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Substituted tren-capped porphyrins: probing the influence of copper in synthetic models of cytochrome *c* oxidase

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Tris(2-aminoethylamine) (tren)-capped porphyrins bearing electron donating or withdrawing groups on the amino functions of their tripod have been synthesised and the catalytic activity of each complex was carefully studied both as the iron and the iron–copper complexes: only one of our iron–copper complexes was shown to be active and selective towards the reduction of dioxygen to water.

Introduction

Intensive studies have been devoted to the understanding of the catalytic cycle by which cytochrome *c* oxidase (C*c*O) reduces oxygen to water in the inner membrane of mitochondria. The major effect of this final step in the respiratory chain is the formation of the highly energetic molecule ATP, without any leakage of the deleterious partially reduced intermediates.[1]

The nature of O_2 interaction with the iron and copper metal centers in C*c*O is of paramount importance. Indeed, many issues remain unsolved, such as the role of copper during the catalytic cycle in O_2-bridging, and the type of O_2-bound intermediates, even if some of them were characterized by time-resolved resonance Raman spectroscopy.[2] Most researchers are convinced that the catalytic cycle passes through the formation of a heterobimetallic μ-peroxo intermediate.[3–6] Nevertheless, this very plausible hypothesis did not receive confirmation as the numerous studies with synthetic model molecules did not really clarify the matter. However, in former reports about the activity of tren-capped porphyrins, we have shown that "iron-only" porphyrins are efficient catalysts for the reduction of dioxygen, when the complexes are adsorbed on graphite electrodes in contact with aqueous media at pH close to 7.[7] This crucial observation demonstrates that the postulated μ-peroxo complex is not an essential intermediate for the four-electron reduction of oxygen, as long as electrons and protons can be rapidly delivered to the catalytic site of a synthetic model.[8] Our conclusion, in agreement with recent spectroscopic results,[9] is supported by Yoshikawa *et al.* who suggested that the peroxo derivative could be protonated by Tyr_{244}, located in a closed environment of the coordination sphere of copper, forming a hydroperoxo adduct.[10] In 1997, Collman *et al.* reported the electrocatalytic reduction of dioxygen, using heterobimetallic Fe–Cu or Co–Cu compounds, tethered with TACN (triazacyclononane) or tren, in order to investigate the influence of the presence of the two metals on the ratio of $2e^-/4e^-$ processes.[11] Their conclusion was that the iron-only catalysts studied were not active and that Cu was essential to the $4e^-$ process. However, in a recent study, with different molecules, these authors came to the conclusion that the presence of Cu is not needed for the reduction of O_2 to water.[12]

Binding of the Cu centre to the oxygen–iron porphyrin complex could possibly be dependent on the coordination sphere of the metal. Various nitrogenous ligands (triazacyclononane, tren, imidazole and quinoline pickets, benzyl-tren) differing simultaneously by several structural parameters such as the geometry, the flexibility, or the nature of the nitrogen atoms have already been tested. So far, with our first generation complexes,[7] we have only probed the effect of the relative position of the two metal centres, but the environment of copper has never been investigated. The aim of the present work is to examine the influence of substituents on the *para* position of benzyl-tren. It is reasonable to expect these to modify the electron density on the Cu^I site and thus its ability to interact with the oxygen molecule bound to the iron porphyrin.

Results and discussion

Herein are reported the synthesis and electrochemical behaviour of a new series of molecules in which electronically active ligands have been attached to the amino groups of the tren moiety. This functionalisation of the secondary amines of the cap should affect the interaction with dioxygen. The interest of such a derivatisation is that it modifies the electronic properties of the copper chelate, without any modification of the basic backbone, the steric hindrance or the relative position of the two metals.

Three molecules have been synthesised and these differ only by the *N*-substitution of the tren amino functions bearing either a tribenzyl, a tris[4-(dimethylamino)benzyl], or a tris(4-nitrobenzyl) group. These benzyl moieties should have different electronic effects on the copper ion in the distal position and, consequently, modify the electron density of the bound O_2. Since it has been previously demonstrated that a nitrogen base covalently bound to the porphyrin is not critical for the efficiency of the catalyst,[8,13] 1,2-dimethylimidazole (1,2-diMeIm) was simply added as the fifth ligand of iron in the present study. The synthesis of the iron five-coordinate complexes and heterobimetallic iron–copper catalysts is depicted in Scheme 1.

