Purification and Characterization of EDTA Monoxygenase from the EDTA-Degrading Bacterium BNC1

JASON W. PAYNE, 1,2 HARVEY BOLTON, JR., 2 JAMES A. CAMPBELL, 3 AND LUYING XUN 1,2*

Department of Microbiology, Washington State University, Pullman, Washington 99164-4233, 1 and Environmental Microbiology Group 2 and Advanced Organic Analytical Methods Group, 3 Pacific Northwest National Laboratory, Richland, Washington 99352

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The synthetic chelating agent EDTA can mobilize radionuclides and heavy metals in the environment. Biodegradation of EDTA should reduce this mobilization. Although several bacteria have been reported to mineralize EDTA, little is known about the biochemistry of EDTA degradation. Understanding the biochemistry will facilitate the removal of EDTA from the environment. EDTA-degrading activities were detected in cell extracts of bacterium BNC1 when flavin mononucleotide (FMN), NADH, and O2 were present. The degradative enzyme system was separated into two different enzymes, EDTA monoxygenase and an FMN reductase. EDTA monoxygenase oxidized EDTA to glyoxylate and ethylenediaminetriacetate (ED3A), with the coconsumption of FMNH2 and O2. The FMN reductase provided EDTA monoxygenase with FMNH2 by reducing FMN with NADH. The FMN reductase was successfully substituted in the assay mixture by other FMN reductases. EDTA monoxygenase was purified to greater than 95% homogeneity and had a single polypeptide with a molecular weight of 45,000. The enzyme oxidized both EDTA complexed with various metal ions and uncomplexed EDTA. The optimal conditions for activity were pH 7.8 and 35°C. Km values were 34.1 μM for uncomplexed EDTA and 8.5 μM for MgEDTA 2−; this difference in Km indicates that the enzyme has greater affinity for MgEDTA 2−. The enzyme also catalyzed the release of glyoxylate from nitrilotriacetate and diethylenetriaminepentacetate. EDTA monoxygenase belongs to a small group of FMNH2-utilizing monoxygenases that attack carbon-nitrogen, carbon-sulfur, and carbon-carbon double bonds.

Synthetic chelating agents have a wide variety of uses, from household cleaners, water treatment, and pulp and paper bleaching to rubber and metal processing (2, 23, 30). About 70% of the chelating agents used worldwide are aminopolycarboxylic acids, primarily EDTA, diethylenetriaminepentacetate (DTPA), and nitrilotriacetate (NTA), with annual production about 372 million lb in the United States, western Europe, and Japan in 1994 (30). EDTA is the most commonly used chelating agent. In the environment, EDTA has some undesirable environmental consequences such as the remobilization of radionuclides and heavy metals from soils and sediments (6, 9, 24). The mobilized radionuclides and toxic heavy metals can cause direct health problems or can be accumulated by plants and then transferred to human beings through the food chain. EDTA is recalcitrant in soils (25, 34, 35) and sediments (3, 34, 35). Photodegradation of FeEDTA plays a major role in the fate of EDTA in surface waters (13, 14). Other species, such as CuEDTA, ZnEDTA, and NiEDTA, are not degraded by sunlight, whereas CoEDTA is only slightly sensitive (20, 26).

To date, only two microorganisms have been reported to degrade EDTA. The gram-negative bacterium BNC1 was isolated from sewage receiving EDTA-containing wastewater effluents (27); an Agrobacterium sp. growing on Fe(III)EDTA has also been isolated (18). However, little is known about the biochemistry of EDTA degradation by these microorganisms. To facilitate bioremediation of EDTA and reduce the mobility of heavy metals in the groundwater, the biochemistry of EDTA degradation by bacterium BNC1 was studied. We report here the purification and characterization of EDTA monoxygenase (EDTA-Mo), which catalyzed the first step of EDTA degradation by bacterium BNC1.

