Comparative Studies of Structures and Peroxidase-like Activities of Copper(II) and Iron(III) Complexes with an EDTA-Based Phenylene-Macrocycle and Its Acyclic Analogue

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1. INTRODUCTION

The studies of metal–ligand interaction in coordination compounds have constructed an important research area because the interaction causes and controls the chemical and biological activities of metal centers. From this viewpoint, various types of coordination compounds have been designed and their applications have been exploited in many fields.1−3 In the human organism, metal ions encompass several crucial functions through metalloproteins. Among the metalloenzymes that have attracted attention in relation to the development of biomimetic agents, our special interest is directed to peroxidases (POx), which are heme proteins that catalyze the degradation of H2O2 when the human body defends itself against oxidative damage by superoxide anions. Although the active site of POx contains iron(III) coordination centers, the peroxidase-inactive Fe3+ complex consists of a mononuclear complex ion [Fe(edtabz)(H2O)]+, the metal ion of which is suited in a distorted pentagonal bipyramid to be protected from environmental oxygen. The copper(II) complexes, which have mononuclear structures with high thermodynamic stability compared with the iron(III) complexes, show no peroxidase activity. The steric effects play a fundamental role in the biomimetic activity.

Supporting Information

ABSTRACT: With the objective of studying the conformational and macrocyclic effects of selected metal chelates on their peroxidase activities, Cu2+ and Fe3+ complexes were synthesized with a macrocyclic derivative of ethylenediaminetetraacetic acid and a-phenylenediamine (abbreviated as edtaodH2) and its new open-chain analogue (edtabzH2). The Fe3+ complex of edtaodH2 has a peroxidase-like activity, whereas the complex of edtabzH2 does not. The X-ray study of the former shows the formation of a dimeric molecule [{Fe(edtaod)}2O] in which each metal with an octahedral coordination is overposed over the macrocyclic cavity, as a result of rigid macrocyclic frame, to form an Fe−O−Fe bridge; the exposure of the central metal to the environment facilitates the capture of oxygen to drive the biomimetic activity. The peroxidase-inactive Fe3+ complex consists of a mononuclear complex ion [Fe(edtabz)(H2O)]+, the metal ion of which is fitted in a distorted pentagonal bipyramid to be protected from environmental oxygen. The copper(II) complexes, which have mononuclear structures with high thermodynamic stability compared with the iron(III) complexes, show no peroxidase activity. The steric effects play a fundamental role in the biomimetic activity.
edge of the structural and physicochemical factors that control their biomimetic activity; the major conceivable factors include the structural type of the ligand, size of the cavity, and number and nature of the metal in the complexes, all of which can be readily controlled by modifying EDTA with selected groups. In the present work, we have chosen a macrocycle consisting of EDTA and aromatic units (edtabzH₂ in Scheme 1), for the following reasons: the phenylene group integrated in the macrocyclic frame results in the high rigidity which strictly defines the coordination geometry around the central metal; moreover, electronic interaction between the coordinated metal ion and aromatic group favors a change in the oxidation state that may occur in the intermediates of the peroxidase process. To study the effect of the steric effect due to the macrocyclic frame, a new open-chain ligand (edtabzH₂ in Scheme 1) has been synthesized, which consists of a chelating EDTA unit linked with phenyl group in a sterically less-restricted manner. Their Fe³⁺ and Cu²⁺ complexes have been subjected to the tests of peroxidase-like activity; only the iron(III) complex of edtabzH₂ is active. In order to study the correlation of the biomimetic activity with the structural and thermodynamic properties, the metal complexes have been characterized by X-ray diffraction, calorimetric analysis, and UV-spectrometry. On the basis of the results of these studies, we can verify that a greater thermodynamic stability of a complex is not necessarily translated into a better biomimetic activity, but the steric effects may play a more fundamental role in the biomimetic activity.

2. RESULTS AND DISCUSSION

2.1. Characterization of edtabzH₂. Reaction of EDTA dianhydride with aniline gave acyclic ligand edtabzH₂ (Scheme 1), which was characterized by ¹H and ¹³C NMR spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, and electrospray mass spectrometry as described in the Experimental Section. The water solubility is high enough for potentiometric and NMR studies in the pH range 2–12. The logarithmic protonation constants of edtabz⁻⁻ were determined by potentiometry in 0.1 M KCl at 25 °C as log_{[LH]}/[L][H] = 6.23 (±0.01) and log_{[LH]²}/[L][H][H] = 3.64 (±0.02); Figure 1 presents the calculated species distribution. These values are close to the corresponding values 6.07 and 3.7 reported for edtaodH₂ in Scheme 1. The pD dependence of the ¹H NMR chemical shifts observed for edtabzH₂ is shown in Figure 1. The protons labeled a, b, and c in Scheme 1 shift downfield with decreasing pD in the range 5.5–8.5, indicating that the amino nitrogen atoms are protonated. Only the proton b shifts downfield with decreasing pD from 5 to 2, indicating protonation at carboxylate oxygen. The NMR δ of proton i is expressed by the following function of pD:

δ_i(pD) = δ_i,0 + Σ β_n,0 10^(-n(pD)) / 1 + Σ β_n 10^(-n(pD))

(1)

where β_n is the overall protonation constant of n-fold protonation in D₂O media, 10^(-n(pD)) gives D⁺ concentration, δ_n is the chemical shift of proton i in the completely deprotonated species L⁺², and δ_o is the chemical shift in the n-fold protonated species. The shifts of all proton signals are well reproduced by eq 1 with the values of β_n, δ_o, and δ_i,0 presented in the legend of Figure 1. The obtained deuterium constants, log K_D¹ = 6.74 (±0.02) and log K_D₂ = 3.26 (±0.02), are similar to the corresponding values 6.94 and 3.89 reported for edtaodH₂.

