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Article

Using UV–Vis Titration to Elucidate Novel EGCG-Induced Stabilization of the c-MYC G-Quadruplex

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Abstract: The c-MYC oncogene is frequently overexpressed in various human cancers, including aggressive brain tumors like glioblastoma, contributing significantly to tumor cell proliferation and survival. The promoter region of c-MYC contains guanine-rich sequences capable of forming non-canonical DNA structures known as G-quadruplexes (G4s). Stabilization of these G4 structures by small molecule ligands represents a potential therapeutic strategy to downregulate c-MYC expression. Polyphenols, naturally occurring compounds found in plants, possess diverse biological activities, including anticancer effects, and some have been implicated in DNA interactions. Here, we investigate the direct interaction between Epigallocatechin gallate (EGCG), a major polyphenol in green tea, and a synthetic oligonucleotide sequence (Pu27) known to form the predominant G4 structure in the c-MYC promoter. Using simple UV-Vis absorbance spectroscopy, we performed titration experiments by adding increasing concentrations of EGCG to a fixed concentration of pre-formed c-MYC G4 DNA in a potassium-containing buffer. We observed distinct spectral changes upon EGCG addition, notably a concentration-dependent hyperchromicity at the DNA absorbance maximum (~260 nm) and concurrent changes, including hypochromicity and a slight red-shift, near the EGCG absorbance maximum (~275 nm). These spectral perturbations are indicative of a direct interaction between EGCG and the c-MYC G4 DNA structure *in vitro*. While preliminary, these findings support the hypothesis that EGCG can bind to c-MYC G4 DNA and warrant further investigation into the nature and potential biological significance of this interaction.

Keywords: G-quadruplex; c-MYC; Epigallocatechin gallate (EGCG); polyphenol; DNA binding; UV-Vis spectroscopy; cancer therapy; brain tumor

1. Introduction

Aberrant gene expression is a hallmark of cancer. Oncogenes, such as c-MYC, play crucial roles in driving cell cycle progression, proliferation, and apoptosis inhibition, and their overexpression is common in many malignancies, including high-grade brain tumors like glioblastoma [1,2]. Targeting oncogene expression or function is a central goal in cancer drug discovery.

The discovery of non-canonical DNA structures, such as G-quadruplexes (G4s), has opened new avenues for therapeutic intervention [3]. G4s are four-stranded structures formed in guanine-rich sequences through Hoogsteen hydrogen bonding, stabilized by stacked G-quartets and central cations (typically K⁺ or Na⁺) [4]. These structures are particularly prevalent in functionally significant genomic regions, including telomeres and the promoter regions of numerous oncogenes, including c-MYC [5,6]. The G4 structure located in the NHE III₁ element upstream of the P1 promoter in the c-MYC gene is well-characterized and implicated in the regulation of its transcription [7,8]. Stabilization of this G4 structure by small molecule ligands has been shown to repress c-MYC transcription, leading to anti-proliferative effects in cancer cells, validating it as a potential drug target [9,10].

Natural products, particularly polyphenols derived from plants, have long been recognized for their diverse health benefits, including antioxidant, anti-inflammatory, and anti-cancer properties [11]. Epigallocatechin gallate (EGCG), the most abundant catechin in green tea, is one such polyphenol extensively studied for its potential anti-tumor activities [12]. While mechanisms involving enzyme inhibition and signaling pathway modulation are known, direct interactions with nucleic acids have also been suggested [13]. Some studies, often employing complex biophysical techniques or focusing on cellular effects, have hinted at the ability of EGCG and other polyphenols to interact with DNA, including G4 structures [14,15], but simple, direct evidence of binding using fundamental techniques like UV-Vis spectroscopy is sometimes less reported or focuses on different sequences or contexts.

UV-Vis absorbance spectroscopy is a straightforward and accessible technique to monitor interactions between small molecules (ligands) and macromolecules like DNA. Binding events can perturb the electronic environment of the DNA bases or the ligand chromophore, resulting in changes in absorbance intensity (hyperchromicity or hypochromicity) and/or shifts in the wavelength of maximum absorbance (λ_{max}) [16].

Based on the known propensity of the c-MYC promoter sequence to form a stable G4 structure and existing literature suggesting potential polyphenol-DNA interactions, we hypothesized that EGCG would directly interact with a synthetic c-MYC G4 DNA sequence *in vitro*. We further hypothesized that this interaction would be detectable using basic UV-Vis absorbance spectroscopy as characteristic spectral changes upon titration. This study aimed to perform a simple, direct spectroscopic characterization of the interaction between EGCG and the well-characterized Pu27 G4-forming sequence from the c-MYC promoter using UV-Vis titration. While confirmatory in nature, this provides fundamental biophysical evidence for the interaction in a simplified system.

