SPR Studies of Carbohydrate–Protein Interactions: Signal Enhancement of Low-Molecular-Mass Analytes by Organoplatinum(II)-Labeling

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The relatively insensitive surface plasmon resonance (SPR) signal detection of low-molecular-mass analytes that bind with weak affinity to a protein—for example, carbohydrate–lectin binding—is hampering the use of biosensors in interaction studies. In this investigation, low-molecular-mass carbohydrates have been labeled with an organoplatinum(II) complex of the type [PtCl-(NCN–R)]. The attachment of this complex increased the SPR response tremendously and allowed the detection of binding events between monosaccharides and lectins at very low analyte concentrations. The platinum atom inside the organoplatinum(II) complex was shown to be essential for the SPR-signal enhancement. The organoplatinum(II) complex did not influence the specificity of the biological interaction, but both the signal enhancement and the different binding character of labeled compounds when compared with unlabeled ones makes the method unsuitable for the direct calculation of biologically relevant kinetic parameters. However, the labeling procedure is expected to be of high relevance for qualitative binding studies and relative affinity ranking of small molecules (not restricted only to carbohydrates) to receptors, a process of immense interest in pharmaceutical research.

Introduction

Introduced in the early 1990s, biosensors based on surface plasmon resonance (SPR) have become a well-established tool for studying biomolecular interactions in real time.[1,2] Major applications have been reported, not only for protein–protein interactions, including in conjunction with mass spectrometry, but also in SPR studies on nucleic acid–protein, carbohydrate–protein, and carbohydrate–carbohydrate interactions.[3–6]

Qualitative SPR applications range from orphan-ligand and small-analyte screening to epitope mapping and complex assembly studies, whereas quantitative experiments include concentration measurements of active molecules in solution, evaluation of competition/inhibition events, and determination of rate and affinity constants. Nevertheless, since the SPR response is proportional to the accumulation of mass on the sensor surface, a serious constraint imposed by this technique concerns the dimensions of the molecules to be employed as analytes.[7]

In recent years, several groups have focused on SPR as an emerging technique to detect protein–carbohydrate interactions,[8–20] key steps in many biological events.[21,22] SPR studies of these biological events are hampered by the low availability of high-molecular-mass oligosaccharides and by the weakness of protein–carbohydrate interactions. To overcome these problems, more accessible low-molecular-mass carbohydrate epitopes are multivalently presented to the lectin to increase both their binding affinity and overall mass (thereby enhancing the SPR response). Thus, glycan epitopes can either be immobilized on the surface of a sensor chip[23] or, when used as analytes, conjugated to carrier proteins[24–26] or, in the case of carbohydrate–carbohydrate interactions, clustered on gold glyconanoparticles.[27]

Although recent improvements in signal-to-noise ratio have made it possible to measure the binding of monovalently presented low-molecular-mass analytes directly,[28] relevant control surfaces for blank subtraction and high surface concentrations of active immobilized ligands are needed. This is often difficult to achieve, and mass transport limitations and rebinding events may complicate interaction analysis at such high ligand densities.[29] Moreover, to obtain sufficient SPR signal for the weak-affinity binding of a carbohydrate to a protein with analytes of molecular mass < 1000 Da, high analyte concentrations (up to the millimolar range) are required.[15,30–34] Under these conditions, the contribution from the bulk refractive index to the specific response becomes significant, with an apparent loss in specific binding.

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This study presents a method that allows facile qualitative SPR detection of low-molecular-mass carbohydrate epitopes at low concentrations with a Biacore 2000 instrument. In our experimental setup, low-molecular-mass carbohydrates (mono- and disaccharides) are labeled with an organoplatinum(II) complex. These compounds, when allowed to flow at very low analyte concentrations (0.5–20 μM range) across suitable lectin surfaces, give rise to intense SPR signals. The crucial role of the platinum atom for this SPR signal enhancement is discussed.