The X-ray structure presented in Figure 2 shows that edtabzH₂ is crystallized as zwitterionic dihydrate, in which one of the carboxyl hydrogen atoms is transferred to the adjacent amino group. The aromatic substituents are pointed to the same direction; this orientation is attributed to crystal packing.
Table 1. Selected Crystallographic Data for \{[\text{Fe(edtaod)}]_{2}O\}\cdot 4.4\text{H}_2\text{O}, \text{edtabzH}_2\cdot 2\text{H}_2\text{O}, \text{and} \ [\text{Fe(edtabz)}]\text{NO}_3\cdot 5.5\text{H}_2\text{O}

|                              | \{[\text{Fe(edtaod)}]_{2}O\}\cdot 4.4\text{H}_2\text{O} | \text{edtabzH}_2\cdot 2\text{H}_2\text{O} | [\text{Fe(edtabz)}]\text{NO}_3\cdot 5.5\text{H}_2\text{O} |
|------------------------------|------------------------------------------------------|------------------------------------------|----------------------------------------------------------|
| formula                      | C_{32}H_{36}Fe_{2}N_{8}O_{13} \cdot 4.4\text{H}_2\text{O} | C_{22}H_{26}N_{4}O_{6} \cdot 2\text{H}_2\text{O} | 2(C_{22}H_{26}FeN_{10}O_{8}) \cdot 8\text{O}, 3\text{H}_2\text{O} |
| MW (g mol\(^{-1}\))          | 931.66                                               | 478.50                                   | 1334.70                                                  |
| T (K)                        | 100                                                  | 293                                      | 125                                                      |
| space group                  | Pbca                                                 | P\(_2\)\(_1\)\(_2\)/c                    | Cu K                                                     |
| radiation                    | Mo Kα                                                | Mo Kα                                    | Cu K                                                     |
| a (Å)                        | 18.020(18)                                           | 7.3272(5)                                | 12.1188(8)                                               |
| b (Å)                        | 18.028(2)                                            | 13.6323(10)                              | 18.1908(12)                                              |
| c (Å)                        | 22.855(3)                                            | 23.8535(18)                              | 14.0536(9)                                               |
| α (deg)                      | 90                                                   | 90                                       | 90                                                       |
| β (deg)                      | 90                                                   | 90                                       | 105.176(2)                                               |
| γ (deg)                      | 90                                                   | 90                                       | 90                                                       |
| V (Å\(^3\))                  | 7424.9(14)                                           | 2382.6(3)                                | 2990.1(3)                                                |
| Z                             | 8                                                    | 4                                        | 2                                                        |
| μ (mm\(^{-1}\))              | 0.873                                                | 0.102                                    | 4.759                                                    |
| \(\rho_{\text{calc}}\) (g cm\(^{-3}\)) | 1.667                                               | 1.334                                    | 1.482                                                    |
| \(R_1\) (\(F_o >4\)\(σ_F\)) | 0.0301                                               | 0.0778                                   | 0.0394                                                   |
| \(wR_2\) (all data)          | 0.0721                                               | 0.1468                                   | 0.1059                                                   |
| GOF                           | 1.095                                                | 1.274                                    | 1.061                                                    |
| REFCODE/CCDC                 | 1920517                                              | 1920512                                  | 1920516                                                  |

Figure 3. UV–vis spectra of edtabzH\(_2\) and edtaodH\(_2\) in titration with copper(II) and iron(III) nitrates at pH 3.0: (A) Fe\(^{3+}\)–edtabz\(^{2-}\), (B) Cu\(^{2+}\)–edtabz\(^{2-}\), (C) Fe\(^{3+}\)–edtaod\(^{2-}\), and (D) Cu\(^{2+}\)–edtaod\(^{2-}\). The insets present changes in absorbance at 246 nm for M–edtabz and at 302 nm for M–edtaod: the solid line is the fitting curve generated by program AFFINIMETER.

Table 2. Thermodynamic Parameters Determined by ITC for the 1:1 Complexation of edtaodH\(_2\) and edtabzH\(_2\) with Copper(II) and Iron(III) Nitrates in Potassium Hydrogen Phthalate Buffer (pH 3) and at 298.15 K—Stability Constant (\(K_{\text{ITC}}\)), Standard Free Energy (\(\Delta G^\circ\)), Enthalpy (\(\Delta H^\circ\)) and Entropy Change (\(T\Delta S^\circ\)), and Conditional Stability Constant (\(K_{\text{UV–vis}}\)) Determined by UV–Vis Titration at the Same pH

| complex                        | n   | \(K_{\text{ITC}}\) (M\(^{-1}\)) | \(\Delta G^\circ\) (kcal/mol) | \(\Delta H^\circ\) (kcal/mol) | \(−T\Delta S^\circ\) (kcal/mol) | \(K_{\text{UV–vis}}\) (M\(^{-1}\)) |
|--------------------------------|-----|---------------------------------|--------------------------------|--------------------------------|---------------------------------|----------------------------------|
| \([\text{Fe(edtabz)}]\)^{2+}   | 1.04 ± 0.01 | 1.20 (±0.08) \times 10\(^7\) | −9.66 ± 0.11                  | −2.55 ± 0.01                   | −7.11                            | 9.52 (±1.12) \times 10\(^1\)    |
| \([\text{Cu(edtabz)}]\)        | 1.10 ± 0.02 | 5.19 (±0.81) \times 10\(^7\)  | −10.52 ± 0.10                 | −4.86 ± 0.08                   | −5.67                            | 3.45 (±0.98) \times 10\(^3\)    |
| \([\text{Fe(edtaod)}]\)^{2+}   | 1.09 ± 0.03 | 8.75 (±0.52) \times 10\(^6\)  | −6.74 ± 0.14                  | 1.49 ± 0.03                    | −8.23                            | 1.16 (±0.048) \times 10\(^5\)   |
| \([\text{Cu(edtaod)}]\)        | 1.10 ± 0.09 | 2.64 (±0.19) \times 10\(^6\)  | −8.76 ± 0.06                  | −1.58 ± 0.01                   | −7.18                            | 1.34 (±0.26) \times 10\(^6\)    |

