cellular lectins on the one hand and cell surface carbohydrate epitopes on the other hand, synthetic glycosylated surfaces, so-called glycoarrays, have become valuable tools to study details of carbohydrate-specific cell adhesion in a supramolecular setting.[7] To date, glycoarrays have mainly been utilized to study carbohydrate specificity of lectins and cell adhesion, respectively. However, it must be assumed that also carbohydrate orientation on cell surfaces is crucial for the adhesion of cells. To be able to study the effect of carbohydrate orientation for cell adhesion on a glycosylated surface, we have recently introduced a system which permits the control of the orientation of surface-bound carbohydrate ligands.[8] Azobenzene glycosides were immobilized onto gold surfaces to form photoswitchable glyco-SAMs (self-assembled monolayers). The advantage of this reversible system is that the distinction of the switching effect from other, irreversible effects, such as photolytic damage of the monolayer,[9] is possible. Azobenzene derivatives have favorable photochromic properties, allowing for reversible photoisomerization of the azobenzene N=N double bond in a biological context.[10] The defined change going along with the *E*/*Z* isomerization has already been extensively exploited to adjust the shapes of ligands (recognition sites) for the switching of biological on/off events in solution as well as on surfaces.[11] If the azobenzene units are attached to surfaces, the orientation of the exposed phenyl ring, and everything that is attached to it, changes significantly.

Herein, we wish to present for the first time that such a different orientation of the ligand within an otherwise inert environment can alter its recognition without changing the

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ligand itself. For this, we investigated the specific adhesion of type 1-fimbriated *E. coli* to α-D-mannosyl residues, the orientation of which was determined by the configuration of surface-bound azobenzene units.

It has been shown earlier that azobenzene mannoside derivatives serve as good ligands for the type 1 fimbrial lectin FimH.[12] Computer-assisted docking experiments performed within this project (see Supporting Information) suggest that both isomers of α-D-mannosyl ligated azobenzene are recognized by FimH equally well (score values of −9.7 and −9.3 for the *E* and *Z* form of azobenzene α-D-mannoside, respectively). This result is supported by experimental studies, which show that in solution *E*- or *Z*-form azobenzene mannosides have the same capability to suppress adhesion of *E. coli* to mannan-coated surfaces.[12] Thus, FimH-mediated bacterial adhesion to an azobenzene mannoside monolayer forms an ideal and well-defined system to study the effect of the orientation of a surface-bound carbohydrate ligand on the adhesion of cells. For the formation of such a photoswitchable glyco-SAM, an azobenzene mannoside derivative was designed (see Supporting Information) consisting of a thiol group for the anchoring of the molecule to gold surfaces, an undecane chain to promote the order and packing density of the SAM,[13] an oligoethylene glycol (OEG) chain to suppress unspecific surface recognition, the photoswitchable azobenzene unit, and finally the α-D-mannosyl ligand (see Figure 1, center).

Deposition of this mannoside onto Au films from ethanolic solutions provided dense films, as could be demonstrated by ellipsometry and infrared reflection absorption spectroscopy (IRRAS; see Supporting Information). The ellipsometric thickness was determined to be 3.91 nm. Hence, based on the length of the molecule (4.07 nm), a tilt angle of 16° can be estimated. In the IRRA spectra (Figure 2) the signal at 2919 cm^{-1} (asymmetric CH_2 vibration) is indicative

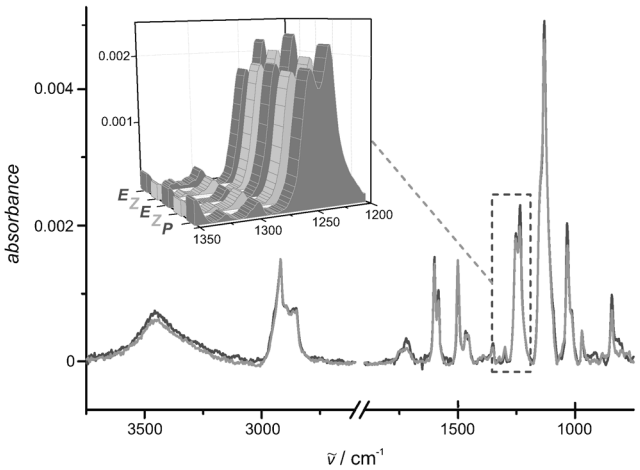

Figure 2. IRRA spectra of the azobenzene glycoside monolayer on gold. During switching, the intensities of many signals vary, with the effect being particularly pronounced for the signals at around 1240 cm^{-1} (see Inset). *P*: pristine SAM, *Z*: SAM after irradiation with 365 nm light (2 $J\,cm^{-2}$), *E*: SAM after irradiation with 450 nm light (100 $J\,cm^{-2}$).

