A strategic approach for the synthesis of new porphyrin rings, attractive for heme model purpose

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Abstract—Novel complexes have been efficiently synthesized with a facile route using two different atropisomers of the same porphyrin. These compounds feature a tridentate binding site, a tyrosine molecule, and a proximal base, all bound to the porphyrin ring in different fashions, making them attractive for heme modeling purposes.

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1. Introduction

Cytochrome c oxidase (CcO) is an important metalloenzyme of the respiratory chain in many aerobic organisms. It catalyzes the four electron, four proton reduction of oxygen to water, without releasing toxic, reactive intermediates (e.g., H2O2), conserving the energy required for the biosynthesis of ATP.1 The catalytic active site of the enzyme consists of a heme (heme a3) and a tricoordinated copper (CuB) in close proximity (~4.4–5.3 Å), depending on the protein and its oxidation state.2 Recent X-ray crystallographic studies revealed that one of the copper-bound histidines (H240) is covalently connected to a tyrosine residue (Y244).3 The presence of a phenol residue is proposed to act either as an electron and proton donor to the oxygen reduction cycle3a,4 or to help the enzyme to adopt a favorable structural conformation.5 Despite all the above detailed information derived from the natural enzyme, the precise role of CuB in mediating O2 reduction remains unresolved. Therefore, in order to elucidate the mechanism of O2 reduction, a number of synthetic heme-based binuclear complexes have been prepared.6 Despite the large number of catalysts that have been synthesized so far, it is not clear which elements influence the selective reduction of O2 to H2O over H2O2.

2. Results and discussion

In this new synthetic approach, we have developed new complexes in which it is easy to modify possible factors that are responsible for the reduction of oxygen. These new complexes 1–4 are easily made, based on the two different atropisomers of the same porphyrin. All complexes have the same porphyrin base and differ in three ways: (i) the coordination environment of a second metallic center; (ii) the presence or absence of a proximal base; (iii) the existence, or not, of a tyrosine molecule as well as the mode by which the tyrosine is linked to the porphyrin. Finally, various physicochemical studies of these molecules can give an insight into whether one of the above features will influence the catalytic activity of these compounds.

Complex 1 is based on the z,a-atropisomer of porphyrin 11a and has a tridentate ligand covalently attached on one site of the porphyrin ring and a tyrosine molecule on the opposite site. In compounds 2–4, tyrosine and bis(2-(pyridin-2-yl)-ethyl)amine (BPEA)5 are covalently linked to the porphyrin. The hydroxyl group of all compounds can be protected/deprotected in order to investigate the role of this tyrosine mimic. Moreover, in complexes 3 and 4 an axial pyridine ligand is covalently attached to the porphyrin in order to block the lower face of the porphyrin ring.

The synthesis of tridentate ligands is shown in Scheme 1. BPEA 5 and axial ligand 6 were prepared by the reaction of 2-vinylpyridine and ammonium chloride in 50 and 30%, respectively.7 Reaction of commercially available protected tyrosine 7 and BPEA 5, followed by deprotection of the 9-fluorenylmethoxycarbonyl (Fmoc) group afforded ligand 9 in good yield.8 After preparation of compounds 5 and 9, a suitable trans-substituted porphyrin such as 11 was desired in order to proceed with the synthesis of superstructured porphyrins9 (Scheme 2). Dipyromethane 10 was synthesized by reacting 2,4,6-trimethyl-benzaldehyde, pyrrole, and trifluoroacetic acid in 70% yield. A mixture of two porphyrin atropisomers 11...
was prepared in 20% yield by the reaction of dipyrromethane 10 with 2-nitrobenzaldehyde, in the presence of TFA, followed by oxidation of the resulting porphyrinogen with DDQ. The two atropisomers were separated by column chromatography. The α,α-atropisomer was used for the synthesis of 1, while for 2 the α,β-atropisomer was required. Interestingly, compound 12a can be selectively reduced by SnCl₂ in the presence of concentrated HCl in CH₂Cl₂ at 0 °C, to give α,α-monoaminoporphyrin 12a in 65% yield. Acylation of 12a with chloroacetyl chloride gave 13 (93%). Condensation of ligand 5 in the presence of DIPEA gave 14 (88%), followed by reduction to give 15 in 94% yield. Then 15 was coupled with protected tyrosine molecule 7 in the presence of DCC, yielding 16 (75%). The synthesis of compound 1 is completed with deprotection of the Fmoc group in the presence of piperidine in DMF (92%), and deprotection of the tert-butyl group using TFA affording 1 in 90% yield.