1 and **2** have been synthesised by grafting the corresponding trisubstituted trens **6** and **7** on the three acryloyl pickets of porphyrin **5** under mild conditions.[14] In model molecule **2**, the secondary amine groups are functionalised by an electron donor substituent ($X = NMe_2$).† Unfortunately, attempts towards synthesis of a compound possessing an electron-withdrawing substituent ($X = NO_2$) failed, plausibly, because of the lower nucleophilicity of the amine. Actually, the preparation of **3**† required the reaction of *p*-nitrobenzyl bromide, under more drastic conditions, on the tren-capped porphyrin **4**, the synthesis of which has been previously reported.[7]

The electroreduction of dioxygen was examined by rotating ring-disk voltammetry (RRDE).‡ The iron-only complexes corresponding to **1** (Fig. 1) and **2** (Fig. 2, dashed line) are efficient catalysts of the $4e^-$ reduction when adsorbed on the graphite electrode, in contact with an aqueous solution at pH = 6.86. This is similar to what has been oberved for other model

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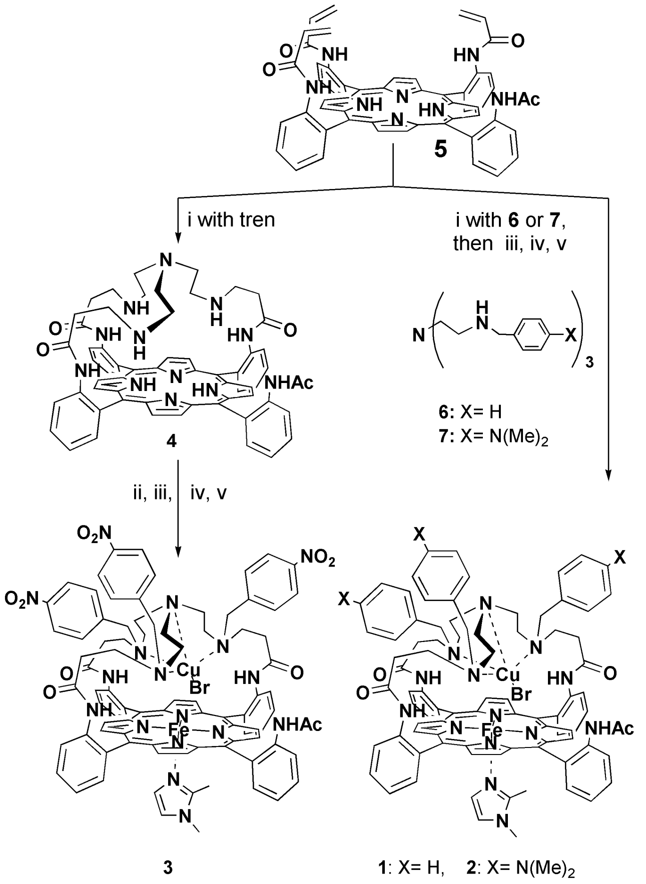

Scheme 1 Synthesis of substituted tren-capped porphyrins. *Reagents*: i, tren, **6** or **7**, MeOH–$CHCl_3$, 50 °C; ii, *p*-nitrobromobenzyl, DMF, 90 °C; iii, iron bromide, THF, 55 °C; iv, copper bromide, CH_3OH, 80 °C; v, excess of 1,2-diMeIm.

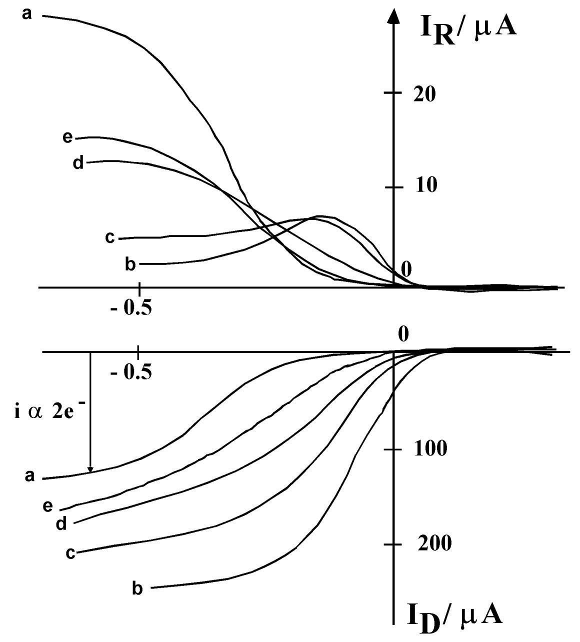

Fig. 1 Catalysis of O_2 reduction by **1Fe**. a: bare graphite electrode; b, c, d, e: four consecutive cycles after adsorption of **1Fe** on the graphite disc of the RRDE.

