Facile Uptake of Manganese(III) by Apo-Transferrin: Possible Origin of Manganism

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We have investigated the mechanism of manganese ion uptake by apo-transferrin using a capillary electrophoresis method, and obtained clear evidence that oxidation state +3 and the binuclear unit of a manganese chelate are essential factors for the facile uptake by apo-transferrin, similar to that observed for Fe(III) chelates. These results may give valuable information to understand the pathogenesis of manganism and to develop new countermeasures for the neurotoxicity by manganese ions.

Key words: Manganism, Capillary Electrophoresis, Transferrin, Binuclear Mn(III) Unit

Introduction

Metal speciation (oxidation state and ligand environment) is important in determining the functionality and toxicity of trace elements in biological systems. This may be particularly true for manganese which is an essential trace element in biology. Concern over possible neurotoxic effects associated with chronic exposure to moderate levels of manganese in mixed oxidation states may be justified by recent studies reporting that workers in the manganese alloy industry are exposed to manganese aerosols of mixed oxidation state and by the growing use of MMT, a manganese-containing antiknock additive in gasoline, in a number of developed countries (Reaney *et al*., 2006; Crossgrove *et al*., 2003).

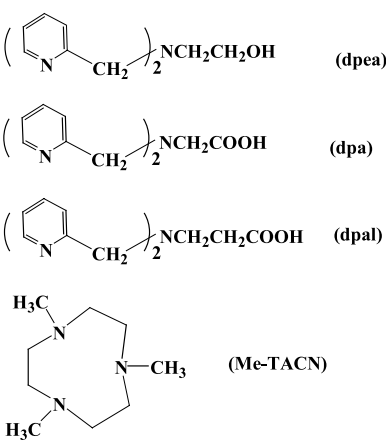
Elevated occupational exposures to manganese are known to cause significant neurotoxicity, and epidemiologic studies have suggested a relationship between high manganese exposure and an increased risk for parkinsonian disturbances, called *manganism*, although the exact mechanisms underlying the neurotoxic effects of manganese remained unclear (Dobson *et al.*, 2004). It has been reported that rats exposed to very elevated manganese levels via drinking water from an early age displayed increased brain manganese levels and altered copper and iron levels in the striatum and in the basal ganglia. Transport of manganese ions into the central nervous system has been directly investigated in a limited number of studies, and it has become evident that the likeliest modes of transport are by transferrin (Tf)/transferrin receptor and divalent metal transporter 1 (DMT-1) (Dobson *et al*., 2004). It is generally believed that iron and manganese ions are able to be complexed and carried by transferrin/transferrin receptor, with iron being far more prevalent under normal circumstances. Several authors strongly suggested the transport of trivalent manganese complexed to transferrin into the brain capillary endothelium, although the exact mechanisms underlying the transport of manganese into the brain by transferrin remained unclear (Heilig *et al*., 2006).

In this study we have investigated the mechanism of manganese ion uptake by apo-transferrin in terms of the capillary electrophoresis (CE) method, and obtained clear evidence that oxidation state +3 and the binuclear unit of a manganese chelate are critical factors for the facile uptake by apo-transferrin, similar to that observed for Fe(III) species (Nishida *et al.*, 2007).

Materials and Methods

Reagents

Apo-transferrin (bovine: T1428–100 MG) and holo-transferrin (bovine: T1283–100 MG) were purchased form Sigma. Manganese compounds were prepared according to published methods



Scheme I.

(Okuno and Nishida, 1996; Kobayashi *et al*., 1996; Sutoh *et al*., 2005), Mn(II)(dpea)Cl_2, Mn(II)(dpa)$ClClO_4$, Mn(II)(dpal)Cl_2, $Mn(III/IV)_2O_2(dpa)_2ClO_4$, $Mn(IV)_2O_3$ $(Me\text{-}TACN)_2(PF_6)_2$. The chemical structures of the ligands are illustrated in Scheme I. All the manganese chelate solutions used in this study were prepared by dissolving the crystalline manganese compounds in tris [tris-(hydroxymethyl)aminomethane] buffer solution. Buffer solution (10 mM, pH 7.3) was prepared by diluting the tris solution (1 M, pH 7.6; NACALAI TESQUE, Kyoto, Japan) with distilled water.

Measurements

Capillary electrophoregrams (CE profiles) of the solutions containing apo-transferrin and a manganese chelate were obtained with a Beckman/Coulter P/ACE MDQ instrument: zone electrophoresis; temperature, 298 K; buffer solution, 10 mM tris buffer (pH 7.3); voltage, 20 kV; uncoated column with internal diameter of 50 μm and length of 50 cm; detection, 214 nm. Two solutions, apo-transferrin (100 μL, 2 mg/mL) and manganese complex (10 μL, 5 mM), were mixed and eluted with tris buffer solution (pH 7.3, 10 mM).

Results

Under the present experimental conditions (column with internal diameter 50 μM) two proteins, apo-transferrin (apo-Tf) and holo-transferrin (holo-Tf), can be discriminated by the CE method (Nishida *et al*., 2007; capillary column with internal diameter of 75 μm), *i. e*., holo-Tf, which is obtained by the addition of Fe(ida) complex [H_2(ida) = iminodiacetic acid] to the apo-transferrin solution (Nishida *et al*., 2007), is detected at slightly longer retention time and with higher peak intensity than apo-Tf (cf. Fig. 1).

