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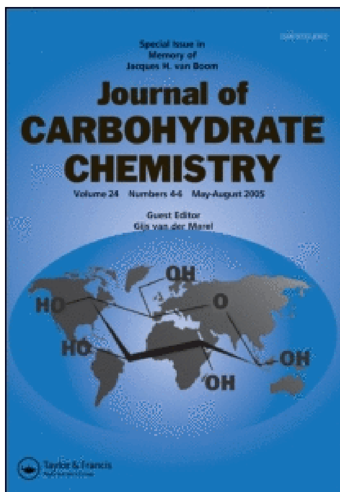

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COMMUNICATION

LOW AFFINITY CARBOHYDRATE LECTIN INTERACTIONS EXAMINED WITH SURFACE PLASMON RESONANCE

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The recognition of carbohydrates by proteins is an important element in many biological events like cell targeting, tumor invasion, immune response or bacterial and viral adhesion to host cells.[1,2] Besides structural data obtained from X-ray crystallography or NMR spectroscopy, the analysis of carbohydrate protein interactions should also take into consideration other biophysical parameters such as dissociation constants or kinetic rate constants of the complex. However, these parameters are often difficult to determine due to the rather low affinity of the systems under investigation.

Two lectins where the structures of bound carbohydrates are being studied in our laboratory by NMR spectroscopy are the lectins *Viscum album* agglutinin I (VAA I) and *Aleuria aurantia* agglutinin (AAA). VAA I is a β-D-Gal specific lectin for which no equilibrium dissociation constants (K_D) of carbohydrate complexes have yet been determined. AAA has a high specificity for α-L-fucosylated oligosaccharides but only the K_D for α-L-fucose complexed by AAA is reported to be $1.6*10^{-5}$ M.[3] Transferred NOE (trNOE) experiments, from which the structures of the bound carbohydrates can be

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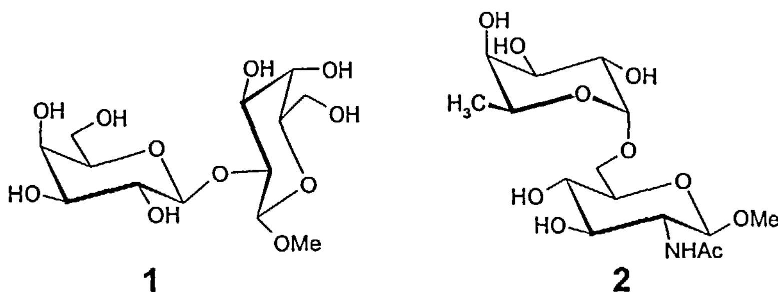
deduced, have been reported for the complexes of VAA I and β-D-Gal-(1→2)-β-D-Gal-(1→O)-Me (**1**)[4] and AAA and α-L-Fuc-(1→6)-β-D-GlcNAc-(1→O)-Me (**2**).[5] To further probe the binding specificity of the lectins and to interpret the trNOE derived structures of the lectin bound carbohydrates, the equilibrium dissociation constants of these complexes had to be compared to the corresponding monosaccharide complexes of the lectins with β-D-Gal-OMe (**3**) and α-L-Fuc-OMe (**4**).



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Surface plasmon resonance (SPR) technology appeared to be an attractive method to achieve this goal since it can be used to study the interactions between biomolecules by monitoring complex formation through the increase of mass of a surface where one molecule is immobilized and a binding partner is present in the mobile phase.[6-8] Due to the optical nature of SPR, the technique is very sensitive and does not need any labels while studying the interaction event. The time dependence of the SPR signal can be used to derive the kinetic rate constants and the equilibrium dissociation constant of a complex. So far, some SPR experiments with lectin-oligosaccharide complexes have been published.[9-17] Except for two reports,[13,17] the oligosaccharide was usually immobilized onto the sensor surface and the lectin injected over this surface. The immobilization of oligosaccharides has the distinct disadvantage that these molecules cannot be coupled onto the surface as they are but have to be derivatized first. Also, when different oligosaccharides are to be studied with one protein, each carbohydrate needs to be immobilized on a separate surface. Finally, the amount of protein required for such experiments might easily reach the milligram level and many proteins may not be available in these quantities. Since it was shown recently for an immobilized monoclonal antibody against α-D-Glc-(1→4)-D-Glc-motifs that modern SPR instruments are capable of detecting the formation of complexes with analytes[18] smaller than 1000 Da,[19,20] we decided to immobilize the lectins on the surface of the sensor-chip.