DOI: 10.1021/acsomega.9b03164
ACS Omega 2019, 4, 22487–22496

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2.2. Cu$^{2+}$ and Fe$^{3+}$ Complexation in Aqueous Solution. Coordination of edtabz$^{-2}$ and edtaod$^{-2}$ with Cu$^{2+}$ and Fe$^{3+}$ ions in solution was studied at pH 3.0 by UV–vis spectrometric titrations and ITC. At this pH, the two ligands are in equilibrium between LH$^{-}$ and LH$_{2}$; the amino group is protonated completely, and the carboxylate group is protonated partially (Figure 1). Despite the mixed protonation state, the pH 3.0 was selected to prevent the formation of Fe(OH)$_{3}$ so that the complexation of the two metals can be compared. Figure 3 shows the near-UV spectra in the presence of Cu$^{2+}$ or Fe$^{3+}$ ion at different concentrations. All complexes exhibit intense ligand-to-metal charge-transfer transition in the range 280–375 nm. The absorbance of every new band increases almost linearly with the total metal concentration up to the ratio [M]/[L]$_{t}$ ≈ 1. Larger changes in the charge-transfer bands occur in the formation of Fe$^{3+}$ complexes. Every titration curve is interpreted by the metal-to-ligand ratio of 1:1, and it cannot be reproduced by other stoichiometry. The conditional stability constants were determined using the AFFINIMETER program; the results are presented in Table 2. The constants are ranged from 10$^{5}$ and 10$^{6}$ M$^{-1}$ (M = mol dm$^{-3}$), and higher stability is found for the Cu$^{2+}$ complexes.

The coordination of edtabz$^{-2}$ with copper in basic media was also studied at pH 8.5 at which the L$^{2-}$ species is present at almost 100% level (the observed spectra are included in the Supporting Information). A large hypochromic effect is observed for the 246 nm band, and a hyperchromic effect is observed for the CT band; both changes in absorbance are linear up to [M]/[L]$_{t}$ = 1. This greater spectral change is due to that fact that all donor atoms in L$^{2-}$ can form coordinate bonds without proton dissociation. For the same reason, the conditional stability constant is as high as the order of 10$^{13}$ M$^{-1}$.

The curves obtained for the isothermal titrations of edtabz$^{-2}$ with Cu$^{2+}$ and Fe$^{3+}$ ions are presented in the panels A and B of Figure 4, respectively (the curves for edtaod$^{-2}$ are given in Supporting Information). The raw data of both (A) Cu$^{2+}$–edtabz$^{-2}$ and (B) Fe$^{3+}$–edtabz$^{-2}$ systems present exothermic peaks, which indicate the saturation of the association until small peaks appear due to heat generated by dilution in the last injections. The lower panel (C) of Figure 4 presents the integrated data. The thermal change of copper complexation is steeper with the higher plateau than is the iron complexation, suggesting that the former is accompanied by the higher affinity and greater enthalpic contribution. Thermal parameters determined by curve fittings are summarized in Table 2. The n value of ≈1 is consistent with the 1:1 stoichiometry determined by the UV–vis titrations. It is interesting that, in the acidic environment, both the edtabz$^{-2}$ complexes have comparable affinities with ∆G around −10 kcal/mol, while [Cu(edtabz)] bears almost double the ∆H value of the Fe complex (i.e., the former is driven by the entropy effect completely). The stability constants determined by the calorimetry are higher than those found by the UV–vis technique. The ITC technique detects all of the thermal processes involved in the formation of the complex. On the other hand, the spectrometric technique traces only the spectral change caused by competition between the complexation and protonation to determine a conditional constant, which is usually smaller than the proper constant. Despite the difference, the relative stabilities determined by the same techniques under identical conditions can be compared and evidently indicate that the Cu$^{2+}$ complexes are more stable than the corresponding Fe$^{3+}$ complexes and the acyclic ligand forms more stable metal complexes.

2.3. Characterization in Solid. The copper(II) and iron(III) complexes with the ligands edtaodH$_{2}$ and edtabzH$_{2}$ were isolated in satisfactory yields. The structures of the metal complexes have been established by various techniques. Some informative FTIR bands of the ligands and their Cu$^{2+}$ and Fe$^{3+}$ complexes are given in Table 3. The characteristic C=O stretching band of carboxylic acid in the free ligand appears around 1700 cm$^{-1}$ and moves to a lower wavenumber side by at least 30 cm$^{-1}$ upon complex formation. This band shift indicates that the carboxylate groups participate in the
formation of the complexes. Other bands that are displaced to smaller wavenumber sides upon complexation are the vibrational bands of amide C=O and C–N bonds, which indicate that the amide groups are also involved in the coordination with the metal ions. The X-ray photoelectron spectra (XPS) are shown in Supporting Information. The Fe\(^{3+}\) complexes of edtaod\(^2^-\) and edtabz\(^2^-\) exhibit 2p\(^{3}/2\) core-electron peaks at binding energies of 709.9 and 710.5 eV, respectively; the energies are consistent with the trivalent state. Each peak is accompanied by a broad, intense peak at 4–5 eV higher energy. This additional peak might be due to multiplet splitting caused by five electrons in the 3d level, but they are normally difficult to observe clearly because the net effect would broaden the 2p\(^{3}/2\) and 2p\(^{1}/2\) peaks. More viable is a shake-up satellite.\(^{16}\) In the case of the copper(II) complexes, a 2p\(^{3}/2\) peak is observed at 932.7 and 932.8 eV for the complexes of edtaod\(^2^-\) and edtabz\(^2^-\), respectively. Both peaks are accompanied by shake-up satellites which appear characteristically of the 3d\(^5\) open-shell in Cu\(^{2+}\) ion.