All the manganese(II) complex solutions used in this study were colourless. Mn(II)(dpea) in the buffer solution became pale-brown when the solution stood for more than one day, demonstrating that the Mn(II) ion in the Mn(II)(dpea) chelate was readily oxidized to a Mn(III) ion in the atmosphere. As the oxidation to Mn(III) did not proceed in the compounds with (dpa) and (dpal) under the same conditions, it seems likely that oxidation to the Mn(III) state may occur in the Mn(II)(dpea) compound *via* the formation of an alkoxo-bridged binuclear structure, because facile oxidation to Mn(III) ions proceeds in the alkoxo-bridged binuclear Mn(II) complex with H(HPTP), $[Mn_2(HPTP)Cl_2]^+$ (Scheme II).

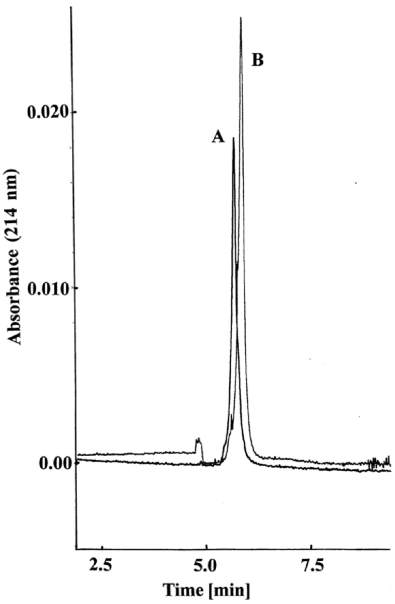
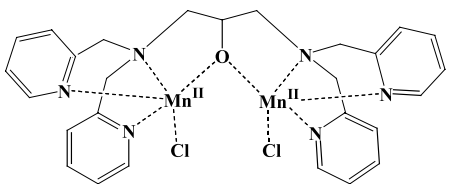


Fig. 1. CE profile of the solution; A, apo-transferrin; B, brown Mn(dpea) solution was added to apo-transferrin.



$[Mn(II)_2(HPTP)CI_2]^+$

Scheme II.

When the colourless solution containing Mn(II) ions, such as $MnCl_2$, Mn(dpea)Cl_2, Mn(dpal)Cl_2, was added to the apo-transferrin solution, no change was observed in the CE properties. In contrast with this, the CE peak due to apo-Tf moved to the longer retention time, when the pale-brown Mn(dpea) complex was added, as exemplified in Fig. 1. Addition of colourless apo-transferrin to the pale-brown Mn(dpea) complex immediately induced a colour change to dark-brown, and the spectral properties (Fig. 2) are quite similar to those obtained by the addition of hydrogen peroxide to the $[Mn(II)_2(L)(Cl_2)]^+$ complex, a binuclear manganese(II) complex of dimeric structure with a phenoxo-bridge (see Scheme III) (Sasaki *et al*., 1998). These findings clearly imply that the dark-brown solution derived from apo-transferrin and pale-brown Mn-dpea solution should be due to the formation of an Mn(III)-phenolate bonding, dem-

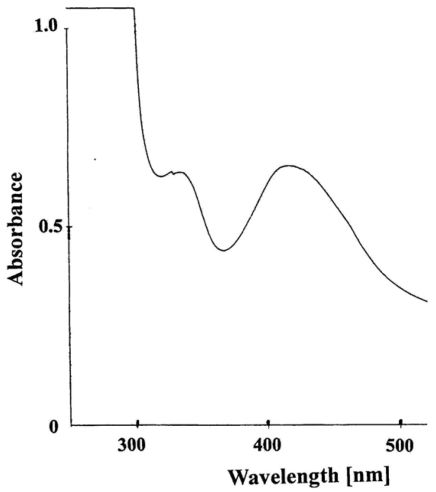
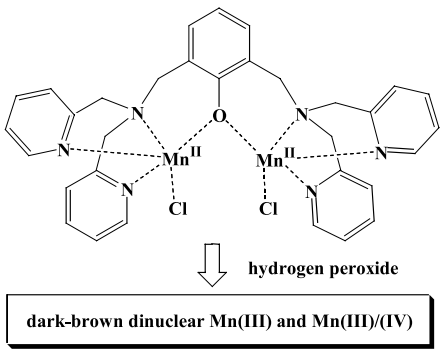


Fig. 2. Absorption spectrum of the dark-brown solution consisting of 0.1 mL of pale-brown Mn(dpea) solution and 1 mL of apo-transferrin (10 mg/mL).

onstrating that the Mn(III) ion of the pale-brown Mn(III)(dpea) chelate is transferred to the transferrin. We believe that this is the first report on the spectral absorption of Mn(III)-transferrin.

A similar absorption spectrum and CE changes were observed when the binuclear Mn(III)/Mn(IV) complex, $Mn_2O_2(dpa)_2ClO_4$, was added to the apo-transferrin solution, whereas no CE and colour change were observed on the addition of mononuclear Mn(II)(dpa) to apo-transferrin. Little transport of Mn(IV) ion to apo-transferrin was detected when the binuclear Mn(IV) compound, Mn_2O_3 $(Me\text{-}TACN)_2(PF_6)_2$, was added. These facts indicate that the presence of the Mn(III) ion in the binuclear unit is the essential factor for a ready uptake by apo-transferrin.

As stated in Introduction, it is generally believed that iron and manganese ions are able to be complexed and carried by a transferrin/transferrin receptor, with iron being far more prevalent under normal circumstances. But, this is only true under the circumstance that the manganese ion exists as a manganese(II) ion, *i.e.*, the prevalency of the iron ion over the manganese ion is not valid when Mn(III) ions are in excess; and such condition may be induced by the abnormal metabolism of iron ions (Nishida, 2004, 2006). Thus the present results may give useful information to understand the mechanism of the manganese ion transport to the brain, which should induce manganism.



Scheme III.

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