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Kinetic Inertness of the Mn^{2+} Complexes Formed with AAZTA and Some Open-Chain EDTA Derivatives

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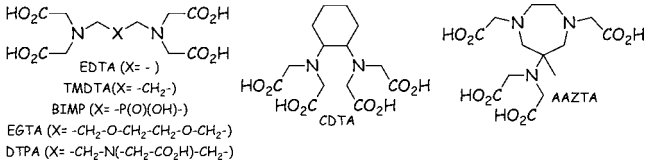
Supporting Information

ABSTRACT: The results of systematic equilibrium, kinetic, and relaxometric investigations carried out on the Mn^{2+} complexes of open-chain and AAZTA ligands indicate that the $[Mn(CDTA)]^{2-}$ complex has satisfactorily high kinetic inertness ($t_{1/2}$ = 12 h at pH = 7.4), which, in turn, may allow its use as a contrast agent in the field of magnetic resonance imaging (as a replacement for Gd^{3+}-based agents).

The recent discovery and association of the disease called Nephrogenic Systemic Fibrosis (NSF) with gadolinium deposition originating from the use of Gd^{3+}-based contrast agents (CAs) in patients with severe renal failure or following liver transplantation have pointed out that the rules of the application of paramagnetic metal complexes in magnetic resonance imaging (MRI) investigations have to be more strict.[1] Parallel with the recognition of NSF, there is a growing interest in the development of the CAs in order to design safer candidates. To obtain harmless CAs, one possibility is to change the paramagnetic metal center for the one that is better tolerated in the living systems such as Mn^{2+}. The biogenic Mn^{2+}, with its half-filled electron shell and slow electron-spin relaxation, is a good candidate to replace the Gd^{3+} ion in CAs because it is an endogenous metal and biological systems have developed effective routes to control its homeostasis.[2−8] Unfortunately, the lack of ligand-field stabilization, which can be traced back to the symmetric d^5 electron configuration system of the Mn^{2+} ion, results in thermodynamically less stable complexes than those of other transition metals, while its lower positive charge makes the Mn^{2+} complexes less stable than the complexes of the lanthanide ions. Additionally, even the most highly stable Mn^{2+} complexes, such as the $[Mn(DTPA)]^{3-}$, were found to be kinetically labile.[9] On the other hand, the use of the only Mn^{2+}-containing CA Mangafodipir, $[Mn(DPDP)]^{4-}$, is also based on its fast dissociation under in vivo conditions.[10]

In a sharp contrast to the avenue represented by open-chain ligands, recent studies have shown that the kinetic inertness of some Mn^{2+} complexes of macrocyclic ligands makes them suitable for in vivo applications.[4−6,11] The lack of systematic investigations carried out on the Mn^{2+} complexes of open-chain ligands made the basis of the current study. For this reason, the thermodynamic stability and kinetic inertness of some Mn^{2+} complexes formed with open-chain and AAZTA ligands have been investigated (Chart 1). The relaxivity values of the Mn^{2+} complexes were also determined at 20 MHz magnetic field strength, and a simple model calculation was carried out for the $[Mn(CDTA)]^{2-}$ complex to approximate the rate and extent of its dissociation in plasma.

Chart 1. Structure of the Ligands Studied in the Current Work



The stability of the complexes is characterized by the stability constants of the complex species and by a report on their pMn values defined by the conditional stability constant of the complexes using conditions suggested recently by Drahos et al. (pH = 7.4; $c_{Mn} = c_L = 10^{-5}$ M). The pMn values calculated for the Mn^{2+} complexes of EDTA, CDTA, TMDTA, BIMP, DTPA, EGTA, and AAZTA ligands are 7.83, 9.90, 5.81, 6.30, 7.95, 6.91, and 8.29, respectively. These values are similar to those reported for the most inert Mn compexes of macrocyclic ligands, NOTA and DOTA (pMn = 7.94 and 9.09 were calculated from the stability data reported by Cortes et al.[12] and Bianchi et al.[13] for $[Mn(NOTA)]^-$ and $[Mn(DOTA)]^{2-}$, respectively). While these data did not differ substantially, the kinetic inertness values of the complexes of open-chain and macrocyclic ligands are known to differ by orders of magnitude. Furthermore, nowadays, the kinetic inertness is recognized to be a more important property of the complexes considered for in vivo use.