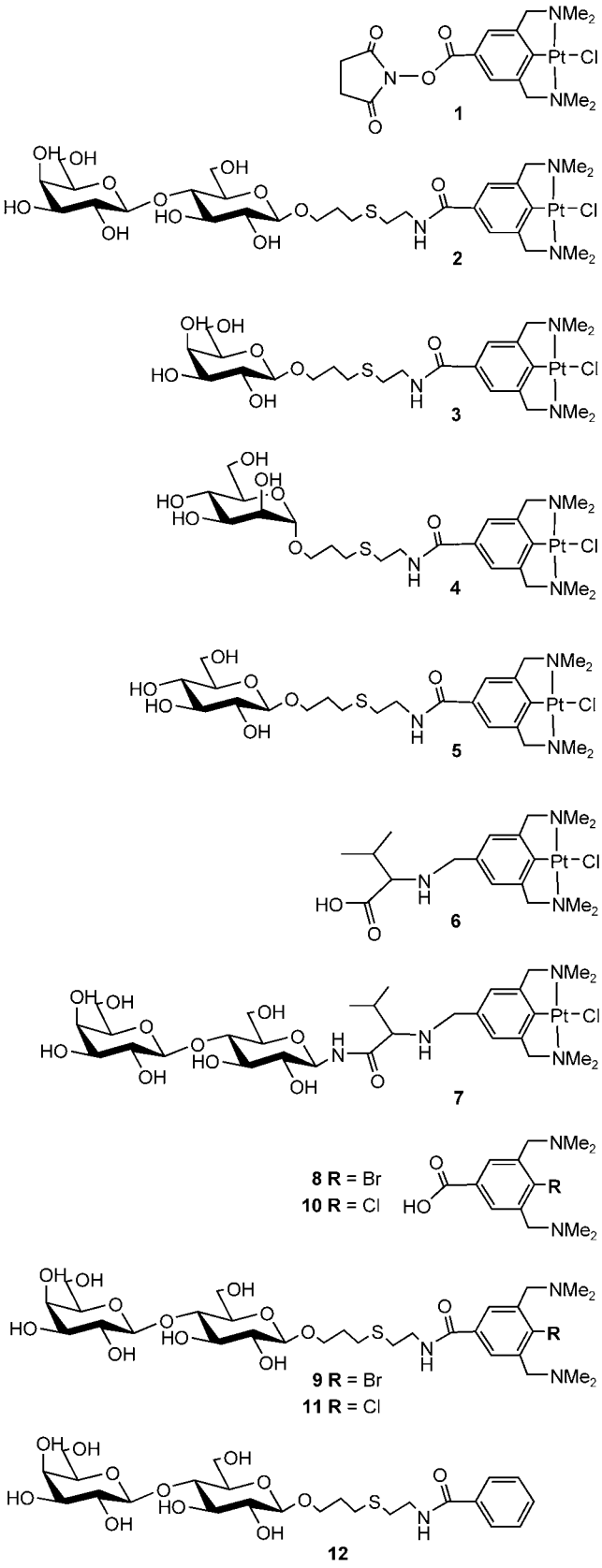
Results

PtCl(NCN–R)-labeling ensures higher sensitivity in SPR detection

RCA_{120} (specific for galactose/lactose) and Con A (specific for mannose and displaying a weak binding affinity for glucose) were chosen as model carbohydrate-binding proteins for SPR analysis of the interactions between low-molecular-mass saccharides and immobilized lectins. Each lectin was immobilized on two channels of a CM5 sensor chip (≈11 000 RU for dimeric Con A, ≈11 500 RU for RCA_{120}), and one channel of each lectin was denatured to serve as a blank surface. Firstly, the mono- and disaccharides D-mannose, D-glucose, D-galactose, methyl α-D-mannopyranoside, methyl β-D-galactopyranoside, and lactose were tested for their SPR responses on the lectin surfaces. The same series of free mono- and disaccharides were then labeled with the organoplatinum(II) complex of the type [PtCl(NCN–R)] with the aid of the activated ester **1** (**2**–**5**;) and allowed to flow over the same lectin surfaces (NCN–R is an abbreviation for the terdentate, monoanionic, 4-substituted 2,6-bis(dimethylaminomethylene)phenyl "pincer" ligand[35,36]).

Despite their well known specificity for these lectins, none of the free saccharides or methyl glycopyranosides showed any detectable binding either to Con A or to RCA_{120} in the 1–600 μM concentration range. In contrast, lactose labeled with the organoplatinum(II) complex PtCl(NCN–R) (**2**), injected at 9 μM concentration over RCA_{120}, produced a strong SPR signal (Figure 1). To examine whether such a high response could be attributed to specific binding, compound **2** (9 μM) was also injected simultaneously onto denatured RCA_{120}, Con A, and denatured Con A. As depicted in Figure 1, only the active RCA_{120} surface gave a strong SPR signal, while the responses on the other surfaces were comparable to one another and very low. Similar sensorgrams, demonstrating the signal-enhancing properties of the organoplatinum(II) complex, were obtained for galactose derivative **3** on the RCA_{120} surface (90 RU at 2.5 μM after blank subtraction), and for mannose derivative **4** and glucose derivative **5** on the Con A surface (50 and 10 RU at 2.5 μM after blank subtraction, respectively).

Figure 2A shows the concentration-dependent overlay plot for **2** (1.1–17.5 μM), injected over RCA_{120}. The question of whether the length of the linker between the saccharide and the organoplatinum(II) complex would influence the signal-enhancing qualities of the complex or prevent the biomolecular interaction was also investigated. For this purpose, compound **7** was synthesized and analyzed by SPR. As illustrated in the sensorgram in Figure 2B, a signal-enhancing SPR response similar to that observed for **2** (Figure 2A) was seen; this establishes that the binding of **7** to RCA_{120} was not disturbed by the



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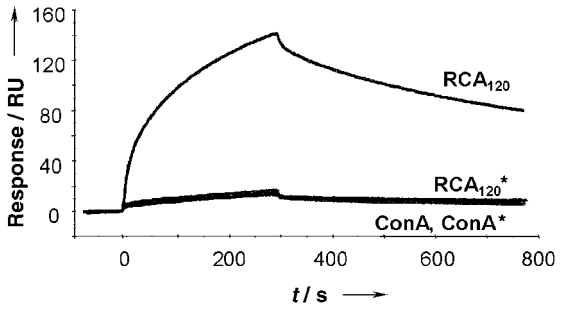

Figure 1. Sensorgrams of PtCl(NCN–R)-labeled lactose **2** (9 μM) flowing across RCA_{120}, denatured RCA_{120}*, Con A, and denatured Con A*. An asterisk denotes a denatured component.