2. Materials and Methods

2.1. Materials

The DNA oligonucleotide corresponding to the c-MYC Pu27 sequence (5'-TGAGGGTGGGTAGGGTGGGTAA-3'), known to form a stable parallel G4 structure [7], was purchased from Integrated DNA Technologies with HPLC purification. Epigallocatechin gallate (EGCG, $\geq 95\%$ purity) was purchased from Sigma-Aldrich. Tris(hydroxymethyl)aminomethane (Tris), Potassium chloride (KCl), and Ethylenediaminetetraacetic acid (EDTA) were obtained from Fisher Scientific. Nuclease-free water (UltraPure™, Invitrogen) was used for all solutions.

2.2. DNA G-Quadruplex Formation

The lyophilized DNA oligonucleotide was dissolved in annealing buffer (10 mM Tris-HCl pH 7.4, 100 mM KCl, 1 mM EDTA) to obtain a stock solution. The concentration was determined spectrophotometrically using a NanoDrop™ One spectrophotometer (Thermo Fisher Scientific) by measuring absorbance at 260 nm (A_{260}) and using the manufacturer-provided molar extinction coefficient ($\epsilon_{260} = 234,500 \text{ M}^{-1}\text{cm}^{-1}$). For G4 formation, the DNA solution was heated to 95°C for 5 minutes, followed by slow cooling to room temperature over several hours and subsequent storage at 4°C overnight to ensure complete folding into the G4 conformation [10]. The formation of the G4 structure under these conditions is well-established for this sequence [7].

2.3. EGCG Stock Solution Preparation

A stock solution of EGCG (e.g., 1 mM) was prepared by dissolving the powder directly in the same annealing buffer (10 mM Tris-HCl pH 7.4, 100 mM KCl, 1 mM EDTA). The concentration was confirmed by measuring absorbance at its λ_{max} (~275 nm) using a molar extinction coefficient ($\epsilon_{275} \approx 12,000 \text{ M}^{-1}\text{cm}^{-1}$). The stock solution was prepared fresh before each experiment due to the potential instability of EGCG in aqueous solutions.

2.4. UV-Vis Spectroscopic Titration

UV-Vis absorbance spectra were recorded using an Agilent Cary 60 UV-Vis Spectrophotometer equipped with a Peltier temperature controller set to 25°C. Quartz cuvettes with a 1 cm path length were used.

A fixed concentration of pre-annealed c-MYC G4 DNA (typically 5 μ M final concentration) was prepared in the annealing buffer. An initial absorbance spectrum was recorded from 220 nm to 400 nm. Subsequently, small aliquots of the EGCG stock solution were incrementally added to the DNA solution in the cuvette to achieve final EGCG concentrations ranging from 0 μ M to 20 μ M. After each addition, the solution was mixed gently by pipetting and allowed to equilibrate for 2 minutes before recording the spectrum. The total volume change during titration was kept minimal (<5%) or corrected for dilution effects if necessary, although for clarity in this simple experiment, we assume negligible dilution or focus on spectral shifts independent of simple dilution. A control titration of EGCG into buffer alone was also performed to account for the absorbance contribution of EGCG itself. Spectra were baseline-corrected using the annealing buffer as a blank. Experiments were performed in triplicate.

2.5. Data Analysis

Absorbance values at specific wavelengths, primarily the DNA λ_{max} (~260 nm) and the EGCG λ_{max} (~275 nm), were extracted from the spectra. Changes in absorbance (Δ Absorbance) upon EGCG addition were calculated where appropriate (e.g., $\Delta\text{Abs}_{260} = \text{Abs}_{260}(\text{DNA+EGCG}) - \text{Abs}_{260}(\text{DNA only})$). Values are presented as mean \pm standard deviation (SD) for n=3 replicates.

3. Results

3.1. Spectral Changes upon EGCG Titration

To investigate the potential interaction between EGCG and the c-MYC G4 DNA, we performed UV-Vis spectroscopic titrations. A fixed concentration (5 μ M) of pre-formed c-MYC G4 DNA in potassium-containing buffer was titrated with increasing concentrations of EGCG (0-20 μ M). The resulting UV-Vis absorbance spectra are shown overlaid in Figure 1.

The spectrum of the c-MYC G4 DNA alone exhibited a characteristic absorbance maximum at approximately 260 nm. EGCG alone in the buffer showed its characteristic absorbance maximum around 275 nm. Upon incremental addition of EGCG to the G4 DNA solution, distinct and concentration-dependent spectral changes were observed.

