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Synthesis, structure and interactions with DNA of novel tetranuclear, [Mn_4(II/II/II/IV)] mixed valence complexes

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Abstract

Reaction of Mn(II) with phenoxyalkanoic acids and di-2-pyridyl ketone oxime (Hpko) leads to neutral tetranuclear complexes of the general formula $Mn_4(O)(pko)_4$(phenoxyalkanoato)$_4$ (phenoxyalkanoic acids: H-mcpa = 2-methyl-4-chloro-phenoxy-acetic acid, H-2,4,5-T = 2,4,5-trichloro-phenoxy-acetic acid or H3,4-D = 3,4-dichloro-phenoxy-acetic acid). The compounds were synthesized by adding di-2-pyridyl ketone oxime to $MnCl_2$ in the presence of the sodium salts of the alkanoic acids in methanol. The crystal structure of Mn_4(II/II/II/IV)$(O)(pko)_4(2,4,5\text{-}T)_4 \cdot 2.5CH_3OH \cdot 0.25H_2O$ **1** shows that the complex consists of a $[Mn_4(\mu_4\text{-}O)]^{8+}$ core with a Mn(IV) and 3 Mn(II) ions in octahedral environment and a μ_4-O atom bridging the four manganese ions. Spectroscopic studies of the interaction of these tetranuclear clusters with DNA showed that these compounds bind to dsDNA. The binding strength of the Mn_4(II/II/II/IV)$(O)(pko)_4(2,4,5\text{-}T)_4$ complex for calf thymus DNA is equal to $1.1 \times 10^4\ M^{-1}$. Among the deoxyribonucleotides they bind preferentially to deoxyguanylic acid (dGMP). Competitive studies with ethidium bromide (EthBr) showed that the Mn_4(II/II/II/IV)$(O)(pko)_4(2,4,5\text{-}T)_4$ complex exhibited the ability to displace the DNA-bound EthBr indicating that the complex binds to DNA via intercalation in strong competition with EthBr for the intercalative binding site. Additionally, DNA electrophoretic mobility experiments showed that all three complexes, at low cluster concentration, are obviously capable of binding to pDNA causing its cleavage (relaxation) at physiological pH and temperature. At higher cluster concentration, catenated dimer forms of pDNA was formed.

Keywords: Manganese complexes; Mixed valence; Phenoxyalkanoic acids; DNA interaction

1. Introduction

The interest in interactions of metal complexes with DNA is first of all stimulated by their biological activity, which is focusing on their involvement in DNA function or in other biological processes or systems in the living cell. Mn^{2+} ions were found to be an essential element for a number of DNA binding proteins, which possess structural rather than enzymatic activity or are able to change the enzymatic activity of some nuclear proteins. It has become accepted that the catalytic site for water oxidation in the oxygen evolving complex (OEC) of photosystem II contains one calcium and four manganese ions that are bridged together by water-derived O^{2-} and OH^- ligands [1–3].

Several tetranuclear mixed valence manganese complexes have been prepared as potential models for the OEC of PSII [4–15]. To date, the lowest mixed valence tetranuclear clusters that have been reported have average oxidation state 2.5+/Mn and (II,III,II,III) or (II,II,II,IV) formulations, with a linear $(\mu\text{-}O)Mn_2(\mu_3\text{-}O)Mn$ bridging