The dissociation mechanisms of the Mn^{2+} complexes do not differ basically from those of the Gd^{3+} complexes.[4−6,11] The dissociation of the metal complexes applied in vivo may occur via the following pathways: spontaneous, acid-catalyzed, metal ion-initiated decomplexation (with the direct attack of the exchanging metal ion). Some endogenous ligands may also accelerate the dissociation of the complexes.[14] For the dissociation of the Mn^{2+} complexes in the presence of Cu^{2+}, a general reaction scheme can be established (Scheme 1).

In order to obtain information on the rate of dissociation, usually transmetallation reactions, which occur between the paramagnetic complex and a suitable exchanging metal ion such as Mg^{2+}, Ca^{2+}, Zn^{2+}, or Cu^{2+}, are studied. The metal-exchange

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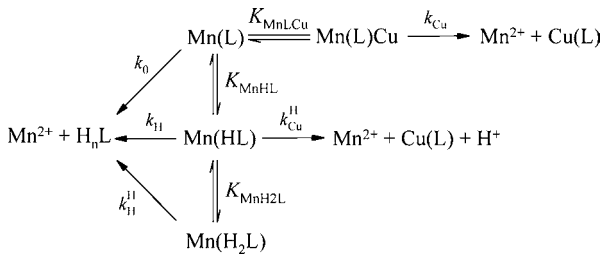
Scheme 1. Assumed Reaction Mechanisms of the Decomplexation of the Mn^{2+} Complexes



reactions of the Mn^{2+} complexes were investigated by spectrophotometry, in the presence of a high (10–40-fold) excess of exchanging Cu^{2+} ion, ensuring pseudo-first-order conditions. Under these conditions, the rate of the reaction can be expressed as follows: $-d[MnL]_t/dt = k_{obs}[MnL]_{tot}$, where k_{obs} is the pseudo-first-order rate constant and $[MnL]_{tot}$ is the total concentration of the Mn^{2+} complex.

Taking into account the different pathways (characterized by the rate constants k_0, k_H, k_H^H, k_{Cu}, and k_{Cu}^H; Scheme 1) and the equations of protonation and stability constants of the intermediates (K_{MnHL}, K_{MnH_2L}, and K_{MnLCu}), the pseudo-first-order rate constant (k_{obs}) can be expressed by eq 1. Equation 1 is a general equation for describing the rates of the metal-exchange reactions of the Mn^{2+} complexes (more details can be found in the Supporting Information).

$$k_{obs} = \frac{k_0 + k_1[H^+] + k_2[H^+]^2 + k_3[Cu^{2+}] + k_4[Cu^{2+}][H^+]}{1 + K_{MnHL}[H^+] + K_{MnHL}K_{MnH_2L}[H^+]^2 + K_{MnLCu}[Cu^{2+}]} \quad (1)$$

The pseudo-first-order rate constants characterizing the dissociation of the Mn^{2+} complexes increase with increasing H^+ ion concentration in almost all cases (k_1 and k_2) and increase with increasing Cu^{2+} concentration (k_3) or remain unaffected by the Cu^{2+} concentration ($[Mn(EDTA)]^{2-}$) except in the case of $[Mn(CDTA)]^{2-}$, where the k_{obs} values were found to be inversely proportional to the Cu^{2+} concentration (the fittings of the k_{obs} values is shown in the Supporting Information). The results of the fitting are summarized and compared in Table 1. The data fitting for the $[Mn(CDTA)]^{2-}$ complex returned the stability constant of the dinuclear intermediate, but the rate constant of the metal-assisted dissociation had to be neglected. This phenomenon can be explained by considering the dinuclear intermediate as a "dead-end" complex.