molecules.[7] After adsorption of **3Fe** at the electrode, the voltammogram is equivalent to that observed for the bare graphite electrode; one notices exclusively the $2e^-$ reduction of O_2. This could result from a very low concentration of **3Fe** at the surface of graphite due to leaching from the contact of the electrode with the aqueous solution; **3Fe** is somewhat soluble in water.

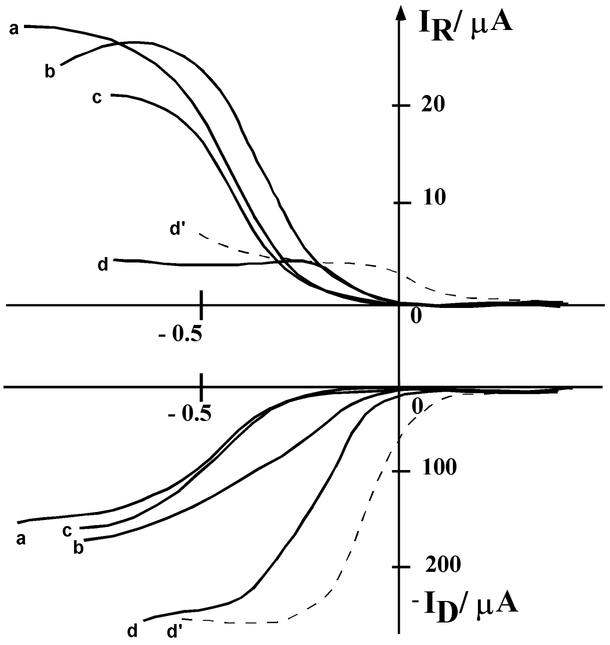

Fig. 2 RRDE voltammograms of FeCu complexes **1** (b), **2** (d) and **3** (c) and of Fe complex **2Fe** (d′) for comparison. a: bare graphite electrode.

As shown in Fig. 1, **1Fe** is almost a selective catalyst for the $4e^-$ reduction of dioxygen (curve b). The number of electrons exchanged per O_2 molecule has been derived from the limiting current on the bare graphite electrode (curve a). The production of hydrogen peroxide at the platinum ring is very low when the limiting current of the reduction process is reached. Some hydrogen peroxide is produced in the activation region of the O_2 reduction process, at the foot of the wave; this has been observed in our previous work[7,8] and is also apparent in the results reported by others.[9] The $4e^-$ reduction process takes over at larger overpotentials. The reduction starts at a much more positive value (+0.05 V) than those corresponding to various compounds we have previously studied.[7] From successive potential scans (Fig. 1), it appears that the catalyst is not stable, consequently preventing Koutecky–Levich analysis. Along its degradation, the wave shifts to more negative potentials and the reduction current decreases, which is attributed to the diminution of the surface concentration of the catalyst.

The FeCu nitro-derivative complex **3FeCu** does not exhibit a catalytic activity (Fig. 2, curve c), probably for the same reasons as those mentioned above for **3Fe**. **1FeCu** exhibits a poor catalytic activity, forming almost exclusively hydrogen peroxide; the reduction of dioxygen begins at a potential only slightly more positive than that on the graphite surface. **2FeCu**, the complex bearing NMe_2 electrodonor groups, has a modest catalytic activity towards O_2 reduction with respect to its reduction potential; the reduction starts at about −0.05 V, the plateau being reached at −0.4 V (Fig. 2, d). However, it induces mainly the four electron reduction of dioxygen to water. This has to be pointed out as, from the various **PFeCu** and **PFe** examined, **2FeCu** is the first heterodinuclear compound having a catalytic efficiency equivalent to that of the iron-only complex, **2Fe**.

