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MEETING ABSTRACTS

ORGANOPHOSPHATE HYDROLASE (OPH) DESIGNED AS A TETHERED MONOMER

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Organophosphate hydrolase (OPH) mutants have shown potential use as a medical countermeasure against organophosphorus compounds (OPs). OPH is typically expressed in bacteria as a homodimer. Two separate subunits (35 kDa each) self-assemble through non-covalent bonding at the enzyme face close to the putative active site. OPH homodimers do not secrete expediently from mammalian cells. This causes potential problems when trying to express the protein from a heterologous plasmid or viral delivery system. To enhance secretion of OPH from mammalian cells, we sought to increase protein solubility without catastrophic detriment to activity and without addition of fusion proteins. To this end, we designed OPH to be expressed as a tethered monomer by joining two OPH subunits with a poly-glycine linker. We created the single polypeptide OPH with a tether 10 or 35 amino acids in length between the two halves, and named them T10 and T35 respectively. Western blot analysis and paraoxon hydrolysis assays revealed that T10 was being produced and retained some activity against paraoxon. This was a surprise as we expected T10 to have no enzymatic activity. T35 monomer (75 kDa) was also being produced and retained 71% of specific activity against paraoxon compared to untethered OPH. T10 and T35 showed no significant decrement in activity against the nerve agent sarin. Both constructs showed high molecular weight aggregates greater than 250 kDa in dynamic light scattering and native polyacrylamide gels. These tethered constructs are the first attempts known for producing OPH as a single polypeptide.

Keywords: Organophosphate hydrolase; tether; monomer; sarin

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