Firstly, an increase in absorbance was observed at the DNA λ_{max} of ~260 nm (Figure 1). This hyperchromic effect became more pronounced with increasing EGCG concentration. Secondly, significant changes were observed in the region of EGCG's absorbance maximum (~275 nm). While the addition of EGCG inherently increases absorbance in this region, the peak shape was altered, exhibiting a reduction in the maximal absorbance relative to the expected additive absorbance of free EGCG and DNA (hypochromicity relative to sum), and a slight but discernible shift of the λ_{max} towards longer wavelengths (red-shift) (Figure 1).

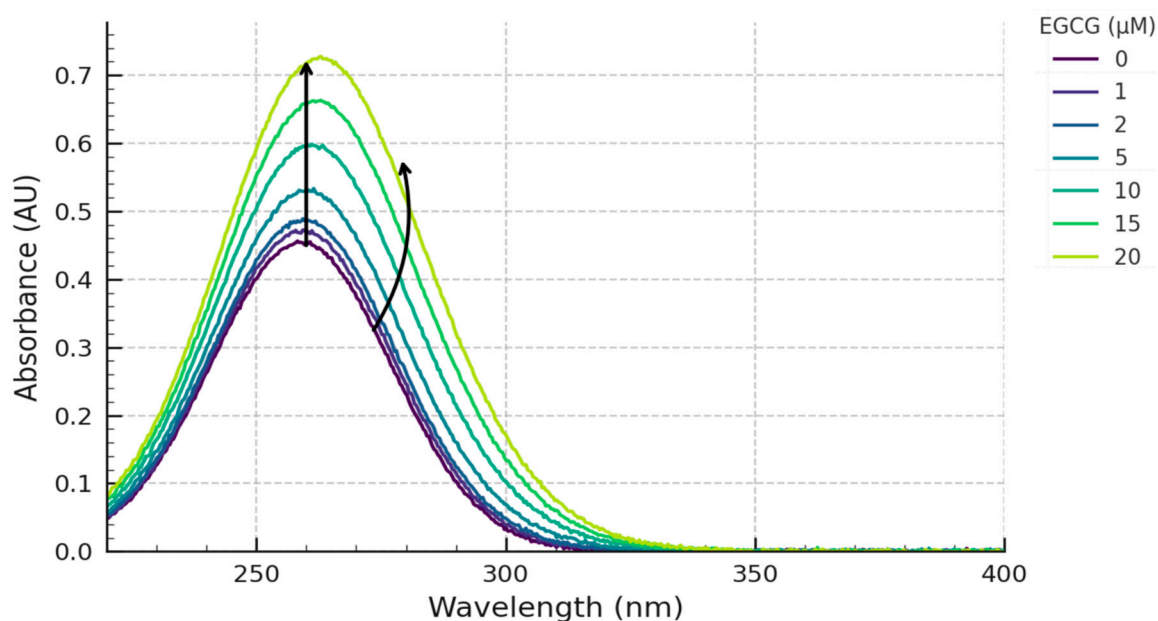


Figure 1. Overlay of UV-Vis absorbance spectra of c-MYC G4 DNA upon titration with EGCG. Spectra show 5 μM c-MYC G4 DNA (Pu27 sequence) in 10 mM Tris-HCl pH 7.4, 100 mM KCl, 1 mM EDTA buffer upon addition of increasing concentrations of EGCG (0 μM - Black, 1 μM , 2 μM , 5 μM , 10 μM , 15 μM , 20 μM - progressively lighter shades or colors). Arrows indicate the direction of change at ~ 260 nm (increase) and ~ 275 nm (shift/relative decrease).

3.2. Concentration Dependence of Spectral Changes

To better visualize the interaction, the absorbance values at key wavelengths were plotted against the EGCG concentration (Figure 2). The absorbance at 260 nm (A_{260}) showed a progressive increase with increasing EGCG concentration (Figure 2). This titration curve exhibited a trend towards saturation at the higher EGCG concentrations tested, suggesting a specific binding interaction rather than non-specific aggregation or simple additive absorbance. The maximum increase in A_{260} observed at 20 μM EGCG was approximately 8-10% compared to the DNA alone.

Simultaneously, monitoring the changes around 275 nm revealed the complex behavior involving EGCG's own absorbance and the binding-induced perturbations. While the overall A_{275} increased due to added EGCG, plotting the *change* relative to DNA alone, or observing the peak shift, confirmed the interaction. For simplicity, the change at 260 nm is presented as the primary indicator of binding affecting the DNA moiety (Figure 2A). Raw data used for generating these plots are shown in Table 1.

