Disaccharide recognition by binuclear copper(II) complexes†

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The sugar recognition by binuclear copper(II) complexes in solution is strongly dependent on secondary interactions and cannot be predicted from the intermetallic Cu···Cu distance.

The molecular recognition of carbohydrates in water is an intriguing subject due to the important role of saccharides in biological activities, such as intercellular recognition, signal transduction, fertilization or as targets of bacterial/viral infection of cells.[1–5] Considerable efforts have been directed towards understanding and mimicking these recognition events, as well as developing effective agents to control such processes.[2,6–9] Selective carbohydrate binding in water, however, remains one of the most significant challenges in the field of molecular recognition in spite of all efforts.[6]

While exploring the use of transition metal complexes for carbohydrate binding and differentiation in aqueous solution, we identified a sugar-discriminating binuclear Cu(II) complex.[10] Based on these results, *we hypothesized that the ligand backbone structure has a significant effect on the complex geometry, and therefore on its sugar recognition ability*. As a proof of concept, we studied the binding of selected disaccharides to three structurally related binuclear Cu(II) complexes, namely the symmetric complexes Cu_2(TEGbsdpo)(OAc) (**1**)[11] and Cu_2(bpdpo)(OAc)$(ClO_4)_2$ (**2**),[10] and asymmetric complex Cu_2(bpdbo)(OAc)$(OH_2)(ClO_4)_2$ (**3**).‡

Although crystal structures of the symmetric complexes **1** and **2** are not known yet, single crystals of their slightly modified derivatives Cu_2(EGbsdpo)(OAc) (**1a**)§ and Cu_2(bpdpo)-(DPP)$(ClO_4)_2(CH_3OH)$ (**2a**, DPP = diphenyl phosphate)[12] are characterized by X-ray diffraction (Fig. 1a and ESI†).[13]

The crystal structure analysis of complex **1a** reveals a symmetric square planar arrangement of both Cu(II) ions. Gajda *et al.* showed, by contrast, a significantly distorted geometry around the Cu(II) ions for complex **2a** in spite of the symmetry of the pentadentate bpdpo ligand.[12] One of the Cu(II) ions in **2a** is bound in a distorted octahedral structure; the geometry around the second Cu(II) ion is distorted square pyramidal.[12] Both derivatives **1a** and **2a** show a comparable intermetallic Cu···Cu distance in the solid state, which should be preserved for complexes **1** and **2** used in this investigation (Cu···Cu = ~3.50 Å).

The complex Cu_2(bpdpo) **2** and asymmetric complex Cu_2(bpdbo) **3** have similar pentadentate pyridine-based backbone ligands, but different intramolecular Cu···Cu distances due to the asymmetry in **3** (Cu···Cu = 3.39 Å).§ The structural analysis of complex **3** reveals a square pyramidal geometry for one of the Cu(II) ions and a distorted square planar geometry around the other Cu(II) ion (Fig. 1b and ESI†).

While the backbone ligands of the complexes **2** and **3** are different by one methylene group altering the intermetallic distance (*vide infra*), the overall geometries of both complexes are alike. Provided that the observed structures and intermetallic Cu···Cu distances in the solid state are maintained in solution, a comparison of the sugar recognition abilities of the complexes **1–3** will reveal the contribution of the ligand backbone structures to their carbohydrate recognition ability.

Prior to investigating the sugar recognition ability in solution, the speciation of the complexes **1–3** in water was derived from UV/Vis spectra by the method of Zuberbühler (see ESI†).[10,11,14] The study revealed a different amount of hydroxyl ions coordinated to the Cu(II) centers in **1–3** and different resulting overall charges of the complexes at pH 10 that was subsequently used for the investigation of the carbohydrate coordination (Fig. 2). Isothermal titration calorimetry (ITC) was employed to characterize the *stoichiometry of complexation* and the *binding sites of selected disaccharides* during coordination to **1–3**.