2.3.1. X-ray Diffraction Analyses. The most relevant crystallographic data of \{Fe\text{(edtaod)\}_2O\} are summarized in Table 1. Figure 5 shows the molecular structure in which two Fe\text{(edtaod\}_2O\} chelate labeled A and B are bridged by an oxygen atom. As seen from the perspective views given for each chelate in Figure 6, the iron(III) ions are each embedded in distorted octahedral coordination environments. In the equatorial plane, the Fe1/Fe2 atoms are coordinated by atoms O2A/O2B, N2A/N2B, and N3A/N3B from the ligand molecules and the bridging oxygen atom O9 so that a distorted square plane is generated around each central metal ion. The distortion is seen from the O–Fe–O, O–Fe–N and N–Fe–N bond angles formed between neighboring atoms in the plane; the angles range from 77.50(5) to 104.15(5)° (Table 4). The axial positions of each square plane are occupied by oxygen atoms from the second carboxylate group (O4A/O4B) and the amide group (O1A/O1B) to construct an elongated distorted octahedron. As expected, the Fe–O\text{carboxylate\} bond distances [2.0094(12) and 2.0182(12) Å] are shorter than the Fe–O\text{amide\} distances [2.0184(12) and 2.0358(12) Å]. The Fe–N distances are in the range of 2.2338(15) to 2.3012(14) Å. Among the Fe–O and Fe–N distances, the shortest is found for Fe–O9 with 1.7919(12) and 1.7930(12) Å, which are comparable to those found in other octahedral complexes of chelating N- and O-donor ligands with Fe–O–Fe bridging. The interatomic distance between the bridged Fe(III) ions is 3.4692(7) Å, which falls well within the normal range observed for μ-oxo monobridged Fe(III) dimers.\(^{17}\) The Fe(III)–O–Fe(III) angle is 150.80(7)°. The crystallographic structures of the ligand edtaod\(_2\) and its copper complex \{Cu\text{(edtaod)\}_2\}·H\(_2\)O have been reported already.\(^{13}\) Both metal complexes of this ligand crystallize with the same space group (Pbca), but there are major differences in the coordination geometries. In \{Cu\text{(edtaod)\}_2\}, the octahedral coordination sphere is accomplished with the oxygen atom from a carboxyl group of an adjacent chelate molecule, whereas in \{Fe\text{(edtaod)\}_2\}·4.4H\(_2\)O the \{Fe\text{(edtaod)\}_2\} complex ions are coordinated to an oxide anion forming the μ-oxo bridge between them. The connectivity is strengthened further by two intramolecular N–H···O hydrogen bonds formed between one of the amide hydrogens and a carbonyl oxygen atom from one of the metal-coordinated carboxylate groups (N4A–H4A···O2B and N4B–H4B···O2A; not shown in Figure 6).

The iron(III) complex of edtabz\(^2^-\) is isolated in the form of the nitrate salt and crystallized as hydrate with composition Fe\text(edtabz\}_2NO\(_3\)·5.5H\(_2\)O. This metal complex is mononuclear...

Table 3. IR and XPS Data of edtabzH\(_2\) and edtaodH\(_2\), and Their Cu\(^{2+}\) and Fe\(^{3+}\) Complexes\(^{a}\)

| ligand/complex | υN–H\(_\text{est}\) (cm\(^{-1}\)) | υCOOH (cm\(^{-1}\)) | υCONH(I) (cm\(^{-1}\)) | υCONH(II) (cm\(^{-1}\)) | υC–N (cm\(^{-1}\)) | peak 1 (eV) | peak 2 (eV) | peak 3 (eV) | peak 4 (eV) |
|----------------|-------------------------------|-----------------|------------------------|------------------------|----------------|-------------|-------------|-------------|-------------|
| edtabzH\(_2\) | 3273                          | 1700            | 1611                   | 1548                   | 1219           | 932.8       | 940.5       | 944.4       | 952.7       |
| [Cu\text(�dtabz\}_2| 3318                          | 1670            | 1598                   | 1544                   | 1103           | 710.5       | 715.3       | 723.4       | NV          |
| [Fe\text(dedtaod\}_2| 3277                          | 1612            | 1568                   | 1495                   | 1111           | 932.7       | 939.0       | 943.8       | 952.6       |
| edtaodH\(_2\) | 3261                          | 1692            | 1659                   | 1509                   | 1160           | 709.9       | 714.5       | 723.0       | 729.3       |
| [Cu\text(edtaod\}_2| 3292                          | 1643            | 1593                   | 1499                   | 1090           | 932.7       | 939.0       | 943.8       | 952.6       |
| [Fe\text(edtaod\}_2| 3239                          | 1569            | 1612                   | 1498                   | 1084           | 932.7       | 939.0       | 943.8       | 952.6       |

\(^{a}\)NV = no value obtained for that peak.
with the coordination of a water molecule to form [Fe(edtabz)(H2O)]+, in contrast to μ-oxo bridge formation in [Fe(edtaod)]2O·4.4H2O. The iron(III) center in [Fe(edtabz)(H2O)]+ exhibits seven-fold coordination with the FeO5N2 core embedded in a distorted pentagonal-bipyramidal geometry (Figure 7).

In the coordination polyhedron, the amino nitrogen atoms and the amide oxygen atoms of the edtabz2− ligand occupy four positions in the equatorial plane, while the carboxylate oxygen atoms reside at the axial sites. The metal-coordinated water molecule comprises the remaining equatorial site (Figure 7). The O−Fe−O, O−Fe−N and N−Fe−N bond angles formed between neighboring atoms in the equatorial plane range from 70.13(5) to 74.03(5)° (Table 5). The O−Fe−O bond angle between the axial substituents is 160.71(6)°. The Fe−Namine, Fe−Oamide, Fe−Ocarboxylate, and Fe−Ow distances are 2.3276(16)−2.3534(15), 2.1892(13)−2.1477(13), 1.9435(13)−1.9609(13), and 2.0282(14) Å, respectively (Table 5); these distances are similar to the values established for [Fe(edtaod)]2O·4.4H2O and other structurally related iron(III) complexes reported previously.18,19 In the crystal structure, adjacent [Fe(edtabz)(H2O)]+ molecules are linked together through strong O−H···O hydrogen-bonding interactions between the Fe−OH2 water molecules and one of the carboxylate oxygen atoms [OW1···O6 = 2.687(2) Å], yielding dimeric aggregates with a Fe···Fe distance of 5.3181(5) Å. There are no direct intermolecular contacts between the metal chelate and nitrate ions.

