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Synthesis and superoxide dismutase-like activity of new manganese(III) complexes based on tridentate N_2O ligands derived from histamine

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Abstract

We have obtained two new manganese(III) complexes based on tridentate ligands bearing imidazole and phenol moieties. The structure of the chosen ligands favored the Mn(III) oxidation state due to the compatibility of the geometry of the complexes with a Jahn–Teller tetragonal distortion, as was shown by X-ray diffraction for one of the complexes. This induced a lowering of the oxidation potential for the Mn(III)/Mn(II) couple, which can be correlated to the superoxide dismutase-like (SOD-like) activity of the complexes, compared with a previously published Mn(III) complex.

Keywords: Manganese(III); SOD mimic; Histamine; Electrochemistry; Tetragonal distortion; N_2O ligand

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1. Introduction

In the past years, a series of Mn-SOD mimetic complexes based on tertiary or secondary amines as tetradentante or tridentate ligands was synthesized in our laboratory. The whole series showed a SOD-like activity, according to the results of the indirect McCord–Fridovich test [1] and from pulsed radiolysis experiments [2,3]. We showed, in the series, that the anodic potential associated with the oxidation from Mn(II) to Mn(III) is correlated with the SOD activity [2]. The most oxidizable complex, which was indeed isolated in the Mn(III) state, was obtained from the 2-hydroxybenzyl-imidazol-2-yl-methylamine (PIH) ligand (see Fig. 1) and was the most active. Similar correlations were observed by other research groups in the series of Mn porphyrins [4–6] and iron complexes [7].

In order to further lower the potential of the Mn(II)/Mn(III) couple, we have used two ligands derived from histamine. The first one is 4-(2-salicylideneamino-ethyl)-imidazole (abbreviated PhIImH in this work) [8], and the second one is its amine analog 4-(2-salicylamino-ethyl)-imidazole (abbreviated PhIImH in this work) [9]. We expected a supplementary stabilization of the Mn(III) state in $[Mn^{III}L_2]^+$ (**1**, L = PhIIm; **2**, L = PhI) cationic complexes in comparison to the $[Mn(PI)_2]^+$ (**3**) complex [2] due to the fact that PhI and PhIIm ligands, including a supplementary carbon atom in the arm bearing the imidazole moiety, should favor a Jahn–Teller distortion (elongation along the N_{imid}–Mn–N_{imid} axis) for their ML_2 complex.

2. Experimental

NMR spectra were recorded on a Bruker AC 250 spectrometer. IR spectra (KBr pellets) were recorded on a Bruker IFS 66 FT-IR spectrometer. Microanalysis was performer by Service de Microanalyse de l'ICSN (Gif-sur-Yvette) for C, H and N, and by Service Central d'Analyse du CNRS (Vernaison) for other elements. Cyclic voltammetry was recorded with an EGG PAR potentiostat (M273 model).

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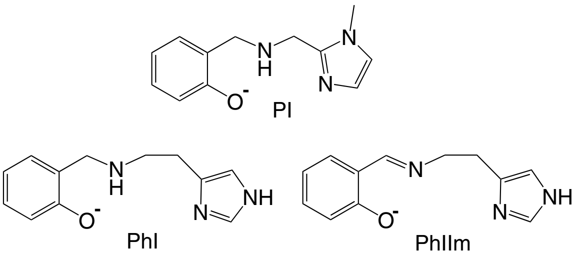

Fig. 1. The tridentate PI, PhI, PhIIm ligands.

The counter electrode was a Pt wire. The working electrode, a glassy carbon disk, was polished before each voltammogram with 3 μm diamond paste The reference electrode was a SSCE electrode isolated by a fritted bridge. Chemical reagents and solvents were purchased from Acros and used without further purification. For electrochemistry, acetonitrile was distilled on $CaCl_2$ just before use.

Safety note: Although no particular problems were encountered during the preparation of the perchlorate salt of complex **2**, perchlorate salts of metal complexes with organic ligands are potentially explosive and should be handled with care.

McCord–Fridovich assay or Xanthine–Xanthine Oxidase-Cytochrome c (cytc) assay: The superoxide anion was supplied to the system from the xanthine–xanthine oxidase reaction. To check that the tested compounds do not inhibit the production of superoxide by xanthine oxidase, the rate of conversion of xanthine to urate (see below) was determined by measuring the increase in absorbance at 290 nm over a 2-min period with and without the tested compounds. At concentrations higher than the *IC*50 value, no inhibition of this conversion was recorded.

