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Redox-responsive and calcium-dependent switching of glycosyldisulfide interactions with Concanavalin A

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Abstract—Glycosyldisulfides can interact efficiently with carbohydrate-binding entities. This has been shown for a range of thiosaccharide dimers when tested for their effects against the lectin Concanavalin A using a modified quartz crystal microbalance-technique. Contrary to the thiosaccharide monomers, showing no significant binding up to 10 mM, several of the dimers showed IC_{50}-values in the low millimolar range. Three of the glycosyldisulfides tested also displayed very high positive apparent cooperativity effects that were found to be both calcium-dependent and redox-responsive.

Thiosaccharides, where a hydroxyl group is replaced by a thiol functionality constitute an interesting group of compounds in glycochemistry, possessing unique characteristics compared to their oxygen-containing counterparts. These compounds are often used as efficient glycoside donors and acceptors in oligosaccharide- and neoglycoconjugate synthesis,[1–8] owing to the fact that the thiolate is a potent nucleophile and a weak base that react easily and selectively with soft electrophiles.[9] The resulting thioglycosides possess furthermore increased resistance to degradation by glycosidases and hence have potential as therapeutics.[10] Thiol-containing carbohydrates may also be subjected to mild oxidation in forming disulfide-bridged dimers, and glycosyldisulfides have more recently been identified as efficient glycosyl donors and potentially useful glycomimetics.[11–13] Glycosyldisulfides can occupy a larger conformational space, as compared to natural glycosides, due to the increased flexibility and extended length of the disulfide bond.[11,12] This, together with their differences in electronic properties, make glycosyldisulfides interesting in exploring their potential in biological interactions. However, the recognition properties of thiol- and disulfide-containing carbohydrates have not been evaluated. For this reason, a range of 1-thio- or 6-thiosaccharides were tested in the present study for their inhibitory effects against the common plant lectin Concanavalin A (Con A).[14–16] The thiol monomers as well as the easily accessible glycosyldisulfides were subjected to the inhibition studies. The recognition properties were simply monitored using a modified quartz crystal microbalance- (QCM-) technique, a highly powerful means to monitor adsorption/binding events to surfaces.[17–20]

Six thiosaccharide monomers, together with their corresponding disulfide-bridged dimers, were evaluated (Fig. 1). These include the 1-thio-derivatives of five common carbohydrates D-mannose (**1**), D-glucose (**2**), D-galactose (**3**), *N*-acetyl-D-glucosamine (**4**) and *N*-acetyl-D-galactosamine (**5**). Since Con A is selective for mannose-containing carbohydrate structures,[14–16,21] methyl-6-thio-α-D-mannopyranoside (**6**) was also evaluated. The syntheses of these compounds were easily performed in few steps from the suitably derivatised carbohydrates by reaction with thioacetate, and subsequent deprotection. The corresponding dimers, **1**–**1** to **6**–**6**, were easily generated from the monomers by mild oxidation with hydrogen peroxide.

The inhibitory effects of the thiosaccharides were monitored by a flow-through QCM-system, using a modified mannan-based protocol commonly used in Con A analysis.[17] Briefly, gold-plated quartz crystals were first coated with polystyrene, and then coated with mannan

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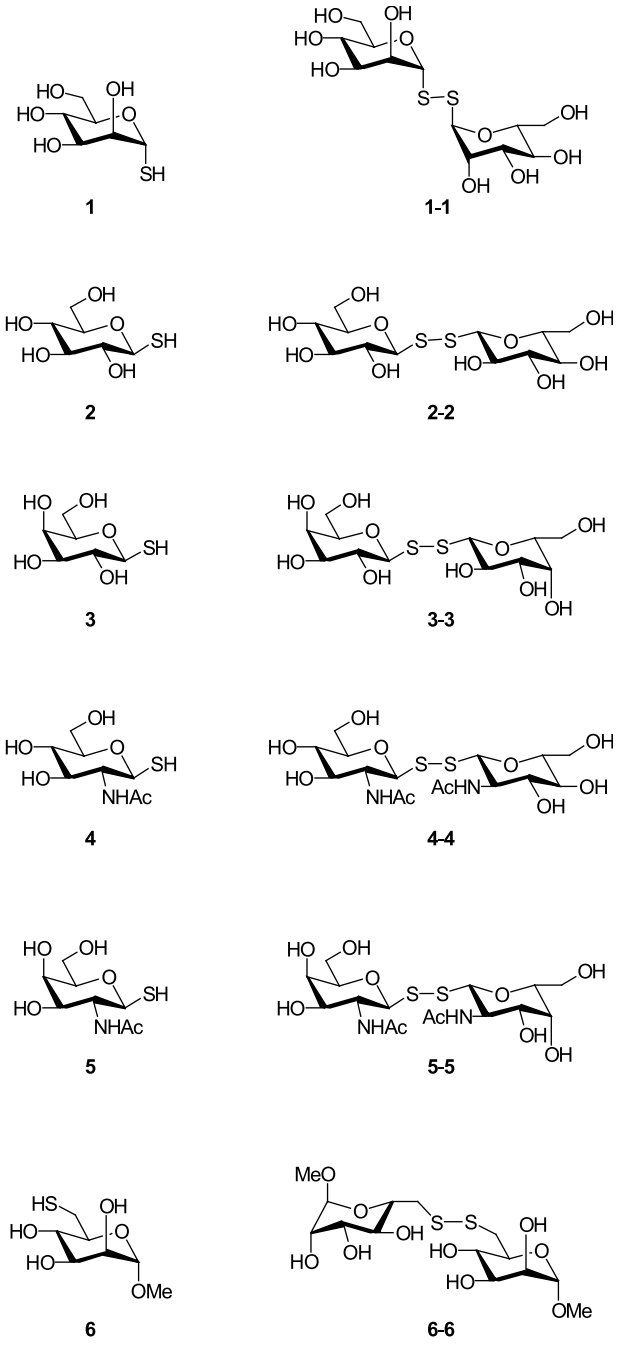