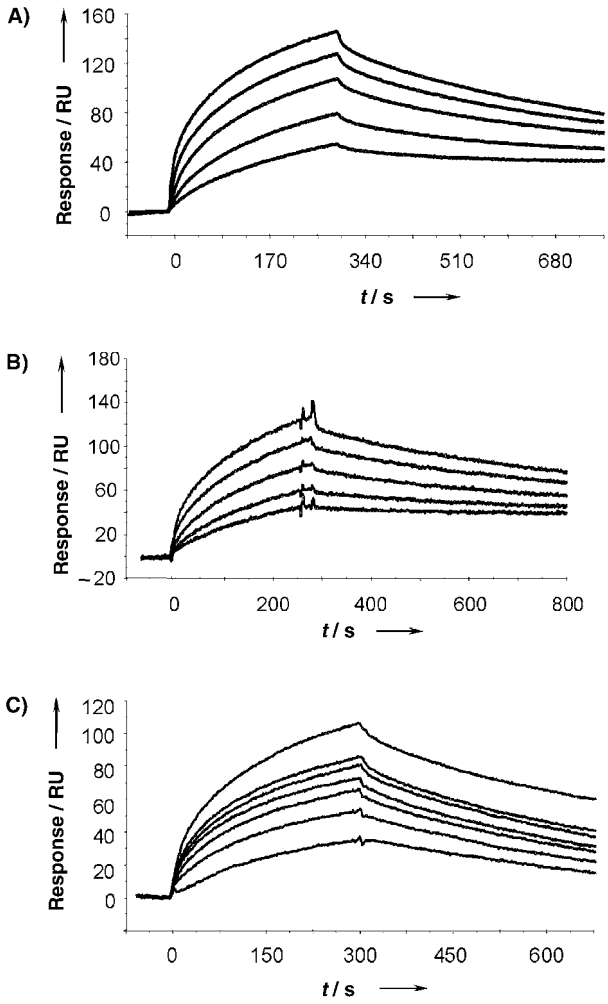

Figure 2. A) Concentration-dependent binding of **2** to RCA_{120}. Concentrations from bottom to top: 1.1, 2.2, 4.4, 8.75, 17.5 μM. B) Concentration-dependent binding of **7** to RCA_{120}. Concentrations from bottom to top: 1.25, 2.5, 5, 10, 20 μM. C) Competition assay between free lactose and PtCl(NCN–R)-labeled lactose **2**. Increasing amounts of free lactose were progressively added to a 7.5 μM solution of **2** (top curve; from top to bottom curve, free lactose concentrations: 0, 0.5, 1, 2, 4, 8, 16 μM).

close proximity of the organoplatinum(II) complex to the carbohydrate.

Subsequently, the binding affinities of free and PtCl(NCN–R)-labeled lactose **2** on RCA_{120} were compared in a competition experiment. Increasing amounts of free lactose were added to a 7.5 μM solution of **2**, and the resulting sensorgrams were measured. The SPR data presented in Figure 2C show that the response decreased progressively with increasing free lactose concentration, resulting in a reduction to half of the original RU for an equimolar free lactose/compound **2** solution. This result demonstrates clearly that PtCl(NCN–R)-labeled lactose competes effectively with free lactose for the same lectin binding site.

Unraveling the characteristics of the organoplatinum(II) complex

To investigate the influence of the different structural components of the organoplatinum(II) complex PtCl(NCN–R) on the signal-enhancing phenomenon, the lactose derivatives **9**, **11**, and **12** were synthesized. The modifications relative to **2** involved the removal of the platinum atom (**9** and **11**) and the removal of both the platinum atom and the pincer arms to yield an unsubstituted phenyl moiety (**12**). Free lactose and compounds **2**, **9**, **11**, and **12** (1.25–40 μM) were allowed to flow across the RCA_{120} surfaces to yield, after blank subtraction, the sensorgrams depicted in Figure 3A–E. The strongest response is clearly that associated with the organoplatinum(II)-containing compound **2** (60–160 RU, Figure 3B). The sensorgrams of compounds **9**, **11**, and **12** (Figure 3C–E) show that the intensity of the SPR signal decreased dramatically whenever the aglycon did not contain a platinum atom.

As would be expected, the platinum-free compounds presented low signals that increased linearly according to their molecular masses. In the chosen concentration range the RU values are close to zero for free lactose ($M_W = 342$), 2–7 RU for **12** ($M_W = 563$), and 5–40 RU for **11** and **9** ($M_W = 711/713$ and 755/757, respectively). Hence, it appears that the response shown by **2** (60–160 RU) cannot be explained simply by the increase in molecular mass of **2** ($M_W = 906/908$). This observation is further supported by comparison of the SPR responses (at 1 μM concentration) of PtCl(NCN–R)-labeled galactose **3** and Br(NCN–R)-labeled lactose **9**, possessing close molecular masses ($M_W = 745$ vs. 755/757), but differing in the presence or absence of the platinum atom. Even though lactose has a higher affinity for RCA_{120} than galactose,[37] the lack of the platinum atom in **9** causes a significant drop in RU relative to **3** (Figure 4). A curve relating the RU values at 26 μM concentration for lactose, **2**, **9**, **11**, and **12** with their respective molecular masses (Figure 5) reveals that the PtCl(NCN–R)-labeled lactose **2** and the PtCl(NCN–R)-labeled galactose **3** not only deviate from the linear trend presented by **9**, **11**, and **12**, but also give responses corresponding to higher-molecular-mass compounds.

Evaluation of the reference surface

To investigate whether denatured lectins could be considered suitable reference surfaces, compound **2** was allowed to flow across RCA_{120}, denatured RCA_{120}, Con A, and denatured Con A for 50 min at 8 μM concentration. Inspection of the sensor-

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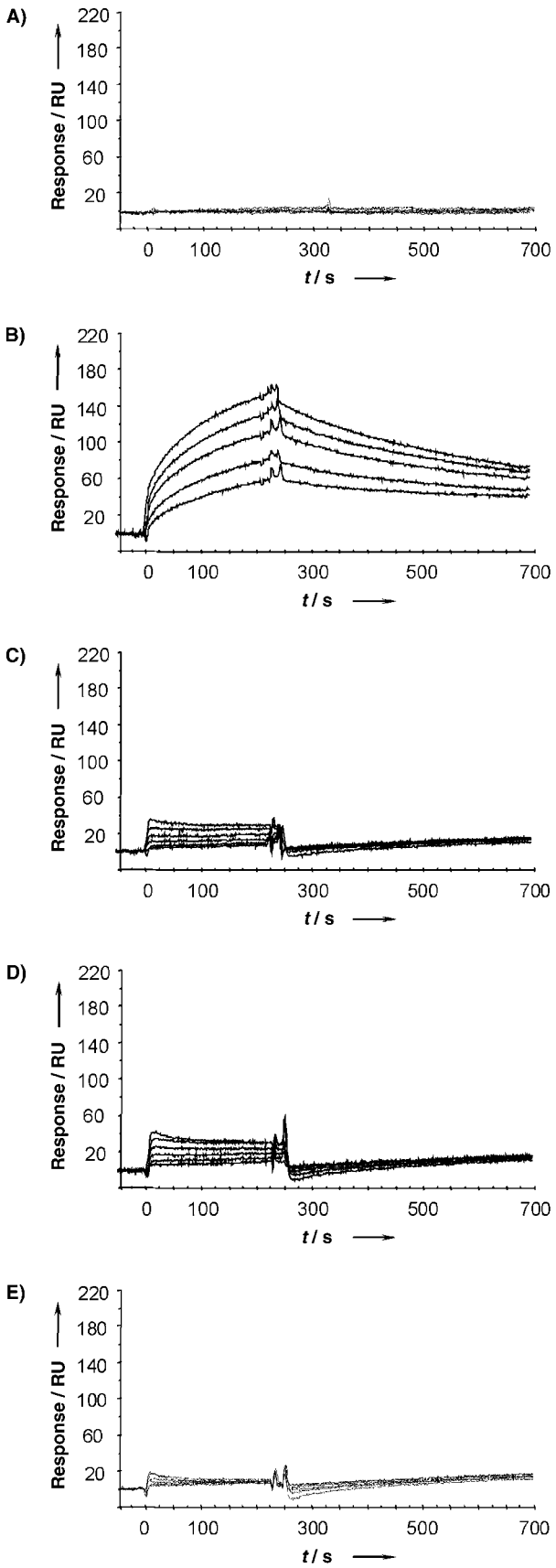

