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The Spectral Properties of (-)-Epigallocatechin 3-O-Gallate (EGCG) Fluorescence in Different Solvents: Dependence on Solvent Polarity

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Abstract

(-)-Epigallocatechin 3-*O*-gallate (EGCG) a molecule found in green tea and known for a plethora of bioactive properties is an inhibitor of heat shock protein 90 (HSP90), a protein of interest as a target for cancer and neuroprotection. Determination of the spectral properties of EGCG fluorescence in environments similar to those of binding sites found in proteins provides an important tool to directly study protein-EGCG interactions. The goal of this study is to examine the spectral properties of EGCG fluorescence in an aqueous buffer (AB) at pH=7.0, acetonitrile (AN) (a polar aprotic solvent), dimethylsulfoxide (DMSO) (a polar aprotic solvent), and ethanol (EtOH) (a polar protic solvent). We demonstrate that EGCG is a highly fluorescent molecule when excited at approximately 275 nm with emission maxima between 350 and 400 nm depending on solvent. Another smaller excitation peak was found when EGCG is excited at approximately 235 nm with maximum emission between 340 and 400 nm. We found that the fluorescence intensity (FI) of EGCG in AB at pH=7.0 is significantly quenched, and that it is about 85 times higher in an aprotic solvent DMSO. The Stokes shifts of EGCG fluorescence were determined by solvent polarity. In addition, while the emission maxima of EGCG fluorescence in AB, DMSO, and EtOH follow the Lippert-Mataga equation, its fluorescence in AN points to non-specific solvent effects on EGCG fluorescence. We conclude that significant solvent-dependent changes in both fluorescence intensity and fluorescence emission shifts can be effectively used to distinguish EGCG in aqueous solutions from EGCG in environments of different polarity, and, thus, can be used to study specific EGCG binding to protein binding sites where the environment is often different from aqueous in terms of polarity.

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Introduction

EGCG (Figure 1A), a major catechin in green tea, exhibits antioxidant [1,2], antimutagenic [3], anticancer [4-6], antiallergic [7,8], and antiatherosclerotic [9,10] properties. EGCG is carried by serum albumin[11] and has been identified as a novel inhibitor of heat shock protein 90 (HSP90)[12], a cytoplasmic chaperone protein, which has recently received much attention as a drug target for treatment of cancer [13,14]. As a chaperone protein, it stabilizes and maintains many client proteins and assists with normal protein folding and trafficking. These functions are essential in cell division and are being widely studied as a target for treatment of cancer[15]. To facilitate studies of the interaction of HSP90 with EGCG and analogs a direct binding assay would be useful and a frequently used very sensitive approach involves fluorescence spectroscopy. It is significantly more efficient to study binding of a ligand to a protein if the ligand is fluorescent using fluorescence polarization[16]. When excited at λ_{Ex}=280 nm, catechin (Figure 1B), one portion of EGCG, has two fluorescence emission maxima, one peak at 314 nm and another peak ranging from 446 nm to 470 nm [17]. Another fragment of EGCG, gallic acid (Figure 1C), when excited at λ_{Ex}=280 nm, has one fluorescence emission maximum ranging from 335 nm to 362 nm depending on the solvent[18]. Since these two fragments of EGCG are fluorescent, it is reasonable to hypothesize that EGCG is also fluorescent, and its fluorescence depends on the solvent. However, this hypothesis requires experimental verification since the two fragments when combined can quench each other's fluorescence. We