Conclusions

A new series of tren-capped porphyrins has been prepared and studied both as their iron and iron–copper complexes. Slight modifications of the electronic properties in the distal coordination site have been achieved through the substitution of the *para* hydrogen atom by NMe_2 and NO_2 groups. The results indicate that closely related catalysts can influence very differently the reduction of dioxygen. For instance, in this new series, only one of the bimetallic complexes is a selective $4e^-$ catalyst. Indeed, in the case of the $-NMe_2$ substituent, the copper might be in a suitable electronic environment to interact properly with the iron-bound O_2 in such a way that the cleavage of the O–O bond occurs. The adsorption of these molecules could also be affected by as light structural modifications as those performed in this work. These results confirm that, for synthetic molecules adsorbed at the surface of an electrode, the presence of a copper center does not improve the catalysis in comparison with iron-only analogues.

These observations demonstrate the fact that precise comparisons should be performed only between very closely related structures. Besides, in order to examine the influence of the Tyr_{244} motif, a third generation of tren-capped catalysts is under investigation. In this series, both the location and the properties of the group mimicking the tyrosine will be modulated.

Notes and references

† Selected data for **1**: ^{1}H NMR δ_H (500 MHz, $CDCl_3$, 323 K): $\delta = 10.13$ (s, 1H, –NHCO), 9.67 (s, 2H, –NHCO), 9.02 (d, $J = 5.0$ Hz, 2H, $H_{\beta\text{-pyr}}$), 8.99 (d, $J = 5.0$ Hz, 2H, $H_{\beta\text{-pyr}}$), 8.92 (d, $J = 5.0$ Hz, 2H, $H_{\beta\text{-pyr}}$), 8.71 (d, $J = 5.0$ Hz, 2H, $H_{\beta\text{-pyr}}$), 8.37 (d, $J = 8.5$ Hz, 2H, H_{aro}), 8.18 (d, $J = 8.0$ Hz, 1H, H_{aro}), 7.86–7.76 (m, 8H, H_{aro}), 7.51 (td, $J = 7.5$ Hz, $J = 1.0$ Hz, 4H, H_{aro}), 7.43 (td, $J = 7.5$ Hz, $J = 1.0$ Hz, 2H, H_{aro}), 7.23–7.16 (m, 4H), 7.13 (m, 5H), 6.82 (d, $J = 7.0$ Hz, 3H, H_{aro}), 6.67 (m, 3H), 2.82 (d, $J = 20.0$ Hz, 4H, $-CH_{2\ benzyl}$), 2.41 (d, $J = 15.0$ Hz, 2H, $-CH_{2\ benzyl}$), 2.02–1.63 (m, 12H, $-CH_2$), 1.31 (s, 3H, $-CH_3$), 0.56 (m, 2H), −0.06 (m, 4H), −1.58 (m, 4H), −2.10 (m, 2H), −2.48 (s, 2H, $-NH_{pyr}$). Anal. Calcd. for $C_{82}H_{78}N_{12}O_4\cdot H_2O$: C, 74.98, H, 6.14, N, 12.80, Found: C, 75.03, H, 6.01, N, 12.62%; **1Fe**: MS (MALDI-TOF, linear mode): *m*/*z* (%): 1348.96 (100) $[M^+]$. **1FeCu**: MS (FAB): $m/z = 1349.55$ $[M - (CuBr) + H]^+$.

2: ^{1}H NMR δ_H (500 MHz, $CDCl_3$, 323 K): $\delta = 10.36$ (s, 1H, –NHCO), 10.05 (s, 2H, –NHCO), 8.97 (d, 2H, $J = 5.0$ Hz, $H_{\beta pyr}$), 8.93 (d, 2H, $J = 5.0$ Hz, $H_{\beta pyr}$), 8.87 (d, 2H, $J = 5.0$ Hz, $H_{\beta pyr}$), 8.66 (d, 2H, $J = 5.0$ Hz, $H_{\beta pyr}$), 8.34 (d, 2H, $J = 8.0$ Hz, H_{aro}), 8.02 (d, 1H, $J = 8.0$ Hz, H_{aro}), 7.83–7.70 (m, 6H), 7.72 (td, 1H, $Jo = 8.5$ Hz, $Jm = 1.5$ Hz, H_{aro}), 7.50 (m, 4H, H_{aro}), 7.39 (td, 1H, $Jo = 7.0$ Hz, $Jm = 1.0$ Hz, H_{aro}), 6.67 (d, 2H, $J = 8.0$ Hz, H_{aro}), 6.49–6.40 (m, 10H), 5.30 (s, 1H, –NHCO), 2.92 (s, 6H, $-CH_3$), 2.83 (s, 9H, $-CH_3$), 2.69 (m, 4H, $-CH_{2benzyl}$), 2.28 (d, 2H, $J = 13.5$ Hz, $-CH_{2benzyl}$), 2.15–1.70 (m, 12H, $2CH_{2benzyl} + 2CH_3$), 1.59 (m, 6H, $-CH_{2benzyl}$), 0.49 (m, 2H, $-CH_{2benzyl}$), −0.25 (m, 4H, $-CH_{2benzyl}$), −1.55 (m, 4H, $-CH_{2benzyl}$), −2.08 (m, 2H, $-CH_{2benzyl}$), −2.56 (s, 2H, $-NH_{pyr}$). Anal. Calcd. for $C_{88}H_{93}N_{15}O_4\cdot CH_2Cl_2$: C, 70.81, H, 6.64, N, 13.92, Found: C, 70.44, H, 6.32, N, 11.91%; **2Fe**: MS (MALDI-TOF): $m/z = 1477.9$ $[M]^+$. **2FeCu**: MS (MALDI-TOF): $m/z = 1478.7$ $[M - (CuBr) + H]^+$.