2.4. Peroxidase-like Activity. The capability of acting as peroxidase mimetics was determined for the ligands and their metal complexes in phosphate buffer solution (pH 7.4), as the assay kit was supplied. At pH 3 which ITC was studied, the reference peroxidase is not stable enough for use. The thermodynamic data, however, are supposed to be informative of the relation between the relative stability and peroxidase-like activity at pH 7.4. As shown in Table 6, the Fe3+−edtaod2− system shows the most significant peroxidase-like capability with a specific activity equivalent to 0.24 ± 0.04 mU/mL horseradish peroxidase (HRP). The activity of the Fe3+−edtaod2− complex is higher than the values 0.13 mU/mL HRP (at a final concentration of 1 × 10−4 M) reported for some binuclear Fe3+ complexes of macrocycles derived from EDTA and aromatic diamines,8 in contrast to the inactiveness of the Fe3+−edtabz2− complex. The catalytic cycle of HRP in cells is initiated by hydrogen peroxide through an electron oxidation of the ferric ground state of the enzyme, and the resulting transient oxo-Fe4+ complex is then reduced by an electron transfer from the substrate. This process can be repeated by a second hydrogen peroxide molecule reacts to return the iron to its state ferric ground state.20 The Fe3+−edtaod2− complex is supposed to form a dimeric structure with a μ-oxo bridge in solution as found by the X-ray study because the mass spectrum exhibits the dimeric-molecular species with well-defined isotope peaks—

Table 4. Selected Bond Lengths (Å) and Bond Angles (deg) for [Fe(edtaod)]2O·4.4H2O

| bond lengths | Fe1 | Fe2 |
|--------------|-----|-----|
| Fe1−O1A      | 2.0184(12) | 2.0358(12) |
| Fe1−O4A      | 2.0094(12) | 2.0182(12) |
| Fe1−O2A      | 1.9974(12) | 2.0125(12) |
| Fe1−O9       | 1.7919(12) | 1.7930(12) |
| Fe1−N2A      | 2.3012(14) | 2.2720(15) |
| Fe1−N3A      | 2.2707(14) | 2.2238(15) |

| bond angles | Fe1 |
|-------------|-----|
| Fe1−O1A−Fe1−N3A | 90.93(5) |
| Fe1−O4A−Fe1−N2A | 161.71(5) |
| Fe1−O2A−Fe1−N3A | 78.95(5) |
| Fe1−O9−Fe1−N2A | 86.52(5) |
| Fe1−O1A−Fe1−N2A | 91.04(5) |
| Fe1−O4A−Fe1−N2A | 91.66(5) |
| Fe1−O2A−Fe1−N3A | 154.20(5) |
| Fe1−O9−Fe1−N2A | 77.50(5) |
| O9−Fe1−O1A | 93.25(5) |
| O9−Fe1−O4A | 103.61(5) |
| O9−Fe1−O2A | 104.15(5) |
| O9−Fe1−N3A | 101.41(5) |
| O9−Fe1−N2A | 169.60(5) |
| N3A−Fe1−N2A | 77.99(5) |

Figure 7. Perspective view of the molecular structure of [Fe(edtabz)(H2O)]+. Atoms are drawn at the 30% probability level.

In the coordination polyhedron, the amino nitrogen atoms and the amide oxygen atoms of the edtabz2− ligand occupy four positions in the equatorial plane, while the carboxylate oxygen atoms reside at the axial sites. The metal-coordinated water molecule comprises the remaining equatorial site (Figure 7). The O−Fe−O, O−Fe−N and N−Fe−N bond angles formed between neighboring atoms in the equatorial plane range from 70.13(5) to 74.03(5)° (Table 5). The O−Fe−O bond angle between the axial substituents is 160.71(6)°. The Fe−Namine, Fe−Oamide, Fe−Ocarboxylate, and Fe−Ow distances are 2.3276(16)−2.3534(15), 2.1892(13)−2.1477(13), 1.9435(13)−1.9609(13), and 2.0282(14) Å, respectively (Table 5); these distances are similar to the values established for [Fe(edtaod)]2O·4.4H2O and other structurally related iron(III) complexes reported previously.18,19
framework (Figure 6). As a result, the Fe$^{3+}$ center can capture for pyridyl based macrocyclic Fe$^{3+}$ complexes.21

The stability constants determined by the ITC correspond to the structural features of the two Fe$^{3+}$ chelates cause the peroxidase action. The inactiveness of the Fe$^{3+}$ ion as to be protected against an O$_2$ environment, owing to the steric e

peroxidase action. The inactiveness of the Fe$^{3+}$ center to mimic the catalytic cycle described by a pseudo-macrocyclic cavity. The macrocyclic ligand edtabzH$_2$ is more stable metal complexes than does the macrocyclic ligand. No evidence was found for the macrocyclic effect on the stability of the complexes. The X-ray diffraction study of the acyclic ligand revealed that two aromatic groups are oriented to the same direction. In solution, a closer interannular contact may be induced by the hydrophobic effect to preorganize a pseudo-macrocyclic cavity. The macrocyclic ligand edtaodH$_2$ is naturally preorganized for complexation, but the small rigid frame generates a high steric hindrance, decreasing the stability even for copper, which normally has the highest affinity with O, N-ligands among transition metals. The X-ray studies of the Cu$^{2+}$ and Fe$^{3+}$ complexes of edtabzH$_2$ ligand do not show any peak attributable to monomeric species (the spectrum is given in the Supporting Information). The metal center of the [Fe(edtaod)]$^+$ unit is displaced from the plane constructed by ligand atoms to be exposed to environment, owing to the steric effect of the rigid macrocyclic framework (Figure 6). As a result, the Fe$^{3+}$ center can capture an O$^{2-}$ ion and can stabilize the transient Fe$^{4+}$ state during peroxidase action. The inactiveness of the trans$^{3+}$ state of the Fe$^{3+}$ complex is attributable to the coordination geometry of [Fe(edtaod)(H$_2$O)]$^+$ in which the metal ion is so well suited in the coordination sphere formed by the donor atoms of the ligand as to be protected against an O$^{2-}$ ion (Figure 7). Thus, the structural features of the two Fe$^{3+}$ chelates cause the difference in their ability to mimic peroxidase, as pointed out for pyridyl based macrocyclic Fe$^{3+}$ complexes.21

The inactiveness of the ligands is predictable because of the lack of transition metal centers. The negligible activity of the Cu$^{3+}$ complexes may be related to the relative stability of the transient complexes $[\text{Cu}^{3+}(\text{edtaod})]^+$ and $[\text{Cu}^{3+}(\text{edtabz})]^+$ formed in the course of peroxidase action because the lower stability of the transient complexes the lower ability of metal center to mimic the catalytic cycle described by a peroxidase$^{20,22}$

