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Spin-labelled cyclodextrins as hosts for large supramolecular assemblies

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EPR spectroscopy was used to study formation of inclusion complexes of monofunctionalised spin-labelled β-cyclodextrins; this method is very sensitive to the interactions of cyclodextrins with large guest molecules.

Cyclodextrins (CDs) are cyclic oligosaccharides with a toroidal shape. For instance, β-cyclodextrin (β-CD) has seven glucopyranose units. CDs have hydrophobic inner cavities and hydrophilic outer surfaces, which makes them capable of complexing a large variety of small hydrophobic molecules or functional groups in larger structures. Formation of such inclusion complexes can enhance solubility of the guest molecules, reduce their volatility or protect them from degradation by light, temperature, oxidation, reduction and hydrolysis. CDs have therefore found applications in the pharmaceutical industry (to control the release of active ingredients in drug formulations), in the food industry (to stabilise aromas and to mask the unpleasant odours), and in analytical chemistry (acting as chiral selectors for separation of enantiomers). CDs can also catalyse certain chemical reactions by supramolecular catalysis, which involves reversible formation of host–guest complexes by non-covalent bonding and are currently studied as enzyme models.[1,2]

Formation of host–guest complexes can be studied by a variety of physico-chemical methods (UV–Vis, fluorescence spectroscopy, conductometry, NMR, surface tension, calorimetry *etc.*). The majority of these methods, however, do not provide direct information about the structure of supramolecular assemblies. Some structural information can be obtained from fluorescence spectroscopy data or NMR studies. The EPR spectroscopy is complementary to NMR, as it is sensitive to the local structure in the vicinity of the spin label and molecular dynamics on the nanosecond timescale. Therefore, EPR is a useful tool for studying supramolecular aggregation.

EPR can only be used to probe complexation if either the host or the guest are paramagnetic (*e.g.*, contain a spin label sensitive to environmental changes). EPR studies of the interactions of unfunctionalised cyclodextrins with spin-labelled guests (*e.g.*, persistent[3–7] or short-lived[8–10] radicals) have provided a wealth of structural and thermodynamic information about the supramolecular complexes. Such studies however cannot be expanded to a broader range of non spin-labelled guest molecules.

Despite recent advances in spin labelling (particularly in biological systems) and a surge of interest in cyclodextrin chemistry, spin labelling of cyclodextrins has not been explored except for one early study which did not probe host–guest complexation.[11] Preparation of spin-labelled cyclodextrins (SL-CD) will open up the possibilities to apply EPR spectroscopy to study a large variety of supramolecular assemblies of CDs. Here, we report the synthesis of three SL-CDs (Fig. 1) and their interactions with different guest molecules.

Spin-labelled cyclodextrins were prepared in two or three steps starting from β-CD. First, β-CD was monotosylated at the C6 position[12] and the resulting 6-*O*-*p*-toluenesulfonyl–β-CD was converted into 6-deoxy-6-mercapto–β-CD[13] in order to obtain CYCAT (by reaction with 4-(2-bromoacetamido)–TEMPO; TEMPO is 2,2,6,6-tetramethylpiperidine-1-oxyl) and MTCYC (by reaction with 4-maleimido–TEMPO). TCYC was obtained by direct substitution reaction of 6-*O*-*p*-toluenesulfonyl–β-CD with 4-NH_2–TEMPO.†

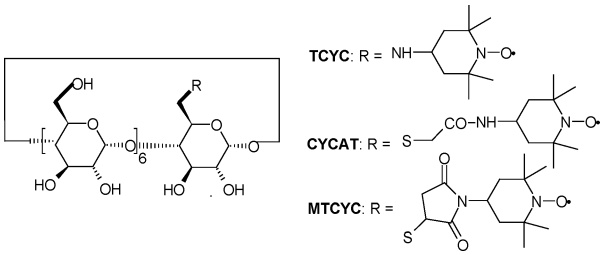

Fig. 1 Schematic representation of SL-CDs.

The aqueous solutions of SL-CDs show typical nitroxide EPR spectra with the high field line broadened due to restricted tumbling. The ^{14}N hyperfine splittings a_N, and the rotational correlation times τ, are shown in Table 1.[14]

The a_N values of SL-CDs are slightly lower than the corresponding value for 4-NH_2–TEMPO, indicating a small reduction in the polarity of the environment of the spin label. The rotational correlation times of SL-CDs, on the other hand, are with an order of magnitude higher than that for 4-NH_2–TEMPO (Table 1). This substantial reduction of the tumbling rate is typical of spin labels attached to large molecules.

In order to characterise the host properties of SL-CDs, we have recorded EPR spectra of solutions containing SL-CDs and several guest molecules, including phenolphthalein (PHE) and 1-adamantyl amine (AA). Inclusion complexes of these compounds with unfunctionalised β-cyclodextrin are characterised by a high binding constant value (*ca.* 10^4 M^{-1}).[15–17] The τ values of SL-CD mixtures with guests (at concentrations which correspond to >50% complex formation provided the binding constant is above 300 M^{-1}) are shown in Table 2.

