The Impact of the C-Terminal Domain on the Interaction of Human DNA Topoisomerase II α and β with DNA

Kathryn L. Gilroy*, Caroline A. Austin*

Institute for Cell and Molecular Biosciences, University of Newcastle Upon Tyne, Newcastle upon Tyne, United Kingdom

Abstract

**Background:** Type II DNA topoisomerases are essential, ubiquitous enzymes that act to relieve topological problems arising in DNA from normal cellular activity. Their mechanism of action involves the ATP-dependent transport of one DNA duplex through a transient break in a second DNA duplex; metal ions are essential for strand passage. Humans have two isoforms, topoisomerase IIα and topoisomerase IIβ, that have distinct roles in the cell. The C-terminal domain has been linked to isoform specific differences in activity and DNA interaction.

**Methodology/Principal Findings:** We have investigated the role of the C-terminal domain in the binding of human topoisomerase IIα and topoisomerase IIβ to DNA in fluorescence anisotropy assays using full length and C-terminally truncated enzymes. We find that the C-terminal domain of topoisomerase IIβ but not topoisomerase IIα affects the binding of the enzyme to the DNA. The presence of metal ions has no effect on DNA binding. Additionally, we have examined strand passage of the full length and truncated enzymes in the presence of a number of supporting metal ions and find that there is no difference in relative decatenation between isoforms. We find that calcium and manganese, in addition to magnesium, can support strand passage by the human topoisomerase II enzymes.

**Conclusions/Significance:** The C-terminal domain of topoisomerase IIβ, but not that of topoisomerase IIα, alters the enzyme’s $K_d$ for DNA binding. This is consistent with previous data and may be related to the differential modes of action of the two isoforms in vivo. We also show strand passage with different supporting metal ions for human topoisomerase IIα or topoisomerase IIβ, either full length or C-terminally truncated. They all show the same preferences, whereby Mg $>$ Ca $>$ Mn.

Introduction

Type II DNA topoisomerases are ubiquitous enzymes that are essential for cellular survival, acting to relieve torsional stress arising in DNA from normal cellular activity such as replication and transcription. Their mechanism of action involves the ATP-dependent transport of one DNA duplex through a transient break in a second DNA duplex. Humans have two isoforms, topoisomerase IIα and topoisomerase IIβ, which are encoded on different chromosomes and have distinct cellular roles. Human topoisomerase IIα is required for chromosome segregation, while topoisomerase IIβ plays a key role in the regulation of transcription [1-6] and is crucial for the late stages of neuronal development [7]. Topoisomerase IIα and IIβ are 60% identical in amino acid sequence and share a similar domain structure that is comparable to other type II enzymes. They have an N-terminal ATPase domain, a central core domain housing the active site, consisting of a pair of semicircular grooves on the B' and A' subdomains that are thought to bind to ‘G’ segment, has been reported following footprinting experiments and structural work [9-10]. The cleavage reaction is carried out via an active site tyrosine residue located in the core of the enzyme, with the cleaved ‘G’ segment interacting with a helix turn helix (HTH) motif in the CAP domain of the core [11]. In particular, the α4 ‘recognition

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* E-mail: caroline.austin@ncl.ac.uk

¤ Current address: Integrative and Systems Biology, Faculty of Biomedical and Life Sciences, University of Glasgow, Glasgow, United Kingdom
helix’ has been shown to interact with substrate DNA [12]. A recent crystal structure of S. cerevisiae topoisomerase II with DNA showed that on binding the ‘G’ segment the enzyme induces a 150° bend in the DNA and undergoes large conformational changes such that an essential metal ion is positioned near to the reactive tyrosine ready for cleavage [13]. Additionally, a second site has been proposed in the N-terminal half of the enzyme, which could potentially interact with the ‘T’ segment, following the discovery of a groove lined with arginines in the crystal structure of E. coli GyrB [14].

Metal ions, in particular divalent cations, are essential in the mechanism of topoisomerase II. Magnesium is required for stabilising the interaction with ATP in the N-terminal domain. The phosphodiester cleavage reaction by a tyrosine in the enzyme core also requires divalent metal ions and thus metal ions are required to support strand passage. Experiments with eukaryotic D. melanogaster topoisomerase II reported that only Mg²⁺ could support strand passage, in contrast to subsequent work with E. coli gyrase which showed that Mg²⁺, Ca²⁺, Mn²⁺ and Co²⁺ could all support activity [15–16], thus the interaction with metal ions differs in topoisomerase II isoforms from different species.