Figure 1 shows typical sensorgrams which are obtained when the analytes are injected over the lectin-coated surfaces. The association as well as the dissociation phase is very fast and creates a rectangular response. Although the kinetic rate constants of the formation of the complex cannot be derived from such a sensorgram, the experiments benefit from this behavior since, after stopping the injection, the baseline of the sensorgram is reached instantaneously, making a regeneration of the surface unnecessary. It has to be mentioned here that fast dissociation rate constants of the complexes are in accord with the trNOE experiments.[4,5] By repeating the experiments with different concentrations of analyte, a saturation binding curve can be obtained from which K_D can be determined by a scatchard plot analysis and/or by fitting the data to a saturation curve (Figures 1 and 2).

From the saturation binding curves, K_D values of $7.3*10^{-5}$ M and $8.5*10^{-4}$ M for the complexes of **1** and **3** with VAA I (figures 1 and 2) were determined. Since the equilibrium dissociation constant for disaccharide **1** is one order of magnitude lower than for monosaccharide **3**, the data indicate that both galactose moieties of **1** are recognized by VAA I.

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The situation is different for the complexes of AAA. Here, K_D values of $5.0*10^{-5}$ M and $3.3*10^{-5}$ M for the complexes of **2** and **4** with AAA were obtained. In this case the monosaccharide binds slightly better than the disaccharide and therefore, the binding site of the lectins recognizes only the fucose moiety. Knowing about the flexible nature of the $1\rightarrow6$ linked disaccharide **2**[22] the rest of the ligand does not seem to interfere with the protein. This observation is in accord with the trNOE data of the bound disaccharide[5] where it was demonstrated that AAA binds **2** in different conformations. It will be interesting to examine the binding of other fucosylated oligosaccharides to AAA by SPR, especially with different linkage types, to probe negative effects on the equilibrium binding originating from steric hindrance.

The obtained K_D for the complex of AAA with α-L-Fuc-OMe ($3.3*10^{-5}$ M) is in very good agreement with the published value of $1.6*10^{-5}$ M from equilibrium dialysis experiments[3] and proves that SPR experiments with immobilized lectins match other biophysical methods where both binding partners are present in solution when equilibrium binding data have to be derived. When using small and fast diffusing analytes, the immobilization of a protein has also the advantage that a simple 1:1 binding model can be assumed for the evaluation and that no rebinding effects have to be considered.

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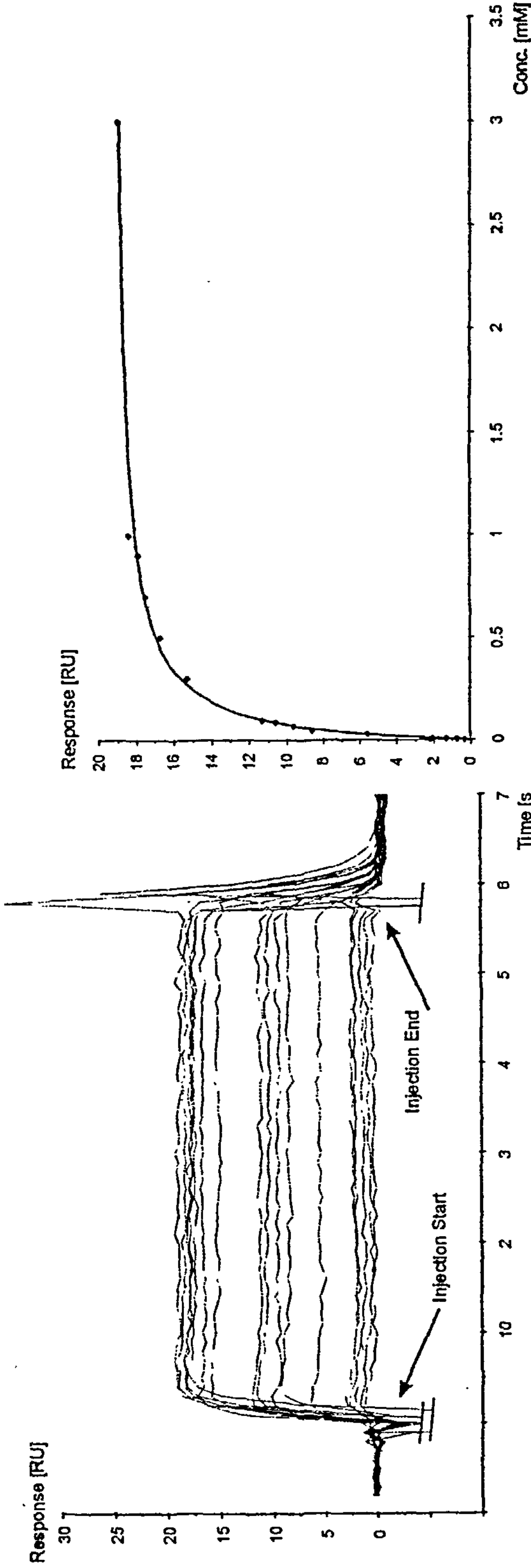

Figure 1. Left: Sensorgrams of different concentrations (1 μM to 10 mM) of disaccharide 1 injected over the dextran surface with immobilized VAA I.[21] The response of the reference cell has been subtracted. **Right:** Steady-state affinity plot of the experimental response with concentrations between 1 μM and 3 mM.