(A preliminary account of this work was presented at the 96th General Meeting of the American Society for Microbiology [28].)

MATERIALS AND METHODS

Bacterium and culture conditions. The EDTA-degrading bacterium BNC1 was kindly provided by Bernd Nortemann (Technical University of Braunschweig, Braunschweig, Germany). BNC1 was cultured with Na2EDTA·2H2O (0.3 g/liter) and potassium acetate (0.25 g/liter) in a mineral medium (27). Large quantities of cells were obtained by culturing BNC1 in a 50-liter carboy containing 30 liters of the medium bubbled with sterile air for 2.5 days at 24°C. Toward the end of log phase, cells were harvested by concentrating the culture to 2 liters in a hollow fiber filtration unit (model DC10L system; Amicon, Beverly, Mass.) and then centrifuged at 17,000 × g for 15 min at 4°C. The cells were stored at −20°C for a maximum of 3 days.

Enzyme assays. EDTA-Mo activity was assayed by measuring the production of glyoxylate. A standard assay mixture contained 20 mM HEPES buffer (pH 7.8), 10 μM flavin mononucleotide (FMN), 2 mM NADH, 500 μM Na2EDTA (or other chelator), 300 μM MgCl2, 0.2 U of NAD(P)H:FMN oxidoreductase from Photobacterium fischeri (Boehringer Mannheim Co., Indianapolis, Ind.), 30 U of catalase (from bovine liver; Sigma), and an appropriate amount of EDTA-Mo preparation in a total volume of 250 μl. The NAD(P)H:FMN oxidoreductase generated FMNH2 by reducing FMN with NAD(P)H. The reaction was initiated by adding NADH. A stock solution of 100 mM NADH was prepared in 10 mM Tris buffer (pH >13). The assay was stopped by adding 100 μl of 0.1 N HCl. The glyoxylate produced was detected by phenylhydradiazine-K3Fe(CN)6, as previously described (37). The aqueous speciation of EDTA among its free acid and complexed forms (e.g., H2EDTA2− and MgEDTA2−) was calculated with an aqueous speciation-solubility model MINTEQA2 (1, 31) as previously described (39).

Purification steps. All operations were performed at 6°C. All buffers except those used for the hydroxylapatite column contained 1 mM dithiothreitol. The levels of ammonium sulfate saturation were those at 25°C.

1. Extraction of cells. About 15 g of frozen cells was thawed at room temperature and suspended in 30 ml of 20 mM Tris buffer (pH 8.0) containing 2.5 mM EDTA. The protease inhibitor phenylmethylsulfonyl fluoride was freshly
prepared in absolute ethanol at a concentration of 30 mM and added to the cell suspension to a final concentration of 0.5 mM. The slurry was passed through a French pressure cell (model FA-030: Aminco, Urbana, Ill.) three times at 260 MPa. The product was centrifuged at 17,000 × g for 15 min. The supernatant was saved and the pellet discarded.

(ii) Protamine sulfate treatment. A 2% solution of protamine sulfate in 20 mM Tris buffer (pH 8.0) was added to the supernatant slowly to 0.05% with stirring. After 5 min, the mixture was centrifuged at 17,000 × g for 15 min, and the supernatant was saved.

(iii) Ammonium sulfate fractionation. Solid ammonium sulfate was added to the supernatant to 33% saturation with constant stirring. The pH of the solution was not adjusted. After 10 min of stirring, the mixture was centrifuged at 17,000 × g for 15 min and the precipitate was discarded. Additional solid ammonium sulfate was added to the supernatant to 70% saturation with constant stirring. After 10 min of stirring, the mixture was centrifuged at 17,000 × g for 15 min. The precipitate was saved, and the supernatant was discarded.

(iv) Ultracentrifugation. The precipitate was suspended in an equal volume of 25 mM potassium phosphate (KPi) buffer (pH 7.0). The suspension was dialyzed against 1 liter of the same buffer for 2 h. The dialyzed sample was centrifuged at 331,000 × g for 40 min, and the supernatant was saved.

