Porphyrin cored hyperbranched polymers as heme protein models†

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The single step synthesis of an Fe(II) porphyrin cored hyperbranched polymer, possessing similar size and topology to the natural heme containing proteins, is reported: UV spectroscopy successfully demonstrated the ability of this polymer to reversibly bind oxygen.

One of the principal tasks of blood is to transport oxygen throughout the body and then to release the oxygen to tissues. This is accomplished by the oxygen-carrying protein hemoglobin, which possesses an Fe(II) porphyrin unit at its active site. It is this Fe(II) porphyrin that is responsible for the (reversible) binding of oxygen.[1] The protein serves to protect and isolate the oxygen binding porphyrin units, as well as to help prevent its deactivation (through u-oxo dimer formation and over oxidation).[2] Therefore, when designing a synthetic replacement for an oxygen binding protein the following key parameters should be considered: (i) a five coordinate iron(II) porphyrin with an N-base axial ligand and a vacant coordination site for oxygen is required; and (ii) bulky groups should be placed around the "active" porphyrin to slow down or prevent its deactivation.[2] Traditional attempts to mimic the reversible oxygen binding properties of heme containing proteins concentrated on constructing simple Fe(II) porphyrins appended with large groups.[3] The current "state of the art" systems embed Fe(II) porphyrins within the globular structure of a highly branched monodispersed macromolecule (known as a dendrimer), whose shape and size closely match those of the natural heme containing proteins.[4] Despite their success with respect to reversible oxygen binding, these macromolecules require lengthy and expensive methods of preparation. These synthetic limitations have restricted their exploitation as a potential artificial blood product. Recently it became possible to construct dendrimer "type" molecules utilizing just one synthetic step. These materials have become known as hyperbranched polymers (HBPs)[5] and despite their structural imperfections, retain a similar three-dimensional globular structure to perfect dendrimers. If the size topology and functionality of a HBP could be tailored to match those of an oxygen binding protein, then these globular macromolecules would represent an exceptionally simple synthetic mimic of the oxygen binding proteins. We proposed that a suitably functionalized porphyrin unit could act as an initiator core and be incorporated at the centre of a HBP. Furthermore, if the environment around the central porphyrin could be controlled, then a hemoglobin/myoglobin mimic could be obtained using just one synthetic step.

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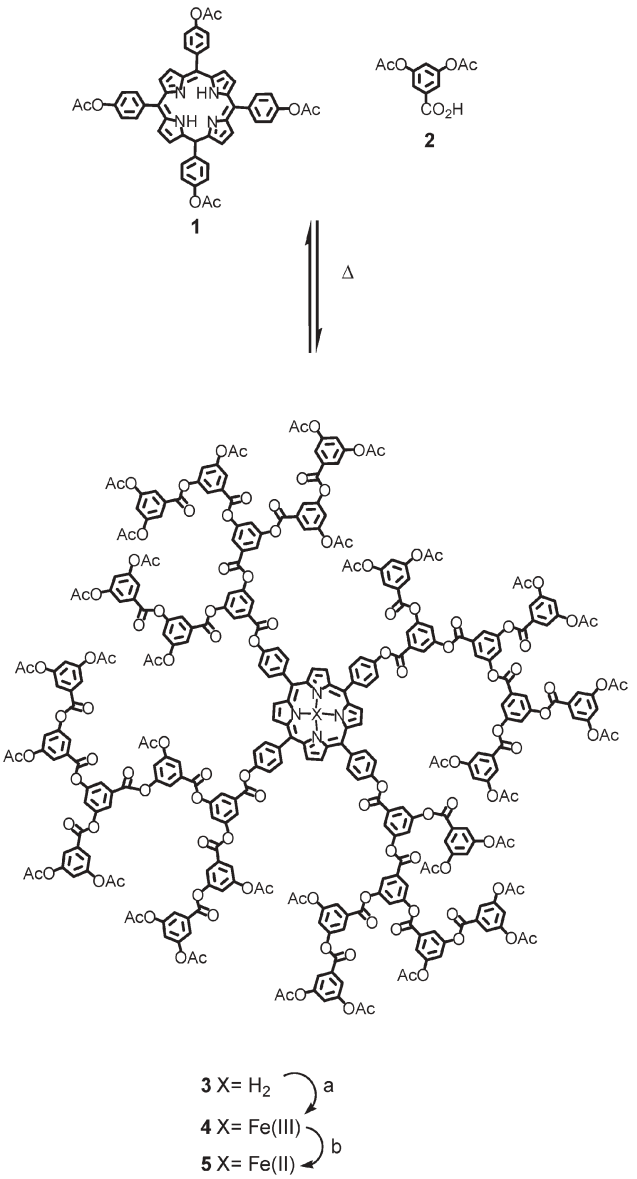
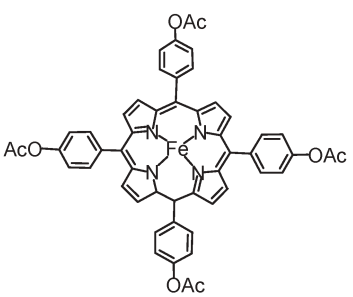
Towards these aims the porphyrin cored hyperbranched polyester **3** was synthesized by reacting an excess of the branching monomer, 3–5 diacetoxybenzoic acid **1** (95 mol%), with a small amount of the core unit tetrakis(4-acetoxyphenyl)porphyrin **2** (5 mol%) under reversible trans-esterification conditions (Scheme 1).[6] Equilibrium chemistry was chosen so as to encourage



