Dynamic Biopolymers

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Glycodynamers: Fluorescent Dynamic Analogues of Polysaccharides**

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Dedicated to Teruaki Mukaiyama on the occasion of his 80th birthday

Constitutional dynamic chemistry (CDC)[1] relies on the implementation of reversible chemical connections of either a covalent or noncovalent nature to link the components of a molecular or supramolecular entity. As a consequence, constitutionally dynamic entities are able to continuously modify their constitution by assembly/disassembly of their building blocks, thus generating a dynamic set of interconvertible constituents, namely, a constitutional dynamic library (CDL).[2,3]

The implementation of CDC in material science, in particular in polymer chemistry, led to the introduction of the notion of dynamic polymers (dynamers), which result from the reversible connection of monomers through either covalent or noncovalent linkages.[4–8] Such equilibrium polymers have the ability to undergo changes in their constitution, length, and sequence which can induce a modification of the properties (for example, mechanical[6] or optical[7]) of the material.

Dynamers based on components of a biological nature may generate dynamic analogues of natural polymers (biodynamers) that offer the possibility to combine the functional properties (recognition, catalysis) of naturally occurring polymers with the adaptive behavior of constitutional dynamic systems. Therefore, in view of the prospects offered, the incorporation of biologically relevant moieties into dynamic polymers deserves close scrutiny.[8]

In addition to proteins and nucleic acids, polysaccharides represent a third class of biopolymers. Oligo- and polysaccharides are involved in a wide range of biological or pathological events such as immune response, inflammation, cancer, cell adhesion, and cell–cell recognition.[9] These recognition processes involve, in particular, multivalent carbohydrate–protein interactions.

Various scaffolds have been used to display sugars in a multivalent fashion so as to mimic or improve the binding efficiency of natural carbohydrate ligands. Glycoclusters[10] have been obtained by appending saccharide residues to β-cyclodextrin[11] and calixarene cores.[10] Other approaches have involved, for example, combinatorial glycosamino acids[12] and oligosaccharide[13] derivatives, dynamic monolayers,[14] a polyrotaxane,[15] self-assembling glycodendrimers,[16] and conjugating carbohydrate residues to a polymerizable scaffold, to prepare glycopolymers.[17]

Extending our work on dynamers[4,5a,6,7] to the biopolymer area,[8b] we were interested in designing a dynamic analogue of such glycopolymers (glycodynamers) that could confer an adaptive character which would enable them to modify their constitution in response to external physical or chemical stimuli (temperature, pH, etc.) or to the presence of a (bio)chemical template.

Such biodynamers may be of three types: 1) main-chain, which result from polycondensation of saccharide residues through reversible reactions; 2) side-chain, where the saccharide residues are either a) appended on a dynamic main chain or b) the saccharide residues are reversibly grafted on to a nondynamic main chain; and 3) "doubly dynamic", which incorporate both main-chain and side-chain dynamics.

A reversible condensation reaction of special interest is the formation of an imine-type double bond by reaction of an amino group and a carbonyl group. The formation of oximes has been used in the appending of multiple saccharide groups[18] and in glycoblotting.[19] The formation of poly(acylhydrazone)s[20] and polyoximes[21] illustrate that polymers incorporating carbohydrate-based monomers can be obtained. Ditopic saccharide monomers yield reversible polymers with a bisboronic acid,[8a] and a dynamic library of cyclic sugar oligomers has been generated by formation of an acylhydrazone.[22]

We present here our results on the generation of glycodynamers of type 2a (above) and describe their remarkable physical properties as well as the dynamic modulation of the properties. The starting monomers consist of aromatic bishydrazides **1a** and **1b** as well as dialdehydes **2a** and **2b** which are decorated with oligosaccharide groups (Scheme 1). Monomer **1a** bears a maltohexaose moiety, derived from α-cyclodextrin, which was chosen, in addition to its biorecognition properties, to confer a well-defined secondary structure to the resulting polymer.

The four possible glycodynamers **1a**·**2a**, **1a**·**2b**, **1b**·**2a**, and **1b**·**2b** were obtained by polycondensation of a dilute solution of dialdehyde **2a** or **2b** and dihydrazide monomers **1a** or **1b** in D_2O (5 mM) under mildly acidic conditions, typically pD = 4–6 (for ^1H NMR spectra see Ref. [23]).

