Inclusion complexes of cyclodextrins with biradicals linked by a polyether chain—an EPR study

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Received 19th March 2007, Accepted 2nd May 2007
First published as an Advance Article on the web 14th May 2007
DOI: 10.1039/b704112h

Complexation of β-cyclodextrin with flexible nitroxide biradicals linked by a polyethylene glycol chain was monitored by EPR spectroscopy. The EPR spectra of the uncomplexed biradicals show an exchange interaction due to the flexibility of the polyethylene glycol chain. Complexation with cyclodextrin leads to the disappearance of the exchange interaction in the EPR spectra. The complexation can be reversed by the addition of competing guests (*e.g.*, adamantane derivatives). At high concentration, the inclusion complexes precipitate, and differential scanning calorimetry (DSC) of the precipitates proved the formation of complexes. Elemental analysis data revealed that the complexes contain several cyclodextrin units per biradical but that the composition was not stoichiometric.

Introduction

Cyclodextrins (CDs) are oligomers of glucose, composed of six or more glucose units bound through an α-1,4-glycosidic linkage which determines a toroidal geometry. The cavity of CDs is *ca.* 7.8 Å deep, while the minimum internal diameter is 4.4 and 5.8 Å for α-CD and β-CD, respectively.[1] Due to their ability to form inclusion complexes with various organic molecules, cyclodextrins are often used as building blocks in supramolecular structures.[2] We were interested in applying spin labelling methods and EPR spectroscopy to study host–guest interactions in such systems. The advantages of this method include its high sensitivity and the ability to probe interspin distances and molecular dynamics. Additionally, EPR spectra are very sensitive to the molecular environment in the immediate vicinity of the spin label.[3] Of course, EPR spectroscopy can only be used if at least one component of the supramolecular system is paramagnetic.

Complexes of unlabelled cyclodextrins with different types of paramagnetic compounds (stable free radicals such as TEMPO derivatives,[4–7] or short-lived organic radicals[8,9]) have been successfully studied by EPR spectroscopy. Functionalisation of cyclodextrin with a suitable spin label can in principle broaden the scope of such studies to include complexes of CDs with any unlabelled guests. We and others reported the synthesis of several spin-labelled cyclodextrin derivatives.[10–12] While the sensitivity of spin-labelled β-CDs to complexation with small molecules was in some cases limited, these compounds were well-suited to probe larger supramolecular assemblies, such as functionalized dendrimers and concentrated PEG solutions.[10,11] Acyclic TIPNO-type nitroxide-functionalised permethylated cyclodextrin reported by Tordo and co-workers showed better sensitivity to host–guest interactions, including formation of self-inclusion complexes and self-association.[12]

Here, we report the EPR study of interactions of cyclodextrins (*e.g.*, α- and β-cyclodextrin and hydroxypropyl-β-cyclodextrin) with flexible biradicals linked by a polyether chain. Both structural fragments of biradicals used in this study can interact separately with the cyclodextrin cavity. It is well known that cyclodextrins form strong inclusion complexes with polyether-type polymers[2,13] generating rotaxanes or pseudorotaxanes.[1,2,13] The paramagnetic TEMPO fragment present in our biradicals can also form inclusion complexes with the cyclodextrin cavity, and the thermodynamic parameters of this complexation have been reported.[4–7] The feasibility of using EPR spectroscopy to probe the interactions in biradical/CD systems has been shown in an early literature report.[14] The exchange interaction between paramagnetic moieties of polyethylene glycol biradicals was found to be sensitive to the presence of some metal ions and γ-CD. A recent elegant report used a similar approach to monitor formation of α-CD-based rotaxanes with a persistent biradical linked by an alkane chain.[15]

Results and discussion

Polyethylene glycol biradicals

Spin-labelled polyethers (Scheme 1) were synthesised by dicyclohexylcarbodiimide coupling of polyethylene glycols with carboxy-TEMPO.