(v) Dye chromatography. The supernatant was loaded onto a 25-mL Cibacron Blue 3GA agarose (Sigma) column (1.5 by 14 cm) previously equilibrated with 25 mM KPi buffer (pH 7.0). A maximum of 150 mg of total protein was loaded per run. After the proteins were loaded, the column was washed with 80 ml of the equilibrating buffer and then washed with 80 ml of 1 M NaCl in 25 mM KPi buffer (pH 7.0). Most proteins did not bind to the column and were washed off with the starting buffer. Both EDTA-Mo and an FMM reductase were eluted with the 1 M NaCl solution. The eluent containing the enzymes was concentrated to 1 ml with a Centricon-10 (Millipore). The sample was desalted to 25 mM KPi (pH 7.0) with a Centriprep-10.

(vi) MonoQ chromatography. The sample from the dye column in 25 mM KPi buffer (pH 7.0) was injected onto a MonoQ HR 5/5 column (Pharmacia) equilibrated with the same buffer. Proteins were eluted with a step and linear gradient of NaCl (percentages of 0.5 M NaCl in the same buffer: 0%, 4 ml; 0 to 15%, 20-ml linear gradient; 100%, 6 ml; and 0%, 3 ml) by a fast protein liquid chromatography (FPLC) system (Pharmacia). EDTA-Mo was eluted associated with a major peak around 80 mM NaCl. The fractions containing the enzyme activity were pooled and concentrated to 1 ml with a Centricon-10. The FMM reductase was not bound to the column and was separated from EDTA-Mo by this step.

(vii) Hydroxyapatite chromatography. The buffer for EDTA-Mo was changed to 8 mM KPi (pH 6.6) with a Centriprep-10. The sample was injected onto a Bio-Scale CHTA-1 hydroxyapatite column (Bio-Rad, Hercules, Calif.) equilibrated with the same buffer. Proteins were eluted with a step and linear gradient of KPi (pH 6.6) (concentrations of KPi: 8 mM, 4 ml; 50 mM, 5 ml; 25 to 50 mM, 20-ml linear gradient; and 500 mM, 8 ml) by FPLC. EDTA-Mo was eluted as a major peak around 165 mM KPi, concentrated to 1 ml with a Centriprep-10, and stored at 4°C.

Analytical methods. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done by the method of Laemmli (17). Gels were stained for proteins with Coomasie brilliant blue R-250. Protein concentrations were determined in a protein reagent (4), with bovine serum albumin as the standard. Gel filtration chromatography was used to estimate the native molecular weight of the purified enzyme. The purified enzyme was injected onto a Superose 12 column (Pharmacia) equilibrated with 25 mM KPi (pH 7.0) containing 150 mM NaCl. The enzyme was eluted with the same buffer by FPLC. The EDTA-Mo complex was eluted off the column as a single peak with a retention volume of 12.5 ml. Gel filtration standards were purchased from Bio-Rad. The nitrogen-containing moiety of enzymic end product from NTA was derivatized with 9-fluorenylmethylchloroformate (7) and then determined by a high-performance liquid chromatography (HPLC) system (Waters). The N-terminal amino acid sequence of the purified protein was determined on an ABI 470 protein sequencer at the Department of Biochemistry and Biophysics, Washington State University, as previously described (38).