Towards this goal, lactulose (**4**), a synthetic disaccharide known as a potent laxative, was used as a model compound (Fig. 3). Disaccharide **4** contains a 1→4 β-linked non-reducing galactopyranosyl and a reducing fructose moiety. The titration of **4** into a solution of **1** (**2** or **3**) indicated coordination of the carbohydrate with multiple *dependent* binding sites to the

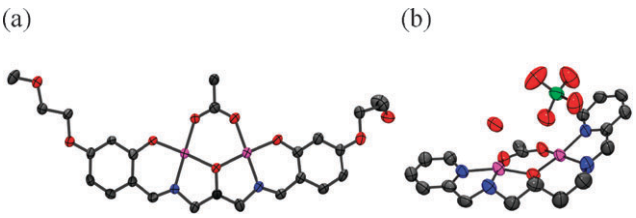

Fig. 1 Structure of (a) complex **1a** and (b) complex **3** in the solid state; ellipsoids with 70% probability; C atoms are shown in gray, Cu in magenta, N in blue, O in red; Cl in green; one weakly coordinated perchlorate ion and all hydrogen atoms are omitted for clarity.

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† Electronic supplementary information (ESI) available: Experimental details for the synthesis of the complexes **1a** and **3**, the ITC study for sugar binding, CD spectra and species distribution curves for complexes **1** and **3**; a table with all binding constants, and figures of the structural analysis of **1a** and **3**. CCDC 699735 and 699736. For ESI and crystallographic data in CIF or other electronic format, see DOI: 10.1039/b813356e

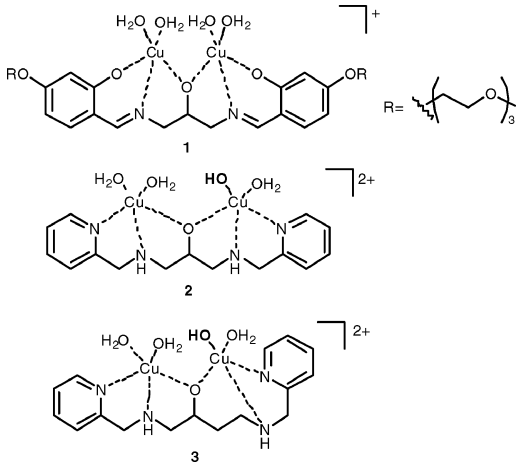
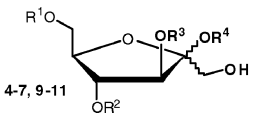


Fig. 2 Composition of the binuclear copper(II) complexes **1–3** in CHES buffer at pH 10.



Compound	R^1	R^2	R^3	R^4
4	H	α-D-gal*p*	H	H
5	H	H	H	H
6	H	α-D-glc*p*	H	H
7	α-D-glc*p*	H	H	H
9	H	H	α-D-glc*p*	H
10	H	H	H	α-D-glc*p*
11	H	H	α-D-glc*p*	α-D-glc*p*

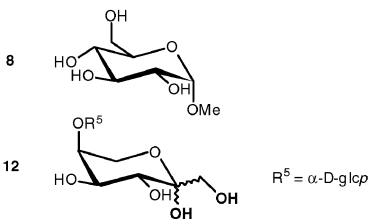


Fig. 3 Structures of selected saccharides; putative binding sites highlighted in bold.

metal complex. Saturation of the metal binding sites is achieved when the molar ratio between carbohydrate and binuclear metal complex exceeds 1 : 1. A similar coordination is observed for complexes derived from **1**, **2** or **3** and other carbohydrates, namely D-fructose (**5**), maltulose (**6**) or palatinose (**7**). Both disaccharides **6** and **7** contain a reducing fructose moiety like **4** (Fig. 3).

