Bis spin-labelled cyclodextrins†

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Two isomers of permethylated β-cyclodextrin, labelled with paramagnetic TEMPO moieties attached on the same rim of the cavity, were synthesised and their host–guest properties investigated by EPR spectroscopy.

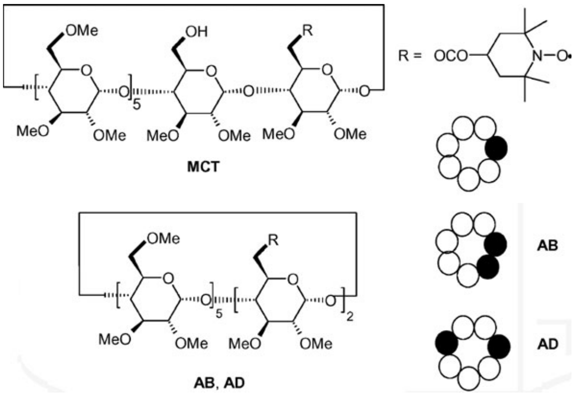
Cyclodextrins (CDs) can be used as building blocks in various supramolecular systems due to their rigid structure, hydrophobic cavity and hydrophilic exterior. They are capable of forming inclusion complexes with low molecular weight compounds or parts of larger molecules in aqueous solution.[1,2] Inclusion complexes of CDs have been studied by a variety of physical methods. For instance, the sensitivity of EPR spectroscopy to changes in the environment around paramagnetic compounds was exploited to probe the formation of inclusion complexes.[3] Monitoring supramolecular complexation by EPR spectroscopy offers significant advantages compared to other spectroscopic techniques, as EPR can provide direct molecular information about the paramagnetic moiety in the system, including distances, polarity and tumbling rates. Additionally, EPR is a very sensitive method that makes it possible to study dilute solutions (down to micromolar concentrations).

Host–guest interactions between nitroxide free radicals and natural CDs were successfully monitored by EPR in early studies.[4–9] Recently, we synthesised some mono spin-labelled cyclodextrins (SL-CDs) and found that the sensitivity of SL-CDs to complexation with small molecules was limited. However, these compounds were well suited to probe interactions with larger supramolecular assemblies, such as functionalised dendrimers and concentrated PEG solutions.[10,11] Tordo *et al.* also reported the synthesis of a mono spin-labelled permethylated CD and analysed the self-inclusion process of the paramagnetic moiety covalently attached to the CD core.[12]

In order to improve the sensitivity of SL-CDs as spin probes, we have synthesised two spin-labelled CD biradicals (Scheme 1). The EPR spectra of flexible biradicals often show additional lines due to an exchange interaction. The strength of the exchange interaction depends on the rate of collisions between the radicals.[13–15] We hoped that the host–guest interactions between the CD biradicals and small molecule guests would increase the conformational rigidity of the assembly, which in turn would affect the strength of exchange. A similar approach has been used to probe supramolecular complexation with other hosts, including resorcinarenes and calixarenes.[16,17]

Selective functionalisation of CDs usually involves the preparation of intermediate adducts with rigid aromatic compounds (*e.g.*, *meta*-dichlorosulfonyl benzene), which makes it possible to achieve high regioselectivity due to geometrical restraints. However, we found that CD derivatives with *meta*-dichlorosulfonyl benzene are difficult to purify; additionally, strong nucleophilic reagents are required for further substitutions.[18–20] Therefore, we chose an alternative method of CD functionalisation that results in the formation of a statistical mixture of different isomers, which can then be separated using silica gel column chromatography.[21] β-CD was partially protected using *tert*-butyldimethylsilyl chloride, and the remaining hydroxy groups were permethylated with methyl iodide. The silyl protecting group was then removed to give a mixture of 6A,6D-, 6A,6C- and 6A,6B-dihydroxy permethylated β-CD. Diols 6A,6B and 6A,6D were separated and reacted with 4-carboxy-TEMPO in the presence of DCC to give bis spin-labelled CDs **AB** and **AD** (Scheme 1). **AB** refers to the isomer with the functional groups attached to adjacent glucose units and **AD** represents the isomer with the functionalities separated by two glucose units (Scheme 1). Mono spin-labelled permethylated β-CD (**MCT**) was also separated from the reaction mixture.