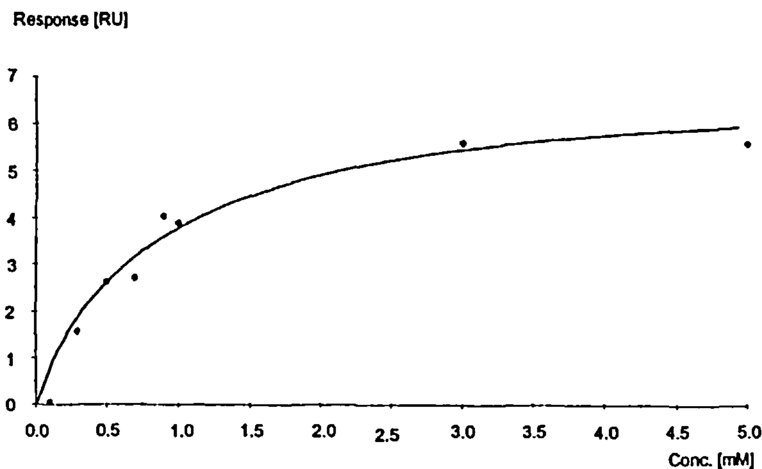

Figure 2. Steady-state affinity plot of the experimental response from SPR experiments of β-D-Gal-OMe bound by VAA I.[21]

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The potential of modern Biacore instruments to examine low affinity interactions with very small ligands is demonstrated in Figure 2. The binding of β-D-Gal-OMe to VAA I is a perfect example where only a few picogram[21] (~ 6 pg) of the monosaccharide ligand are bound by the immobilized protein at saturation. For SPR experiments with VAA I we, like others,[17,20] find that very high analyte concentrations (> 5 mM) lead to a decrease of the resonance signal. The reason for this could be non-specific binding in the reference cell,[20] however we do not observe such phenomena during the experiments with AAA.

Our experiments show that SPR has a high potential to investigate low molecular weight, low affinity complexes and thus we have started to analyze the oligosaccharide recognition of both lectins with SPR and a wider variety of oligosaccharides. The optical detection makes SPR a very sensitive method which uses only low μg quantities of the immobilized protein. Because of this sensitivity, SPR will become more and more important for the investigation of biomolecular interactions where other methods are not applicable, especially when only a limited amount of protein is available.

EXPERIMENTAL

Disaccharides **1** (MW 356) and **2** (MW 381) have been synthesized according to published protocols.[23,24] AAA (MW 72 kDa, dimer of two identical subunits) was

purchased from Vector Laboratories, Burlingame, CA, USA, and VAA I (MW 115 kDa, tetramer of two A and two B subunits) was a gift from Dr. Samtleben, Munich. All SPR experiments were performed with a Biacore 3000 instrument (Biacore AB, Uppsala) at 25 °C using original Biacore HBS-EP buffer (10 mM Hepes, 150 mM NaCl, 0.005 % polysorbat, 3 mM EDTA, pH 7.4) and a flow rate of 10 µL/min. The carboxylated dextran matrix[25] of a CM5-chip was activated with a EDC/NHS solution (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, *N*-hydoxysuccinimide)[26] for 10-20 minutes until the SPR signal increased 200-300 resonance units (RU).[21] Both lectins were injected in immobilization buffer (~ 10 µg/mL, 10 mM NaAc, pH 5.0) to reach levels of 4000-6000 RU for each lectin. To stop the immobilization, all activated flow cells were deactivated with a solution of ethanolamine (1 M) for 15 minutes. Carbohydrate solutions (concentrations between 1 µM and 10 mM) in buffer were injected for 2 minutes into the flow cells using the *kinject* command. The equilibrium response (after subtraction from the response of the reference surface) of each experiment was used to create saturation curves of analyte binding which were fitted to a 1:1 steady-state affinity model with the Biaevaluation software 3.0 (Biacore AB, Uppsala).

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