Equation 1 displays the general equation used in data refinement; however, not all of the pathway was active for the studied complexes. Different dissociation mechanisms make a direct comparison of the data obtained difficult; therefore, to characterize the kinetic inertness, the half-lives ($t_{1/2}$) of the dissociation reactions of Mn^{2+} complexes were calculated at physiological (pH = 7.4 and at 1×10^{-5} M concentration of the exchanging Cu^{2+} ion) conditions (Table 1). The comparison of the $t_{1/2}$ values shows that the kinetic inertness of the $[Mn(CDTA)]^{2-}$ complex is 3–5 orders of magnitude higher than that of the Mn^{2+} complexes formed with the other open-chain ligands, and it also dissociates more slowly than the $[Mn(AAZTA)]^{2-}$ complex. This behavior is clearly related to the more rigid structure of the CDTA ligand, which provides a compact structure and a preorganized coordination cavity suitable for metal-ion encapsulation. The replacement of the ethylene backbone in EDTA for a cyclohexyl bridge results an increase of the kinetic inertness by more than 2 orders of magnitude. The kinetic inertness (characterized by $t_{1/2}$) of $[Mn(CDTA)]^{2-}$ is just 3–6 times less than values obtained in our group recently for the $[Mn(DO2A)]$ complex[15] and published by Tóth et al. for $[Mn(NOTA)]^-$.[6]

The longer backbone of the TMDTA ligand causes an increase of the central chelate ring size from 5 to 6, resulting in an increase in the lability of the complex and a decrease in the kinetic inertness of its Mn^{2+} complex. By comparing the kinetic inertness of the BIMP and TMDTA complexes, one can conclude that the presence of the phosphinate moiety in the BIMP ligand does not increase significantly the kinetic inertness of the Mn^{2+} complex while it does contribute to an increase in the kinetic inertness of the $[Ln(BIMP)]^{2-}$ complexes.[16] The scientific explanation for this phenomena can be obtained by analyzing the X-ray structures of some other transition-metal-ion (Co^{2+} and Cu^{2+}) complexes of the BIMP[17,18] ligand because the coordination of the phosphinate moiety in these complexes is sterically hindered while the coordination of the phosphinate moiety in $[Ln(BIMP)]^{2-}$ complexes is accepted now.[16]

The investigation of the metal-exchange reactions between the $[Mn(DTPA)]^{3-}$ complex and the Cu^{2+} ion was not possible even by a stopped-flow technique. The presence of the highly basic, central amine nitrogen in the DTPA ligand decreases not only the conditional stability of the Mn^{2+} complex but also its kinetic inertness. This gives an explanation of why the dissociation of $[Mn(DTPA)]^{3-}$ was witnessed after its in vivo injection.[9]

With the use of the rate constants characterizing the dissociation of the $[Mn(CDTA)]^{2-}$ complex, it is possible to calculate the percentage of $[Mn(CDTA)]^{2-}$ that would be

Table 1. Rate Constants Characterizing the Dissociation of the Mn^{2+} Complexes (25 °C)

	k_0 (s^{-1})	k_1 (M^{-1} s^{-1})	k_2 (M^{-2} s^{-1})	k_3 (M^{-1} s^{-1})	k_4 (M^{-2} s^{-1})	log K_{MnHL} [b]	K_{MLCu}	$t_{1/2}$ [c] (h)
CDTA[a]		$(4.0 \pm 0.1) \times 10^2$					79 ± 13	12
EDTA[a]		$(5.2 \pm 0.2) \times 10^4$	$(2.3 \pm 0.3) \times 10^8$	45 ± 8				7.6×10^{-2}
TMDTA		$(2.3 \pm 0.4) \times 10^7$		$(8 \pm 2) \times 10^5$	$(3.0 \pm 0.4) \times 10^{10}$	4.90	$(2.1 \pm 0.4) \times 10^3$	2.3×10^{-5}
BIMP	2.1	$(5 \pm 1) \times 10^4$		$(2.6 \pm 0.2) \times 10^3$	$(2.7 \pm 0.5) \times 10^7$			9.0×10^{-5}
EGTA		$(1.9 \pm 0.2) \times 10^6$		$(5 \pm 1) \times 10^3$			317 ± 73	1.5×10^{-3}
AAZTA		$(3.4 \pm 0.2) \times 10^3$	$(5.5 \pm 0.4) \times 10^7$	14 ± 2			147 ± 18	0.7

[a]For $[Mn(CDTA)]^{2-}$, $k_1 = 3.2 \times 10^2$ M^{-1} s^{-1} and $t_{1/2} = 15$ h, while for $[Mn(EDTA)]^{2-}$, $k_3 = 3.0 \times 10^{-1}$ M^{-1} s^{-1}, $k_4 = \sim 4.8 \times 10^1$ M^{-2} s^{-1}, and log $K_{MnHL} = 3.10$ were found in ref 19. [b]Determined by pH-pot. [c]pH = 7.4 and $c(Cu^{2+}) = 1 \times 10^{-5}$ M were used in the calculations.