Figure 1. Thiosaccharides and glycosyldisulfides tested.

while mounted online. Binding studies were performed on the same crystal using competitive binding between the mannan and the tested ligands at physiological pH in phosphate-buffered saline. Samples were prepared with Con A and the different ligands and injected over the QCM crystal consecutively. The frequency shift after each injection was recorded, and the bound Con A was subsequently released from the mannan-surface by washing with phosphate buffer at lower pH.

Competition assays were performed over a concentration range from 1 μM to 10 mM, and the inhibitory effects were quantified as IC_{50}-values and Hill coefficients, respectively. For comparison, both the thiol monomers and the glycosyldisulfides were subjected to the inhibition studies. In order to maintain reducing conditions while analysing the thiol monomers, a minimal amount of dithiothreitol (DTT), showing no influence on the binding in itself, was added to the samples. The resulting inhibitory effects from the thiol monomers were very weak. With the exception for 1-thio-α-D-mannopyranose (**1**), displaying weak inhibition at 10 mM, none of the thiosaccharide monomers displayed any significant inhibition in the concentration range tested. For comparison, the inhibitory effects of D-mannose and methyl-α-D-mannopyranoside (IC_{50}: 5.3 and 1.1 mM, respectively),[17] well indicate the importance of a hydroxyl group in the 1- and 6-position for these structures, as compared to a thiol moiety.

In contrast to the thiol monomers, several of the dimers proved efficient (Table 1). The 1-thio-α-D-mannose dimer (**1**–**1**) showed an IC_{50}-value of 1.2 mM, considerably lower than the 6-thio-derivative (**6**–**6**), which proved essentially inefficient as expected from the monomer effect. On the other hand, 1-thio-β-D-glucose-, 1-thio-β-D-galactose- and *N*-acetyl-1-thio-β-D-glucosamine-based dimers displayed relatively high inhibitory effects, despite the total lack of response of the corresponding monomers. 1-Thio-β-D-glucose- (**2**–**2**), and 1-thio-β-D-galactose- (**3**–**3**) dimers displayed very similar effects with IC_{50}-values approximately of the same order as the 1-thio-α-D-mannose dimer (**1**–**1**), whereas the *N*-acetyl-1-thio-β-D-glucosamine dimer (**4**–**4**) proved slightly less efficient (IC_{50}: 2.0 mM). Interestingly, the corresponding *N*-acetyl-1-thio-β-D-galactosamine derivative (**5**–**5**) showed no inhibition under these conditions.

Similar to D-mannose and methyl-α-D-mannopyranoside, the 1-thio-α-D-mannose dimer (**1**–**1**) displayed a binding pattern with no apparent cooperativity effects (Table 1). On the other hand, ligands **2**–**2**, **3**–**3** and **4**–**4** displayed strong positive apparent cooperativity effects in the binding to Concanavalin A with Hill coefficients up to 6.2 (Fig. 2, Table 1). These unexpected and unprecedented effects suggest that the 4-subunit lectin undergoes a dramatic change upon interacting with these ligands in the present system. Previous investigations with Con A-binding ligands have however not

Table 1. Estimation of IC_{50} values for carbohydrate structure based on consecutively diluted concentrations of tested inhibitors in the QCM-assay with Con A

Glycosyldisulfide	Inhibition IC_{50}/mM[a,b]	Hill coefficient[a,b]
1–**1**	1.2	1.0
2–**2**	1.4	6.1
3–**3**	1.4	6.2
4–**4**	2.0	5.2
5–**5**	na	na
6–**6**	na	na

None of the thiosaccharide monomers **1**–**6** showed any strong inhibitory effect in the concentration range tested.