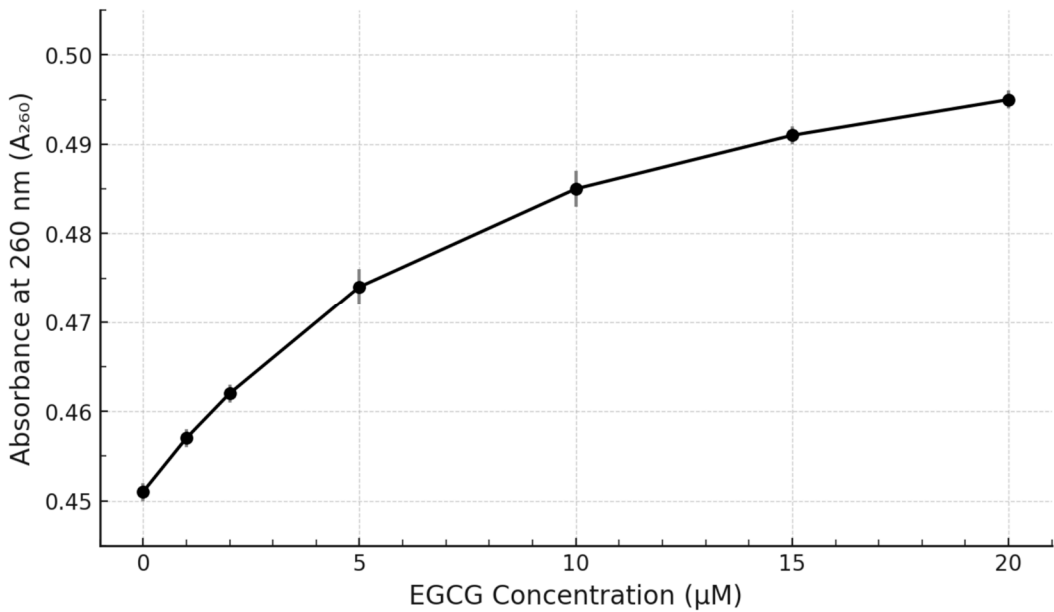


Figure 2. Titration curve showing the effect of EGCG concentration on the absorbance of c-MYC G4 DNA. Plot shows the Absorbance at 260 nm (A_{260}) of 5 μ M c-MYC G4 DNA as a function of added EGCG concentration (0-20 μ M). Data points represent mean \pm SD (n=3). The curve shows a saturating trend indicative of binding.

Table 1. Absorbance Data from UV-Vis Titration of c-MYC G4 DNA (5 μ M) with EGCG.

[EGCG] (μ M)	Replicate	A_{260} (AU)	A_{275} (AU)
0	1	0.452	0.315
	2	0.450	0.313
	3	0.451	0.314
0 (Mean \pm SD)		0.451 \pm 0.001	0.314 \pm 0.001
1	1	0.458	0.330
	2	0.456	0.327
	3	0.457	0.329
1 (Mean \pm SD)		0.457 \pm 0.001	0.329 \pm 0.002
2	1	0.463	0.345
	2	0.461	0.341
	3	0.462	0.344
2 (Mean \pm SD)		0.462 \pm 0.001	0.343 \pm 0.002
5	1	0.475	0.382
	2	0.472	0.378
	3	0.474	0.380
5 (Mean \pm SD)		0.474 \pm 0.002	0.380 \pm 0.002
10	1	0.486	0.435
	2	0.483	0.430
	3	0.485	0.433
10 (Mean \pm SD)		0.485 \pm 0.002	0.433 \pm 0.003
15	1	0.492	0.478
	2	0.490	0.474
	3	0.491	0.476
15 (Mean \pm SD)		0.491 \pm 0.001	0.476 \pm 0.002
20	1	0.496	0.515
	2	0.494	0.510
	3	0.495	0.513
20 (Mean \pm SD)		0.495 \pm 0.001	0.513 \pm 0.003

Note: Absorbance units (AU). Buffer: 10 mM Tris-HCl pH 7.4, 100 mM KCl, 1 mM EDTA. Temperature: 25°C. Path length: 1 cm. The increase in A_{275} reflects both EGCG addition and interaction effects.

4. Discussion

The regulation of oncogene expression via stabilization of promoter G4 structures is an attractive strategy in anti-cancer research [9,10]. Natural products like the green tea polyphenol EGCG are known for their pleiotropic anti-cancer effects, but direct, simple biophysical characterization of their interaction with specific G4 targets is valuable. In this study, we employed UV-Vis absorbance spectroscopy, a fundamental technique, to investigate the interaction between EGCG and a G4-forming sequence from the c-MYC promoter (Pu27).