3. CONCLUSIONS
We have synthesized and characterized a new open-chain ligand edtabzH$_2$ derived from EDTA and carried out the comparative study of the Cu$^{3+}$ and Fe$^{3+}$ complexes and the corresponding complexes of an EDTA-derived macrocycle edtaodH$_2$. The stability constants determined by the ITC technique for the Cu$^{2+}$ and Fe$^{3+}$ complexes of edtabzH$_2$ ligand are higher than those obtained for the corresponding metal complexes of edtaodH$_2$. The stability constants determined by UV-vis also have found that the Cu$^{3+}$ complexes are more stable than the Fe$^{3+}$ complexes, and the acyclic ligand forms more stable metal complexes than does the macrocyclic ligand. No evidence was found for the macrocyclic effect on the stability of the complexes. The X-ray diffraction study of the acyclic ligand revealed that two aromatic groups are oriented to the same direction. In solution, a closer interannular contact may be induced by the hydrophobic effect to preorganize a pseudo-macrocyclic cavity. The macrocyclic ligand edtaodH$_2$ is naturally preorganized for complexation, but the small rigid frame generates a high steric hindrance, decreasing the stability even for copper, which normally has the highest affinity with O, N-ligands among transition metals. The X-ray studies indicate that edtabz$^{2-}$ forms a larger number of coordinate bonds as a result of its flexible frame that can adapt to the ionic radii of iron and copper. The Fe–edtaodH$_2$ complex presents a significant POx biomimetic activity, whereas the Fe–edtabzH$_2$ complex is almost inactive despite a larger number of coordinate bonds. This fact indicates that the activity is controlled not only by stability but also by other factors such as the geometry of the complex. The X-ray structure of $[[\text{Fe}(\text{edtaod})]_2O]$ involves oxygen of the external origin within the first coordination sphere. Probably, the POx biomimetic associates with this oxygen, which exchanges with the oxygen of superoxide radical and makes Fe$^{3+}$ ion responsible for the process of dismutation to create hydrogen peroxide from molecular oxygen. In the Fe–edtabzH$_2$ complex, the coordination sphere of the metal ion is totally occupied by the donor atoms of the ligand. Thus, the acyclic ligand has higher affinity to Fe$^{3+}$ and Cu$^{2+}$ ions so that they may be useful for chelating agents in metal intoxication rather than artificial POx mimics.

4. EXPERIMENTAL SECTION
All reagents used were of analytical grade. N,N-Dimethylformamide (DMF, Fisher Scientific) was dried with molecular sieves for 24 h, and other solvents were used without further purification. The solutions of Cu(NO$_3$)$_2$·2.5H$_2$O and Fe(NO$_3$)$_3$·9H$_2$O were standardized by EDTA complexometry.

Table 5. Selected Bond Lengths (Å) and Bond Angles (deg) for [Fe(edtabz)]NO$_3$·5.5H$_2$O

| bond lengths | Fe1—O1 | 2.1892(13) | Fe1—OW1 | 2.0282(14) |
|--------------|--------|------------|--------|------------|
| Fe1—O2      | 2.1477(13) | Fe1—Fe1 | N1 | 2.3534(15) |
| Fe1—O3      | 1.9609(13) | Fe1—N2 | 2.3276(16) |
| Fe1—O5      | 1.9435(13) |        |        |            |

| bond angles | O1—Fe1—N1 | 70.13(5) | O5—Fe1—O2 | 95.68(6) |
|------------|------------|---------|------------|---------|
| O1—Fe1—N2 | 141.89(5)  | Fe1—Fe1 | 160.7(6)   |
| O2—Fe1—O1 | 146.26(5)  | O5—Fe1 | OW1 | 104.40(6) |
| O2—Fe1—N1 | 143.56(5)  | Fe1—Fe1 | N1 | 85.67(6)   |
| O2—Fe1—N2 | 70.95(5)   | OW1—Fe1 | O1 | 72.71(5)   |
| O3—Fe1—O1 | 96.12(5)   | OW1—Fe1 | O2 | 74.03(5)   |
| O3—Fe1—O2 | 92.06(5)   | OW1—Fe1 | N1 | 140.89(6)  |
| O3—Fe1—OW1 | 94.70(6)  | OW1—Fe1 | N2 | 144.96(6)  |
| O3—Fe1—N1 | 77.68(5)   | OW1—Fe1 | N1 | 73.73(5)   |
| O3—Fe1—N2 | 87.59(6)   | OW1—Fe1 | N1 | 73.73(5)   |
| O5—Fe1—O1 | 87.19(5)   | OW1—Fe1 | N1 | 73.73(5)   |

Table 6. Specific Peroxidase-like Activities (mU/mL HRP) of Fe$^{3+}$ and Cu$^{2+}$ Complexes at pH 7.4 (0.25 M Sodium Phosphate), Compared with Free Metal Ions and Uncoordinated Ligands

|        | Fe$^{3+}$—L | Cu$^{2+}$—L | L         |
|--------|-------------|-------------|-----------|
| edtaod$^{2-}$ | 0.24 ± 0.04 | undetectable | undetectable |
| edtabz$^{2-}$ | undetectable | 0.02 ± 0.009 | undetectable |
| bis(edtapo)$^{4-}$ | 0.13 ± 0.03 | n/a         | undetectable |
| free metal | not active   | not active  | not active |

“Macrocycle derived from two edta and two bis(4-aminophenyl) ether [ref 8].”
4.1. Synthesis of the Ligands. The macrocycle edtaodH2 (Scheme 1) was synthesized by the method reported previously, and the purity was checked by measurement of the decomposition point as well as IR and 1H NMR spectra.12

The acyclic ligand edtabzH2 (Scheme 1) was obtained by a reaction of ethylenediaminetetraacetic dianhydride with aniline. To 2.4 g (9.3 mmol) of EDTA dianhydride dissolved in 8 mL of dry DMF was added 2 mL (20 mmol) of aniline distilled in advance. The resulting reaction mixture was left to stand overnight. Any solids were filtered out. The filtrate was concentrated by a rotary evaporator to 5 mL, into which acetonitrile was added. Precipitates formed were filtered off, washed with acetone several times until a colorless solid was obtained. It was dried in vacuum for 8 h at room temperature. Yield: 3.21 g, 90%. mp 182–183 °C (dec). Found: C, 55.70; H, 5.84; N, 11.58%. Calcld for C23H35O8N2: C, 55.46; H, 5.92; N, 11.76%. 1H NMR (400 MHz, D2O–Na3CO3, pD = 9.0, DSS): δ 2.86 (s, 4H, H1), 3.29 (s, 4H, H2), 3.42 (s, 4H, H3), 7.20 (t, 2H, J = 8 Hz, H4), 7.36 (t, 4H, H5, H6). 13C NMR (100 MHz, D2O–Li2CO3, pD = 9.0, DSS): 175.24 (COOH), 168.75 (CONH), 136.02 (Ar, hipso), 129.02 (Ar), 125.58 (Ar, p), 121.48 (Ar, o), 57.81(Cc), 57.56 (Cb), 52.29 (Ca). Mass spectrum (ESI+): 496.1 (100%), [56FeL]+, 424.0 (100%), [63CuL·H]+.

