

Origin of Renal Proximal Tubular Injuries by Fe(III)-nta Chelate

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Interaction between apo-transferrin and several iron(III) chelates has been investigated in terms of the capillary electrophoresis method. Based on the results, it has been clarified that (i) a binuclear iron(III) unit with an oxo-bridge is necessary for the facile transfer of an iron atom from the iron(III) chelate to apo-transferrin, and (ii) the renal proximal tubular injuries by Fe(III)-nitrilotriacetate (Fe-nta) should be due to the unique binuclear structure of this complex, which gives a peroxide adduct of the binuclear Fe-nta in the presence of glutathione cycle and oxygen.

Key words: Ferric-nitrilotriacetate (Fe-nta), Renal Carcinogen, Renal Proximal Tubular Injury, Capillary Electrophoresis

Introduction

Ferric-nitrilotriacetate (Fe-nta) is a well-known renal carcinogen, and Fe-nta-injected animals have been used as a model of carcinogenesis (Okada, 2003; Toyokuni, 2002; Nishida, 1999). When Fe-nta was intraperitoneally injected into animals, lipid peroxidation and oxidative modification of protein and DNA occurred in the renal proximal tubules and the tubular epithelial cells were damaged (Liu and Okada, 1994). TBARS increased in the kidneys and cold Schiff's staining showed lipid peroxidation in the renal proximal tubules, and the increase of 4-hydroxy-2-nonenal (8-HNE)-modified proteins and 8-hydroxy-deoxyguanosine (8-H-dG) was demonstrated by biochemical and immuno-histochemical methods.

Kawabata *et al.* (1997) have reported that injuries by Fe-nta, such as lipid peroxidation and protein oxidation, were observed in the renal proximal tubules, but no injury was detected in the distal portion, although redox-active iron was still higher in the distal portion of the renal tubules, and proposed that some reducing environments such as glutathione cycle may promote the iron-induced free radical injuries. Okada *et al.* (1993) assumed that Fe-nta *in vivo* is rapidly reduced by cysteine, a component of glutathione, and that this reduced iron initiates lipid peroxidation in the lumen. But, their idea could not explain the observed facts that no damage was found in the

kidneys of rats injected with Fe(III)-pac (Fe-2-pyridylmethylamine-*N,N*-diacetate), and the damage by Fe-edda (Fe-ethylenediamine-*N,N'*-diacetate) was much weaker than that by Fe-nta (for the abbreviations of the ligands see Scheme I) (Nishida, 1999).

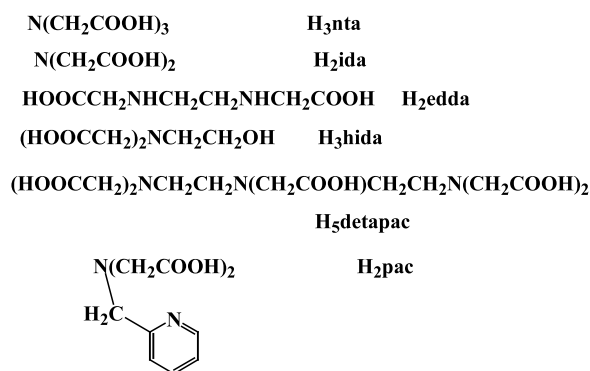
In order to gain a more accurate mechanism of renal carcinoma by iron chelates, we have compared the renal tubular injuries caused by amino-carboxylato-type chelators (see Scheme I); nta, edda, ida (iminodiacetic acid), edta (ethylenediamine-*N,N,N',N'*-tetraacetic acid), and hida (2-hydroxyethylamine-*N,N*-diacetic acid), and found that Fe-ida solution induced acute tubular injuries in the same manner as Fe-nta, but the other chelators, such as hida, did not (Mizuno *et al.*, 2006). The dependency of the renal tubular injuries on the structures of the iron chelate remains unsolved at present.

In this study we have measured the capillary electrophoresis (CE) profiles of the solution containing an iron chelate and apo-transferrin in order to obtain a detailed information on the interaction between the iron chelate and apo-transferrin. Based on the results obtained we have drawn the conclusion that the renal proximal carcinogenesis by Fe-nta should be due to the unique binuclear structure of this complex, which can give a peroxide adduct of the binuclear Fe-nta in the presence of oxygen and glutathione cycle in the renal proximal tubules.