Three very conserved motifs (EDGSA, IMTDQ and PLRGK) are important for type II topoisomerase function [17]. Altered magnesium optima were seen in proteins mutated within these motifs in human topoisomerase IIβ and two possible binding sites were proposed for the metal ion(s) needed for DNA cleavage [18]. Subsequently, a two metal ion mechanism analogous to DNA polymerases was proposed for the cleavage reaction [15,18–19]. A recent crystal structure of the central breakage reunion domain from yeast with DNA in a cleavage competent conformation has two metal ions present, but these are zinc not magnesium [20]. In contrast other crystal structures of core domains of type II topoisomerases show only one metal ion [21–22]. Work with human topoisomerase IIα and human topoisomerase IIβ indicated that interactions with the second metal ion may enhance the ability of topoisomerase IIβ, but not topoisomerase IIα to cleave DNA [23]. Whether one or two ions are needed for cleavage, and which ions these are, requires further study. In addition to a catalytic role, metal ions have also been reported to play a structural role in type II topoisomerase [24–25].

Eukaryotic type II topoisomerases have been shown, in strand passage experiments, to prefer supercoiled substrates over relaxed ones, with selectivity for crossovers, both experimentally via electron microscopy experiments, and in silico. Such selectivity may represent a mechanism for topological recognition [26–28].

Relaxation of supercoiled plasmid DNA by topoisomerases occurs via a strand passage reaction that can produce a series of topoisomers, with differing levels of superhelicity. This pattern may be either processive, in which case the protein stays on the same DNA molecule to relax it further, or distributive, where the enzyme moves onto another supercoiled DNA molecule after one or more rounds of relaxation. Previously, it was reported that topoisomerase IIα gave a more distributive pattern in relaxation assays, while topoisomerase IIβ gave a highly processive pattern [29], consistent with subsequent work showing that topoisomerase IIα and topoisomerase IIβ interact differently with supercoiled substrates. Topoisomerase IIα had a 10-fold preference for positive over negative supercoils whereas topoisomerase IIβ showed no preference [30]. Experiments with C-terminal domain swapped proteins suggested that this geometry sensing was linked to the C-terminal domain of the enzymes [31]. Consistent with this, experiments show topoisomerase II from Paramecium bursaria chlorella virus (PBCV) or chlorella virus Marburg-1 (CVM-1), whose N-terminal and core domains have high homology to eukaryotic topoisomerase II but which naturally lack the C-terminal domain, have no preference for positive or negative supercoils in relaxation [32]. We have previously shown that the C-terminal domain of human topoisomerase IIα and topoisomerase IIβ affects levels of strand passage in vitro as well as levels of cell growth in complementation analysis, and suggested that the topoisomerase IIβ C-terminal domain may act as a negative regulator [33].

In this study we have examined the role of the C-terminal domain in the topoisomerase II-DNA interaction using fluorescence anisotropy. We have also examined the role of divalent metal ions in strand passage activity and in DNA binding. We find that the strength of interaction of human topoisomerase IIβ, but not topoisomerase IIα with DNA is significantly increased in the absence of the C-terminal domain, suggesting that the C-terminal domain of topoisomerase IIβ acts as a negative regulator. Additionally, we find that the supporting metal ion has no impact on the binding of DNA. The strand passage reaction with both human topoisomerase II enzymes can be supported by...
Proteins were dialysed into 50 mM Tris pH 8, 10% glycerol, we reported previously [33]. For anisotropy experiments, YEphTOP2
CATCGTCATCCTCGCGACGCG-3
[35,38], using 400 ng kDNA. Reactions were performed in CCGAGGATGACGATGAGCGCATTGTCAGATTGCG-3.
Protein Preparation

Proteins were overexpressed and purified from Saccharomyces strain JEL1Atop1 as described previously [34–35]. Full length topoisomerase IIα was expressed from plasmid YEphTOP2βKLM [37]. Truncated topoisomerase IIα and truncated topoisomerase IIβ were expressed from plasmids YEphTOP2α (1242) and YEphTOP2β (1263) respectively, as we reported previously [33]. For anisotropy experiments, proteins were dialysed into 50 mM Tris pH 8, 10% glycerol, 1 mM EDTA, 1 mM EGTA, 150 mM KCl with protease inhibitors.