The purified mono- and biradicals thus prepared were characterised by mass-spectroscopy and EPR. In aqueous solution **P3T1** showed a three-line spectrum typical of a nitroxide monoradical. The biradicals **P*n*T2** showed five-line spectra due to the exchange interaction between the paramagnetic fragments linked by the polyether chain, as the exchange coupling constant between unpaired electrons, J, is greater than the hyperfine splitting, a_N.[15,16] This exchange interaction, which leads to the appearance of extra lines in the EPR spectra, depends on the frequency of collisions between the paramagnetic moieties. The complexation of the

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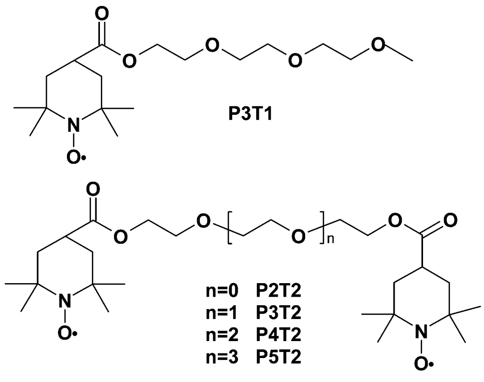

Scheme 1 Structures of radicals.

biradicals with the CDs should significantly reduce the probability of collisions of the nitroxide termini. Therefore, the complexation of the biradicals with the CD units was expected to result in the disappearance of the exchange peaks in the EPR spectra.

EPR spectra of biradicals **P*n*T2** were recorded in solutions of β-CD, α-CD and hydroxypropyl-β-CD (HPB). The concentration of biradicals was kept constant at 10^{-4} M, while the concentration of CDs was varied in the range 10^{-4}–10^{-2} M for β-CD, 10^{-4}–0.1 M for α-CD and 10^{-4}–0.3 M for HPB.

Biradical complexes with β-CD

Fig. 1 shows the EPR spectra of **P3T2** in an aqueous solution and in the presence of β-CD. Addition of β-CD leads to several changes in the EPR spectra. The high-field line (h_{+2}) decreases upon addition of CD. This is due to the slower tumbling of the nitroxide moiety, caused by the complexation with the CD unit. Similar broadening of the high field line was observed for the interaction of **P3T1** with β-cyclodextrin; the rotational correlation time τ increased from 0.49×10^{-10} s in water to 0.86×10^{-10} s in 1.5×10^{-3} M β-CD.

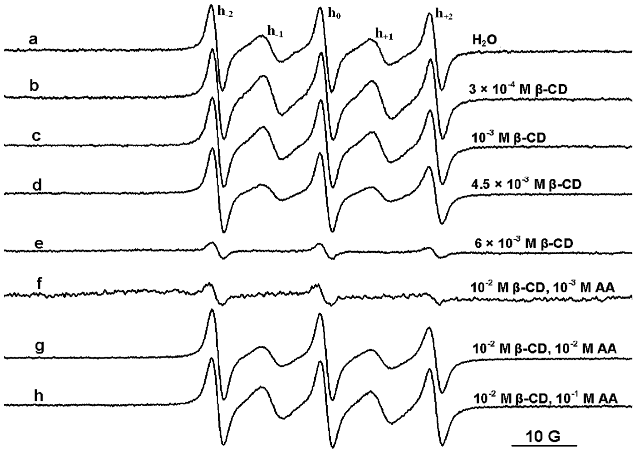

Fig. 1 EPR spectra of **P3T2** in water (a), in the presence of different concentrations of β-CD (b–e), and in mixtures of β-CD +AA (f–h).

The exchange lines in the spectra of **P3T2** (h_{-1} and h_{+1}) also decrease continuously with increased CD concentration until complete disappearance at around 6×10^{-3} M (Fig. 1e). This is consistent with the complexation with the CD unit which reduces the exchange interaction (*vide supra*).

By increasing β-CD concentration still further, the solutions of biradicals became turbid and a precipitate appeared. This is accompanied by the reduction of the overall intensity of the EPR spectra, and the appearance of the broad features typical of the completely immobilised nitroxide in the solid complex (Fig. 1e,f). In the case of the **P3T2**/β-CD system, formation of precipitate was very rapid, while with the other biradicals some induction period was noticed.

The precipitate did not appear when β-CD was reacted with **P3T1**. The formation of the precipitate is most likely due to poor solubility of the β-CD/**P*n*T2** complexes. This is rather unusual. It is known that α-CD forms solid threaded complexes with polyethylene glycols; however, formation of insoluble complexes of β-CD and γ-CD take place with polypropylene glycols but not with low molecular weight polyethylene glycols.[17]

In order to check if the observed changes in the EPR spectra are indeed caused by host–guest interactions and are not due to increased viscosity of the solution, we mixed the solutions/suspensions of the complexes with adamantane amine (AA). Adamantane amine has very strong affinity for the β-CD cavity[18] and can be expected to release the biradical molecule from the complex into the solution. This assumption was confirmed by the re-appearance of the five-line pattern of the biradical in adamantane amine-containing solutions (Fig. 1g,h). Visual inspection also showed the disappearance of the precipitated complexes and formation of clear solutions. We can therefore confidently assign the changes in the EPR spectra to host–guest interactions.