Oxygen consumption. Oxygen consumption by FMNH2 and by EDTA-Mo was determined in a closed reaction vessel (0.6-ml total volume) fitted with a Clarke-type oxygen electrode (Instech, Plymouth Meeting, Pa.). The electrode was calibrated against a Nageen ammonia electrode and NADH (29). The reaction was in 20 mM HEPES buffer (pH 7.8)–10 mM FMN–500 μM Na2EDTA–500 μM MgC2O4. Two sets of experiments were carried out. The first set contained 0.8 U of NAD(P)H:FMN oxidoreductase only. The second set contained 0.8 U of NAD(P)H:FMN oxidoreductase and 580 μg of EDTA-Mo from the MonoQ column in both cases. NADH was added in a 2.5-μl volume to the final concentration of 198 μM to initiate O2 consumption. When O2 consumption stopped, 90 μl of catalase was added to release O2 from H2O2. For the reaction containing EDTA-Mo, the glyoxylate produced was also quantified.

pH and temperature optima. The EDTA-Mo activities were measured at various pH values within the range of 6.6 to 8.4 with 20 mM HEPES buffer. The enzyme assay was performed as described above except that the reaction was stopped by heating at 90°C for 5 min instead of acidification. The temperature optima for the enzyme activity were determined in a similar way at pH 7.7. The reaction mixture without NADH was incubated at the corresponding temperature for 5 min, and then NADH was added to the mixture to start the reaction. The reaction was terminated by acidification.

Product determination by mass spectrometry. A 5-ml aliquot of the reaction mixture was lyophilized with an LPH LOCK (LABCONCO) for approximately 12 h. The dried material was transferred to a Reactivial (VWR Scientific); 2 ml of 12% BF3–methanol (Aldrich) was added, and the sample was heated to 100°C for 1 h. The solution was cooled, and 2 ml of chloroform was added. The entire solution was then quenched in a vial containing 10 ml of 0.4 M KH2PO4 (pH 9.5). The vial was vortexed, and the aqueous and chloroform layers were allowed to separate. The chloroform layer, containing the derivatized organics, was retained, and the aqueous layer was discarded. The chloroform solution was then analyzed by gas chromatography-mass spectrometry (GC-MS) (5, 10).

RESULTS

Identification of EDTA degradation in cell extracts. Cell extracts of BNC1 cultured with EDTA and acetate in a mineral medium are able to degrade EDTA to glyoxylate in the presence of NADH, FMN, and O2. Flavin adenine dinucleotide (FAD) and riboflavin could not replace FMN. The effects of temperature and pH on glyoxylate production by cell extracts were determined. Optimal reaction rates were achieved at 35°C and pH 7.7. The rates of glyoxylate formation were also affected by the metal cation complexed by EDTA in the assay. Dissolved cell extracts were able to release glyoxylate from EDTA complexed with any of the cations tested (Mg2+, Mn2+, Ni2+, Co2+, Zn2+, Fe2+, Cu2+, Cr3+, Sn2+, Ba2+, Cd2+, Sr2+, Pd2+, Al3+, Cr3+, K+, or Na+). MgEDTA2− was found to be the best substrate, having a rate nearly twice that of uncomplexed EDTA. For this reason, MgCl2 was used in the assay.

Enzyme purification. EDTA-Mo was purified by monitoring glyoxylate production from EDTA in reaction mixtures following each purification step. In the cell extract, there was an FMN reductase that was later separated from EDTA-Mo on a MonoQ column. After the separation, the FMN reductase could be substituted by an NADH:FMN oxidoreductase of Chelatobacter heintzii (38) or an NAD(P)H:FMN oxidoreductase of P. fischeri (36) to generate FMN2H3 for EDTA-Mo. Because its activity can be replaced by other NAD(P)H:FMN oxidoreductases, we purified EDTA-Mo with the NAD(P)H:FMN oxidoreductase of P. fischeri to supply FMN2H3. The results of a typical purification of EDTA-Mo are summarized in Table 1.

Enzyme properties and activities. EDTA-Mo was apparently purified to homogeneity as indicated by SDS-PAGE analysis, revealing a single 45-kDa band (Fig. 1). The native EDTA-Mo was estimated to be a monomer by gel filtration chromatography. The N-terminal sequences of EDTA-Mo was determined to be MKNKLMLY.

