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Addition and Correction

Correction to Structural Insights into the Dual Activities of the Nerve Agent Degrading Organophosphate Anhydrolase/Prolidase [(2010) *Biochemistry 49,* 547. DOI: 10.1021/bi9011989]. Nand K. Vyas, Alexei Nickitenko, Vipin K. Rastogi, Saumil S. Shah, and Florante A. Quiocho*

Past characterizations of OPAA preparations at the Edgewood Center have indicated briefly that the enzyme is a monomer in solution (refs 4−6). To take this into account, we reported that the three molecules (A, B, and C) occupying the asymmetric unit of the isomorphous crystals (*I*222 space group) of the native *Alteromonas* sp. strain JD6.5 OPAA and its complex with mipafox or DDP exhibit no local 3-fold rotation axis of symmetry and no significant and uniform total buried surfaces between pairs of them. Here we provide evidence from crystal packing and gel filtration that in fact OPAA exists as a protomer.

Crystal packing consideration generated two identical dodecamers in the unit cell, each displaying 222 symmetry and consisting of six essentially identical dimers (see Figure 1 for one dodecamer). The two monomers in each dimer make extensive interactions. The six dimers can be grouped into two types (Figure 1): two of type 1 between A (x, y, z) and A ($-x$, y, $-z$) molecules (colored red), each related by a crystallographic 2-fold, and four of type 2 between B (x, y, z) (blue) and C ($^1/_2 - x$, $^1/_2 + y$, $^1/_2 - z$) (green), each with local 2-fold symmetry. The observation that the dimer interface of both types buries ~2300 Å^2 of accessible surface per monomer, which is very close to the values of dimers seen in all structures of AMPP [e.g., dimer of dimers of the *Escherichia coli* form (e.g., see refs 31, 35, and 37−39) and a dimer of the *Pyrococcus furiosus* form (ref 15)], other prolidases, and obligate dimers of many unrelated proteins (ref 27), is indicative of a very stable OPAA dimer. Furthermore, two B−C dimers form a tetramer, albeit with only ~300 Å^2 of buried surface per dimer (Figure 1). In contrast, the two crystallographic symmetry related A−A dimers are well separated by intercalations of B molecules and, consequently, make no contacts (Figure 1).

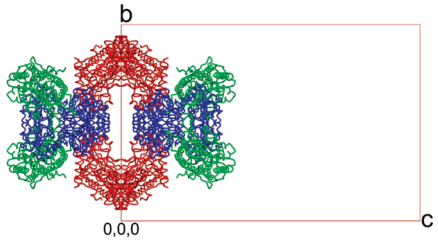

FIGURE 1: One of the two dodecamers of OPAA in the unit cell centered at 0, $^1/_2$, 0. The second dodecamer (not shown) is centered at $^1/_2$, 0, $^1/_2$.

The real likelihood of an oligomeric OPAA (at the very least a dimer or potentially a tetramer) prompted the laboratory in Houston to conduct gel filtration experiments using an analytical column. The result shown in Figure 2 indicates a tetrameric OPAA with a mass of 249 kDa, which would be compatible with a protomer structure of dimer of dimers.

Thus, both OPAA and AMPP not only exhibit a very high degree of structural and functional similarity (described extensively in the paper) but may also act as a protomer. This requires revision of the few references in the paper that describe the monomeric nature of OPAA. For instance, Asp38, Trp88 (misidentified as Trp86), and Arg153 located in one subunit of the most stable AMPP dimer have been suggested to play ancillary roles in catalysis by interacting with potential primary functional residues in the bimetallic center located in the other subunit (refs 39 and 47). These roles are duplicated in the OPAA dimer by the equivalent Asp45 and Trp89 residues, respectively. However, both structures of OPAA, as well as its amino acid sequence, show no residue equivalent or similar to Arg153 of AMPP. That the N-terminal domain of both enzymes deploys the Asp and Trp residues underscores the functional role of the domain.

DOI: 10.1021/bi100138q
Published on Web 02/18/2010

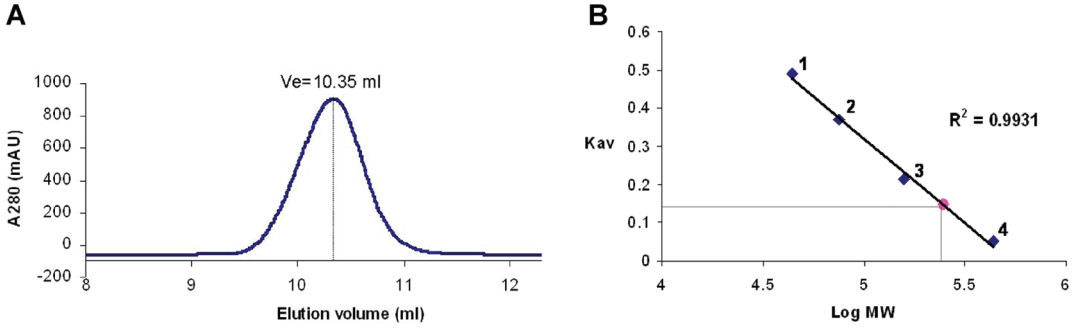

FIGURE 2: (A) Gel filtration profile of OPAA. We loaded 200 μL of 5 mg/mL OPAA onto the Superdex 200 HR (GE Healthcare) analytical column and eluted with 1 mM β-mercaptoethanol and 10 mM Tris (pH 7.2) buffer. A second gel filtration (not shown) of a 10-fold diluted protein solution gave a similar elution profile. (B) Protein standard curve of 1-ovalbumin (44 kDa), 2-conalbumin (75 kDa), 3-aldolase (158 kDa), and 4-ferritin (440 kDa) [$K_{av} = (V_e - V_o)/(V_c - V_o)$, where V_e is the elution volution, V_o is the column void volume, and V_c is the geometric column volume]. The apparent molecular weight of the eluted protein was fit with the linear regression $K_{av} = a \log MW + K_{av0}$, where $a = -0.440$ and $K_{av0} = 2.52$. The V_e of 10.35 mL (panel A) or K_{av} of 0.14 for OPAA (red circle) (panel B) yielded a molecular mass of 249 kDa (experiment kindly performed by S. Wu).