Figure 3. Sensorgrams of lactose variants with different aglycon structures. All binding curves have been corrected for nonspecific binding (RCA_{120}–RCA_{120}*). A) Free lactose. B) Compound **2**. C) Compound **11**. D) Compound **9**. E) Compound **12**. Concentrations: 40–1.25 μM (top to bottom).

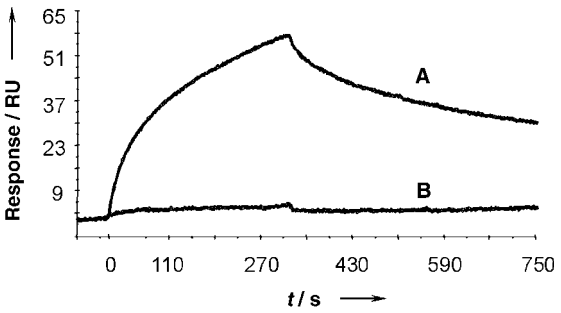

Figure 4. Relative response (RU) versus time for the binding responses of: A) PtCl(NCN–R)-labeled galactose **3** (M_W = 745) and B) Br(NCN–R)-labeled lactose **9** (M_W = 755/757) flowing across immobilized RCA_{120} at 1 μM concentration.

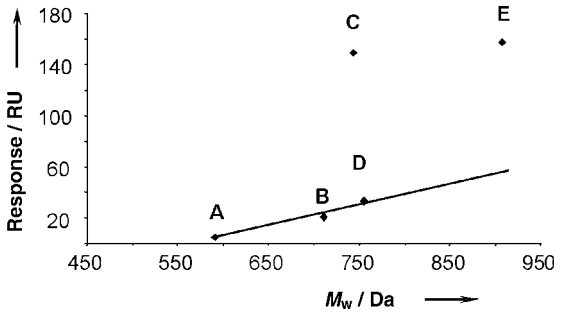

Figure 5. Molecular mass versus SPR response at 26 μM concentration for: A) compound **12**, B) compound **11**, C) compound **3**, D) compound **9**, and E) compound **2**.

grams presented in Figure 6 reveals that the SPR response on active RCA_{120} during the first 5 min of association differed significantly from those observed on denatured RCA_{120}, Con A, and denatured Con A, due to specific binding on this surface. With prolonged injection times, similar linear increases are observed for all the four curves, suggesting nonspecific binding of **2** on each of the sensorchip surfaces. Since both active and denatured surfaces present the same trend, subtraction of reference channels from the active lectin surface could be usable to correct for nonspecific binding, refractive index changes, and detector drift. The contribution of these phenomena to

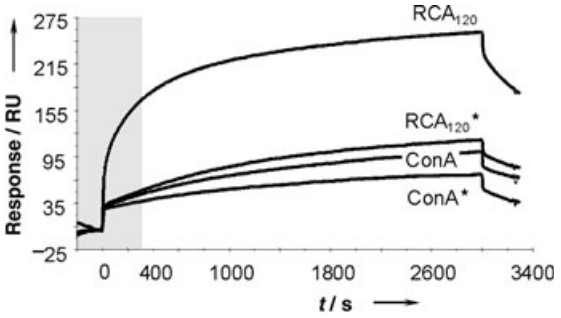

Figure 6. Sensorgrams of PtCl(NCN–R)-labeled lactose **2** (8 μM) flowing across RCA_{120}, denatured RCA_{120}*, Con A, and denatured Con A*. Injection time: 50 min. The shaded part indicates the injection time usually considered in Biacore analysis.

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the measured SPR response could be further minimized by use of low analyte concentrations (0.5–20 μM) and short injection times (3–5 min).

Discussion and Conclusions

SPR is an optical phenomenon that is generated at a noble metal-coated interface (a 10 nm gold film in Biacore biosensors) between two media of different refractive index (RI), by a beam of monochromatic, plane-polarized light. Under conditions of total internal reflection, an evanescent wave will penetrate into the medium of lower RI, causing free electrons in the metal layer to oscillate, resulting in the generation of the so-called surface plasmon waves. These plasmons can be resonantly excited only at a well defined angle of incidence, and can be monitored in the reflected light, since a reduction in its intensity occurs at that angle. The SPR of the system is very sensitive to variations in the refractive index of the media adjacent to the metal layer. For a given number of ligand sites, the response increases linearly with the mass bound to the sensor surface, as the RI changes are stronger for high-molecular-mass analytes.[38]

Oligosaccharides binding to immobilized proteins are not easily detected, due to their low molecular masses and weak binding affinities, so labeling procedures are often required.