EDTA-Mo produced glyoxylate from EDTA, NTA, and DTPA, with the coconsumption of FMN2H3 and O2. FMN2H3 was generated by an FMN reductase with NADH as the reductant in the assay system. In the reaction mixture, O2 consumption was observed even with NAD(P)H:FMN oxidoreductase alone. When 198 μM NADH was added to the reaction mixture in a closed vessel, 225 μM O2 was consumed in 12 min. The consumed O2 was quantitatively converted to H2O2 since 116 μM O2 was released when catalase was added. When the reaction mixture also contained EDTA-Mo, 192 μM glyoxylate was produced and 197 μM O2 was consumed in 4
min. Only 10 μM O₂ was converted to H₂O₂ since only 5 μM O₂ was released when catalase was added.

The end product with the nitrogen moiety from NTA was identified as iminodiacetate by HPLC analysis. The enzyme did not release glyoxylate from commercial iminodiacetate (Sigma). The end product with the nitrogen moiety from EDTA was not detected by the HPLC method and was analyzed by GC-MS. The total ion chromatogram is shown in Fig. 2A. On the basis of the mass spectrum of the component at the retention time of 24.1 min, it was identified as the methyl ester of the lactam of ethylenediaminetriacetate (ED3A) (Fig. 2B). ED3A forms an internal amino carboxylic ester bond to yield the lactam of ED3A during derivatization before GC-MS analysis (10). The compound at the retention time of 26.7 min was identified as the methyl ester of EDTA by its mass spectrum (data not shown). Neither unsymmetric nor symmetric ethylenediaminediacetate (EDDA) was detected. The first peak in Fig. 2A was caused by HEPES used as a buffer for the enzymatic assay. The nitrogen-containing end product from DTPA was not identified because the enzyme did not produce enough of it for GC-MS analysis.

The effects of temperature and pH on EDTA-Mo activity were determined. The optimal temperature for EDTA-Mo activity was 35°C, with 75, 93, and 96% of the optimal activity retained at 25, 30, and 40°C, respectively. The optimal pH was 7.7 in 20 mM HEPES buffer; the activities were 75, 83, and 70% of the optimum at pH 6.9, 7.3, and 8.1, respectively. The best activity was obtained in 20 mM HEPES buffer (pH 7.7) at 35°C.

The kinetic parameters were determined for EDTA-Mo with an excess of NAD(P)H:FMN oxidoreductase of *P. fischeri* to generate FMNH₂. *Kₘ* and *Vₘₐₓ* values were determined for EDTA-Mo from Lineweaver-Burke plots of initial reaction rates. The degradation of EDTA or MgEDTA²⁻ was measured based on the formation of glyoxylate. In the assay mixture without Mg²⁺, EDTA did not associate with Na⁺ and was present as uncomplexed EDTA in the forms of 94% HEDTA⁻⁻ and 6% H₂EDTA⁻. For uncomplexed EDTA, *Kₘ* was 34.1 μM, *Vₘₐₓ* was 3.7 μM mg⁻¹ min⁻¹, *kₐₜ* was 2.8 s⁻¹, and *kₐₜ/Kₘ* was 0.08 μM⁻¹ s⁻¹. When equimolar Mg²⁺ and EDTA were added to the reaction mixture, 98.8% of the EDTA was present as MgEDTA²⁻. For MgEDTA²⁻, *Kₘ* was 8.5 μM, *Vₘₐₓ* was 4.7 μM mg⁻¹ min⁻¹, *kₐₜ* was 3.6 s⁻¹, and *kₐₜ/Kₘ* was 0.43 μM⁻¹ s⁻¹. The difference in *kₐₜ/Kₘ* for the two substrates (MgEDTA²⁻ 5.3 times higher) was primarily due to the difference in *Kₘ*, indicating that the enzyme has a greater affinity for the chelate when it is complexed with Mg²⁺.

