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Abstract

Bio-Incorporation of TePhe, a Tellurium-Containing Phenylalanine Analogue, Preserves Protein Structure and Stability †

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Abstract: The heavy chalcogen tellurium (^{52}Te) is a versatile element with many potential applications in chemical biology and biochemistry, including mass cytometry, fluorescence imaging, and protein structure determination. Using L-tellurienylalanine (TePhe), a mimic of the natural amino acid L-phenylalanine (Phe) in which the phenyl side chain is replaced by a nearly isosteric tellurophene ring, tellurium can be covalently incorporated into the proteome of prokaryotes and eukaryotes by endogenous translation machinery. Our goal is to generate proteins with near stoichiometric levels of Phe to TePhe substitutions, verify preservation of protein structure and activity upon TePhe incorporation, and ultimately exploit the site-specific tellurium centres as handles for crystallographic phasing, protein NMR spectroscopy, and bio-orthogonal reactivity. Here we report conditions for the expression of TePhe containing proteins in a standard *E. coli* expression system and validate the ability of TePhe to act as an effective Phe analogue within a folded protein. Our target for TePhe incorporation is the streptococcal immunoglobulin-binding Protein G B1 domain (GB1), a remarkably heat-stable 56-residue domain containing 2 Phe residues which pack against one another within the domain's hydrophobic core. In Phe-deficient media containing glyphosate as an inhibitor of aromatic amino acid biosynthesis, we obtained a GB1 mixture in which approximately 1 in 2 Phe sites were substituted by TePhe. Fractionation by reverse-phase HPLC allowed us to obtain a sample with 85% TePhe substitution as evidenced by amino acid analysis. Using ^{1}H-^{15}N HSQC and circular dichroism spectroscopy, we find that TePhe effectively takes on the role of Phe, and alters the melting temperature of the protein by less than 5 °C.