For the preparation of porphyrin 2, the α,β-atropisomer 11b was selectively reduced with SnCl₂ in the presence of concentrated HCl in CHCl₃ at room temperature yielding 12b (21%). Urea-substituted porphyrin 18 was synthesized by a useful method introduced by Collman and co-workers in which the amino group of the monoaminoporphyrin 12b is converted to isocyanate under mild conditions with triphosgene. The intermediate porphyrin can then be reacted with a nucleophile, such as amine 9, to form 18 in 62% yield. The reason that ligand 9 is attached on the porphyrin ring via urea link is because the affinity of dioxygen might be stronger compared to an amide link, as observed for other oxyhemoglobin models. Finally, compound 2 was obtained by deprotection of the tert-butyl group, using TFA in 96% yield.

The synthesis of complexes 3 and 4 is shown in Scheme 3 and they both bear groups attached to the porphyrin via urea linkers. A tridentate ligand is directly attached to a tyrosine molecule and a proximal base. The only difference between them is the coordinating base that will be used later as a fifth ligand for the iron atoms of porphyrin. Thus, two different substituted pyridine molecules were used in order to investigate which one will better coordinate to the iron. The synthesis of these molecules was achieved by reduction of α,β-dinitroporphyrin 11b to obtain diaminoporphyrin 19 in 99% yield. Therefore, selective reaction of amine 6 or 3-(2-aminoethyl)pyridine hydrobromide with porphyrin 19 afforded compounds 20 and 21 in 35 and 48% yield, respectively. According to the above reaction, the isocyanate was generated in situ by reacting diaminoporphyrin 19 with 1/3 of an equivalent of triphosgene in the presence of Et₃N at room temperature in dry dichloromethane. When the pyridine amine 6 was added 2 equiv of Et₃N was needed to neutralize the HCl generated, but in case of 3-(2-aminoethyl)pyridine hydrobromide, 4 equiv was used. Compounds 20 and 21 were also prepared following an alternative route. Porphyrin 11b was selectively reduced to give 12b and then reacted with 6 or 3(2-aminoethyl)pyridine hydrobromide. Finally, the nitro group was reduced to afford the desired molecules 20 and 21. Following this route the overall yield was much lower compared to the overall yield obtained when reduced porphyrin 11b selectively reacted with the substituted pyridines. The overall yield following the above route (selective reduction) was 16% for 20 and 17% for 21, while the overall yield of the route that was finally followed (selective reaction) was 35 and 48%, respectively. Subsequently, reaction of aminoporphyrins 20 and 21 with triphosgene in the presence of Et₃N and addition of amine 9 afforded urea-linked porphyrins 22 and 23. Finally, the compounds were obtained by deprotection of the tert-butyl group with TFA to produce 3 and 4 in 97% yield.

Structural information from the above compounds in solution was obtained by comparison of the 1H NMR spectra of the free ligands and final molecules. In the first simple approach, a comparison of the chemical shifts was performed between ligands 5 and 1 (Table 1).

Protons H₁–H₄ of compound 1 are found to be shifted upfield in comparison to BPEA 5, with values varying from Δδ=−2.6 ppm for H₄ to Δδ=−0.3 ppm for H₂. This upfield shift observed is due to the porphyrin ring current, which means that the tridentate ligand is well situated on top of the porphyrin plane. On the other hand, comparison of chemical
shifts of free ligand 9 and porphyrin 2 showed a slight upfield shift. This indicates that the tridentate ligand is not suspended over the porphyrin plane. However, the structures shown in Schemes 2 and 3 are not a good representation of 2–4. This is due to the small $\Delta\delta$ values of the phenol protons between free ligand 9 and porphyrins 18, 22, and 23. Moreover, in order to investigate the stereo orientation of the proximal base attached on 3 and 4, the non-porphyrin analogs 24 and 25 were synthesized and their chemical shifts were compared (Scheme 4 and Table 2). Compounds 24 and 25 were synthesized following Collman’s isocyanate method. Reaction of aniline with triphosgene in the presence of triethylamine in dry dichloromethane, followed by the addition of 6 or 3-(2-aminoethyl)pyridine hydrobromide and stirring for 1 h at room temperature, afforded 24 and 25 in 78 and 76% yield, respectively.

In porphyrin 3, protons $H_{a-d}$ and $H_{f,g}$ are shifted upfield in comparison to 24 with a maximum value of $\Delta\delta=0.9$ ppm for $H_a$. This indicates that the proximal base is situated close
to the porphyrin ring. In addition, for compounds 4 and 25 there is a more pronounced upfield trend for all protons with a maximum value of $\Delta \delta = 1.2$ ppm for $H_2$, implying that 3-pyridine base is oriented closer under the porphyrin ring compared to 2-pyridine.

3. Conclusion

In conclusion, a new series of porphyrins 1–4 have been efficiently synthesized, featuring covalently attached tridentate ligand sites, a tyrosine molecule, and a proximal base. Urea and amide linkers have been used to build-up the superstructures, in order to examine whether different linkers can affect binding and reduction of oxygen. Furthermore, protection or deprotection of tyrosine molecule can be used as a switch to probe the role of tyrosine in these compounds. Finally a preliminary structural investigation has been attempted comparing the $^1$H NMR chemical shifts of free and bound ligands.