In this study it has been shown that attachment of an organoplatinum(II)-containing aglycon of the PtCl(NCN–R) type to a saccharide produces a strong SPR signal enhancement, allowing binding studies of low-molecular-mass saccharides to lectins at very low analyte concentrations: monosaccharide analytes containing such an aglycon give rise to intense SPR signals even at 1.25 μM concentration. The PtCl(NCN–R)-labeled saccharides are completely water-soluble, do not aggregate in the buffer solutions conventionally used in SPR experiments, and can be stored for long periods. Competition experiments between free saccharides and their organoplatinum(II)-containing analogues have demonstrated that the labeling does not affect the specificity of the biomolecular interaction. In addition, the binding experiments with organoplatinum(II)-labeled glucose and mannose over the Con A surface show that the relative affinity order of unlabeled glucose and mannose is preserved. The presence of a shorter spacer than the 3-(amidoethylthio)propyl spacer between saccharide and organoplatinum(II) complex gave the same lectin binding profile. This demonstrates that although the organoplatinum(II) complex is in close proximity to the binding site of the lectin, it does not influence the binding of the analyte to it.

Since this signal-enhancing property cannot be explained simply in terms of the molecular mass increase of the carbohydrate, a possible explanation has to be inferred from the SPR phenomenon itself. The observation that the platinum atom is essential for conferring signal-enhancing properties to the aromatic aglycon raises the possibility that the noble metal atom may cause more complex effects than simply inducing bulk changes in the RI close to the gold layer.[39] Introductory experiments, performed in our group, with compounds in which the Pt atom is replaced by a Pd atom have shown the same signal-enhancing effect (data not shown). A significant interaction between the platinum electrons and the evanescent wave produced in the proximity of the sensorchip surface is believed to be responsible for the observed phenomenon.

The inaccuracy involved in relating the response of PtCl(NCN–R)-labeled saccharides directly to their molecular masses may cause overestimation of calculated thermodynamical parameters, such as their affinity constants toward lectins. Exploratory kinetic binding studies indicate that, as a consequence of the signal enhancement, values calculated for the PtCl(NCN–R)-labeled lactose/RCA_{120} interaction ($K_D = 2$ μM) are between 10 and 30 times higher than values previously determined for the lactose/RCA_{120} interaction by isothermal titration calorimetry[37] or equilibrium dialysis calculation.[40,41] Discrepancies in K_D values can also be observed between the PtCl(NCN–R)-labeled mannose/Con A affinity measured by SPR ($K_D = 0.6$ μM) and values obtained from titration microcalorimetry,[42] fluorescence anisotropy,[42] and SPR[19,43] for the methyl α-D-mannopyranoside/Con A interaction. In addition, careful inspection of the SPR sensorgrams of organoplatinum(II)-labeled lactose and unlabeled lactose derivatives (e.g., **9** or **11**) shows that both the association with and dissociation from the lectin are slower for the organoplatinum(II)-labeled compound. The discussed limitations associated with the use of the organoplatinum(II) label make quantitative SPR binding studies unfeasible.

To conclude, labeling of low-molecular-mass saccharides with the PtCl(NCN–R) aglycon ensures high SPR responses. The organoplatinum(II) complex is therefore an excellent label for the qualitative detection of binding events taking place on the gold surface of the biosensor. Although the signal enhancement causes overestimation of the calculated affinities, specificity and affinity ranking between compounds are preserved. Therefore, relative values, more than absolute ones, can furnish a clear picture of the different affinities of labeled oligosaccharides for the tested proteins. We envision that this labeling procedure could be applicable to establishing the carbohydrate-binding specificity of unknown lectins with biosensors, a method traditionally hampered by the low availability of high-molecular-mass oligosaccharides. More generally, qualitative binding studies of synthetic or isolated small molecules to receptors, and their relative affinity ranking, by organoplatinum(II) labeling of analytes has become a possibility. SPR screening of organoplatinum(II)-labeled plant/animal extracts or chemical libraries (e.g., peptide, carbohydrate, DNA, or heterocycles) allows the identification of biologically active lead compounds, a process of immense importance in pharmaceutical research.

Experimental Section

General: Surface plasmon resonance studies were carried out on a Biacore 2000 instrument, with CM5 sensor chips and Biaevaluation 3.0 software (Pharmacia Biosensor AB, Uppsala, Sweden). *N*-Hydroxysuccinimide was purchased from Merck (NJ, USA), *N*-ethyl-*N*′-(dimethylaminopropyl)carbodiimide and ethanolamine from Sigma (St. Louis, USA), cysteamine hydrochloride and *N*-ethylmorpholine from Fluka (Buchs, Switzerland), and *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-

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tetramethyluronium tetrafluoroborate (TBTU) from NovaBiochem (Breda, The Netherlands). C-18 Extract-Clean columns were purchased from Alltech (Breda, The Netherlands) and Dowex 50 W×2 (H^+, 200–400 mesh) from Fluka (Buchs, Switzerland). *Ricinus communis* agglutinin from castor bean (RCA_{120}) and concanavalin A lectin from *Canavalia ensiformis* (Con A) were supplied by Sigma (St. Louis, USA). Compounds **1**[44] and **6**[35] were synthesized by procedures similar to those used for their iodide and bromide analogues, respectively. Compounds **8** and **10** were synthesized as described earlier.[45]

Reactions were monitored by TLC on silica gel (60 F_{254}, Merck); after examination under UV light, compounds were visualized by heating with methanolic H_2SO_4 (10% *v/v*), orcinol (2 mg mL^{-1}) in methanolic H_2SO_4 (20% *v/v*), or ninhydrin (1.5 mg mL^{-1}) in BuOH/H_2O/HOAc (38:1.75:0.25). Vacuum line column chromatography (VLC) was performed on silica gel (Merck 60, 0.040–0.063 mm). UV irradiation for synthetic purposes was performed in quartz vials at 254 nm with a grid tube lamp (VL-50 C, 50 W, Vilber Lourmat). Organic solvents were removed under reduced pressure at 30–50 °C on a water bath. ^{1}H NMR spectra were recorded at 300 K with a Bruker AMX 500 (500 MHz) spectrometer; δ_H values are given in ppm relative to the signal for internal Me_4Si ($\delta_H = 0$, $CDCl_3$) or internal acetone ($\delta_H = 2.22$, D_2O). Two-dimensional ^{1}H–^{1}H TOCSY (mixing time 7 ms) spectra were recorded at 300 K with a Bruker AMX 500 spectrometer. Exact masses were measured by matrix-assisted laser desorption ionization time-of-flight mass spectrometry with a Voyager-DE Pro (Applied Biosystems) instrument in the reflector mode at a resolution of 5000 FWHM. α-Cyano-4-hydroxycinnamic acid (Fluka Chemie GmbH, Buchs, Switzerland) in H_2O (5 mg mL^{-1}) was used as a matrix. A ladder of maltose oligosaccharides (G3–G13) was added as internal standard.