In order to estimate the stoichiometry of the solid precipitates formed upon mixing **P*n*T2** with β-CD, we characterised these materials by elemental analysis. The analysis was complicated by the fact that the complexes were hygroscopic, and complete removal of water proved difficult. The molar ratios of β-CD to **P*n*T2** were therefore calculated by least-squares fitting of the composition of the tricomponent mixtures (β-CD, **P*n*T2**, and water) to the elemental analysis data. We believe these calculations to be very reliable as nitrogen is only present in the **P*n*T2** molecules. The β-CD:**P*n*T2** ratios established from the elemental analysis data were as follows: 1.4 : 1 (**P2T2**), 1.95 : 1 (**P3T2**), 3.65 : 1 (**P4T2**) and 1.6 : 1 (**P5T2**); the samples also contained *ca.* 5% water.

Although no clear trend emerged from the composition data for different biradicals, it is worth noting that all complexes were non-stoichiometric but contained more than one CD unit per biradical. We speculate therefore that 1 : 1 complexes are more soluble and are preferentially formed at low CD concentrations. At higher CD concentrations, several CD units bind to the biradicals, until the non-stoichiometric mixture of complexes precipitates. As no more than 2 CD units can complex the TEMPO units of the biradical, the high β-CD:**P4T2** ratio unambiguously shows that the ethylene glycol units are also involved in the complexation.

Fig. 2 shows EPR spectra of neat **P3T2** (a) and its insoluble complex with β-CD (b). The collapse of the hyperfine interaction in pure **P3T2** (which results in the appearance of a single broad line in the spectra) indicates very short interspin distances in the neat biradical. The much broader appearance of the spectrum of the precipitate (Fig. 2b) is consistent with the increased distance between the nitroxides in the complex.

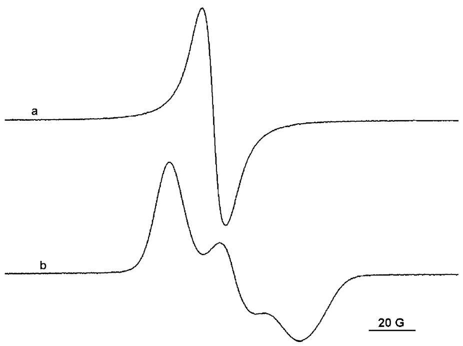

Fig. 2 EPR spectra of neat **P3T2** (a) and the insoluble complex with β-CD (b).

In order to further confirm that the insoluble complex is not a co-precipitated mixture of the cyclodextrin and biradical, we have used DSC to evaluate the thermal stability of these compounds. Fig. 3 shows DSC traces for β-CD, **P4T2**, their physical mixture and their insoluble complex.

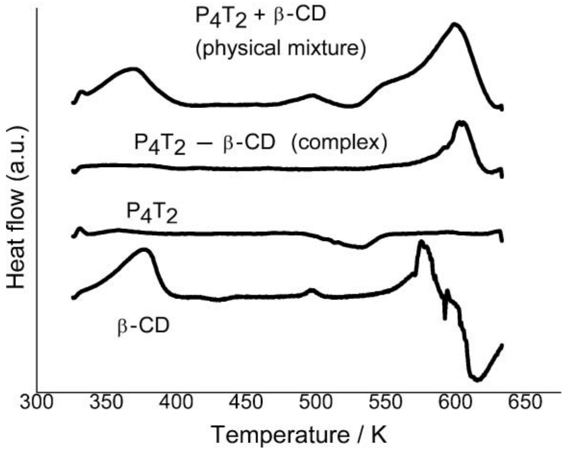

Fig. 3 DSC traces for **P4T2**, β-CD, their physical mixture and their insoluble complex.

The DSC curve profile for β-CD shows an endo peak in the range 325–400 K attributed to water loss (this was confirmed by the absence of this peak at the second scan). A small endo peak between 500 and 530 K is attributed to a molecular reorganisation of β-CD.[21,22] The peak above 550 K corresponds to the thermal decomposition of β-CD. In the case of **P*n*T2**, thermal decomposition occurs below 550 K (*e.g.*, endo peak at 500–550 K in Fig. 3 for **P4T2**). The DSC traces of physical mixtures exhibit all the peaks present in β-CD and **P*n*T2** curves. The DSC traces for the insoluble complex, however, are distinctly different from the physical mixture. The peak corresponding to water loss from the complex is very broad, the peak corresponding to reorganization of β-CD is completely absent and the decomposition occurs at a higher temperature. The presence of the broad peak in DSC diagrams of complexes due to water loss support the results from elemental analysis. These results confirm that the insoluble material is a true complex between the biradical and cyclodextrin.