The τ values of SL-CD complexes appear to be somewhat lower than those of free SL-CDs (Tables 1, 2). Unfortunately, this difference is insufficiently large for accurate determination of the binding constant by EPR titration experiments.

The equilibrium constants for the host–guest complex formation between SL-CDs and PHE can be determined from the UV–Vis measurements, as the strong absorption peak of

Table 1 EPR parameters of spin probes in 10^{-3} M aqueous solution at pH 7 (phosphate buffer)

Spin probe	a_N/G	$\tau \times 10^{10}$/s
4-NH_2–TEMPO	16.90	0.56
TCYC	16.70	5.87
MTCYC	16.78	7.74
CYCAT	16.89	3.90

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Table 2 Rotational correlation times τ ($\times 10^{-10}$ s) of 5×10^{-3} M aqueous spin-labelled CDs in the presence of different guests

Guest	TCYC	MTCYC	CYCAT
PHE (5×10^{-3} M)[a]	4.87	3.89	2.20
AA (5×10^{-3} M)[b]	5.33	5.86	2.49

[a] pH 9.2, borate buffer. [b] pH 7, phosphate buffer.

PHE (553 nm) at high pH is reduced upon complexation with cyclodextrins. We have recorded a series of UV–Vis spectra of an alkaline solution of PHE (7×10^{-4} M) in the presence of each SL-CD at different concentrations. The data were analysed using the Benesi–Hildebrand equation,[18] and the binding constant values were determined as 454, 745 and 160 M^{-1} for TCYC, MTCYC and CYCAT, respectively. These values of binding constant are more than an order of magnitude lower than the corresponding value for the interaction of PHE with unfunctionalised β-CD.[16,17] Such dramatic reduction of the binding affinity of SL-CDs may be due to the competing formation of self-inclusion complexes by these compounds (Fig. 2).

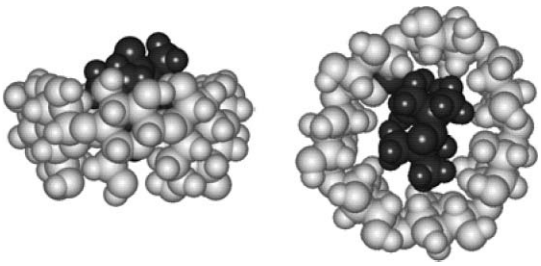

Fig. 2 Side and top views of a possible structure of a self-inclusion complex by TCYC. TEMPO unit is shown in black, while cyclodextrin moiety is grey.

Formation of self-inclusion complexes of SL-CDs is indirectly supported by other observations. For instance, a slight reduction in the a_N values of SL-CDs compared to 4-NH_2–TEMPO (Table 1) may be due to the less polar environment around the nitroxide group in the interior of the cyclodextrin moiety in the self-inclusion complexes. Reduction of τ values upon complexation with guest molecules (which indicate an *increase* in the rate of tumbling, Table 2) is also consistent with the release of the TEMPO unit from the cyclodextrin during complex formation. Unfortunately, we were unable to obtain good quality crystals of SL-CDs which would enable unequivocal structure assignment using X-ray crystallography.

Binding of small molecule guests to the SL-CDs only leads to small changes of the molecular dynamics (*e.g.*, τ values in Table 2). This is probably due to the fact that the rate of molecular tumbling depends on the hydrodynamic radius of the SL-CD molecule, and it does not undergo significant changes upon binding a small molecule.

Binding of larger molecules to SL-CDs, however, may significantly reduce the mobility of the TEMPO unit in the complex. In order to test this hypothesis, we have explored the complexation of SL-CDs with adamantane-functionalised dendrimers. General characteristics of dendrimers (globular shape, size monodispersity, multivalent periphery which enables easy functionalisation) make them highly suitable building blocks for a variety of supramolecular assemblies.[19] For instance, interactions of dendrimers with biological structures (*e.g.* amino acids, proteins, vesicles) were studied using EPR methods.[20–22] The relatively large molecular size of even low generation dendrimers should make it possible to monitor complexation of adamantane-derivatised dendrimers with SL-CDs using EPR spectroscopy.

We have prepared poly(propylene imine) dendrimers DAB-dendr-$(NH_2)_{16}$ functionalised with a different number of

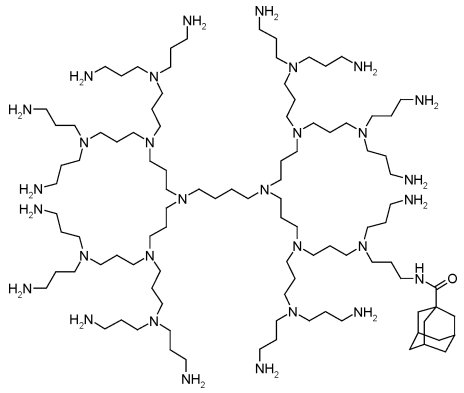

Fig. 3 Adamantane-functionalised dendrimer DAB-dendr-$(NH_2)_{16}$.

adamantane groups (Fig. 3) following a procedure described in the literature for per-adamantane-functionalised dendrimers.[23,24] The degree of functionalisation was determined by quantitative analysis of the ^{1}H NMR spectra. The dendrimers containing more than 4 adamantane moieties were found to be insoluble in water. Functionalisation of DAB-dendr-$(NH_2)_{16}$ with one or two adamantane groups, however, did not significantly reduce its solubility in water.