Material and Methods

Extraction of parthenin

Apo-transferrin (human: T-2252) and holo-transferrin (T-1283) were purchased from Sigma. Nitrilotriacetic acid (H_3nta), H_2edda , H_3hida were purchased from Sigma. Iron(III) compounds of nta , $Cs_4Fe_2O(CO_3)(nta)_2 \cdot 4H_2O$ (Fujita *et al.*, 1994); pac , $Cs_2Fe_2O(CO_3)(pac)_2 \cdot 7H_2O$ (Nishida *et al.*, 1994); $edda$, $Na_2Fe_2O(CO_3)(edda)_2 \cdot 2.5H_2O$ (Nishida and Ito, 1995); $detapac$ (diethylenetriamine- N,N',N'' -pentaacetate), $K_2Fe(detapac) \cdot 2H_2O$; ida , $K_4Fe_2O(ida)_4 \cdot 10H_2O$ (Mizuno *et al.*, 2006); and $hida$, $Fe_2(hida)_2(H_2O)_2$ (Hearth and Powell, 1992) were prepared according to the published methods. All the iron chelate solutions used in this study were prepared by dissolving the crystalline iron(III) compounds in [tris = tris(hydroxymethyl)-aminomethane] buffer solution. The chemical structures of the ligands used in this study are illustrated in Scheme I.



Scheme I.

Measurements

The capillary electrophoregrams of the solutions containing apo-transferrin and iron 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); 20 kV; uncoated column (I.D. 75 μm , 50 cm); detection, 214 nm. Two solutions, apo-transferrin (100 μL , 0.5 mg/mL) and iron(III) complex (10 μL ; 1/60 M), were mixed and eluted with tris buffer solution (pH 7.3, 10 mM). Buffer solution (10 mM, tris, pH 7.3) was prepared by diluting the tris solution (1 M, pH 7.6) with distilled water; the latter two were purchased from NACALAI TESQUE (Osaka, Japan).

Results

Under our experimental conditions two proteins, apo-transferrin (Apo-Tf) and holo-transferrin (holo-Tf), can be discriminated in terms of the CE method, as shown in Fig. 1, *i.e.*, holo-Tf is detected at a slightly longer retention time than Apo-Tf. Addition of Fe- nta or Fe- ida chelate solution to the Apo-Tf solution induced the shift of the peak to a longer retention time as exemplified by the Fe- ida compound in Fig. 2 (B), and this is consistent with the fact that the iron atoms in Fe- nta readily transfer to the Apo-Tf *in vivo* (Bates *et al.*, 1967; Bates and Schallbach, 1973).

In contrast, the CE profile of Apo-Tf does not change by the addition of Fe- $detapac$, Fe- $edta$, Fe- pac , and Fe- $hida$ chelate solutions (data not shown). This indicates that the iron atoms in these four iron(III) chelates do not move to the Apo-Tf, which may be consistent with Liu and Okada's and Mizuno's reports that no damage was found in the kidneys of rats injected with Fe- pac , Fe- $edta$, and Fe- $hida$ solutions (Liu and Okada, 1994; Mizuno *et al.*, 2006).

Under the same experimental conditions, the addition of freshly prepared Fe- $edda$ chelate solution gave the CE profile as illustrated in Fig. 3 (B); this indicates that the iron atom of the Fe- $edda$ chelate partially transfers to apo-transferrin. Increasing of the concentration of Fe- $edda$ chelate solution induced more reduction of the CE peak strength due to Apo-Tf. The little change in the CE profile of Apo-Tf was observed when the old

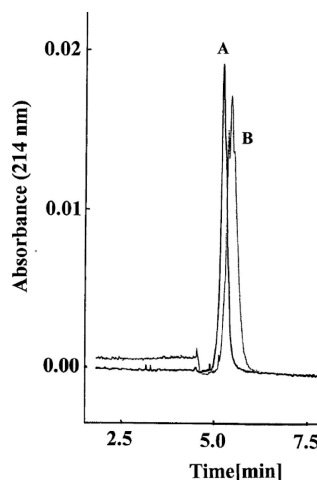


Fig. 1. CE profiles of (A) apo-transferrin and (B) holo-transferrin.