In vitro activity assays

Decatenation assays were performed as described previously [35,38], using 400 ng kDNA. Reactions were performed in ‘relaxation buffer’ (50 mM Tris-HCl pH 7.5, 10 mM MgCl2, 0.5 mM EDTA, 30 μg/mL bovine serum albumin (BSA), 1 mM DTT, 100 mM KCl). To assess the impact of different metals, 10 mM MgCl2 was replaced by 10 mM CaCl2, 10 mM MnCl2, 10 mM NiCl2 or 10 mM CoSO4 as appropriate. In all cases reaction products were quantified using TINA version 2.09d densitometry software. The ratio of decatenated DNA to the total DNA was measured in each case and compared to a control lane. Statistical significance was assessed using a one-sample t-test measuring variance from 100%, with p<0.05 considered significant.

DNA-binding measurements using fluorescence anisotropy

DNA binding capacity was determined with purified protein and a hexachlorofluorescein (HEX) labelled 40 bp double stranded DNA oligo using fluorescence anisotropy. Measurements were carried out at 20°C using a SLM-Amnicco 1000 spectrofluorometer (SLM-Amnicco, Urbana, IL). The excitation wavelength was 530 nm with an excitation slit width of 8 nm and the emission wavelength was 570 nm with an emission slit width of 3 nm. A 1 mL fluorescence cuvette was used with excitation and emission paths each of 10 mm. Assays were carried out in anisotropy buffer (50 mM Tris pH 8, 5% glycerol, 50 mM KCl, 1% Triton X-100) supplemented with 100 μg/mL acetylated BSA, and topoisomerase II proteins were matched for buffers and salt concentration. 1 μM HEX-labelled oligo was added to the buffer and a baseline reading taken. Protein was added and an average anisotropy of 12 readings over 99 seconds measured for each titration point. 10 mM MgCl2 or 10 mM CaCl2 was added to the buffer where described. A one-binding site hyperbola was fitted to data and the Bmax and Kp measured for each titration point. 10 mM MgCl2 was replaced by 10 mM CaCl2, 10 mM MnCl2, 10 mM NiCl2 or 10 mM CoSO4 as appropriate. The two binding curves plateaus indicating that the maximum anisotropy or maximum binding (Bmax) has been reached. Differences in Bmax indicate a difference in binding mode, although specific mechanistic details cannot be inferred from this data. The Kp is the concentration of ligand required to give half maximal binding. An example plot is shown in Figure 2a. Measurements were determined as described in Materials and Methods.

Four different proteins were used, full length topoisomerase IIα, full length topoisomerase IIβ, and each isoform without its C-terminal domain, referred to as truncated topoisomerase IIα and truncated topoisomerase IIβ. A minimum of three independent experiments was done for each enzyme variant; for each protein concentration 12 readings were taken over 99 seconds and averaged. The average anisotropy readings were plotted versus protein concentration, and the resultant curves analysed to give dissociation constants (Kp) and maximum anisotropy indicating the maximal binding (Bmax). The measured values are shown in Table 1 and Figure 2.

The Kp of full length topoisomerase IIβ was found to 72.9 nM which while lower than that of full length topoisomerase IIα at 189 nM (implying stronger binding), is not a significant difference, with a p value of 0.0542 by Student’s t-test. There was no significant difference in binding strength between the full length and truncated versions of topoisomerase IIα. The difference in Kp between truncated topoisomerase IIβ and the other isoforms is more striking, with truncated topoisomerase IIβ having a Kp of 18 nM, showing more than 6 times stronger binding than truncated topoisomerase IIα which has a Kp of 116 nM (p<0.0001). Additionally, truncated topoisomerase IIβ gave significantly stronger binding than the full length version, with a p value of 0.0189 by Student’s t-test. Our unpublished data with 3 other 40 bp oligonucleotides and all four proteins confirmed that truncated topoisomerase IIβ bound most strongly.

