Binding of Various Aminopolycarboxylates

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Synthetic aminopolycarboxylates like ethylenediaminetetraacetate (EDTA) are common chelating agents. EDTA-degrading bacterium *Chelativorans* sp. BNC1 uses an ABC-type transporter for the uptake of free EDTA into its cells for biodegradation. The key component of the transporter is a periplasmic EDTA-binding protein, EppA, and the structural and functional analyses indicate that EppA is a general binding protein for the uptake of free aminopolycarboxylates, suggesting that stable metal-chelate complexes are not transported into the cells for biodegradation and explaining the persistence of stable metal-EDTA complexes in the environment.

Keywords: EDTA ; periplasmic binding protein ; ABC transporter ; crystal structure ; isothermal titration calorimetry

1. Aminopolycarboxylate chelators (APCs)

Aminopolycarboxylate chelators (APCs) are amine-containing polycarboxylic acids that are used as metal chelators. Ethylenediaminetetraacetic acid, better known as EDTA, is the most widely used chelator in science, industry, medicine, and consumer goods due to its ability to chelate metals to form stable, water-soluble metal–chelate complexes [1][2]. The stability of metal–EDTA complexes leads to EDTA's persistence and accumulation in the environment, making it a significant anthropogenic pollutant [3][4][5]. Concerns about EDTA's potential to mobilize heavy metals, and radionuclides in particular, have led many countries to regulate its use [2][6][7]. Besides EDTA, similar APCs with more specialized applications exist. Both 1,2-bis(2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid (BAPTA) and ethylene glycol-bis(β-aminoethylether)-*N,N,N′,N′*-tetraacetic acid (EGTA) are used as selective calcium chelators; ethylenediamine-*N,N′*-bis(2-hydroxyphenylacetic acid) (EDDHA) is used to increase the bioavailability of iron for plant fertilization; diethylenetriaminepentaacetic acid (DTPA) is used in numerous applications, such as a contrasting agent in magnetic resonance imaging; *N*-(2-hydroxyethyl)ethylenediamine-*N,N′,N′*-triacetic acid (HEDTA) is used as an iron-based herbicide; and nitrilotriacetate (NTA) is often used in laundry detergents. Their effluence into water supplies may also contribute to persistent APC pollutants. Environmentally friendlier alternatives like the naturally occurring EDDS ((S,S)-ethylenediamine-*N*,*N′*-disuccinic acid) exist [8], and others are under development [9], but the effectiveness and affordability of EDTA have so far ensured its continued use.

2. Bioremediation

One promising method for removing environmental EDTA contamination is bioremediation. For this purpose, multiple bacterial species have been identified that can subsist on EDTA as a sole source of carbon, nitrogen, and energy [10][11][12][13][14]. Being related, the EDTA-degrading bacteria were assigned to the novel genus *Chelativorans* [15]. In *Chelativorans* species, the genes encoding an ATP-binding cassette (ABC)-type transporter system for EDTA uptake and EDTA-degrading enzymes are co-located in a single operon [16]. *Chelativorans* sp. BNC1 (formerly *Mezorhizobium* sp. BNC1), isolated from industrial sewage [17], uses the ABC transport system to uptake EDTA. Inside the cell, EDTA is catabolized [18]. In the first step, an $FMNH_2$-dependent EDTA monooxygenase, EmoA, together with its partner NADH:FMN oxidoreductase, EmoB, oxidizes EDTA to ethylenediamine-*N,N′*-diacetate (EDDA) [16][19][20]. Next, iminodiacetate oxidase (IdaA) oxidizes EDDA to ethylenediamine (ED) [21][22]. EmoA and EmoB also oxidize nitrilotriacetate (NTA) to iminodiacetate, and IdaA oxidizes the latter to glycine [16][21]. Each oxidative cleavage produces a glyoxylate molecule, which is used as a carbon source, and the ethylenediamine can be used as a nitrogen source [15].

The EDTA transporter system is composed of a periplasmic binding protein (PBP), EppA, and a type I ABC-type importer consisting of a heterodimer of its transmembrane domain components, EppB and EppC, and a dimer of its nucleotide binding domain component, EppD. By sequence comparison, EppA belongs to the periplasmic binding protein PBP2 NikA/DppA/OppA-like superfamily, which is a family in the Class II Cluster C PBP [18]. Class II Cluster C PBPs contain two large polypeptide lobes connected via flexible tethers, allowing them to undergo a large and reversible conformational change, known as the "Venus fly-trap'' model, in which ligand binding in the cleft between the two lobes induces a closed

conformation [23][24]. EppA binds free EDTA, but not metal–EDTA complexes, restricting the ability of *Chelativorans* sp. BNC1 to use only weak metal–EDTA complexes that can dissociate to free EDTA [18], making it imperative to determine the biophysical mechanism of EppA's binding specificity before any improvements to its binding capabilities can be engineered. We have been delineating the underlying substrate specificity, catalytic mechanism and molecular interactions of key metabolic enzymes—EmoA [20], EmoB [19], and IdaA [22]—in the EDTA-degradation pathway of *Chelativorans* sp. [16]. To understand the first step of EDTA catabolism by *Chelativorans* sp. BNC1 and how it may act as a gatekeeper for all enzymes downstream, here we report structural characterization of EppA and thermodynamic characterization of its binding of EDTA and other APCs.

Structural and binding assays indicate that EppA is a general binding protein for aminopolycarboxylates. It may originally binds naturally occurring aminopolycarboxylates, such as ethylenediaminedisuccinate [25], and also binds synthetic chelating agents when recently introduced [26]. Binding of ligands by PBPs is a prerequisite for their import to the bacterial cytoplasm by the PBP's cognate ABC transporter. EppA only binds free aminopolycarboxylates. In the case of weak metal-chelate complexes, EppA facilitates dissociation of the weak chelates with the binding and transporting the free aminopolycarboxylates into the cells for biodegradation. However, the stable metal-chelate complexes will not be subject to EppA-dependent uptake and biodegradation. Thus, stable metal-chelate complexes have accumulated in surface waters as the most dominant pollutants [3][4][5].

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