General procedure for the synthesis of [PtCl(NCN)]-3-(amidoethylthio)propyl glycosides: Cysteamine hydrochloride (1 equiv) was added to a solution of an allyl glycoside in water (3 mL). The mixture was transferred to a quartz vial and irradiated with UV light for 2 h, after which TLC analysis (dichloromethane/methanol 8:2) showed the formation of a new spot on the baseline and some remaining allyl glycoside. The mixture was applied to a Dowex 50 W×2 (H^+) column (50 mm×6 mm), and after the elution of contaminants with water, the 3-(aminoethylthio)propyl glycoside was eluted with aq. ammonia (6%). The product was lyophilized twice from water, and was directly used in the next reaction step.

A solution of **1** (1.5 equiv) in tetrahydrofuran (0.3 mL) was added to a solution of the 3-(aminoethylthio)propyl glycoside in aq. $NaHCO_3$ (0.25 M)/acetonitrile (1:1, 0.6 mL, *v/v*), and the mixture was agitated gently overnight. After concentration in vacuo, the residue was dissolved in water (15 mL) and washed with dichloromethane (3×15 mL), and the aqueous layer was concentrated to a volume of approximately 3 mL, and then loaded on a C-18 Extract-Clean column. The remaining 3-(aminoethylthio)propyl glycoside was eluted with water (15 mL) and the [PtCl(NCN)]-3-(amidoethylthio)propyl glycosides with methanol (10 mL). After concentration in vacuo, followed by lyophilization from water, the products were obtained as solids.

[PtCl(NCN)]-3-(amidoethylthio)propyl β-lactoside (**2**): White solid, 11.0 mg, overall yield 59%. ^{1}H NMR (500 MHz, D_2O): $\delta = 1.93$ (m, 2H; $OCH_2CH_2CH_2S$), 2.73 (brt, 2H; $OCH_2CH_2CH_2S$), 2.80 (brt, 2H; SCH_2CH_2ND), 3.02 and 3.14 (2s, each 6H; $2CH_2N(CH_3)_2$), 3.28 (dd, $J_{1,2} = 7.9$ Hz, $J_{2,3} = 8.1$ Hz, 1H; H-2), 3.48 (m, 1H; H-5), 3.66 (m, 1H; H-5′), 3.72 and 3.89 (2m, each 1H; $OCH_2CH_2CH_2S$), 3.73 (dd, $J_{5',6a'} = 4.5$ Hz, $J_{6a',6b'} = 11.4$ Hz, 1H; H-6a′), 3.80 (dd, $J_{5',6b'} = 7.7$ Hz, 1H; H-6b′), 3.84 (dd, $J_{5,6b} = 4.6$ Hz, $J_{6a,6b} = 12.3$ Hz, 1H; H-6b), 3.87 (brd, $J_{3',4'} = 2.4$ Hz, $J_{4',5'} < 1$ Hz, 1H; H-4′), 3.93 (dd, $J_{5,6a} = 2.2$ Hz, 1H; H-6a), 4.15 and 4.16 (2s, each 2H; $2CH_2N(CH_3)_2$), 4.36 (d, 1H; H-1), 4.42 (d, $J_{1',2'} = 6.4$ Hz, 1H; H-1′), 7.32 and 7.34 (2s, each 1H; $2CH_{arom}$). High-resolution MS data for $C_{30}H_{50}{}^{35}ClN_3O_{12}{}^{195}PtS$ (M = 906.245): $[M+H-HCl]^+$: found 871.273; calculated 871.276.

[PtCl(NCN)]-3-(amidoethylthio)propyl β-D-galactopyranoside (**3**): Light yellow solid, 10.7 mg, overall yield 72%. ^{1}H NMR (500 MHz, D_2O): $\delta = 1.91$ (m, 2H; $OCH_2CH_2CH_2S$), 2.71 (brt, 2H; $OCH_2CH_2CH_2S$), 2.83 (brt, 2H; SCH_2CH_2ND), 2.90 (brs, 12H; $2CH_2N(CH_3)_2$), 3.26 and 3.92 (2m, each 1H; $OCH_2CH_2CH_2S$), 3.50 (dd, $J_{1,2} = 7.7$ Hz, $J_{2,3} = 9.7$ Hz, 1H; H-2), 3.59 (m, 2H; SCH_2CH_2ND), 3.63 (dd, $J_{3,4} = 3.5$ Hz, 1H; H-3), 3.91 (brd, $J_{4,5} < 1$ Hz, 1H; H-4), 4.19 (brs, 4H; $2CH_2N(CH_3)_2$), 4.40 (d, 1H; H-1), 7.41 (brs, 2H; CH_{arom}). High-resolution MS data for $C_{24}H_{40}{}^{35}ClN_3O_7{}^{195}PtS$ (M = 744.192): $[M+H-HCl]^+$: found 709.229; calculated 709.224.

[PtCl(NCN)]-3-(amidoethylthio)propyl α-D-mannopyranoside (**4**): White solid, 9.9 mg, overall yield 46%. ^{1}H NMR (500 MHz, D_2O): $\delta = 1.91$ (m, 2H; $OCH_2CH_2CH_2S$), 2.69 (brt, 2H; $OCH_2CH_2CH_2S$), 2.82 (brt, 2H; SCH_2CH_2ND), 2.99 (brs, 12H; $2CH_2N(CH_3)_2$), 3.59 and 3.80 (2m, each 1H; $OCH_2CH_2CH_2S$), 3.61 (brt, 2H; SCH_2CH_2ND), 3.89 (brd, $J_{1,2} < 1$ Hz, $J_{2,3} = 3.2$ Hz, 1H; H-2), 4.18 (brs, 4H; $2CH_2N(CH_3)_2$), 4.82 (brs, 1H; H-1), 7.31 (s, 2H; CH_{arom}). High-resolution MS data for $C_{24}H_{40}{}^{35}ClN_3O_7{}^{195}PtS$ (M = 744.192): $[M+H-HCl]^+$: found 709.222; calculated 709.224.