The overall binding strength K_{app} for the complexes is described by the sum of all apparent association constants of all sites.[15] The apparent binding strength of complex **1–4** ($K_{app}^{1-4} = 2.3 \times 10^4\ M^{-1}$) is slightly higher than those determined for the complexes **2–4** ($K_{app}^{2-4} = 1.3 \times 10^4\ M^{-1}$) or **3–4** ($K_{app}^{3-4} = 9 \times 10^3\ M^{-1}$). However, the overall binding strength of the resulting complexes remains identical, when **1** (or **2**) coordinate to the disaccharides **4** (**6** or **7**), and is typically about half an order of magnitude higher than for complexes formed with **5**. The complexes derived from the asymmetric complex **3** and the carbohydrates **4** (**5**, **6**, or **7**) show comparable binding strengths ($K_{app}^{3-n} \approx 4.5 \times 10^3\ M^{-1}$; n = **4–7**) that are in general slightly lower than those for complexes derived from symmetric **1** or **2**. The observed apparent binding constants, binding enthalpies and entropies of all titrations are summarized in Table 1 (ESI†).

Titration of α-methyl-D-glucopyranoside (**8**) or of disaccharides with glucose as the reducing sugar does not result in detectable complexation with the complexes **1–3**. These observations indicate that the fructose moiety in **4**, **6** or **7** promote coordination to the binuclear metal core in **1–3**. Weak binding of turanose (**9**) and negligible complexation with the non-reducing disaccharide sucrose (**10**) and the trisaccharide melizitose (**11**) furthermore indicate coordination of the disaccharides **4**, **6** or **7** to the complexes **1–3** *via* the hydroxyl groups at C-1, C-2 and C-3 of the reducing sugar.

In subsequent studies we focused on evaluating, which of the equilibrium structures is predominantly involved in complexation to the metal complexes **1–3**. Due to mutarotation, a hexose exists in solution in equilibrium structures including pyranoses, furanoses and an open chain form. Fructose (**5**), the reducing sugar moiety in the disaccharides **4**, **6** or **7**, exists in solution preferably in the pyranose form (α-pyranose 2.5% and β-pyranose 65%), and is in equilibrium to a smaller extent present in a furanose (α-furanose 6.5% and β-furanose 25%) and open-chain (0.8%) form.[16] Coordination between a metal complex and a carbohydrate in the open-chain form has been previously shown to be insignificant.[17] Fixing a sugar moiety in one of the equilibrium structures and evaluating the remaining complexation ability therefore provides valuable insight into the sugar form during coordination.

Towards this goal, leucrose (**12**), α-D-glc*p*(1→5)fru, was employed. The fructose moiety of **12** exists in β-pyranose form only,[18] while the 1→5 linkage of the sugar moieties inhibits the formation of a furanose form. Weak or no binding interaction between **12** and the metal complexes **1–3** is observed under the provided conditions rendering the contribution of the fructopyranose form to the complexation insignificant. The result gives furthermore indirect evidence for the complexation between **4**, **6** or **7** and **1–3** in the *fructofuranose* form of the disaccharides.

To evaluate the overall complex structure upon coordination, circular dichroism (CD) studies were employed during complexation of the binuclear complexes **1–3** with 5 to 10-fold molar excess of all binding mono- and disaccharides. Surprisingly, *different* Cotton effects, and thus distinctly different overall structures for the resulting sugar–**1** and sugar–**2** complexes were observed, while those for sugar–**2** and sugar–**3** are comparable (Fig. 4).

This observation is remarkable in several aspects: (i) the binding sites of the carbohydrates **4**, **5**, **6** and **7** upon chelation to the metal complexes are identical; (ii) the binding strength of the resulting complexes is of the same order of magnitude as revealed by ITC (*vide supra*); and (iii) the distance between the copper(II) ions in the complex cores is identical for **1** and **2** in the solid state, while the distance between the copper(II) ions in the core of **2** and **3** is different. Thus, the intermetallic Cu···Cu distance is less significant for the resulting overall complex structure than the geometry of the binuclear Cu(II) complexes, which discriminates **1** from **2** and **3** (ESI†).