for an *all-trans* conformation and thus a high order within the alkane chain[14] while the signal at 1350 cm^{-1} (wagging mode) is typical for the gauche conformation around the -CH_2CH_2- bond in the ethylene glycol chain and thus its bioresistant arrangement.[15]

IRRAS also allowed monitoring the reversible photoswitching of the carbohydrate orientation within the SAM as shown earlier in a similar system.[8] Owing to the selection rules for the IR spectroscopy in the vicinity of metallic surfaces, the observability of vibrational bands strongly depends on their relative orientation. Thus, when the substrates were illuminated with light of 365 nm (2 $J\,cm^{-2}$),

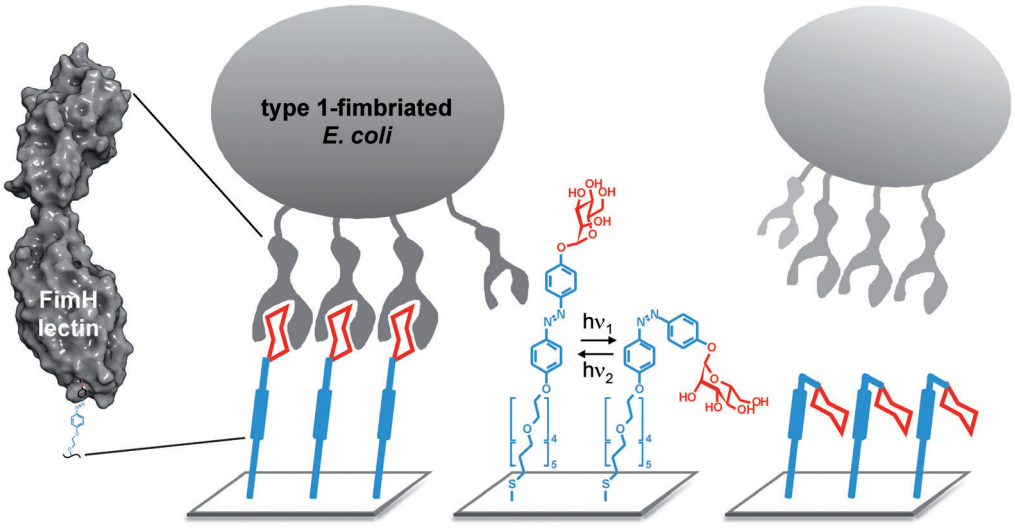

Figure 1. Adhesion of type 1-fimbriated *E. coli* cells is mediated by the fimbrial protein FimH, a two-domain lectin found at the fimbrial tips. The orientation-dependent recognition of immobilized α-D-mannoside ligands by FimH determines adhesion of *E. coli*. To control their orientation, the α-mannoside ligands were attached to solid surfaces in the form of SAMs, which contain photoswitchable azobenzene units. A short OEG spacer provided a hydrophilic environment and suppressed unspecific surface adhesion. Reversible *E*/*Z* photoisomerization was achieved by employing light of two different wavelengths.

$E \rightarrow Z$ isomerization was induced, as demonstrated by the intensity change of several IRRAS peaks (Figure 2). Significantly, the band at 1240 cm^{-1}, which is indicative for the aryl-O(-mannosyl) vibration and thus for the orientation of the ligand system, is affected most (Inset in Figure 2). When the layers were then irradiated again, but with light of 450 nm, the IR spectra slowly reverted, reaching the original state at area doses of 100 $J\,cm^{-2}$. This switching could be repeated several times without deterioration of the system.