[PtCl(NCN)]-3-(amidoethylthio)propyl β-D-glucopyranoside (**5**): White solid, 16.1 mg, overall yield 68%. ^{1}H NMR (500 MHz, D_2O): $\delta = 1.93$ (m, 2H; $OCH_2CH_2CH_2S$), 2.72 (brt, 2H; $OCH_2CH_2CH_2S$), 2.78 (brt, 2H; SCH_2CH_2ND), 3.02 and 3.14 (2s, each 6H; $2CH_2N(CH_3)_2$), 3.21 (dd, $J_{1,2} = 7.9$ Hz, $J_{2,3} = 9.1$ Hz, 1H; H-2), 3.42 (brt, 1H; H-3), 3.34 (m, 2H; H-4 and H-5), 3.56 (t, 2H; SCH_2CH_2ND), 3.72 (m, 1H; H-6b), 3.72 and 3.99 (2m, each 1H; $OCH_2CH_2CH_2S$), 3.88 (dd, $J_{5,6a} = 1.3$ Hz, $J_{6a,6b} = 12.1$ Hz, 1H; H-6a), 4.14 and 4.15 (2s, each 2H; $2CH_2N(CH_3)_2$), 4.33 (d, 1H; H-1), 7.32 and 7.33 (2s, each 1H; $2CH_{arom}$). High-resolution MS data for $C_{24}H_{40}{}^{35}ClN_3O_7{}^{195}PtS$ (M = 744.192): $[M+H-HCl]^+$: found 709.228; calculated 709.224.

[PtCl(NCN)]-valine-β-lactosylamide (**7**): A solution of **6** (59.1 mg, 111 μmol) in dry dimethylformamide (1 mL), preactivated for 5 min with TBTU (34.7 mg, 107 μmol) and *N*-ethylmorpholine (21.2 μL, 185 μmol), was added to a solution of β-lactosylamine[46] (12 mg, 37.0 μmol) in dimethylformamide/dimethyl sulfoxide (1:1, 500 μL, *v/v*). The mixture was stirred overnight, and then concentrated in vacuo and coconcentrated with toluene (4×10 mL). A solution of the residue in H_2O (20 mL) was washed with dichloromethane (3× 15 mL), and was then concentrated to a volume of approximately 3 mL and loaded onto a C-18 Extract-Clean column. Side products and salts were eluted with water (15 mL), and **7** with methanol (10 mL). After concentration in vacuo, followed by lyophilization from water, **7** was obtained as a slightly yellow solid (10.1 mg, 32%). ^{1}H NMR (500 MHz, D_2O): $\delta = 0.93$ (d, $J_{H\beta,H\gamma a} = 6.8$ Hz, 3H; CH_3-γa), 0.96 (d, $J_{H\beta,H\gamma b} = 7.0$ Hz, 3H; CH_3-γb), 2.00 (m, 1H; H-β), 2.76 (brs, 12H; $2CH_2N(CH_3)_2$), 3.21 (brs, 1H; H-α), 3.47 (brt, 1H; H-2), 3.56 (dd, $J_{1',2'} = 7.8$ Hz, $J_{2',3'} = 9.9$ Hz, 1H; H-2′), 3.93 (brd, $J_{3',4'} = 3.4$ Hz, $J_{4',5'} < 1$ Hz, 1H; H-4′), 4.24 (s, 4H; $2CH_2N(CH_3)_2$), 4.47 (d, 1H; H-1′), 5.06 (d, 1H; $J_{1,2} = 9.2$ Hz, H-1), 6.98 (s, 2H; $2CH_{arom}$). High-resolution MS data for $C_{30}H_{51}{}^{35}ClN_4O_{11}{}^{195}Pt$ (M, 873.289): $[M+H-HCl]^+$: found 838.324; calculated 838.320.

[Br(NCN)]-3-(amidoethylthio)propyl β-lactoside (**9**): A solution of **8** (29.2 mg, 93.9 μmol) in dry dimethylformamide (1 mL), preactivated for 5 min with TBTU (29.1 mg, 90.8 μmol) and *N*-ethylmorpholine (17.9 μL, 156 μmol), was added to a solution of 3-(amino-

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ethylthio)propyl β-lactoside (15 mg, 31.3 μmol) in dry dimethylformamide (1 mL). The mixture was stirred overnight, and was then concentrated in vacuo and coconcentrated with toluene (4× 10 mL). The product was purified by VLC (dichloromethane/methanol/triethylamine 98:1.5:0.5→95:4:1→90:9:1) to afford **9** as an amorphous white solid (12.4 mg, 54%). ^{1}H NMR (500 MHz, D_2O): δ = 1.91 (m, 2H; $OCH_2CH_2CH_2S$), 2.29 (s, 12H; $2CH_2N(CH_3)_2$), 2.71 (brt, 2H; $OCH_2CH_2CH_2S$), 2.84 (brt, 2H; SCH_2CH_2ND), 3.26 (dd, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.3 Hz, 1H; H-2), 3.54 (dd, $J_{1',2'}$ = 7.8 Hz, $J_{2',3'}$ = 9.9 Hz, 1H; H-2′), 3.66 (dd, $J_{3',4'}$ = 3.3 Hz, 1H; H-3′), 3.73 (s, 4H; $2CH_2N(CH_3)_2$), 3.74 and 3.95 (2m, each 1H; $OCH_2CH_2CH_2S$), 3.92 (brd, $J_{4',5'} < 1$ Hz, 1H; H-4′), 4.42 (d, 1H; H-1), 4.43 (d, 1H; H-1′), 7.68 (brs, 2H; CH_{arom}). High-resolution MS data for $C_{30}H_{50}{}^{79}BrN_3O_{12}S$ (M = 755.230): $[M+H]^+$: found 756.237; calculated 756.238.