At low β-CD concentrations, the changes in the EPR spectra can be used to estimate the equilibrium constants for the complexation with biradicals **P*n*T2**. This was achieved by fitting the ratios of the lines h_{+2}/h_0 (see Fig. 1) at different β-CD concentrations with the speciation equation for a 1 : 1 complex. This approach is a clear simplification, as the EPR spectra are affected by the presence of higher stoichiometry complexes (*e.g.*, 2 : 1 *etc.*). Unfortunately, accurate separation of the contributions of complexes of different stoichiometry was not possible. Nonetheless, the simple assumption of 1 : 1 stoichiometry makes it possible to estimate the values of equilibrium constants. Fig. 4 shows the dependence of the h_{+2}/h_0 ratio on the β-CD concentration for **P3T2** and the line of best fit.

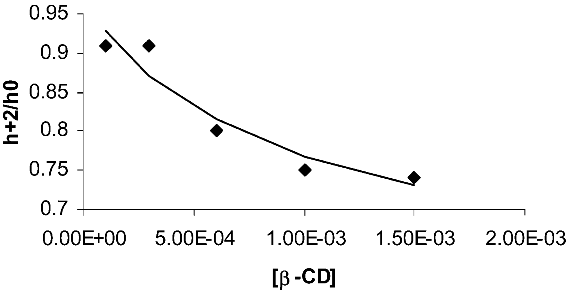

Fig. 4 Experimental h_{+2}/h_0 values and fitted curves as a function of [β-CD] for the **P3T2**/β-cyclodextrin system.[24]

The insensitivity of the EPR spectra to the low concentration of CD (first 2 points in Fig. 4) was found for all biradicals and is not due to experimental error. We believe that this is caused by formation of higher stoichiometry complexes (*e.g.*, 1 : 2 *etc.*), which cannot be accounted for in our simple model.

The binding constants for all biradicals were close to $10^3\ \mathrm{l\,mol^{-1}}$. For comparison, the equilibrium constants for complexation of TEMPO unit and β-CD are usually around $10^3\ \mathrm{l\ mol^{-1}}$.[19] The complexation of oligoethylene glycols with β-CD is usually characterised by a higher equilibrium constant, around $10^5\ \mathrm{l\ mol^{-1}}$.[20] It is therefore likely that the initial complexation of **P*n*T2** with β-CD involves formation of a pseudorotaxane, with the oligoethylene glycol chain threading the CD cavity. At higher β-CD concentration, the terminal TEMPO units associate with the CD cavity, which leads to precipitation of the complexes.

Complexes with other cyclodextrins

The addition of α-CD to the biradical solutions showed no changes until very high concentration (*e.g.*, 0.1 M). At these concentrations, the EPR spectra of the biradicals showed only three lines; however, this effect is at least partially due to the increased viscosity of the solution, and cannot be unambiguously linked to the complexation. This behaviour is consistent with the weaker complexation of most guests with the smaller α-CD cavity.[18] Additionally, we believe that the TEMPO unit cannot pass through the α-CD cavity, and hence complexation with the ethylene glycol units is impossible. The smallest ring the TEMPO unit can pass through is *ca.* 6 nm in diameter. Geometrical characteristics of CDs[1,23] suggest that the TEMPO unit can only thread through β- but not α-CD. Similar conclusions were reached by Lucarini *et al.*; these authors were able to prepare stable rotaxanes using TEMPO as a capping group for α- but not β-CD.[15]

EPR spectra of biradicals in the presence of hydroxypropyl-β-CD (HPB) showed the gradual disappearance of exchange lines at concentrations around 10^{-2} M. (Fig. 5). However, HPB