4. Experimental section

4.1. General

$^1$H and $^{13}$C NMR spectra were recorded unless otherwise specified, as deuteriochloroform solutions using the solvent peak as internal standard on a Bruker AMX-500 MHz spectrometer. UV–vis spectra were recorded on a Shimadzu Multispec-1501 instrument. All electrospray mass spectrometric experiments were performed using an LCQ Advantage (ThermoElectron, San Jose, CA) mass spectrometer. High-resolution mass spectra were performed on a MS/MS ZABSpec TOF spectrometer at the University of Rennes I (C.R.M.P.O.). Thin layer chromatography was performed on silica gel 60 F$_{254}$ plates. Chromatography refers to flash chromatography and was carried out on SiO$_2$ (silica gel 60, SDS, 70–230 mesh ASTM). All dry solvents used were dried by the appropriate technique. Organic extracts were dried over magnesium sulfate unless indicated otherwise.
Evaporation of the solvents was accomplished on a rotary evaporator.

4.1.1. Synthesis of ligands 5 and 6. A solution of 2-vinylpyridine (25.4 mL, 0.23 mmol) and ammonium chloride (24.6 g, 0.46 mmol) in water (70 mL) and methanol (10 mL) was heated for 8 h under reflux. Then the reaction mixture was cooled at 0°C and basified with 30% w/w aqueous NaOH solution (60 mL). The water layer was then washed with chloroform (5 × 20 mL). The combined organic extracts were dried over sodium sulfate, filtered, and the solvent was evaporated to obtain a yellow oil. The resulting mixture was then distilled under reduced pressure (<0.07 Torr) to give first at 100–120°C 2-(pyridin-2-yl)ethanamine 6 (8.43 g, 30%) as a colorless oil and then at 130–150°C bis(2-(pyridin-2-yl)ethyl)amine (BPEA) 5 (26.1 g, 50%) as a pale yellow oil. Compound 5. 1H NMR (500 MHz, CDCl3): δ 8.37 (m, 2H), 7.43 (td, J = 7.5 Hz, J = 2.0 Hz, 1H), 7.02 (d, J = 8 Hz, 2H), 6.96 (td, J = 5 Hz, J = 1 Hz, 2H), 2.94 (t, J = 6.5 Hz, 4H), 2.85 (t, J = 6.5 Hz, 4H), 1.64 (br s, 1H). 13C NMR (125 MHz, CDCl3): δ 160.6 (C), 149.6 (CH), 136.6 (CH), 123.6 (CH), 121.5 (CH), 49.6 (CH3), 38.8 (CH2). Rf (CH2Cl2/MeOH/Et3N 9:1:0.3): 0.37. Anal. Calcd for C14H17N3: C, 73.98; H, 7.54; N, 18.49. Found: C, 73.91;

4.1.2. Synthesis of ligand 9. A solution of 2-vinylpyridine (25.4 mL, 0.23 mmol) and ammonium chloride (25.4 mL, 0.23 mmol) and triphosgene (20 mg, 0.07 mmol) in dry dichloromethane (150 mL) was stirred for 1 h under argon at room temperature, after which ligand 9 (89 mg, 0.2 mmol) was added and the stirring continued overnight. The reaction was monitored by thin layer chromatography (4% methanol in dichloromethane). The residue was purified by column chromatography (1–3% methanol in dichloromethane). The product was purified by column chromatography (1–3% methanol in dichloromethane). The product was purified by column chromatography (1–3% methanol in dichloromethane).

4.1.3. Synthesis of porphyrin 14. A mixture of α,β-monophenylaminoporphyrin 12b (89 mg, 0.2 mmol), triethylamine (56 μL, 0.4 mmol), and triphosgene (20 mg, 0.07 mmol) in dry dichloromethane (150 mL) was stirred for 1 h under argon at room temperature, after which ligand 9 (89 mg, 0.2 mmol) was added and the stirring continued overnight. The reaction was monitored by thin layer chromatography (4% methanol in dichloromethane). The residue was purified by column chromatography (1–3% methanol in dichloromethane). The product was purified by column chromatography (1–3% methanol in dichloromethane). The product was purified by column chromatography (1–3% methanol in dichloromethane). The product was purified by column chromatography (1–3% methanol in dichloromethane). The product was purified by column chromatography (1–3% methanol in dichloromethane). The product was purified by column chromatography (1–3% methanol in dichloromethane). The product was purified by column chromatography (1–3% methanol in dichloromethane). The product was purified by column chromatography (1–3% methanol in dichloromethane). The product was purified by column chromatography (1–3% methanol in dichloromethane).
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Supplementary data

Experimental procedures, characterization, 1H NMR, and 13C NMR spectra, for all new compounds. For synthesis of compounds 10, 11a, 11b, 12a, 12b, and 19, see Ref. 9. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.01.036.

References and notes

13. 1H NMR chemical shifts of pyridine analogs 24 and 25 are provided in the Supplementary data.