**DISCUSSION**

This is the first report that EDTA-Mo has been identified, purified, and characterized. When supplied with FMNH₂, the

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**TABLE 1. Purification scheme for EDTA-Mo**

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Vol (ml)</th>
<th>Protein (mg)</th>
<th>Activity (U)</th>
<th>Recovery (%)</th>
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<tr>
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<td></td>
<td></td>
<td>Activity (U)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td>Specific</td>
</tr>
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<td>65.29</td>
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<td>66.80</td>
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<td>Ammonium sulfate fractionation</td>
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<td>1.442</td>
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<tr>
<td>Hydroxyapatite column</td>
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<td>0.64</td>
<td>3.78</td>
<td>5.913</td>
</tr>
</tbody>
</table>

* One unit is defined as the release of 1 μmol of glyoxylate per min from EDTA.

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**FIG. 1.** SDS-PAGE of purified EDTA-Mo. Lane 1, low-range molecular weight standards (Bio-Rad); lane 2, 1 μg of EDTA-Mo.

**FIG. 2.** GC-MS analysis of the end products of EDTA degradation by EDTA-Mo. (A) Total ion chromatogram of the sample; (B) mass spectrum of lactam ED3A.
envelope appears to break the N-C bond in EDTA, NTA, and DTPA, with one oxygen atom appearing in glyoxylate. The enzyme was able to degrade EDTA, NTA, and DTPA with a variety of metal cations. With cell extracts, the highest rate was found when Mg was added. The purified enzyme showed similar preferences for MgEDTA$^{2-}$ and free EDTA. Kinetic analyses indicate that the enzyme has a higher affinity for MgEDTA$^{2-}$. This finding coincides with Mg$^{2+}$ being the most abundant intracellular cation in several tested microorganisms (12), with MgEDTA$^{2-}$ the likely form of EDTA in the cytoplasm of BNC1.

HPLC, GC-MS, and colorimetric analyses showed that EDTA was oxidized to glyoxylate and ED3A, and NTA was oxidized to glyoxylate and iminodiacetate by EDTA-Mo (Fig. 3). Upon examination of the structures of NTA and ED3A, we wondered whether EDTA-Mo could also degrade ED3A to EDTA. However, GC-MS analysis did not detect any trace of either symmetric or unsymmetric ED3A. We are currently cloning the gene encoding EDTA-Mo by using a DNA probe generated from the N-terminal amino acid sequence. Successful production of a functional EDTA-Mo in an expression host will allow us to confirm the identities of enzyme end products from EDTA and NTA as well as to accumulate enough end products from DTPA for identification. Characterization of the gene sequence will reveal the relationships among related enzymes, especially between EDTA-Mo and NTA monoxygenase (NTA-Mo).

The stoichiometry of EDTA oxidation by EDTA-Mo was studied. One molecule of FMN was reduced to one FMNH$_2$ by NAD(P)H:FMN oxidoreductase at the expense of one NADH. Then one FMNH$_2$ reacted with one O$_2$ chemically to generate one H$_2$O$_2$. When catalase was added, one O$_2$ was produced from two H$_2$O$_2$. When EDTA-Mo was also present in the reaction mixture, the enzyme consumed one O$_2$ and one FMNH$_2$ to oxidized EDTA and produced one glyoxylate. It took 12 min to complete O$_2$ consumption in the absence of EDTA-Mo but only 4 min in the presence of EDTA-Mo. Ninety-five percent of FMNH$_2$ generated by NAD(P)H:FMN oxidoreductase was added to EDTA-Mo to oxidize EDTA, and only 5% reacted with O$_2$ to produce H$_2$O$_2$. Although the concentrations of EDTA and ED3A were not determined, the conversion of EDTA to ED3A was determined by GC-MS analysis. On the basis of these data, the reaction catalyzed by EDTA-Mo is proposed (Fig. 3).