Solution EPR spectra of **MCT** show three lines (Fig. 1a) due to the coupling of the unpaired electron with the nitrogen atom (characterised by hyperfine spitting constant a_N). The presence of two paramagnetic moieties in bis-labelled CDs lead to the appearance of additional lines due to a spin–spin



Scheme 1 Mono and bis spin-labelled permethylated β-CDs.

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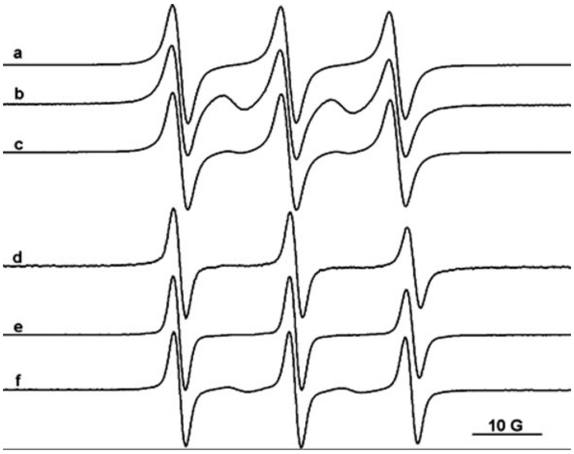

Fig. 1 EPR spectra of (a) **MCT**, (b) **AB**, (c) **AD** in DCM, (d) **AB** in water and (e) **AD** in water at room temperature; (f) EPR spectrum of **AB** in water at 350 K.

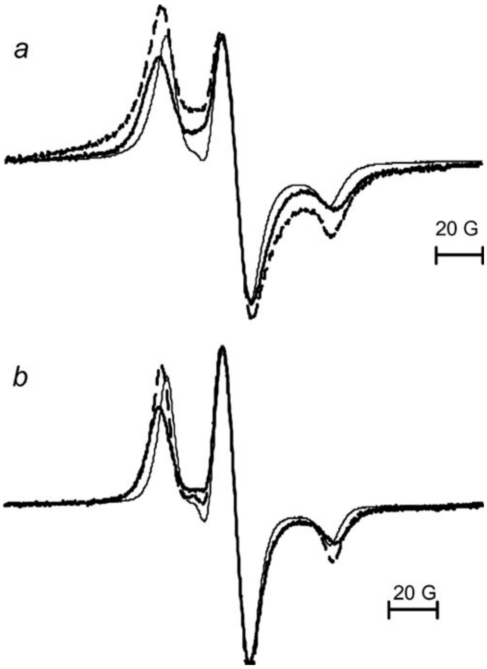

Fig. 2 EPR spectra of (a) **AB** and (b) **AD** at 120 K in 9 : 1 v/v water : glycerol (thick line), PPG 425 (dashed line) and DCM (thin line).

exchange interaction (characterised by exchange coupling constant J). For both **AB** and **AD** isomers, J is much greater then a_N, and the EPR spectra of biradicals in a DCM solution show five lines (Fig. 1b and c). Spin–spin interactions (which depend on the frequency of collisions, *vide supra*) are sensitive to various factors, such as the distance between the paramagnetic moieties, the solvent nature and viscosity, and conformational flexibility. The relative intensities of the lines corresponding to the exchange interaction in Fig. 1b and c suggest a higher frequency of collisions between the nitroxide groups in the **AB** isomer. This is consistent with the shorter distance between the paramagnetic moieties in this isomer. The shorter interspin distance in the **AB** isomer (as compared to **AD**) was further independently confirmed by the stronger dipole–dipole broadening in the powder spectra, which depends only on the interspin distance (Fig. 2). This effect was observed in several solvents of different polarity.

The EPR spectra of solutions of the **AB** and **AD** isomers at room temperature show selective broadening of the high field line due to restricted tumbling of the paramagnetic moieties attached covalently to the CD cavity (Fig. 1a–e). Similar broadening was also observed with previously reported SL-CDs.[10,11] Interestingly, the biradical spectra do not show the spin–spin exchange interaction in aqueous solution (Fig. 1d and e). This is probably due to a more open conformation of the biradicals. However, by increasing temperature (which leads to reduced viscosity and increased mobility), spin–spin interactions were observed for **AB** in water (Fig. 1f), but not for **AD**.