[a] na = not active.

[b] The program GraphPad Prism (GraphPad Software) was adopted for nonlinear regression analysis determining IC_{50} values and Hill coefficients.

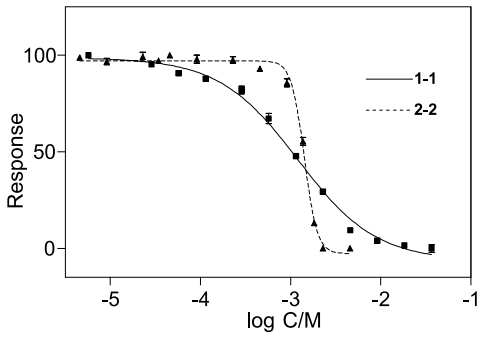

Figure 2. Inhibitory responses of Con A/mannan binding in presence of substances **1**–**1** and **2**–**2**. All experiments were carried out in at least duplicate at ambient temperature using an Attana 80 QCM instrument (Attana AB, Stockholm, Sweden).

shown strong cooperativity effects, and rather a negative effect have been found for oligovalent ligands.[22]

Since Concanavalin A is a metal ion-dependent lectin, with metal ion-binding sites for, preferentially, calcium and a transition metal ion (e.g., manganese) situated in close proximity to the carbohydrate-binding pocket,[14,21] the calcium-dependence of the binding was further evaluated. Carbohydrates may form complexes with calcium,[23,24] and the effects observed could potentially arise from interactions with the calcium binding site, or with the calcium ions per se. Demetallisation of Con A strongly influences the carbohydrate binding, and may also induce structural changes.[25,26] Thus, all active ligands were also tested in presence of excess calcium ions. The resulting effects indeed indicated a strong calcium-dependence, where none of the cooperativity-inducing ligands showed any inhibitory effect in the presence of calcium (Fig. 3). These results suggest that these three thiosaccharide dimers interfere primarily with the calcium binding, and not with the carbohydrate-binding site.

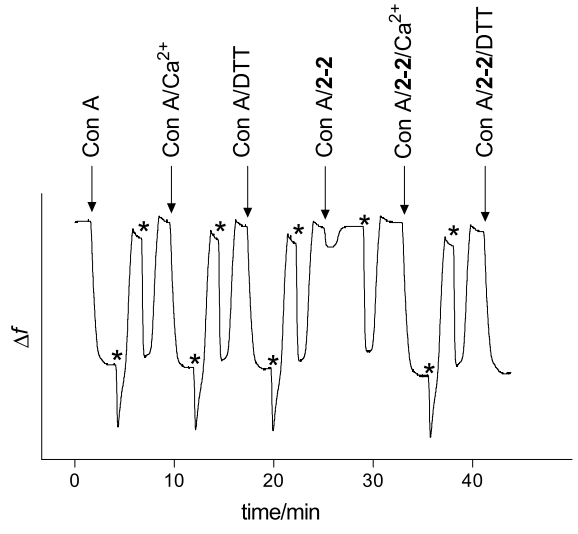

Figure 3. QCM frequency shifts upon addition of Con A in combination with ligand **2**–**2** (2.3 mM). Negative frequency shifts indicate binding. Arrows indicate different injections: Con A alone (Con A), and in combination with Ca^{2+} (Con A/Ca^{2+}), or DTT (Con A/DTT), show identical response indicating no calcium- or DTT dependence. Con A in presence of **2**–**2** results in complete inhibition (Con A/**2**–**2**). Con A in presence of **2**–**2** and Ca^{2+} eliminates inhibition and restores full QCM response (Con A/**2**–**2**/Ca^{2+}). The inhibition could also be reversibly eliminated by addition of DTT (Con A/**2**–**2**/DTT). * indicates washing steps.

The inhibition associated with the strong apparent cooperativity was also found to be fully reversible. When compound **3**–**3** was first allowed to interact with Con A at a concentration showing complete inhibition, and subsequently treated with DTT, the inhibitory effect was eliminated and the mannan-binding activity of Con A fully restored (Fig. 3). This redox controlled phenomenon could potentially be used to specifically turn on or turn off the ligand binding ability of Con A, creating a carbohydrate binding switch.