In addition to GPC measurements on **1a**·**2a**, **1a**·**2b**, **1b**·**2a**, and **1b**·**2b**,[23] the structure of these dynamic glycopolymers was characterized in more detail[23] by small-angle neutron scattering (SANS) and cryo-TEM experiments on polymer **1a**·**2b**.[24] Analysis of the scattering data for polymer **1a**·**2b**

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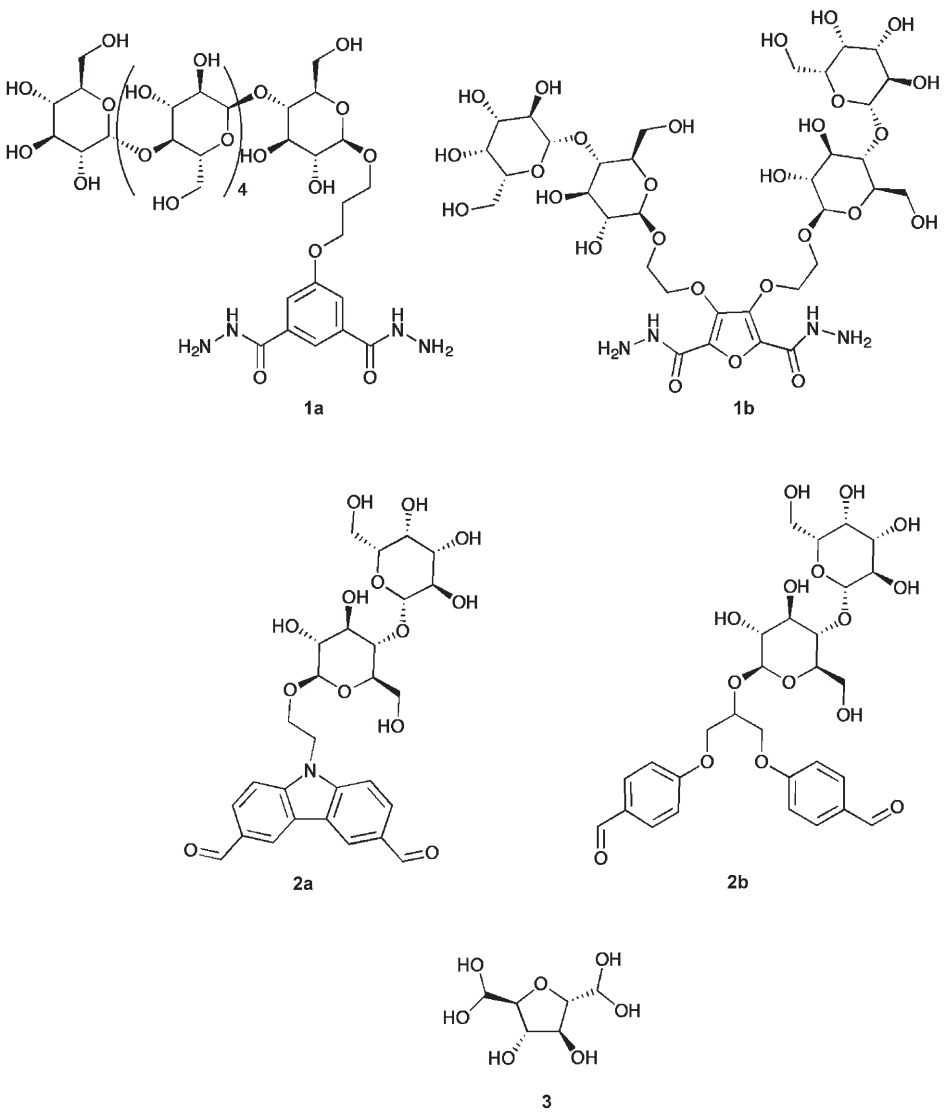

Scheme 1. Molecular structures of the bishydrazides **1a**,**b** and of the dialdehydes **2a**,**b** and **3**.

agrees with it having a rigid rodlike structure with a radius of gyration of 187 Å and an average contour length of 65 nm. Its average molecular weight was about 511 000 g mol^{-1}, which corresponds to 275 monomeric units for an initial monomer concentration of 5 mM at pD = 4. Cryo-TEM observations showed wormlike structures of 40–50 Å diameter[23] together with spherical aggregates of similar diameter. These dimensions are in agreement with the expected diameter of a single polymer chain with a bottlebrush-like shape, as reported for single polystyrene chains decorated with similar oligosaccharide side chains.[25] Such a shape can be expected to confer interesting biorecognition properties to the resulting glycopolymer, as previous examples of this type of polymer were found to have the potential to bind lectin[26] and were used as cell-culture media.[27]

Whereas **2a** was the only monomer to show significant fluorescence, all four polymers displayed remarkable fluorescence, with emission ranging from blue for **1a·2b** (λ_{max} = 457 nm) to green for **1a·2a** (λ_{max} = 495 nm) and **1b·2b** (λ_{max} = 499 nm), and to red for **1b/2a** (λ_{max} = 567 nm; Figure 1).