Xanthine to urate assay: To measure the rate of conversion of xanthine to urate, xanthine oxidase (30 μL of a 0.77 U mL^{-1} solution) was added to a solution of potassium phosphate buffer (pH 7.8; 50 mmol L^{-1}) containing xanthine (150 μmol L^{-1}) at a final volume of 1.0 mL at 25 °C. Urate production was monitored at 290 nm. No difference in the slope was recorded with or without the putative SOD mimics.

Activities were measured using ferricytochrome *c* (cyt*c*-Fe(III)) reduction. The assay was performed at 25 °C in 3 mL of reaction buffer (50 mmol L^{-1} potassium phosphate buffer, pH 7.8) containing cyt*c*-Fe(III) (22 μmol L^{-1}), xanthine (200 μmol L^{-1}), and an amount of xanthine oxidase such as to give a rate of $\Delta A_{550\,\text{nm}} = 0.025\ \text{min}^{-1}$ about 0.01 U mL^{-1} in the absence of a putative SOD mimic. The reduction of cyt*c*-Fe(III) was monitored at 550 nm. After 2 min, different amounts of the putative SOD mimic were added. Rates were linear for at least 6 min. Both rates in the absence and in the presence of the complex were determined for each concentration of the complex added.

The *IC*50 value represents the concentration of putative SOD mimic that induces a 50% inhibition of the reduction of cyt*c*-Fe(III). If s_1 is the slope before addition of the putative SOD mimic and s_2 is the slope after addition of the putative SOD mimic, the inhibition percentage is given by $I\,(\%) = (s_1 - s_2)/s_2 \cdot 100$. For an *IC*50 value determination, *I* is measured for several concentrations in the range of the *IC*50. *IC*50 is obtained for $I = 50$.

As *IC*50 values depend on experimental details (nature and concentration of the marker), we calculated k_{McCF}, an apparent catalytic rate constant for the dismutation of superoxide [2]

$$k_{\text{McCF}} = \frac{k_{\text{cyt}c}[\text{cyt}c]}{[\text{SODm}]}$$

where [cyt*c*] and [SODm] are, respectively, the concentrations of cyt*c* and SOD mimic and $k_{\text{cyt}c}$ is the known rate constant of the bimolecular reaction between O_2^- and cytochrome *c* [10]. These calculated k_{McCF} values allow the comparison of SOD activities obtained by the McCord–Fridovich test with different concentrations of cyt*c* or using another marker such as NBT.

2.1. Synthesis of ($\mathbf{1}(PF_6) \cdot 2H_2O$)

This synthesis was inspired from [9,8]. Histamine dihydrochloride (1.73 g, 9.36 mmol, 1 equiv.) was added to a solution of sodium ethanolate prepared by the addition of 0.431 g of sodium (18.4 mmol, 2 equiv.) in 100 mL of absolute ethanol. NaCl immediately precipitated. As 1 mL (1.15 g, 9.4 mmol 1 equiv.) of salicylaldehyde was added, a yellow color appeared. The resulting mixture was heated to reflux for 20 min, then allowed to cool to room temperature and filtered. $MnBr_2$ (1.01 g, 4.7 mmol 0.5 equiv.) and 0.6 mL of triethylamine (0.489 g, 2.4 mmol, 0.25 equiv.) were added to the resulting solution which turned immediately dark brown. A solid precipitated and was redissolved by heating the mixture to 50 °C. NH_4PF_6 was added to this solution which was allowed to cool down slowly to rt. After a few weeks of slow evaporation, 2.72 g (yield = 85%) of dark-brown crystals was isolated by filtration and washed with cold EtOH.

ES-MS, *m/z* (% intensity): 483.2 $\mathbf{1}^+$ (100), 216.2 $(\text{PhIImH} + \text{H})^+$ (48).

IR ($\nu_{\max}$, cm^{-1}), KBr: 771, $\delta_{\text{imidazole}}$; 856, PF_6; 1297, $\nu_{\text{C–O}_{\text{phenol}}}$; 1446, $\nu_{\text{C=N}_{\text{imidazole}}}$; 1617, $\nu_{\text{C=N}_{\text{imine}}}$.

Microanalysis ($[\text{Mn(PhIIm)}_2](PF_6) \cdot 3H_2O$% calc./found): C:42.24/42.47, H: 4.43/4.12, N: 12.31/12.71, P: 4.54/4.20, Mn 8.05/7.83.[1]

2.2. Synthesis of ($\mathbf{2}(ClO_4) \cdot H_2O$)

2.2.1. Ligand synthesis

PhIImH was synthesized (without isolation) as described above. After NaCl filtration, 0.443 g (11.8 mmol,

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[1] The best correspondence between experimental and calculated values for $\mathbf{1}(PF_6) \cdot nH_2O$ was obtained with three water molecules even though only two appeared in the diffraction structure. The supplementary water molecule could results from non-specific hydration of the ground sample which was analyzed.