The permethylated spin-labelled CDs have a high solubility in organic solvents. Unfortunately, the EPR spectra showed no evidence for the formation of inclusion complexes with various guests in organic solvents. This is not unexpected as the host–guest complexation of CDs is usually driven by hydrophobic interactions.

In order to test if the nitroxide group in the permethylated spin-labelled CDs can form inclusion complexes with another CD cavity, we recorded EPR spectra of **AB**, **AD** and **MCT** in the presence of unlabelled β-CD. We found that the a_N values decreased appreciably in case of **AB** and **AD**, and to a smaller degree for **MCT.** Fig. 3 shows the differences between a_N values in β-CD solution (relative to water) for **AB**, **AD** and **MCT**.

Similar changes were observed in the rotational correlation times, τ, upon formation of inclusion complexes between **AB**, **AD** and **MCT** with unlabelled β-CD. Encapsulation of the TEMPO group within the β-CD cavity leads to more restricted tumbling (consistent with the higher τ) and a lower polarity (consistent with lower a_N).

In order to evaluate the thermodynamic parameters of host–guest complexation, EPR spectra were recorded for a range of β-CD concentrations at different temperatures. Binding constants were calculated from the rotational correlation times, τ; analysis of the a_N values gave identical results. Thermodynamic parameters obtained from Gibbs–Helmholtz equation were $\Delta S = -57$ (**MCT**) and -110 (**AB**) J mol^{-1} K^{-1}, and $\Delta H = -30$ (**MCT**) and -110 (**AB**) kJ mol^{-1}. Negative values of ΔS are in agreement with the immobilisation of the

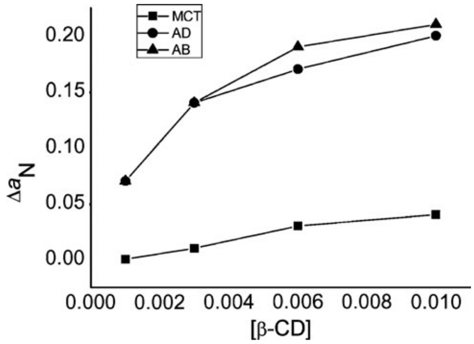

Fig. 3 Effect of β-CD concentration on the a_N values for **AB**, **AD** and **MCT** (relative to water).

paramagnetic moieties during host–guest complexation. The interaction of spin-labelled permethylated cyclodextrins with β-CD is thus driven by a favourable enthalpy change, consistent with literature data for similar processes.[22,23]

At room temperature, addition of known strong guests for CDs such as adamantane derivatives or methyl orange[24,25] did not lead to any significant changes in EPR spectra of **AB** and **AD**. We have observed similar behaviour with mono SL-CDs.[10,11] In order to probe if complexation takes place at all, we monitored interactions between permethylated CDs and methyl orange by UV-Vis spectroscopy. Hypochromic and hypsochromic shifts were observed upon increasing the concentration of CDs, consistent with formation of inclusion complexes.[24] Equilibrium constants were evaluated for inclusion complexes between methyl orange and CDs using Benesi-Hildebrand equation:[26] 632 l/mol (β-CD), 1230 l/mol (permethylated β-CD), 983 l/mol **(MCT)**, and 1077 l/mol **(AB)**. One can see that permethylated CDs bind methyl orange stronger than unfunctionalised β-CD, presumably due to the greater flexibility and hydrophobicity of the cavity induced by derivatisation. Importantly, spin labelling did not reduce the host properties of the permethylated cyclodextrin.

At high temperatures (>323 K), the formation of inclusion complexes of **AB** in water can be monitored by following changes in the spin–spin interactions (as exchange lines are visible at this temperature). Unfortunately, spin–spin interactions for **AB** in water at 323 K are not affected by the presence of adamantane derivatives (adamantane amine, adamantane carboxylic acid) or oligoethylene oxides (containing 1–4 repeat units). The distance between the two spin labels did not change according to powder spectra recorded at 120 K. Presumably, binding of these guests does not significantly change the steric environment around the lower rim of the cavity; this lack of conformational change upon complexation means that EPR could not detect the formation of inclusion complexes.

However, we found that the spin–spin interactions in **AB** are strongly affected by an adamantane end-capped diethylene glycol (**A2P2**, Fig. 4). The almost complete disappearance of the exchange lines in the presence of **A2P2** suggests that the frequency of collisions between the TEMPO units is significantly reduced upon complexation, presumably due to steric hindrance in the complex (Fig. 4). Powder spectra recorded at 120 K in a 9 : 1 (v/v) water : glycerol mixture confirmed that the distance between the TEMPO units in **AB** increased substantially upon addition of **A2P2** (Fig. 5).