These fluorescence properties likely result from the tightly packed structure of the polymer, with the aromatic chromophores isolated and rigidly held in the hydrophobic core. Indeed, the ^{1}H NMR signals of the main chain are so broadened that they can no longer bee seen, whereas those of the flexible saccharide side chains are still observable. Furthermore, the fluorescence intensity markedly decreases (by a factor of more than three) upon heating the polymer solution from 23 °C to 85 °C; this effect is presumably due to the destabilization of the highly packed structure.[28] A similar result is obtained on dissolving polymer **1a·2b** in DMSO, a solvent which is expected to lead to "denaturation".[23]

Demonstration of the dynamic character of the present glycopolymers was achieved by adding an equimolar amount of the dihydrazide monomer **1b** to the polymer **1a·2b** and monitoring its incorporation into the backbone of the polymeric structure by ^{1}H NMR spectroscopy.[23]

The proton signals corresponding to the aromatic region of the polymer are extremely broad, but as **1b** replaces **1a** in the polymer during exchange the release of the latter species in solution was evident by the appearance of

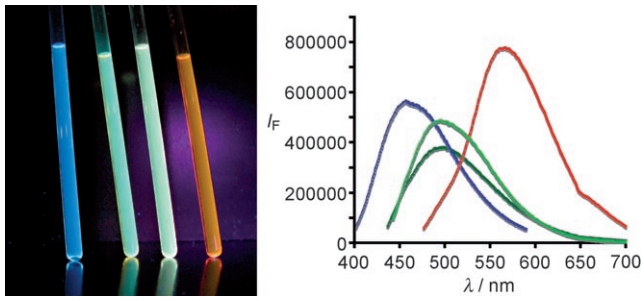

Figure 1. Left: photograph of samples of the fluorescent polymers **1a·2b** (blue), **1a·2a** (green), **1b·2b** (bright green), and **1b·2a** (red) under UV irradiation (365 nm) in D_2O at pD4, with an initial monomer concentration of 5 mM. Right: emission spectra of the same polymer samples (for polymer **1b·2a** a slit width of 3 nm was used instead of the 1 nm width used for the others).

sharp signals for its aromatic and anomeric protons. The aqueous polymer solution was diluted six times in [D_6]DMSO before recording the ^{1}H NMR spectrum so as to enable the signals corresponding to the aromatic and imine protons to be assigned and integrated.[23]

The half-life for exchange was 90 min at 80 °C and 25 h at room temperature at pD = 4 with an initial monomer concentration of 5 mM. No trace of exchange was detected at pD = 7 at room temperature, even after three weeks.

It is also possible to follow the same exchange reaction by fluorescence spectroscopy. Figure 2 shows the evolution of the fluorescence spectrum of polymer **1a·2b** mixed with an equivalent of dihydrazide monomer **1b**. The emission maximum shifts from the blue to the green region as a consequence of the incorporation of the new dihydrazide **1b** building block into the polymer to give **1b·2b** (see also Figure 1 and the text). Extension to biological applications would require finding milder conditions, with an exchange half-life on the order of hours or less at room temperature. To achieve this goal, we chose to decrease the formation efficiency of the imine linkage by using the strongly hydrated dialdehyde monomer **3**. The polymer **1a·3** was prepared from equimolar amounts of **1a** and **3** (5 mM each, pD = 4).

The polymer had a fluorescence profile very similar to that of **1a·2b**, with $\lambda_{max} = 444$ nm for emission and 383 nm for excitation. As for the **1a·2b** polymer (see above), the reversible character of **1a·3** was demonstrated by adding one equivalent of the dihydrazide **1b** to its equilibrated form in aqueous solution and following the covalent exchange reaction by ^{1}H NMR spectroscopy. The release of free **1a** was again observed and could be quantified by integration of the signal corresponding to its two equivalent aromatic protons in [D_6]DMSO/D_2O (5:1). The half-life for exchange at room temperature is 34 minutes at pD = 6, and 12 minutes at pD = 5, with an initial concentration of monomers of 10 mM; thus the exchange is about 125 times faster than in the case of **1a·2b**.