A control experiment showed that the EPR spectrum of TCYC is not affected by the addition of unfunctionalised dendrimer, thus indicating the absence of host–guest interactions. Addition of an adamantane-functionalised dendrimer (containing on average *ca.* 1.72 adamantane units per dendrimer molecule) to an aqueous solution of TCYC at pH 7, however, led to significant changes of the EPR parameters. Figs. 4a and b show the EPR spectra of TCYC before and after addition of the adamantane-functionalised dendrimer, respectively. The reduction of the rate of tumbling (*e.g.*, increased linewidth of all lines, but the high field line in particular) upon complexation is clearly evident from the spectra.

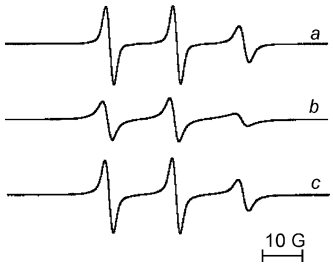

Fig. 4 EPR spectra of a) TCYC (5×10^{-3} M), b) mixture of TCYC (5×10^{-3} M) and adamantane-functionalised dendrimer (5.4×10^{-3} M), c) mixture of TCYC (5×10^{-3} M), adamantane-functionalised dendrimer (5.4×10^{-3} M) and β-CD (10^{-2} M). All solutions were prepared in a phosphate buffer (pH 7). The spectra are normalised by spin count.

The binding of the adamantane-functionalised dendrimer to TCYC is reversible as demonstrated by the following competition experiment. Complexation of TCYC with the adamantane-functionalised dendrimer leads to an increase of rotational correlation time τ from 5.87×10^{-10} (Fig. 4a) to 13.48×10^{-10} s (at dendrimer and TCYC concentrations of 5.4×10^{-3} and 5×10^{-3} M, respectively; Fig. 4b). However, addition of unfunctionalised β-CD (at 10^{-2} M) to the above mixture leads to a reduction of the τ value to 7.52×10^{-10} s, which indicates the release of the TCYC molecule from the complex with the dendrimer (Fig. 4c).

In conclusion, we have prepared three spin-labelled cyclodextrins. Although the EPR parameters of these materials are not very sensitive to the complexation of small molecules, formation of large host–guest complexes leads to substantial reduction of the rate of tumbling. Spin-labelled cyclodextrins are thus highly suitable probes for studying formation of large supramolecular assemblies.

Acknowledgements

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Notes and references

† *Synthesis.* TCYC: 4-NH_2–TEMPO (1.0 g) was added to a solution of 6-monotosylated β-CD (1.289 g) dissolved in dry DMF (10 mL), and the mixture was stirred under argon for one week at 80 °C. After addition of acetone (200 mL), the precipitate was centrifuged and washed several times with acetone. Analytical samples were obtained by preparative TLC. $R_f = 0.38$ (silica gel TLC plate, eluent MeOH–H_2O 3 : 1). ESI-MS, *m*/*z*: 1289.5 (100%, [TCYC + H^+]); 1311 (30%, [TCYC + Na^+]). EPR, a_N: 16.70 G (water).
MTCYC: 6-Deoxy-6-mercapto–β-CD (72 mg) was added to a solution of 4-maleimido–TEMPO (15.8 mg) in dry DMF (5 mL). The reaction mixture was stirred under argon for one day at room temperature and then worked up as described previously. $R_f = 0.43$ (silica gel TLC plate, eluent MeOH–H_2O 3 : 1). ESI-MS, *m*/*z*: 1424.5 (100%, [MTCYC + Na^+]). EPR, a_N: 16.78 G (water).
CYCAT: 4-(2-Bromoacetamido)–TEMPO (25 mg) and a drop of pyridine were added to a solution of 6-deoxy-6-mercapto–β-CD (98 mg) in dry DMF (5 mL). The reaction mixture was stirred under argon at room temperature for 2 days. The final product was isolated after a similar work-up. $R_f = 0.36$ (silica gel TLC plate, eluent MeOH–H_2O 3 : 1). ESI-MS, *m*/*z*: 1385.5 (100%, [CYCAT + Na^+]). EPR, a_N: 16.89 G (water).

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14 The rotational correlation time τ (s) was determined using the following equation:

$$\tau = 6.5 \times 10^{-10} \Delta H_0 \left[\sqrt{\frac{h_0}{h_{-1}}} + \sqrt{\frac{h_0}{h_{+1}}} - 2 \right]$$

Here ΔH_0 is the peak-to-peak width (in Gauss) of the central line; h_{-1}, h_0 and h_1 are the heights of the low field, central, and the high-field lines, respectively.
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