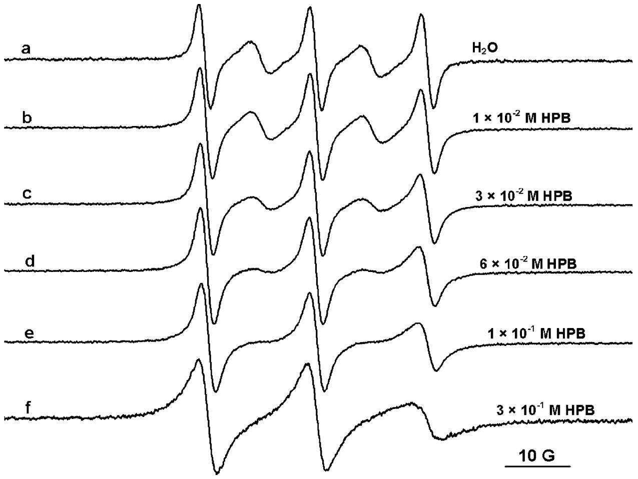

Fig. 5 EPR spectra of **P3T2** in water (a) and in aqueous solutions of HPB of varying concentration (b–f).

solutions are quite viscous and the effect of viscosity can mask the complexation.

The high field line in the EPR spectrum of biradicals in a 10^{-1} M solution of HPB shows asymmetry which suggests the presence of two components (Fig. 5e). This is likely due to complexation. At higher HPB concentrations, the solution became very viscous and the presence of two components in the systems was no longer visible (Fig. 5f). Unfortunately, it was not possible to obtain quantitative information about HPB complexation.

In order to separate the effect of viscosity from the complexation, we have carried out competitive experiments with adamantane amine (AA). We noticed that biradicals are released completely from the complexes by increasing the concentration of AA from 10^{-3} to 10^{-1} M.

Fig. 6 shows spectra of biradicals **P*n*T2** in the presence of 10^{-1} M HPB in the absence of AA (A) and with 0.1 M AA (B). In the presence of 10^{-2} M AA, the high-field line was still asymmetrical, but the lines due to exchange interactions became observable. At 10^{-1} M AA, the high-field line became symmetrical for all biradicals and the exchange interactions were prominent. This indicates that biradicals are released from the complexes with HPB (Fig. 6B). Comparison of the spectra in Fig. 6A and B clearly shows how the EPR spectra are affected by both increased viscosity of the HPB solutions and host–guest interactions.

Conclusions

We have found that nitroxide biradicals linked by a polyether chain form complexes with cyclodextrins. The complexation process can be conveniently monitored by EPR spectroscopy as the exchange interaction between the nitroxide units in the biradicals disappears in the complex. Reversibility of the complexation process was confirmed using competitive reaction with adamantane derivative. The complexes have poor aqueous solubility and are non-stoichiometric. The binding constants were estimated as *ca.* 1000 $l \times mol^{-1}$. Complexation with α-CD is much weaker which is probably due to the inability of the TEMPO unit to pass through the α-CD cavity.

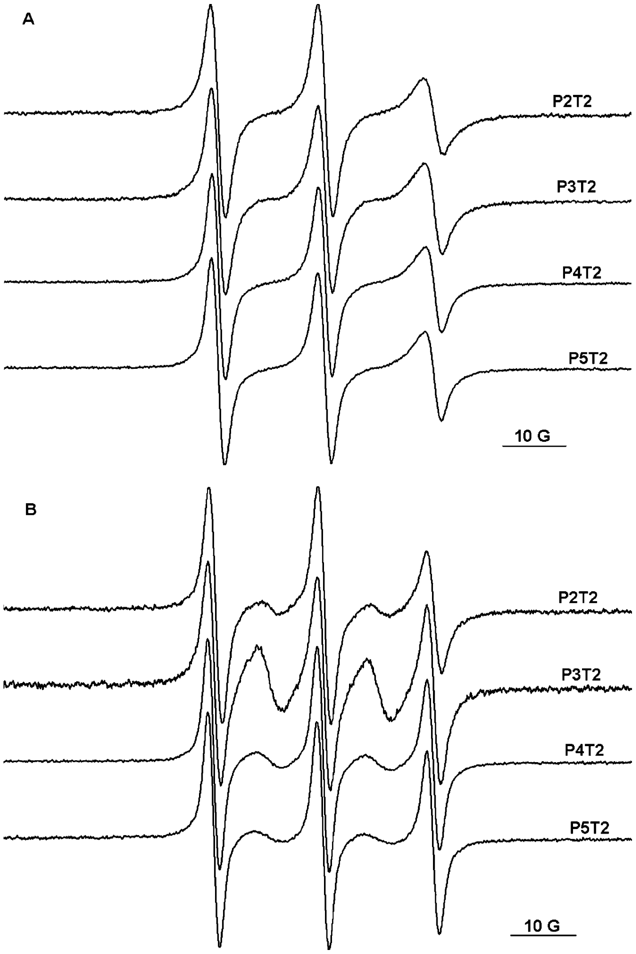

Fig. 6 EPR spectra of **P*n*T2** (A) in HPB (0.1 M), and (B) in a mixture of HPB (0.1 M) and AA (0.1 M).