3: ^{1}H NMR δ_H (500 MHz, $CDCl_3$, 323 K): $\delta = 9.48$ (s, 1H, –NHCO), 9.04 (d, 2H, $J = 5.0$ Hz, $H_{\beta pyr}$), 9.00 (d, 2H, $J = 5.0$ Hz, $H_{\beta pyr}$), 8.97 (s, 2H, –NHCO), 8.94 (d, 2H, $J = 5.0$ Hz, $H_{\beta pyr}$), 8.79 (d, 2H, $J = 5.0$ Hz, $H_{\beta pyr}$), 8.46 (d, 2H, $J = 8.0$ Hz, H_{aro}), 8.22 (d, 1H, $J = 8.0$ Hz, H_{aro}), 8.03 (d, 2H, $J = 9.0$ Hz, H_{aro}), 7.93 (d, 4H, $J = 9.0$ Hz, H_{aro}), 7.91–7.79 (m, 6H, H_{aro}), 7.67 (dd, 1H, $Jo = 7.5$ Hz, $Jm = 1.5$ Hz, H_{aro}), 7.63–7.53 (m, 3H, H_{aro}), 7.51 (td, 1H, $Jo = 7.5$ Hz, $Jm = 1.0$ Hz, H_{aro}), 7.37 (t, 1H, $J = 7.5$ Hz, H_{aro}), 7.00 (d, 2H, $J = 8.5$ Hz, H_{aro}), 6.73 (d, 4H, $J = 8.5$ Hz, H_{aro}), 6.39 (s, 1H, –NHCO), 2.25 (d, 2H, $J = 22.5$ Hz), 2.10–2.00 (m, 4H), 1.98–1.89 (m, 4H), 1.86–1.74 (m, 4H), 1.62 (m, 2H), 1.53 (s, 2H), 1.19 (s, 3H), 0.57 (m, 2H), 0.08 (m, 2H), −0.05 (m, 2H), −1.36 (m, 2H), −1.83 (m, 2H), −1.99 (m, 2H), −2.49 (s, 2H, $-NH_{pyr}$). Anal. Calcd. for $C_{82}H_{75}N_{15}O_{10}\cdot 2CHCl_3$: C, 59.79, H, 4.72, N, 12.45, Found: C, 59.34, H, 4.84, N, 12.44%. **3Fe**: MS (FAB): $m/z = 1484.6$ $[M]^+$. **3FeCu**: HR-MS (LSI-MS): calcd. $m/z = 1484.5092$ for $C_{82}H_{74}N_{15}O_4Fe$ $[M - (CuBr) + H]^+$, Found 1484.5088.

‡ The diameter of the highly oriented graphite electrode (edge plane: EPGE) disk is 6 mm, the collecting efficiency of the ring-disk electrode being 27% (Pine Instrument Co.). The graphite electrode is modified by dipping it 5 minutes in the solution of the catalyst in $CHCl_3$. A bi-potentiostat (Solea-Tacussel) pilots the disk potential, when the platinum ring is maintained at 0.8 V *vs.* SCE for experiments at pH 6.86 (KH_2PO_4 0.025 M + Na_2HPO_4 0.025 M).

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