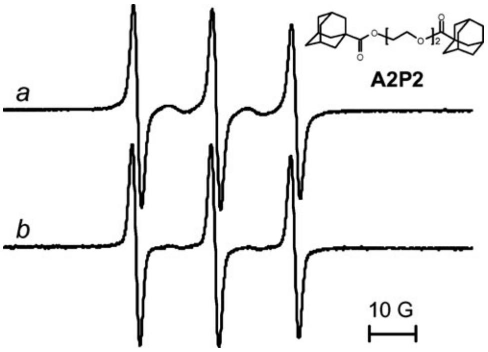

Fig. 4 EPR spectra of (a) **AB** at 323 K in water and (b) in the presence of **A2P2**.

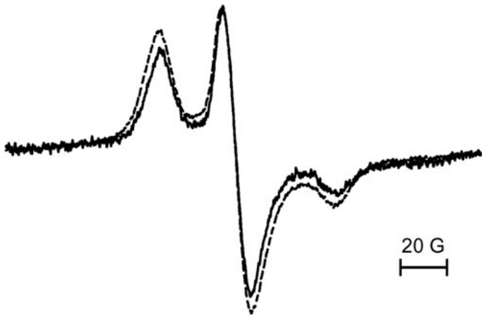

Fig. 5 Powder EPR spectra of **AB** at 120 K in 9 : 1 (v/v) water : glycerol mixture (dashed line) and in the presence of 3 mM **A2P2** (solid line).

β-CDs are known to have a high affinity for adamantane derivatives.[25] The increased distance (and reduced frequency of collisions) between the TEMPO units in the **AB**–**A2P2** complex suggests that the **A2P2** molecule protrudes through the lower rim of the CD cavity, thus acting as a shield that prevents the two spin labels from coming close to each other. In this case, the sensitivity of EPR to interspin distances makes it possible to monitor the subtle geometrical changes of the CD molecule induced by complexation.

In conclusion, we have prepared two isomeric spin-labelled β-CDs. These molecules were found to form strong host–guest complexes with unlabelled CDs and small hydrophobic molecules. The formation of complexes of SL-CD biradicals with adamantane-capped diethylene glycol led to conformational changes (*e.g.*, increased distance between the two spin labels) that made it possible to monitor this process by EPR.

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Experimental

The spin-labelled CD biradicals were prepared as shown for **AB**. Dihydroxy permethylated β-CDs were synthesised by following a literature procedure.[21] Carboxy-TEMPO (440 mg, 2.2 mmol) was added to a solution of dihydroxy 6A,6B-permethylated β-CD (1.4 g, 1 mmol) in dichloromethane (50 ml), followed by the addition of dicyclohexylcarbodiimide (495 mg, 2.4 mmol) and 4-dimethylaminopyridine (120 mg, 1 mmol). After stirring for 1 d at room temperature, the reaction mixture was washed successively with 0.1 M aq. HCl and 0.1 M aq. $NaHCO_3$. The organic layer was dried (Na_2SO_4), the solvent removed on a rotary evaporator and the crude reaction mixture purified by column chromatography using 10% ethyl acetate/dichloromethane as an eluent. Monoradical **MCT** was obtained as a by-product. TLC (10% ethyl acetate/dichloromethane, silica gel): R_f = 0.6 (**AB**), 0.6 (**AD**) and 0.4 (**MCT**). Yields: 20% (**AB** and **AD**) and 30% (**MCT**).

ESI-HR-MS data for **AB**: calc. for $[C_{81}H_{140}N_2O_{39} + NH_4]^+$ 1782.9377, found for $[M + NH_4]^+$ 1782.9306. ESI-HR-MS data for **AD**: calc. for $[C_{81}H_{140}N_2O_{39} + NH_4]^+$ 1782.9377, found for $[M + NH_4]^+$ 1782.9316. ESI-HR-MS data for **MCT**: calc. for $[C_{71}H_{124}NO_{37} + NH_4]^+$ 1600.8196, found for $[M + NH_4]^+$ 1600.8189.

The synthesis of **A2P2** and further experimental details are given in the ESI.†

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