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dissociated in the human body after the intravenous administration. Assuming that the half-life of the excretion of the $[Mn(CDTA)]^{2-}$ complex is the same as that of the Gd^{3+} complexes used in MRI (1.6 h at 37 °C), the rate of the excretion can be given by a first-order rate constant of k_e = 0.433 h^{-1}. The k_{obs} value characterizing the decomplexation of the $[Mn(CDTA)]^{2-}$ complex at physiological conditions (k_d) can be calculated by means of the k_1 and K_{MnLCu} values determined in the metal-exchange reactions. The value of k_d is 5.72×10^{-2} h^{-1}.

The excretion of $[Mn(CDTA)]^{2-}$ from the body through the kidneys and the dissociation of the complex could be regarded as parallel first-order reactions. For such reactions, the ratio of the concentrations of the products depends on the ratio of the first-order rate constants (Supporting Information). For the $[Mn(CDTA)]^{2-}$ complex, the ratio would be $k_d/(k_d + k_e)$ = 0.117 after 6−7 half-lives of the excretion, which means that 11.7 % of the injected dose would dissociate. Although the amount of the released Mn^{2+} ion from the $[Mn(CDTA)]^{2-}$ complex is approximately 7 times higher than that calculated for the $[Gd(DTPA)]^{2-}$ complex, 1.71%, the living system has routes to eliminate the released Mn^{2+} ion like in the case of $[Mn(DPDP)]^{4-}$, so $[Mn(CDTA)]^{2-}$ can be regarded as an acceptable CA for in vivo applications.[10] Experiments performed in human blood serum are in agreement with the results of the kinetic studies (Supporting Information).

The relaxivity values [the relaxivity ($r_{1,2}$, mM^{-1} s^{-1}) is the relaxation enhancement in the 1 mM solution of the paramagnetic metal complex] of $[Mn(EDTA)]^{2-}$, $[Mn(CDTA)]^{2-}$, $[Mn(TMDTA)]^{2-}$, $[Mn(DTPA)]^{3-}$, $[Mn(BIMP)]^{2-}$, $[Mn(EGTA)]^{2-}$, and $[Mn(AAZTA)]^{2-}$[20] were determined and found to be 3.2, 3.6, 2.2, 1.7, 2.1, 1.6, and 1.6[20] mM^{-1} s^{-1}, respectively. From these data, we concluded that EDTA and CDTA form monoaquated (q = 1) complexes with the Mn^{2+} ion, which is highly desired for in vivo applications.

The results of our studies indicate that not all of the Mn^{2+} complexes of open-chain ligands are kinetically labile. It has been proven that some rigid open-chain ligands modeled by the tetraacetate derivative of cyclohexylene diamine (e.g., CDTA) can form a kinetically inert complex with the Mn^{2+} ion. The most promising Mn^{2+} complex, formed with a macrocyclic ligand that possesses at least one inner-sphere water molecule and therefore has high relaxivity, is the $[Mn(15\text{-py-aneN}_5)]^{2+}$,[5] but it is thermodynamically less stable than $[Mn(CDTA)]^{2-}$ and the ligand commercially not accessible. Obviously, more studies are needed to design ligands for Mn^{2+} complexation that would display high thermodynamic stability, acceptable kinetic inertness, and proper water-exchange rates, which, in turn, allows one to obtain high relaxivities. Among the open-chain ligands studied to date, clearly CDTA display the best features for the in vivo applications.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Details of the equilibrium, kinetic, and relaxivity measurements and equations used to calculate the extent of $[Mn(CDTA)]^{2-}$ complex dissociation. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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