No significant difference was seen in maximal binding for the full length topoisomerase IIα, the full length topoisomerase IIβ or the C-terminally truncated topoisomerase IIα, their Bmax values being 0.213, 0.268 and 0.229 respectively. However the Bmax of the truncated topoisomerase IIβ at 0.173 was significantly lower than either the full length topoisomerase IIβ (p = 0.0002) or the truncated topoisomerase IIα (p = 0.0045), suggesting that the lack of the topoisomerase II C-terminal domain significantly alters the
maximal binding of this protein. While it is not possible to ascertain specific mechanistic details from this data, the lower Bmax of truncated topoisomerase II\textsubscript{b} indicates that this enzyme has a different mode of binding to the DNA substrate, in addition to the stronger binding shown by lower K\textsubscript{D}, when compared with the other isoforms.

![Figure 2](image-url)

**Figure 2. Binding of full length and C-terminally truncated topoII\textsubscript{a} and topoII\textsubscript{b} to a 40 bp DNA oligo.** (A) Typical anisotropy curve, measuring topoisomerase II\textsubscript{b} binding to oligo AB. (B) K\textsubscript{D} of binding (in nM). (C) Maximum anisotropy (Bmax). The average of at least three independent experiments is shown with error bars representing standard error from the mean. Statistical significance is indicated with * representing p<0.05, ** representing p<0.01 and *** representing p<0.001. doi:10.1371/journal.pone.0014693.g002

### Table 1. Binding of full length and C-terminally truncated topoII\textsubscript{a} and topoII\textsubscript{b} to a 40 bp DNA oligo, showing binding affinity (K\textsubscript{D}) and maximum anisotropy (Bmax).

<table>
<thead>
<tr>
<th>Topoisomerase</th>
<th>K\textsubscript{D} (nM)</th>
<th>Bmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full length topoII\textsubscript{a}</td>
<td>189±57.4</td>
<td>0.2156±0.034</td>
</tr>
<tr>
<td>Full length topoII\textsubscript{b}</td>
<td>56.3±14.8</td>
<td>0.2579±0.013</td>
</tr>
<tr>
<td>Truncated topoII\textsubscript{a}</td>
<td>116.3±3.48</td>
<td>0.2292±0.0016</td>
</tr>
<tr>
<td>Truncated topoII\textsubscript{b}</td>
<td>17.8±2.72</td>
<td>0.1726±0.0069</td>
</tr>
</tbody>
</table>

The average of at least three independent experiments is shown with standard error from the mean indicated.

doi:10.1371/journal.pone.0014693.t001

**Strand Passage of human topoisomerase II with different metals**

Previous work with *E. coli* gyrase has shown that Ca\textsuperscript{2+}, Mn\textsuperscript{2+} and Co\textsuperscript{2+}, in addition to Mg\textsuperscript{2+}, can support the strand passage reaction [15], in contrast to *D. Melanogaster* topoisomerase II where only Mg\textsuperscript{2+} could support strand passage [16], indicating that there may be species specific differences in this regard. Strand passage by full length and C-terminally truncated topoisomerase II\textsubscript{a} and topoisomerase II\textsubscript{b} was investigated using Mg\textsuperscript{2+}, Ca\textsuperscript{2+}, Mn\textsuperscript{2+}, Co\textsuperscript{2+} and Ni\textsuperscript{2+} as potential supporting ions. For all four proteins the degree of strand passage varied with supporting ion. Figure 3 and Table 2 show the average decatenation of full length and truncated isoforms in the presence of magnesium, calcium or manganese. In each case decatenation with magnesium was set to 100% and that with calcium and manganese is expressed relative to this. In all cases the preference of ions for strand passage was similar; greatest decatenation was found with magnesium, then calcium, and then manganese, an order that is consistent with the results reported with *E. coli* gyrase [15]. Nickel and cobalt supported no detectable strand passage under the conditions used here.