[Cl(NCN)]-3-(amidoethylthio)propyl β-lactoside (**11**): A solution of **10** (25.0 mg, 93.9 μmol) in dry dimethylformamide (1 mL), preactivated for 5 min with TBTU (29.1 mg, 90.8 μmol) and *N*-ethylmorpholine (17.9 μL, 156 μmol), was added to a solution of 3-(aminoethylthio)propyl β-lactoside (15 mg, 31.3 μmol) in dry dimethylformamide (1 mL). The mixture was stirred overnight, and was then concentrated in vacuo and coconcentrated with toluene (4× 10 mL). The product was purified by VLC (dichloromethane/methanol/triethylamine 98:1.5:0.5→95:4:1→90:9:1) to afford **11** as a white amorphous solid (8.9 mg, 41%). ^{1}H NMR (500 MHz, D_2O): δ = 1.94 (m, 2H; $OCH_2CH_2CH_2S$), 2.35 (s, 12H; $2CH_2N(CH_3)_2$), 2.75 (brt, 2H; $OCH_2CH_2CH_2S$), 2.88 (brt, 2H; SCH_2CH_2ND), 3.31 (dd, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 9.3 Hz, 1H; H-2), 3.58 (dd, $J_{1',2'}$ = 7.7 Hz, $J_{2',3'}$ = 9.9 Hz, 1H; H-2′), 3.69 (dd, $J_{3',4'}$ = 3.3 Hz, 1H; H-3′), 3.79 (s, 4H; $2CH_2N(CH_3)_2$), 3.75 and 4.00 (2m, each 1H; $OCH_2CH_2CH_2S$), 3.96 (brd, $J_{4',5'} < 1$ Hz, 1H; H-4′), 4.47 (d, 2H; H-1 and H-1′), 7.77 (brs, 2H; CH_{arom}). High-resolution MS data for $C_{30}H_{50}{}^{35}ClN_3O_{12}S$ (M = 711.280): $[M+H]^+$: found 712.290; calculated 712.288.

Benzoyl-3-(amidoethylthio)propyl β-lactoside (**12**): Benzoyl chloride (29.2 μL, 208.4 μmol) was slowly added at 0 °C to a solution of 3-(aminoethylthio)propyl β-lactoside (20 mg, 41.6 μmol) in dry pyridine (4 mL). After 2 h, when TLC analysis (dichloromethane/methanol 9:1) showed the formation of a faster moving spot (R_f 0.23), the mixture was concentrated and then coconcentrated with toluene (4×10 mL). The product was purified by VLC (dichloromethane/methanol 99:1→95:5) to yield **12** as a white solid (20.2 mg, 89%). ^{1}H NMR (500 MHz, D_2O): δ = 1.92 (m, 2H; $OCH_2CH_2CH_2S$), 2.71 (brt, 2H; $OCH_2CH_2CH_2S$), 2.85 (brt, 2H; SCH_2CH_2ND), 3.28 (dd, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 9.5 Hz, 1H; H-2), 3.54 (dd, $J_{1',2'}$ = 7.8 Hz, $J_{2',3'}$ = 9.9 Hz, 1H; H-2′), 3.61 (brt, 2H; SCH_2CH_2ND), 3.63 (dd, $J_{3,4}$ = 9.5 Hz, 1H; H-3), 3.66 (dd, $J_{3',4'}$ = 3.3 Hz, 1H; H-3′), 3.77 and 3.98 (2m, each 1H; $OCH_2CH_2CH_2S$), 3.92 (brd, $J_{4',5'} < 1$ Hz, 1H; H-4′), 4.43 (d, 1H; H-1′), 4.44 (d, 1H; H-1), 7.53, 7.61, and 7.77 (3 m, 2H; 1H; and 2H; 5 CH_{arom}). High-resolution MS data for $C_{24}H_{37}NO_{12}S$ (M = 563.204): $[M+Na]^+$: found 586.189; calculated 586.193.

Preparation of sensor surfaces: CM5 sensor surfaces were equilibrated with Tris-HCl buffered saline (pH 7.5, 10 mM), containing NaCl (150 mM), $CaCl_2$ (2 mM), and $MgCl_2$ (2 mM), and were then activated with a 10 min pulse of a mixture (1:1 *v*/*v*) of *N*-hydroxysuccinimide (0.05 M) and *N*-ethyl-*N*′-(dimethylaminopropyl)carbodiimide (0.2 M), at a flow rate of 5 μL min^{-1}. Con A lectin was attached to channels 1 and 2 by two injections of 7 min (200 μg mL^{-1} in 10 mM sodium acetate buffer, pH 4.8; ≈11 000 response units (RUs) each); remaining *N*-hydroxysuccinimide esters were blocked by a 10 min pulse of ethanolamine hydrochloride (1.0 M, pH 8.5). In a similar way, ≈11 500 RU of RCA_{120} lectin were immobilized to channels 3 and 4. To measure the level of nonspecific binding and to serve as blank channels for mathematical data treatment, Con A bound to channel 2 and RCA_{120} bound to channel 4 were denatured by a 8 min injection of guanidinium chloride (6 M, pH 1.0), followed by a 4 min injection of SDS (0.5%).

SPR detection of saccharides: Free and derivatized saccharides, dissolved at various concentrations (see Results) in Tris-HCl buffered saline (10 mM, pH 7.5) containing NaCl (150 mM), $CaCl_2$ (2 mM), and $MgCl_2$ (2 mM), were allowed to flow across the lectin surfaces for 5 min at a flow rate of 5 μL min^{-1}, and were allowed to dissociate for 8 min. To restore the response level to zero, a regenerating solution (20 μL) was used. To this end, several regenerating solutions were screened for their effectiveness towards the release of PtCl(NCN–R)-labeled oligosaccharides. The best results were obtained with a mixture of methyl α-D-mannopyranoside (25 μM; Con A) and methyl β-D-galactopyranoside (25 μM; RCA_{120}) in Tris-HCl buffer (pH 7.5).

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