In conclusion, the present study has shown that thiosaccharide dimers display inhibitory effects against the common plant lectin Concanavalin A. The 1-thio-α-D-mannose dimer showed an IC_{50}-value in the low millimolar range but with no apparent cooperativity. On the other hand, 1-thio-β-D-glucose-, 1-thio-β-D-galactose- and *N*-acetyl-1-thio-β-D-glucosamine-based dimers displayed very high positive apparent cooperativity effects. Contrary to these findings, the corresponding *N*-acetyl-1-thio-β-D-galactosamine-based dimer was inefficient at the concentration levels tested. These unprecedented effects was shown to depend mainly on the calcium-levels of the system, with no effects observed in the presence of calcium ions. The results also indicate that glycosyldisulfides may indeed be useful glycomimetics in exploring carbohydrate-binding entities. These structures are easily accessible from the corresponding thiols, which can be used as building blocks in the rapid formation of extended libraries. Thiosaccharides also offer potential to reversible systems, where thiol-disulfide exchange can be used as a means to generate dynamic chemistry.[27,28]

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References and notes

1. Rich, J. R.; Bundle, D. R. *Org. Lett.* **2004**, *6*, 897–900.
2. Rye, C. S.; Withers, S. G. *Carbohydr. Res.* **2004**, *339*, 699–703.
3. Zhu, X.; Stolz, F.; Schmidt, R. R. *J. Org. Chem.* **2004**, *69*, 7367–7370.
4. Liakatos, A.; Kiefel, M. J.; von Itzstein, M. *Org. Lett.* **2003**, *5*, 4365–4368.
5. Greffe, L.; Jensen, M. T.; Chang-Pi-Hin, F.; Fruchard, S.; O'Donohue, M. J.; Svensson, B.; Driguez, H. *Chem. Eur. J.* **2002**, *8*, 5447–5455.
6. Knapp, S.; Myers, D. S. *J. Org. Chem.* **2002**, *67*, 2995–2999.
7. Marcaurelle, L. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2001**, *123*, 1587–1595.
8. Crich, D.; Li, H. *J. Org. Chem.* **2000**, *65*, 801–805.

9. Norberg, T. In *Modern Methods in Carbohydrate Synthesis*; Khan, S. H., O'Neill, R. A., Eds.; Harwood Academic: Amsterdam, 1996, pp 82–106.
10. Driguez, H. *ChemBioChem* **2001**, *2*, 311–318.
11. Davis, B. G.; Ward, S. J.; Rendle, P. M. *Chem. Commun.* **2001**, 189–190.
12. Szilagyi, L.; Illyes, T.-Z.; Herczegh, P. *Tetrahedron Lett.* **2001**, 3901–3903.
13. Hummel, G.; Hindsgaul, O. *Angew. Chem., Int. Ed.* **1999**, *38*, 1782–1784.
14. Rudiger, H.; Gabius, H. J. *Glycoconj. J.* **2001**, *18*, 589–613.
15. Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637–674.
16. Elgavish, S.; Shaanan, B. *Trends Biochem. Sci.* **1997**, *22*, 462–467.
17. Pei, Z.; Anderson, H.; Aastrup, T.; Ramstrom, O. *Biosens. Bioelectron.*, in press, doi:10.1016/j.bios.2004.10.006.
18. Marx, K. A. *Biomacromolecules* **2003**, *4*, 1099–1120.
19. Janshoff, A.; Galla, H. J.; Steinem, C. *Angew. Chem., Int. Ed.* **2000**, *39*, 4004–4032.
20. O'Sullivan, C. K.; Guilbault, G. G. *Biosens. Bioelectron.* **1999**, *14*, 663–670.
21. Naismith, J. H.; Field, R. A. *J. Biol. Chem.* **1996**, *271*, 972–976.
22. Dam, T. K.; Roy, R.; Page, D.; Brewer, C. F. *Biochemistry* **2002**, *41*, 1351–1358.
23. Yang, L.; Su, Y.; Xu, Y.; Wang, Z.; Guo, Z.; Weng, S.; Yan, C.; Zhang, S.; Wu, J. *Inorg. Chem.* **2003**, *42*, 5844–5856.
24. Bugg, C. E. *J. Am. Chem. Soc.* **1973**, *95*, 908–913.
25. Bouckaert, J.; Poortmans, F.; Wyns, L.; Loris, R. *J. Biol. Chem.* **1996**, *271*, 16144–16150.
26. Brown, R. D., III; Koenig, S. H.; Brewer, C. F. *Biochemistry* **1982**, *21*, 465–469.
27. Pei, Z.; Larsson, R.; Ramström, O. In *15th International Conference on Organic Synthesis (ICOS-15)*; Nagoya, Japan, 2004.
28. Ramström, O.; Lehn, J.-M. *Nat. Rev. Drug Disc.* **2002**, *1*, 26–36.