The significance of the degree of activity relative to that with magnesium was assessed using a one-sample t-test measuring
Figure 3. Relative decatenation of full length and C-terminally truncated topolII or topolIIb with different supporting metal ions. Mg – Magnesium, Ca – Calcium, Mn – Manganese. For each protein, strand passage with magnesium ions was taken as 100% and other values calculated relative to this. The average of at least two independent experiments is shown, with error bars representing standard error from the mean. Statistical significance is indicated with * representing p<0.05 and *** representing p<0.001.
doi:10.1371/journal.pone.0014693.g003

Table 2. Relative decatenation of full length or C-terminally truncated topolII or topolIIb with magnesium (Mg\(^{2+}\)), Calcium (Ca\(^{2+}\)) or Manganese (Mn\(^{2+}\)) as supporting ion.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Mg(^{2+})</th>
<th>Ca(^{2+})</th>
<th>Mn(^{2+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topoisomerase IIa</td>
<td>100</td>
<td>46.12±27.9</td>
<td>13.91±13.91</td>
</tr>
<tr>
<td>Topoisomerase IIb</td>
<td>100</td>
<td>46.59±40.02</td>
<td>13.59±5.16</td>
</tr>
<tr>
<td>Truncated topoisomerase IIb</td>
<td>100</td>
<td>40.07±15.25</td>
<td>3.41±2.08</td>
</tr>
<tr>
<td>Truncated topoisomerase IIb</td>
<td>100</td>
<td>24.9±9.05</td>
<td>16.14±16.14</td>
</tr>
</tbody>
</table>

For each protein, strand passage with magnesium ions was taken as 100% and other values calculated relative to this. The average of at least two independent experiments is shown, with standard error from the mean indicated.
doi:10.1371/journal.pone.0014693.t002

Table 3. The \(K_D\) of binding of topolIIa or topolIIb (in nM) to DNA with no metal, 10 mM MgCl\(_2\), or 10 mM CaCl\(_2\).

<table>
<thead>
<tr>
<th></th>
<th>(K_D) TopolIIa (nM)</th>
<th>(K_D) TopolIIb (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No metal</td>
<td>160.5±49.66</td>
<td>46.35±18.09</td>
</tr>
<tr>
<td>10 mM MgCl(_2)</td>
<td>150.3±65.01</td>
<td>55.39±10.7</td>
</tr>
<tr>
<td>10 mM CaCl(_2)</td>
<td>177.7±90.89</td>
<td>84.33±19.45</td>
</tr>
</tbody>
</table>

The average of at least two independent experiments is shown with standard error of the mean indicated.
doi:10.1371/journal.pone.0014693.t003

DNA binding to 40 bp oligo in the presence of metal ions
The level of strand passage by topoisomerase IIa and topoisomerase IIb varies significantly with supporting metal ion (above). To determine whether this was due to differences in interaction strength with DNA, the interaction of human topoisomerase IIa or topoisomerase IIb with the 40 bp DNA oligo described above, in the absence of ions as well as the presence of magnesium or calcium ions, was assessed.

Data are shown in Figure 4 and Table 3. The DNA binding affinity of neither topoisomerase IIa nor topoisomerase IIb is significantly altered by the presence of magnesium or calcium, as seen previously by gel mobility shift assay [39]. As above, in all cases the topoisomerase IIa protein binds to the oligo less strongly than the topoisomerase IIb protein. In the absence of metal ions, topoisomerase IIa binds with an affinity of 160.5±49.7 nM, while topoisomerase IIb binds with an affinity of 46.4±18.1 nM, a difference that is statistically significant (p=0.0276). In the presence of magnesium ions topoisomerase IIa binds with an affinity of 55.4±10.7 nM, and topoisomerase IIb with an affinity of 55.4±10.7 nM, a difference that is also statistically significant (p=0.0401). While topoisomerase IIa binds less strongly, again, than topoisomerase IIb in the presence of calcium ions, with \(K_D\) values of 177.7±90.9 and 84.3±19.5 respectively (p=0.2867), the difference did not reach significance with 3 replicates.

Discussion
We have used fluorescence anisotropy to investigate the DNA interaction between the two human topoisomerase II isoforms, and the impact of the C-terminal domain on this interaction. Firstly, the relative binding affinities of topoisomerase IIa and IIb, variance from 100%. Manganese supported significantly less decatenation than that of magnesium when topoisomerase IIb (p = 0.0379), truncated topoisomerase IIa (p = 0.0005) and truncated topoisomerase IIb (p = 0.0351) were considered. Considering calcium, only truncated topoisomerase IIb gave significantly less decatenation than with magnesium (p = 0.0142). There was no significant difference in the relative response to metals by isoform or upon removing the C-terminal domain.