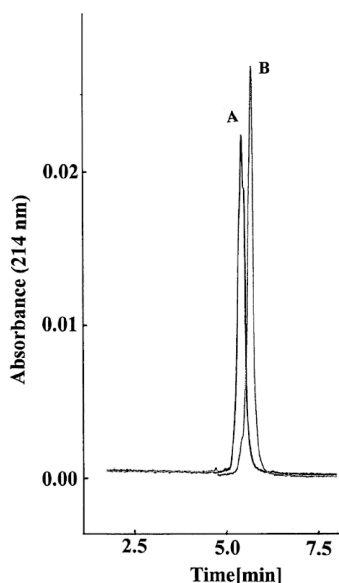


Fig. 2. CE profiles of (A) apo-transferrin and (B) Apo-Tf-containing Fe-ida chelate obtained immediately after mixing.

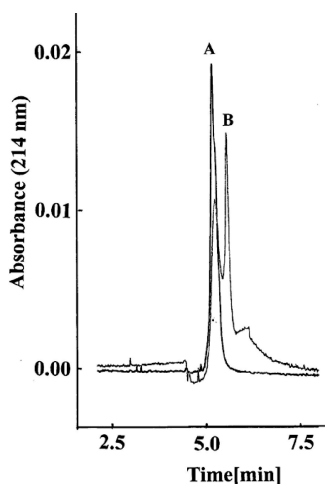


Fig. 3. CE profiles of (A) apo-transferrin and (B) Apo-Tf-containing Fe-edda chelate obtained immediately after mixing the freshly prepared iron(III) chelate solution.

solution (after 24 h) of Fe-edda chelate solution was added, but the complete transfer of the iron atoms in the old Fe-edda solution occurred when NaHCO_3 was added to the old solution. The absorption spectrum of the freshly prepared Fe-edda chelate solution (1 mM) shows a smaller peak around 630 nm, but this peak disappeared after

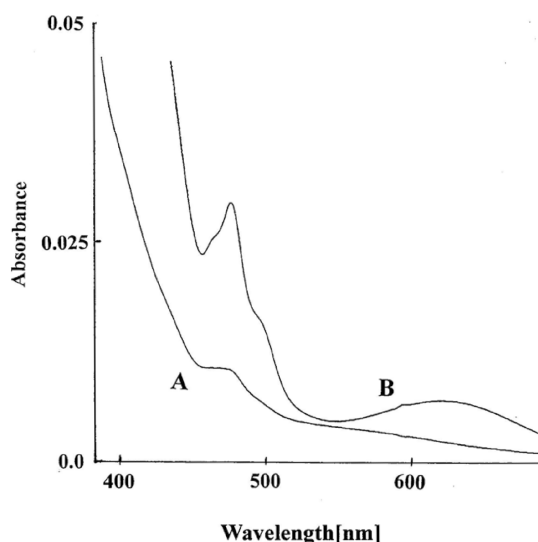


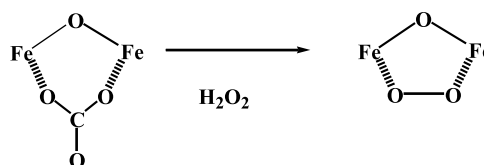
Fig. 4. Absorption spectra of the solution containing $\text{K}_2\text{Fe}_2\text{O}(\text{CO}_3)(\text{edda})_2 \cdot 2.5\text{H}_2\text{O}$ (1 mM); (A) measured 24 h after preparation; (B) NaHCO_3 was added to the above solution.

one day [see Fig. 4 (A)], and re-appeared after the addition of NaHCO_3 [Fig. 4 (B)]. Since the absorption bands around 630 and 480 nm are diagnostic for the binuclear iron(III) with oxo- and carbonato-bridges (Nishida and Ito, 1995), the present results demonstrate that the binuclear structure with an oxo-bridge is necessary for the transfer of the iron atoms from the iron(III) chelate into Apo-Tf.