Scheme 1 Synthesis and idealized representation of an oxygen binding Fe(II) cored HBP. (a) $FeBr_2$, 2,6-lutidine, THF. (b) $Na_2S_2O_4$, CH_2Cl_2/H_2O.

an even distribution of porphyrin core across all molecular weights.[7] The bulk polymer **3** obtained had an average molecular weight of 16 500 (M_n determined by GPC calibrated with linear polystyrene standards),[8] a relatively narrow polydispersity of 2.25 (narrow relative to HBPs synthesised without core units[6]) and a degree of branching equal to 49%.[6] The level of core incorporation was assessed by fractionating the sample[9] and comparing the M_n values obtained from core (M_n-*max*) and bulk (M_n-*min*) properties (as previously reported).[7] For each fraction obtained the ratio of M_n-*min* to M_n-*max* returned a similar level of incorporation (60 $\pm$ 3%).[10] These results clearly indicate that the porphyrin core is evenly distributed across the complete molecular weight range of the hyperbranched polymer. More information regarding the extent of core incorporation can be obtained through mass spectrometry. The polymer is propagated through an ester interchange reaction between a terminal acetoxy group and the carboxylic acid group of the monomer. The addition of each monomer is therefore accompanied by the loss of acetic acid. This corresponds to an increase in mass of 178 for each monomer residue, *i.e.* the additional mass of one monomer unit (238) and the loss of acetic acid (60). This is reduced by a further 60 when cyclisation occurs. Examination of the mass spectra (ESI†) for the most narrowly dispersed fraction showed a Gaussian shaped series of peaks separated by 178 Da (see above). Further analysis revealed that each peak corresponded to an individual polymer molecule possessing a single porphyrin core plus *n*-monomer units. Peaks corresponding to polymeric products without porphyrin core were *not* detected.[11] When considering this information alongside the M_n ratios of the fractionated samples described above, we can conclude that *almost all* polymer molecules possess a porphyrin core (*i.e.* a level of core incorporation approaching 100% has been achieved for HBP **3**).[12] Insertion of iron was carried out by reacting HBP **3** with $FeBr_2$ in the presence of 2,6-lutidine in THF. Purification by precipitation from methanol gave the oxidized Fe(III) porphyrin cored polymer **4** (possessing a MeOH axial ligand). GPC analysis of the product returned values of 16 650 and 2.08 for M_n and polydispersity respectively, confirming that the HBPs structure had remained intact during the metalation process. The inactive Fe(III) porphyrin was reduced to the active Fe(II) species **5** (in the presence of the axial ligand 1,2-dimethylimidazole) using sodium hydrosulfite under two phase reaction conditions.[13] Successful reduction was confirmed spectroscopically by observing a shift in the Soret band from 418 nm for the Fe(III) porphyrin cored HBP **4**, to 435 nm for the Fe(II) species **5** (Fig. 1). Due to the sensitivity of the Fe(II) product, the reduction was carried out immediately prior to the gas binding studies described below.