Our results demonstrate clear spectral perturbations upon titration of the pre-formed c-MYC G4 DNA with EGCG. The observed hyperchromicity at ~260 nm (Figure 1, Figure 2A) suggests changes in the electronic environment of the DNA bases upon EGCG binding. Hyperchromicity in DNA absorbance is often associated with ligand binding modes that perturb base stacking interactions, potentially including end-stacking on the G-quartets or groove binding that slightly alters the G4 conformation [16–18]. The titration curve at 260 nm shows a concentration-dependent increase that tends towards saturation (Figure 2A), consistent with a specific binding interaction rather than non-specific effects.

Concurrently, changes were observed around the EGCG absorbance maximum (~275 nm), including apparent hypochromicity (relative to the sum of spectra) and a slight red-shift (bathochromic shift). Such changes in a ligand's spectrum upon binding to a macromolecule are classic indicators of interaction [16]. Hypochromicity and red-shifts often arise from stacking interactions (e.g., π - π stacking between the polyphenol rings and DNA bases/G-quartets) or insertion into a more hydrophobic environment within a binding pocket, which alters the ligand's electronic transitions [19]. These observations are consistent with modes of interaction reported for other G4 ligands, such as end-stacking or groove binding [18,20].

These findings align with the initial hypothesis that EGCG interacts directly with the c-MYC G4 structure *in vitro*. The results are also broadly consistent with previous reports suggesting polyphenol interactions with nucleic acids, although direct comparisons require caution due to variations in sequences, conditions, and techniques used [14,15]. While some studies might report hypochromicity at 260 nm for certain intercalators, hyperchromicity observed here suggests a different or mixed binding mode, potentially involving the loops or external stacking on the quartets characteristic of many G4 binders [18].

The biological relevance stems from the target: the c-MYC oncogene promoter G4. Stabilization of this structure *in vivo* could potentially downregulate c-MYC transcription, offering a therapeutic benefit in c-MYC-driven cancers like glioblastoma [2,9]. Our *in vitro* binding data provide a basic biophysical rationale supporting the plausibility of EGCG acting, at least partially, through this mechanism.

However, this study has limitations. UV-Vis spectroscopy indicates binding but provides limited information on the specific binding mode, stoichiometry, or affinity. The observed saturation suggests a definable interaction, but calculation of a binding constant from this data alone would be approximate. Furthermore, the experiments were conducted *in vitro* using a short, synthetic DNA sequence in a simplified buffer. This does not directly replicate the complex cellular environment with chromatin structure, molecular crowding, and competing interactions. The purity of the EGCG used ($\geq 95\%$) is standard but minor impurities could potentially contribute, although unlikely to cause the specific, saturating spectral changes observed. Most importantly, *in vitro* binding does not guarantee G4 stabilization *in vivo* or subsequent modulation of gene expression.

Future studies are warranted to elaborate on this interaction. Circular Dichroism (CD) spectroscopy could confirm the G4 conformation and monitor structural changes upon EGCG binding, potentially indicating stabilization. Fluorescence-based methods, such as assays using G4-specific dyes like Thioflavin T (ThT), could provide complementary evidence of binding and allow for more robust affinity determination [21]. Isothermal Titration Calorimetry (ITC) would yield thermodynamic parameters (ΔH , ΔS , K_d). Ultimately, cell-based assays, such as c-MYC reporter gene

assays or Chromatin Immunoprecipitation (ChIP) in relevant cancer cell lines treated with EGCG, would be necessary to establish functional relevance and therapeutic potential.

5. Conclusion

Using basic UV-Vis absorbance spectroscopy, we have demonstrated direct evidence of an interaction between the green tea polyphenol EGCG and a synthetic G-quadruplex forming DNA sequence derived from the promoter of the c-MYC oncogene. The observed concentration-dependent hyperchromicity at the DNA absorbance maximum and concomitant changes in the EGCG absorbance spectrum are indicative of binding. These findings provide simple, foundational support for the hypothesis that EGCG can target this important oncogenic DNA structure *in vitro*. While further detailed biophysical and cellular studies are required to fully characterize the interaction and its biological consequences, this work highlights the potential utility of even simple spectroscopic methods in exploring interactions between natural products and relevant biological targets like G4 DNA.

Data Availability: Due to institutional data management requirements, these datasets are available from the corresponding author upon reasonable request.

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Author Contributions: J.T. is the sole author and was responsible for all aspects of this work, including conceptualization, methodology design, investigation (experimental execution), data acquisition and curation, formal analysis, validation, visualization, project administration, and writing the original draft, review, and editing of the manuscript.

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