REVOLUTIONARY DIAGNOSTICS – NONTRADITIONAL APPROACHES FOR DEVELOPING BREAKTHROUGH CAPABILITIES AGAINST EMERGING THREATS

Solution Nmr Structure Of Dfpase: Prospects For Molecular Level Detection And Large-scale Decontamination Of Organophosphorus Compounds

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The enzyme diisopropyl fluorophosphatase (DFPase) from the squid Loligo vulgaris is a 314 amino acid, 35.0 kDa protein which is able to hydrolyze a wide range of highly toxic organophosphorus compounds, including sarin, soman, and tabun, in addition to the pesticide DFP. The enzyme is highly stable, functional at high temperatures, and is resistant to organic compounds and harsh environments. These properties make it an ideal candidate for a range of applications including large-scale decontamination, but also as a means of sensitive detection of organophosphorus compounds. As part of an ongoing effort to understand the reaction mechanism, and to re-engineer the enzyme for expanded detoxification properties, the solution state NMR structure has been solved, complementing existing X-ray and neutron crystal structures of DFPase. As such, this is one of the most extensively studied enzymes. The NMR structure clearly demonstrates the gene duplication events that give rise to the fold, and also reveals critical spectral regions that can be used as potential diagnostics of substrate binding. In particular, catalytic residue E23 has chemical shifts in an isolated part of the 15N-HSQC spectrum, and other nearby residues have chemical shifts that can be easily tracked as signatures of substrate binding. This group of structures also present opportunities for NMR-based detection of organophosphorus binding, through the exquisite sensitivity of chemical shift information to the immediate environment. In addition, protein-engineering based methods and incorporation of fluorophores as a visual diagnostic is a new avenue for detection of these toxic compounds.