Experimental

EPR spectra were recorded on JEOL JES-RE1X spectrometer with 100 kHz modulation frequency, 1 mW microwave power, 0.1 mT modulation amplitude, 328 s scan time, 100 G scan width for spectra at room temperature and 200 G scan width for spectra at 120 K, and 9.49 GHz microwave frequency.

ESI mass spectra were measured on VG Autospec mass spectrometer. Elemental analysis was performed by M. Jennings, Micro Analytical Laboratory, Department of Chemistry, University of Manchester, Manchester, UK.

DSC was performed by using a DSC-2 Perkin–Elmer apparatus connected to a Tektronix TX3 multimeter and optically coupled to the interface RS 232. The scan heating rate employed was 10 K min^{-1} in the range 300–650 K under an argon atmosphere, the apparatus sensitivity was 5 mcal s^{-1} and the multimeter had a maximum display at 50 000 with a 0.03% error on the work domain.

The procedure for biradical synthesis is described for biradical **P3T2**. Carboxy-TEMPO (440 mg, 2.2 mmol) was added to a solution of triethylene glycol (0.150 g, 1 mmol) in dichloromethane (20 ml) in the presence of dicyclohexylcarbodiimide (500 mg, 1.2 mmol) and 4-(dimethylamino)pyridine (60 mg, 0.5 mmol). After stirring for one day at room temperature, the reaction mixture was washed successively with 0.1 M HCl and saturated $NaHCO_3$ aqueous solutions. The organic layer was dried (Na_2SO_4). The solution was concentrated and purified by column chromatography using 10% ethyl acetate–dichloromethane as eluent. Monoradical **P3T1** and other biradicals were obtained in a similar way. TLC (10% ethyl acetate–dichloromethane, silica gel), R_f: 0.47 (**P2T2**), 0.40 (**P3T2**), 0.33 (**P4T2**), 0.33 (**P5T2**), 0.45 (**P3T1**). Yields: 40% (**P2T2**), 35% (**P3T2**), 30% (**P4T2**), 42% (**P5T2**) 47% (**P3T1**). All biradicals have five-line EPR spectra, while monoradical shows a three-line spectrum. ESI–HR-MS data for **P2T2**: calc. for $[C_{24}H_{42}O_7N_2 + H]^+$ 471.3070, found for $[M + H]^+$ 471.3065. ESI–HR-MS data for **P3T2**: calc. for $[C_{26}H_{46}O_8N_2 + H]^+$ 515.3332, found for $[M + H]^+$ 515.3327. ESI–HR-MS data for **P4T2**: calc. for $[C_{28}H_{50}O_9N_2 + H]^+$ 559.3594, found for $[M + H]^+$ 559.3589. ESI–HR-MS data for **P5T2**: calc. for $[C_{30}H_{54}O_{10}N_2 + H]^+$ 603.3857, found for $[M + H]^+$ 603.3851. Preparation and characterisation of **P3T1** has been reported earlier.[11]

Preparation of complexes for elemental analysis and DSC: the complexes were prepared by mixing an aqueous 10^{-3} M solution of **P*n*T2** in water with a 10^{-2} M β-CD solution (in a 1 : 2 molar ratio); the precipitates were isolated by filtration, washed with water and dried.

Acknowledgements

This work was financially supported by Royal Society Short Visit award (2005), grant MCT-CEEX III 2/2005 (Romania), CNCSIS 729/2006 (Romania), EU-funded COST P15 action "Advanced Paramagnetic Resonance Methods in Molecular Biophysics" (STSM 02566).

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24 The fitting in Fig. 4 assumed a linear relationship between h_{+2}/h_0 and the concentration of complex in the mixture. The concentration of complex was calculated using the following equation:

$$C = \frac{Kc_0 - Kc_{CD} - 1 + \sqrt{(Kc_0 - Kc_{CD} - 1)^2 + 4Kc_0}}{2K}.$$

Here C is the concentration of complex, K is the equilibrium constant, and c_0 and c_{CD} are the initial concentrations of the guest and cyclodextrin, respectively. This equation was derived by assuming formation of a 1 : 1 complex between CD and guest molecules.