To investigate the influence of the orientational change on cellular adhesion, the photoswitchable glyco-SAMs were tested in a bacterial-adhesion assay. For this, a type 1-fimbriated *E. coli* strain, which was labelled additionally with GFP (strain pPKL1162),[16] was used in its exponential growth phase at an optical density of 0.5. To distinguish the primary recognition (α-D-mannosyl/FimH) from any secondary surface-recognition events, such as protein plaque formation or pinhole etching, the exposure time was limited to 30 min. The strong adhesion (about 100000 cells cm^{-2}, Figure 3) provided by the carbohydrate ligands became visible by a more than 13-fold increased adhesion on the *E*-configured surfaces as compared to the adhesion on bare Au surfaces, where only non-specific interactions occur (see Supporting Information).[13]

After irradiation of the glyco-SAMs with light of 365 nm, the number of adherent bacteria decreased by a factor of approximately five (to about 18000 cells cm^{-2}). This finding indeed suggests a relationship between the orientation of immobilized α-D-mannoside ligands and the adhesive properties of the respective surface. Indeed, as can be seen in Figure 3, the density of cells (number of cells per area unit) in both states correlates very well with the peak area of the vibrational signal at 1240 cm^{-1}. Keeping in mind that the configuration of the azobenzene N=N bond of an azobenzene mannoside does not change its affinity for the bacterial lectin FimH in solution, we consider this as the first evidence for an orientation-dependence of a ligand by a biological system in an interfacial environment. Note that no pronounced switching behavior could be attained when similar molecules without the OEG part were employed, demonstrating the importance of this part.

The fact that the ligand in this study is a carbohydrate located at the surface of an OEG matrix in *gauche* confirmation makes the situation very similar to the one at the surface of a glycocalyx, where the apparent disorder in the native state requires some reorganization for the recognition to occur. The introduction of an azobenzene unit into the present model system provides the possibility to induce this reorganization by an external stimulus (light) opening the opportunity to compare binding/recognition events in different states. Future work will involve experiments to understand whether the bacterial adhesion remaining after $E \rightarrow Z$ isomerization is caused by some residual *E*-configured azobenzene mannosides (because of the photostationary state), by the recognition of the *Z*-configured glycosides in spite of their reorientation, or by defects within the monolayers.

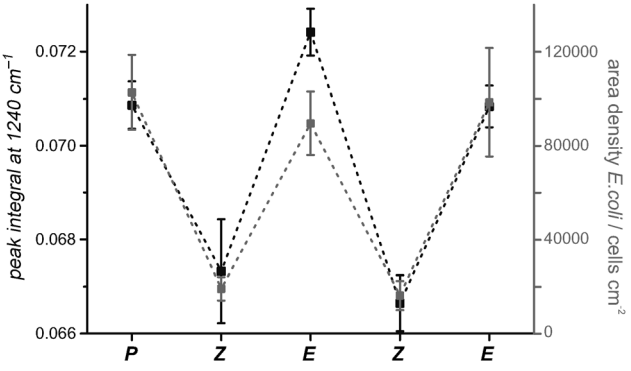

Figure 3. Bacterial adhesion to an azobenzene glycoside monolayer on gold through two $E \rightarrow Z \rightarrow E$ isomerization cycles (*P* signifies the pristine *E* isomer). The alternating amount of attached *E. coli* cells is shown in gray (error bars are 95% confidence intervals), together with the integral of the prominent IR signal at 1240 cm^{-1} (black; cf. Figure 2). The same alternating trend is visible for both observables indicating that adhesion of bacterial cells is switched with changing orientation of the immobilized carbohydrate ligand.

Experimental Section

The azobenzene mannoside was synthesized in three steps starting from a 4′-hydroxyazobenzene mannoside[8] and a commercially available tetraethylene glycol derivative (see Supporting Information for details). Using established techniques,[17] these molecules were deposited onto cleaned gold surfaces.[18] IRRAS spectra were recorded on a Nicolet 6700 FT-IR spectrometer (Thermo) purged with dry and CO_2-free air and equipped with a nitrogen-cooled mercury cadmium telluride detector. Switching of the azobenzene units within the monolayers was achieved by controlled illumination with a filtered mercury-vapor lamp (365 nm) or a high-power LED (450 nm). For adhesion assays, the GFP transfected, type 1-fimbriated *E. coli* strain pPKL1162 was grown in CASO broth until an optical density of 0.5 was reached. Then the *E*/*Z* configured glyco-SAMs were incubated and the area density of adherent bacteria was determined by fluorescence microscopy. For details see Supporting Information.

The computer-assisted docking studies were performed using the FimH structure (pdb code: 1KLF) and the Schrödinger Glide software, version 3.1 (for details see Supporting Information).

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