6 Fe(II)

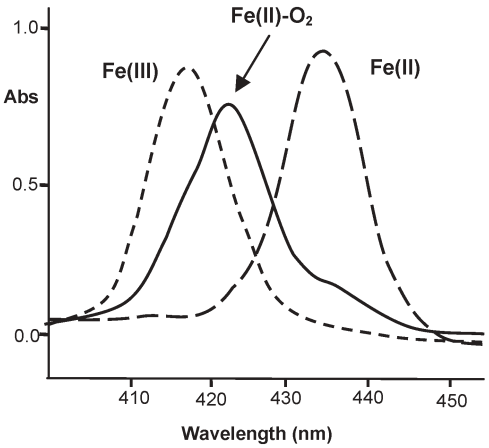


Fig. 1 UV spectra of the Fe(II) porphyrin cored HBP and the Fe(II)–O_2 complex, the Fe(III) spectrum is shown for comparison (all spectra recorded in the presence of 1,2-methylimidazole).

Our initial gas binding experiments investigated the polymers ability to bind oxygen. All of the important intermediates and products involved in oxygen binding have characteristic (and well resolved) peaks in their UV spectra.[14] Consequently, simple UV spectrophotometric techniques were used to assess the oxygen binding potential of the HBP **3**.[15] The Fe(II) cored HBP has a maximum absorption at 435 nm in dicloromethane. After bubbling oxygen through the solution for 1 min, this absorption maxima shifted to 422 nm (Fig. 1). This shift is consistent with an Fe(II)–O_2 complex.[14] After removing the oxygen, by passing nitrogen through the solution for 5 min, the UV spectrum of the original uncomplexed Fe(II) species was restored (maximum absorption of 435 nm). This process of reversible oxygen binding could be repeated 5 or 6 times before a new peak, corresponding to the inactive/oxidized Fe(III) species, began to be noticed at 418 nm. After a further 5 or 6 cycles, the peak at 418 nm dominated the spectrum and reversible oxygen binding became more difficult to observe. The oxidation of the porphyrin within the HBP was also monitored by taking the Fe(II)–O_2 complex and monitoring its UV spectrum over time. After 30 min the Fe(III) signal was significant and the remaining Fe(II)–O_2 peak became hard to distinguish from the tail of the Fe(III) peak. As a control, a similar experiment was carried out using the sterically unhindered porphyrin core, tetra-acetoxyphenylporphyrin **6**. On bubbling oxygen through a solution of the Fe(II) porphyrin we observed direct oxidation to the Fe(III) system, with no evidence of any Fe(II)–O_2 complex. These results represent a clear proof of principle demonstrating how simple HBPs can be used to reversibly bind oxygen, with the potential to be applied as a future artificial blood product. Work is progressing in our laboratory to construct porphyrin cored HBPs with more rigid and sterically demanding porphyrin environments.

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10 Mn values (GPC) of the three fractions were 7000, 15 500 and 22 000, with PDs of 1.35, 1.90 and 2.10 respectively.
11 For comparison a narrowly dispersed ESI mass spectrum of the homopolymer was run (*i.e.* HBPolyesters without porphyrin cores—M_n 1850; PD 1.8). The resulting spectrum contained major peaks 178 mass units apart, separated by slightly smaller peaks corresponding to the cyclic species.
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