4.2. Syntheses of Copper(II) and Iron(III) Complexes. The metal complexes were synthesized by reactions between the appropriate metal nitrates and ligands. The ligands were in 1:1 complexation. In 15 mL of water, 0.46 mmol of an appropriate metal nitrate with the solutions were left to evaporate at ambient temperature. Based on Gran’s plots, pKw was obtained to be 13.78. Precisely weighed 44.2 mg of edtabzH2 was dissolved in a mixture of deaerated 0.1 M KCl solution (100 mL) and 0.1 M HCl (1.5 mL); the initial concentration of edtabzH2 was ca. 1 × 10−3 M. Nitrogen gas humidified by 0.1 KCl was bubbled for 1 h in the sample solution before titration, to minimize the effects of O2 and CO2. Titration was performed by adding standardized 0.10 M KOH in 0.05 mL increments under a nitrogen atmosphere. The computer program HYPERQUAD was used to calculate the protonation constants. The titration curves of the metal–ligand systems were unable to be interpreted probably because of the complexity especially in the iron complexation.

4.3. Crystal Growth. Crystals of edtabzH2 were grown by a method reported previously. Titrations were carried out using Dosimat plus Metrohm semiautomatic titrator consisting of a 20 mL burette and a jacketed titration cell at a controlled temperature of 25.0 ± 0.1 °C under a nitrogen atmosphere. The pH was determined by a Metrohm model 827 pH meter equipped with a combination electrode model 6.00228.010. The electrode was calibrated with 0.01 M HCl and 0.01 M CO2-free KOH in 0.1 M KCl by assuming complete dissociation of the strong acid and base, so as to determine molar hydrogen-ion concentration. Based on Gran’s plots, pKw was obtained to be 13.78. Precisely weighed 44.2 mg of edtabzH2 was dissolved in a mixture of deaerated 0.1 M KCl solution (100 mL) and 0.1 M HCl (1.5 mL); the initial concentration of edtabzH2 was ca. 1 × 10−3 M. Nitrogen gas humidified by 0.1 KCl was bubbled for 1 h in the sample solution before titration, to minimize the effects of O2 and CO2. Titration was performed by adding standardized 0.10 M KOH in 0.05 mL increments under a nitrogen atmosphere. The computer program HYPERQUAD was used to calculate the protonation constants. The titration curves of the metal–ligand systems were unable to be interpreted probably because of the complexity especially in the iron complexation.

4.4. Potentiometric Titration. The potentiometric titration of edtabzH2 was carried out using Dosimat plus Metrohm semiautomatic titrator consisting of a 20 mL burette and a jacketed titration cell at a controlled temperature of 25.0 ± 0.1 °C under a nitrogen atmosphere. The pH was determined by a Metrohm model 827 pH meter equipped with a combination electrode model 6.00228.010. The electrode was calibrated with 0.01 M HCl and 0.01 M CO2-free KOH in 0.1 M KCl by assuming complete dissociation of the strong acid and base, so as to determine molar hydrogen-ion concentration. Based on Gran’s plots, pKw was obtained to be 13.78. Precisely weighed 44.2 mg of edtabzH2 was dissolved in a mixture of deaerated 0.1 M KCl solution (100 mL) and 0.1 M HCl (1.5 mL); the initial concentration of edtabzH2 was ca. 1 × 10−3 M. Nitrogen gas humidified by 0.1 KCl was bubbled for 1 h in the sample solution before titration, to minimize the effects of O2 and CO2. Titration was performed by adding standardized 0.10 M KOH in 0.05 mL increments under a nitrogen atmosphere. The computer program HYPERQUAD was used to calculate the protonation constants. The titration curves of the metal–ligand systems were unable to be interpreted probably because of the complexity especially in the iron complexation.

4.5. Spectroscopic Measurements. The 1H and 13C NMR spectra were obtained with a Bruker AVANCE 400 spectrometer for D2O solutions at a probe temperature of approximately 23 °C. The internal reference was sodium 2,2-dimethyl-2-silapentane-S-sulfonate (DSS). For the study of pD dependence, a minimum quantity of diluted KOD or DCI solution was used to adjust the pD of the sample solutions. The pH value of each sample solution was measured with a long-stem combination electrode inserted into the NMR tube after NMR experiments. The electrode was calibrated with standard aqueous buffers, and the measured pH values were converted to the pD values by the relation pD = pHmeas + 0.44.23

IR spectra were recorded on a PerkinElmer FT-IR Spectrometer Model Frontier equipped with ATR accessory. UV–visible spectroscopy was carried out using PerkinElmer LAMBDA 45. For the studies of complexation at a constant pH, the stock solution of the ligand was made at a concentration of 5 × 10−4 M, and the stock solutions of the metal ions were prepared from the appropriate nitrates so that the concentrations were about 30 times those of the ligand stock solutions. Titrations were carried out at pH 3 adjusted with 0.1 M HCl/NaCl. A quartz cuvette of the spectrometer was loaded with 5 mL of a stock solution of a ligand studied, to which 10 μL aliquots of an appropriate metal stock solution were added successively with a calibrated micropipette so that the ratios of the total concentrations [M]/[L], were in a desired range. Each spectrum was recorded after the sample solution was stirred thoroughly for 3 min. The titration of the Cu–edtabzH2 system was carried out in 0.1 M CAPSO buffer at pH 8.5 as well.