When the kinetic parameters for EDTA oxidation were determined, excess NAD(P)H:FMN oxidoreductase was added in the reaction mixture. Under such conditions, a substantial amount of H$_2$O$_2$ was produced. Catalase was added to the reaction mixture to prevent the buildup of H$_2$O$_2$. This practice provided sufficient amount of FMNH$_2$ to EDTA-Mo. When O$_2$ consumption was studied, excess EDTA-Mo was added to effectively utilize FMNH$_2$ so that the formation of H$_2$O$_2$ was minimized. Because of FMNH$_2$ is highly reactive with O$_2$, we could not use O$_2$ as the limiting factor in the reaction mixture and thus could not determine its kinetic parameters.

There are similarities and differences between EDTA-Mo of BNC1 and NTA-Mo (formerly component A) of C. heintzii (37, 38). Both enzymes require FMNH$_2$ and O$_2$ as cosubstrates, and both are single polypeptides of about 50 kDa. EDTA-Mo oxidizes EDTA to ED3A and glyoxylate, and oxidizes NTA to iminodiacetate and glyoxylate. NTA-Mo oxidizes NTA to iminodiacetate and glyoxylate (37, 38). NTA-Mo degrades only specific metal-NTA complexes (37, 39), while EDTA-Mo degrades EDTA, NTA, and DTPA in the absence or presence of metal cations. NTA-Mo does not degrade EDTA or DTPA.

Flavin-dependent monoxygenases are ubiquitous. FAD and FMN are normally prosthetic groups, not cosubstrates, and they are reduced by the monoxygenases themselves with NADH or NADPH as the reductant (8, 32). EDTA-Mo uses FMNH$_2$ directly. Since the enzyme does not appear to contain any chromophores and does not require any specific transition metal cofactors, the activated oxygen species is likely a C(4a)-flavin hydroperoxide as proposed for other flavin-dependent monoxygenases (15, 22). Thus, FMNH$_2$ acts as both reductant and prosthetic group for EDTA-Mo. An endogenous FMN reductase supplied EDTA-Mo with FMNH$_2$. Since the reductase was replaced by two other FMN reductases, it is unlikely that there is any direct protein-protein interactions between the reductase and oxygenase. These data suggest that EDTA-Mo belongs to a small group of monoxygenases that utilize FMNH$_2$ as both reductant and prosthetic group. Bacterial luciferase of P. fischeri was the first FMNH$_2$-utilizing monoxygenase studied (36). Recently, pristinamycin IIA synthase of Streptomyces pristinaespiralis (33), NTA-Mo of C. heintzii (38), and two monoxygenases involved in desulfurization of dibenzothiophene by Rhodococcus sp. strain IGTS8 (11, 19) have been characterized and shown to use FMNH$_2$ as the cosubstrate. EDTA-Mo appears to be the sixth member of this group. These FMNH$_2$-dependent monoxygenases appear to attack carbon-nitrogen, carbon-sulfur, carbon-carbon, or carbon-oxygen double bonds.

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ADDITIONAL

During review of the manuscript, Witschel et al. reported the identification and characterization of a similar EDTA-degrading enzyme from another bacterial isolate (37a).
REFERENCES


AUTHOR’S CORRECTION

Purification and Characterization of EDTA Monooxygenase from the EDTA-Degrading Bacterium BNC1

JASON W. PAYNE, HARVEY BOLTON, JR., JAMES A. CAMPBELL, AND LUYING XUN

Department of Microbiology, Washington State University, Pullman, Washington 99164-4233, and Environmental Microbiology Group and Advanced Organic Analytical Methods Group, Pacific Northwest National Laboratory, Richland, Washington 99352

Volume 180, no. 15, p. 3823–3827, 1998. Pages 3823–3827: Prior to submission of our article similar findings of an EDTA monooxygenase from a different bacterium were reported by Witschel et al. (M. Witschel, S. Nagel, and T. Egli, J. Bacteriol. 179:6937–6943, 1997). Their work was inappropriately cited in an addendum rather than in the text of our article.