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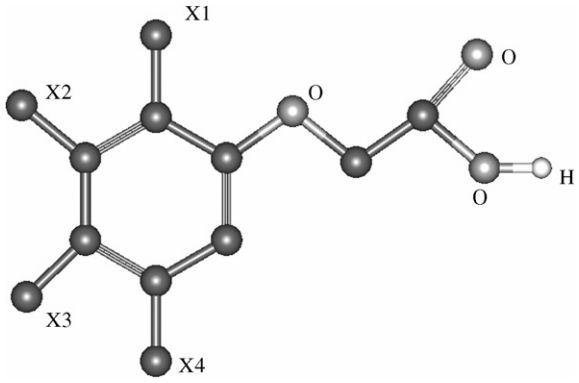
moiety [5,7] or with a (μ_4-O)Mn bridging moiety, respectively [8]. Reports of tetranuclear Mn complexes with average oxidation state 2.75+/Mn (represented by two valence distributions) – [Mn(II)Mn(III)$_3$] [9] and [Mn(II)$_2$Mn(III)Mn(IV)] [6], 3.0+/Mn with only [Mn(III)$_4$] oxidation states [11,12], 3.25+/Mn and 3.50+/Mn with valence distributions [Mn(III)$_3$Mn(IV)] and [Mn(III)$_2$Mn(IV)$_2$], respectively [4,10,12–14], 3.75+/Mn with [Mn(III)Mn(IV)$_3$] formulation, and 4.00+/Mn with [Mn(IV)$_4$] homovalence formulation have also been published [15]. The 3.25+/Mn and 3.50+/Mn oxidation states have the best resemblance of the S_1 state of the OEC while the 3.75+/Mn state is the best model for the S_2 state. Besides the development of such complexes as models for the OEC, an emerging area of interest is the study of the interaction of such metal complexes with DNA, in an effort to identify new agents that can recognize or cleave DNA [16–24]. For this purpose, the knowledge of the mode and the extent of binding of metal complexes to DNA are very important. The interaction of manganese ions with native DNA and its monomers was studied [25], and it was demonstrated that manganese is involved in interactions with phosphate oxygens and with the bases of DNA. At high Mn^{2+} concentrations DNA aggregation was also observed [26]. It is known that metal complexes may interact with DNA either covalently or non-covalently. In covalent binding, the labile part of the complexes is replaced by a nitrogen base of DNA such as guanine N7, as found for cisplatin. On the other hand, non-covalent DNA interactions include intercalative, electrostatic and groove binding of cationic metal complexes along the outside of the DNA helix, in the major or minor groove. Intercalation involves the partial insertion of aromatic heterocyclic rings between the DNA base pairs [27–31]. While many research efforts have been dedicated to the rational design and elaboration of biomimetic systems based on the interaction of nucleobases and their derivatives with a wide range of metal ions, studies of polynuclear metal compounds and especially manganese clusters are quite limited [16–19]. The binding properties of a novel Mn(II) complex, 2*H*-5-hydroxy-1,2,5-oxadiazo[3,4-*f*]1,10-phenanthroline with calf thymus DNA showed that the complex binds to DNA by intercalation [23].

In a previous report [8], we have examined the magnetic and structural features of the first reported example of a $[Mn_4]^{10+}$ (Mn(II,II,II,IV)) system, while studies on whether XANES spectroscopy can be used to accurately assign oxidation states for two valence isomers tetranuclear manganese $[Mn_4]^{10+}$ systems, i.e. Mn(II,II,II,IV) versus Mn(II,III,III,II), are in progress (manuscript in preparation). We have also initiated studies on the co-ordination chemistry of carboxylate-containing herbicides with Mn(II) and Mn(III) metal ions in an attempt to examine their mode(s) of binding and possible biological relevance [32–37]. In addition, we have reported [38–40] studies on the effect of polynuclear metal complexes on the integrity and electrophoretic mobility of nucleic acids. Antifungal and antibacterial properties of a range of metal complexes have been evaluated against several pathogenic fungi and bacteria [41–52].



Scheme 1. H-2,4,5-T: $X_1 = Cl$, $X_2 = H$, $X_3 = Cl$, $X_4 = Cl$, $Y = H$; H-3,4-D: $X_1 = H$, $X_2 = Cl$, $X_3 = Cl$, $X_4 = H$, $Y = H$; H-mcpa: $X_1 = CH_3$, $X_2 = H$, $X_3 = Cl$, $X_4 = H$, $Y = H$.

In this paper, we report the synthesis and characterization of the new mixed valence tetranuclear clusters $Mn_3^{II}Mn^{IV}(O)(pko)_4(L)_4 \cdot XCH_3OH \cdot YH_2O$ (L = 2,4,5-T, X = 2.5, Y = 0.25 (**1**); L = mcpa, X = 3, Y = 0 (**2**) cf. Scheme 1) and the crystal structure of **1**. We also report an investigation of the DNA binding behaviour of the new tetranuclear manganese clusters with DNA in an attempt to explore their potential biological activity with particular focus on their DNA binding mode and affinities or the ability of these clusters to function as DNA intercalators. All tetranuclear clusters reported herein have been tested for their ability to bind/degrade various forms of DNA. Furthermore, DNA binding properties of the complexes with calf thymus DNA by spectroscopic titration were also investigated. Competitive binding studies with ethidium bromide, which is the most widely used intercalative agent and fluorescence probe for DNA structure, have been employed in the examinations of the interaction of the new tetranuclear manganese clusters with DNA in order to investigate a potential intercalative binding mode.

2. Experimental

2.1. Materials

The chemicals for the synthesis of the compounds were used as purchased. Acetonitrile (CH_3CN) was distilled over calcium hydride (CaH_2) and CH_3OH was distilled over magnesium (Mg) and was stored over 3 Å molecular sieves. Dimethylformamide = dmf and dimethyl sulfoxide = dmso were used without any further purification. Hpko = di-2-pyridyl ketone oxime, $MnCl_2 \cdot 4H_2O$, H3,4-D, H2,4,5-T, H-mcpa (cf. Scheme 1) were purchased from Aldrich Co. All chemicals and solvents were reagent grade.

Agarose was purchased from BRL. Tryptone and yeast extract were purchased from Oxoid (Unipath Ltd., Hampshire, UK). The intercalative dye ethidium bromide (EthBr) was purchased from Sigma. Native DNA (dsDNA)