The high-resolution XPS of Fe and Cu 2p electrons were taken using PerkinElmer PHI 5100 with a Mg Kα source under a vacuum of 4 × 10−9 Torr. Each peak was referenced to C 1s (285.0 eV). Curve fitting of the peaks was performed assuming a Gaussian–Lorentzian function with baseline correction.
Mass spectra of electrospray ionization (ESI/MS) were obtained on 6130 Quadrupole LC/MS of Agilent Technologies, in the negative and positive ionization modes.

TGA was carried out on a thermogravimetric analyzer PerkinElmer Pyris I TGA for studying the thermal stability and compositions of the complexes. A sample with an initial weight of 5 mg was set in a ceramic pan and analyzed in a temperature ranging of 25–800 °C under N₂ or O₂ atmosphere.

4.6. Calorimetric Measurements. Microcalorimetry was performed using a MicroCal VP-ITC microcalorimeter at a temperature of 298.15 K, stirrer speed of 307 rpm, filter period of 2 s, and reference power of 10 μl s⁻¹. A ligand solution was titrated with an appropriate metal nitrate solution for four reaction systems, Fe–edtabzH₂O, Cu–edtabzH₂O, Fe–edtaodH₂O, and Cu–edtaodH₂O. The stock solutions 0.02 M of the metal nitrates were standardized by EDTA titration prior to each experiment. To prevent the hydrolysis of Fe(NO₃)₃, the stock solutions were adjusted to a pH 7.4. All measurements were carried out at pH 3.0 in 0.05 M potassium hydrogen phthalate buffer. The influence of the buffer on the complexation was negligible, as confirmed by blank tests: the injection of the metal solution into the buffer alone generated small peaks that were due to dilution unaccompanied by complexation. The raw data were corrected by subtracting the heat of dilution. The titration curves were analyzed with AFFINIMETER application software, which provides thermodynamic and kinetic parameters. The conditions of each titration system are reported in the Supporting Information.

4.7. Single-Crystal X-ray Diffraction Analyses. Diffraction data were obtained on Bruker AXS (for edtabzH₂O, 2H₂O), APEX II DUO (for [Fe(edtaod)₂O].4·H₂O), and D8 QUEST (for [Fe(edtabz)NO₃.5·H₂O] diffractometer systems equipped with a monochromator and a CDD area detector, using either Cu Kr (λ = 1.54178 Å) or Mo Kr (λ = 0.71073 Å) radiation. The measured intensities were reduced to I° and corrected for absorption using the multiscan method SADABS. Corrections were made for Lorentz and polarization effects. Structure solution, refinement, and data output were performed with the OLEX2 program package using SHELXT for the structure solution and SHELXL for the refinement. Nonhydrogen atoms were refined anisotropically. C–H hydrogen atoms were placed in geometrically calculated positions based on the riding model. N–H and O–H hydrogen atoms were located from difference Fourier maps and refined with distance restraints.

The ligand edtabzH₂O crystallizes in the form of a zwitterion hydrate with two crystallographically independent water molecules in the asymmetric unit. In the crystal structure of [Fe(edtabz)]NO₃.5·H₂O, nitrate counterion is disordered over two positions. For the refinement of the disorder, Uᵢ restraints (DELU) and geometric restrictions (FLAT) were employed. Aside from the water molecule (OW1) coordinated to the iron atom, this crystal structure comprises six crystallographically independent sites with uncoordinated water molecules (OW2–OW7) having occupancies of 1.00 (OW2, OW4–OW7) and 0.50 (OW3). Among these, four molecules labeled OW2, OW4, OW5, and OW7 that are disordered over two positions were refined without hydrogen atoms. The asymmetric unit of [Fe(edtaod)₂O].4·H₂O is constituted by the binuclear iron(III) complex and five crystallographically independent uncoordinated water molecules (OW1–OW5), two of which (OW4 and OW5) were refined with an occupancy of 0.70. Crystal structure analyses were performed with Mercury, and Diamond was used for the creation of figures.

Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publications no. CCDC—1920517, 1920512, and 1920516. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk, www: http://www.ccdc.cam.ac.uk).

4.8. Peroxidase-like Activity. Peroxidase-like activity was determined using the Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Invitrogen cat. no. A22188) which is provided in the form of phosphate buffer solution (pH 7.4). The assay was performed pH in a total volume of 100 μL per microplate well. A standard curve was prepared to determine the activity of HRP in a concentration range of 0–2 μM/mL. The wells were loaded individually with 50 μL of the standard material and test materials (edtaodH₂O, edtabzH₂, and their copper(II) and iron(III) complexes), and every sample solution was adjusted to a final concentration of 1 × 10⁻³ M. To each well, 50 μL of a working solution was added to start the reaction; the working solution was composed of a 1 × 10⁻⁴ M Amplex Red reagent (10-acetyl-3,7-dihydroxyphenoxazine) and 2 × 10⁻³ M H₂O₂ in 0.25 M sodium phosphate, pH 7.4. After incubation at room temperature for 30 min under protection from light, the absorbance of each solution in the wells was measured with a microplate reader at 550 nm. The specific activities were determined by correlating the absorbance readings to a peroxidase standard curve prepared with HRP and were reported as mU/mL of HRP activity.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03164.

**Mass spectra of ligands and complexes, IR spectra of ligands and complexes, UV–vis spectrum of edtaodCu at different pH values, ITC edtaod with Cu and Fe, XPS, and X-ray tables of edtabz, edtabzFe, and edtaodFe (PDF)**

Crystallographic data (CIF)

Crystallographic data (CIF)

Crystallographic data (CIF)

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Notes

The authors declare no competing financial interest.
ACKNOWLEDGMENTS

Authors acknowledge support from Consejo Nacional de Ciencia y Tecnología (CONACYT, Mexico); grant CB-10017-236216; a postdoctoral training scholarship under agreement no. 291053-CIAD to Y.S.; grant 294810 "Red Temática en Química Supramolecular"; grant INFRA-2014-01-225455 to acquire the single crystal X-ray diffractometer Bruker D8 QUEST. Also, we thank the Secretaría de Educación Pública, México (SEP) for grant PFCE 2016 and grant Promep/103/S.11/4462.

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