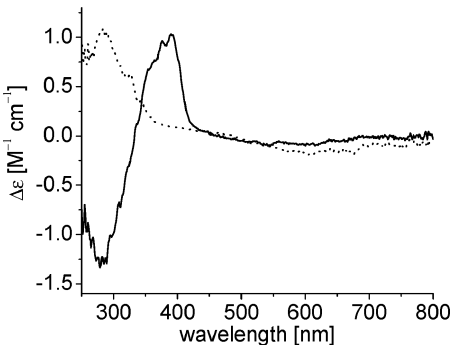


Fig. 4 CD spectra for the complexes **1–4** (solid line) and **2–4** (dotted line).

As the CD spectra reveal a different overall geometry of the complexes formed from the selected saccharides and **1** or **2**, different binding modes of the structures of the resulting complexes are most likely. These might be caused by secondary interactions, such as π–π stacking or hydrophobic interactions, between the carbohydrate and the ligand backbone or the ligand backbone itself. Current efforts are directed towards understanding the discovered phenomenon further and will employ computational methods to predict putative structures in solution.

In summary, this study underlines the significantly different interactions of carbohydrates with structurally related binuclear metal complexes in spite of identical binding sites of the carbohydrate, and comparable intermetallic Cu···Cu distances in two binuclear metal complexes. Minimal changes in the composition of a binuclear Cu(II) complex have therefore far-reaching implications on their molecular recognition abilities with carbohydrates and cannot be predicted from the intermetallic Cu···Cu distance. Thus, the development of potential drugs that interfere with carbohydrate-mediated intercellular recognition, signal transduction, or bacterial and/or viral infection of cells involves careful consideration of actual complex structures in solution, but might in turn allow fine tuning of the resulting recognition events by introducing secondary interactions.

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Notes and references

‡ TEGbsdpo = 6,6′-(1*E*,1′*E*)-(2-hydroxypropane-1,3-diyl)bis(azan-1-yl-1-ylidene)bis(methan-1-yl-1-ylidene)bis(3-(2-(2-(2-methoxyethoxy)-ethoxy)ethoxy)phenol); EGbsdpo = 6,6′-(1*E*,1′*E*)-(2-hydroxypropane-1,3-diyl)bis(azan-1-yl-1-ylidene)bis(methan-1-yl-1-ylidene)bis(3-(2-methoxyethoxy)phenol); bpdpo = 1,3-bis(pyridin-2-ylmethylamino)-propan-2-ol; bpdbo = 1,4-bis(pyridin-2-ylmethylamino)butan-2-ol.

§ X-Ray structural analyses: $Cu_2(EGbsdpo)(OAc)$ (**1a**): green rectangular, crystal dimensions 0.128 × 0.076 × 0.024 mm, triclinic, $P1'$, $Z = 2$, $a = 6.381(2)$, $b = 10.723(4)$, $c = 19.732(7)$ Å, $\beta = 91.898(7)^\circ$, $V = 1283.5(8)$ Å^3 ($T = 193$ K), $\mu = 17.14$ cm^{-1}, $R1 = 0.0523$, w$R2 = 0.1151$; $Cu_2(bpdbo)(OAc)(OH_2)(ClO_4)_2$ (**3**): blue platelet, crystal dimensions 0.304 × 0.250 × 0.168 mm, monoclinic, $P2_1/n$, $Z = 4$, $a = 15.0763(17)$, $b = 12.2502(14)$, $c = 15.3221(18)$ Å, $\beta = 116.020(2)^\circ$, $V = 2543.0(5)$ Å^3 ($T = 153$ K), $\mu = 19.51$ cm^{-1}, $R1 = 0.0584$, w$R2 = 0.1830$; Bruker APEX CCD diffractometer: $\theta_{max} = 56.62^\circ$ (**1a**) and 56.68° (**3**), MoKα, $\lambda = 0.71073$ Å, 0.3° ω scans, 13 126 (**1a**) and 24 008 (**3**) reflections measured, 6310 (**1a**) and 6303 (**3**) independent reflections all of which were included in the refinement. The data were corrected for Lorentz-polarization effects and for absorption (SHELXPREP), solutions were solved by direct methods, anisotropic refinement of F^2 by full-matrix least-squares, 343 parameters. Further details of the crystal structure investigations may be obtained from the CIF files.†

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