Phase V: The Determination of the Active Protein Binding Moiety of EGCG Utilizing Multidimensional NMR Spectroscopy

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The goal of this research is to determine the mechanism by which epigallocatechin-3-gallate (EGCG) and small drug molecules (6-fluoro-4H-1,3-benzodioxine-8-carboxylic acid known as RK038) bind to the glutaredoxin protein from Pseudomonas aeruginosa known as PaGRX. Previous research proved that the EGCG, which is a polyphenol primarily found in green tea, has an anti-cancer effect on breast (MCF7) and cervical (SiHa) cells, but doesn't negatively affect non-cancerous breast epithelial (HMEC) cells. The reason why this occurs is unknown. Plasmids containing the rDNA to express PaGRX were transferred to BL21 (DE3) cells (a strain of E. coli). The PaGRX was 15N-labeled (for use with NMR), extracted from the cells via French Press (lysing), and concentrated. NMR spectra were obtained of the protein sample with 0, 0.5, 1, 1.5, and 3 equivalence points of the RK038 ligand. Other NMR spectra were obtained of the PaGRX with EGCG and its associated moieties. The NMR data shows that the moiety in EGCG that binds to the PaGRX is the gallate portion of the EGCG molecule. The (H,N) chemical shifts for the G65, V53, H70, and A71 amino acids were considered significant because the shifts were 0.05ppm or more. The NMR data sets of the RK038, catechin, and epigallocatechin show no shifting. Because the Rk038 contains a carboxylic acid functional group that didn’t bind, it stands to reason that the acid functional group is not responsible for binding, but the remainder of the gallic acid molecule is the active binding moiety. The green tea polyphenol, epigallocatechin-3-gallate (EGCG) caused the protein to precipitate out of solution, which means that the protein's structure was damaged by the EGCG, and therefore it’s functionality.

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