Replacement of the aromatic dialdehyde monomer **2b** by the aliphatic dialdehyde monomer **3** markedly affects the nature of the resulting polymer, as shown by SANS and cryo-TEM measurements.[24] Polymer **1a·3** was found to be much smaller (radius of gyration 32–34 Å) than **1a·2b**. As **1a** may be assumed to dominate the cross-section of the polymers **1a·2b** and **1a·3**, they should have similar cross-sections, so that a longitudinal semi-axis of about 62 Å can be calculated for the polymer **1a·3** by using an ellipsoidal model.

Furthermore, analysis of the SANS data for the polymer obtained at pD = 5 with an initial concentration of monomers of 5 mM yields an average molecular weight of 39000 g mol^{-1}, which corresponds to 28 monomeric units. These results agree with the cryo-TEM images of a sample of **1a·3**, where the wormlike structures observed in the case of **1a·2b** are absent and only small ellipsoidal structures are seen. Their average diameter of (39 ± 5) Å confirmed the assumption that the cross-section of **1a·3** should be similar to that of **1a·2b**.

The incorporation of **1b** into the polymers **1a·2b** and **1a·3** demonstrates their dynamic nature and results in a modification of their constitution which markedly affects their structural and functional properties.

In conclusion, dynamic analogues of glycopolymers have been generated by using the reversible formation of an acylhydrazone. The reversible character of these glycodynamers was demonstrated by covalent exchange reactions of a monomeric component by another, which resulted in modification of the fluorescence properties of the glycodynamer. The molecular weight as well as the rate of exchange was tuned by modification of the dialdehyde monomers. Dihydrazide monomers in combination with aromatic dialdehydes yield high-molecular-weight glycopolymers that show reversibility under rather harsh conditions, but stable under physiological conditions. This type of dynamer may be of interest for applications where slow decomposition is preferable, such as the slow release of a drug. The use of the aliphatic dialdehyde **3** confers reversibility under milder conditions and yields dynamers with a lower molecular weight which are more applicable for use where adaptive properties are desirable, such as multivalent templating with cell or bacteria receptors. Furthermore, the present fluorescent dynamic glycopolymers could have applications in biosensing,[29] in the binding or labeling of lectin,[30,31c] bacteria,[31] toxins,[32] and viruses,[31a] as well as in the detection of heavy metals.[33]

Finally, the results obtained extend the application of constitutional dynamic chemistry from materials science to

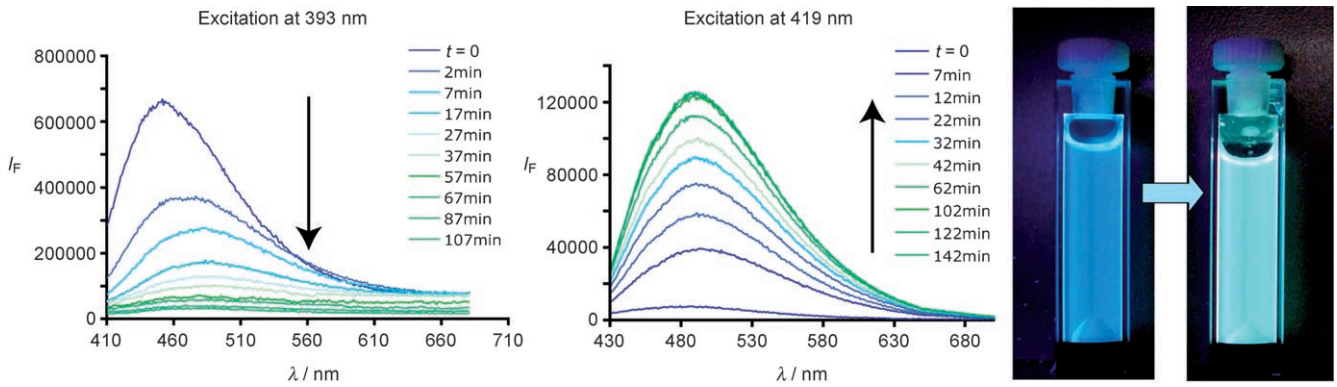

Figure 2. Left and center: Evolution of the fluorescence of **1a·2b** after addition of **1b** under excitation at 393 nm and 419 nm, respectively. Right: Photographs of samples of polymer **1a·2b** before (left fluorescence cell) and after chemical exchange with **1b** (right fluorescence cell).

biopolymers, and illustrate some aspects of the potential of such biodynamers.

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