Discussion

In our previous papers, we have reported that Fe-nta solution [prepared from ferric chloride, $\text{H}_3(\text{nta})$, and KHCO_3 ; pH 7.0] shows high activity towards the degradation of ribose in the presence of hydrogen peroxide. This was contributed to the formation of a highly reactive binuclear iron(III)-peroxide adduct, as illustrated in Scheme II (Nishida and Ito, 1995).

Negligible ability of the Fe-pac solution for the activation of the peroxide ion was attributed to its



Scheme II.

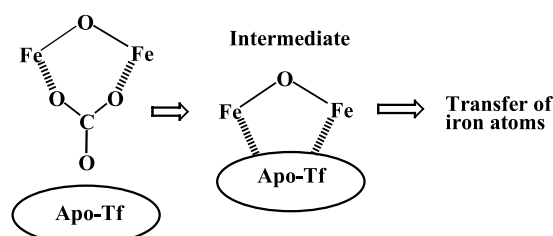
more rigid structural property of this complex; *i.e.*, the bonding of the carbonato ion towards iron(III) is stronger than in the Fe-nta complex, preventing the formation of a peroxide adduct. This is clearly supported by the crystallographic data, *i.e.*, the Fe-O distances with oxo-oxygen and carbonato ion are shorter in the Fe-pac complex than in the Fe-nta complex (Nishida *et al.*, 1994), and in fact Fe-pac chelate solution is green for more than one year, which shows distinct absorption bands around 630 and 470 nm.

In the case of the Fe-edda solution, we reported that the carbonato ion in the binuclear structure is readily released from the compound in the reaction with hydrogen peroxide even in the presence of KHCO_3 , leading to the degradation of the binuclear structure. This may explain the much lower ability of this complex to degrade ribose in the presence of hydrogen peroxide (Nishida and Ito, 1995). Absorption spectral data described above implies that binuclear Fe-edda chelate readily dissociates into a monomer in the solution in the absence of NaHCO_3 (see Results and Fig. 4). Based on the fact that the complete transfer of the iron atoms in the old Fe-edda chelate solution was observed when NaHCO_3 was added to the old solution, we would like to propose that the transfer of the iron atom of the chelates into the Apo-Tf occurs *via* the intermediate formation as shown in Scheme III.

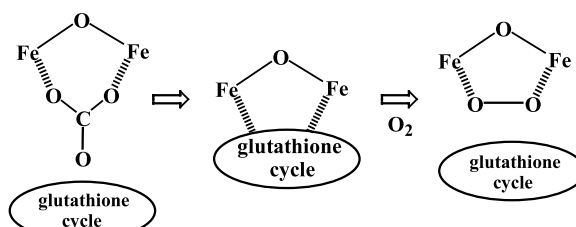
In case of the $[\text{Fe}_2\text{O}(\text{ida})_4]^{4-}$ complex (Mizuno *et al.*, 2006), we have confirmed the presence of the equilibrium in the solution



i.e., release of one of the two ida ligands, probably the bidentate one, occurs in solution, and thus formation of the intermediate in Scheme III by the $[\text{Fe}_2\text{O}(\text{H}_2\text{O})_4(\text{ida})_2]$ species is possible. But the intermediate formation is impossible for the binuclear Fe-hida complex because of the steric hindrance of the chelate structure (Hearth and Powell, 1992).



Scheme III.



Scheme IV.

When the iron(III) chelates approach to the glutathione cycle in the renal proximal region, it is likely that the Fe-nta complex facily interacts with the glutathione reductase as shown in Scheme IV, and produces hydrogen peroxide in the presence of oxygen (Nishida, 2004), and the formed binuclear iron(III)-peroxide adduct (right side in Scheme IV) should destroy the proteins nearby. In the case of Fe-pac and -hida complexes, interaction between the Fe(III) chelate and glutathione cycles is impossible because of the reasons described above. This is quite consistent with the observed fact that the injuries by Fe-nta, such as lipid peroxidation and protein oxidation, were observed in the renal proximal tubules where the glutathione cycle is operating, but no injury was detected in the distal portion in spite of still higher redox-active iron in the distal portion of the renal tubules. Much weaker damage is found in the kidneys of rats injected with Fe-edda that of these injected with Fe-nta (Nishida, 1999). This can be attributed to instability of the binuclear Fe-O-Fe unit of the Fe-edda compound in solution.

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