Figure 4. The \(K_D\) of binding to oligo AB (in nM) of full length topolII and topolIIb, in the absence of metal or the presence of magnesium (Mg) or calcium (Ca) ions. The average of at least two independent experiments is shown, with error bars representing standard error from the mean.
doi:10.1371/journal.pone.0014693.g004

Topoisomerase II

No metal 160.5±49.66 46.35±18.09
10 mM MgCl\(_2\) 150.3±65.01 55.39±10.7
10 mM CaCl\(_2\) 177.7±90.89 84.33±19.45

The average of at least two independent experiments is shown with standard error of the mean indicated.
doi:10.1371/journal.pone.0014693.t003
both full length and C-terminally truncated, were assessed using a 40 bp oligo with an mAMSA cleavage site that has been used previously in DNA binding studies [35,39–40]. The $K_D$ of binding determined by fluorescence anisotropy was comparable to that found by gel mobility shift analysis on the same oligonucleotide substrate (130 nM) [39], but differed from that found by surface plasmon resonance (SPR) (1–73 nM) [40]. SPR differs from the first two assays in that the DNA substrate is fixed a one end, so reducing its ability to diffuse away from the protein, which may account for the difference in $K_D$ seen.

Topoisomerase IIβ, whether full length or C-terminally truncated, gave stronger binding than its topoisomerase IIα counterpart, suggesting topoisomerase IIβ binds DNA with higher affinity, consistent with isoform specific DNA interactions [29–30]. The anisotropy data is most striking when the truncated topoisomerase IIβ isoform is considered, this having significantly lower maximum anisotropy than the other three isoforms. When three other oligonucleotides were tested truncated topoisomerase IIβ had the strongest binding of all four enzymes (unpublished data). The significantly stronger binding of topoisomerase IIβ once the C terminal domain is removed supports the idea that the C-terminal domain may act as a negative regulator of DNA interaction, and that this could provide a rationale for isoform specific functions in vivo [33].

Metal ions are needed in the strand passage reaction both to coordinate ATP for hydrolysis and to polarise the active site tyrosine before the cleavage reaction. Work reported previously indicated that $D. Melanogaster$ topoisomerase IIβ could only perform strand passage in the presence of magnesium ions [16], in contrast to work with bacterial gyrase which showed that magnesium, calcium, manganese and cobalt ions could all support strand passage [15]. We show that magnesium, calcium and manganese ions can all support the strand passage reaction in human topoisomerase IIα, to varying degrees, in an order consistent with previous work [15]. We found no evidence that cobalt ions could support the strand passage reaction of human topoisomerase IIβ, in contrast to an earlier report [41]. The reason for this difference is unknown, but could be related to the metal salt forms used – Baldwin et al. use the chloride salt form, whereas the sulphate form was used in this case. Interestingly cobalt, but not magnesium, calcium or manganese, supported cleavage of the topoisomerase IIβ enzyme using a metal affinity cleavage assay based on Fenton chemistry [42] (unpublished data). No cobalt was found to be associated with either topoisomerase IIα or IIβ when an elemental profile was established using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), although interestingly zinc was found to be consistently associated with both isoforms using this technique (unpublished data).

The strength of interaction of topoisomerase IIα or topoisomerase IIβ with DNA wasn’t affected by the presence of magnesium or calcium ions, indicating that the difference in the level of strand passage supported by different ions may be due to differences in their ability to support the phosphohydrolysis-transfer reaction of cleavage, rather than differences in DNA substrate interaction. The presence or absence of the C-terminal domain of either isoform had no effect on the relative levels of strand passage with each protein, indicating that the C-terminal domain is not involved in the co-ordination of metal ions for strand passage. This is consistent with the proposed location of the metal ion binding sites in the B’ region of the core of the enzyme [15,18–19], and recent topoisomerase II-DNA structures [20–22,24].

In conclusion, we have shown that the C-terminal domain of topoisomerase IIβ, but not topoisomerase IIα, has a significant effect on the $K_D$ with DNA, providing further evidence that this region of topoisomerase IIβ may have a negative regulatory role [33].

Author Contributions
Conceived and designed the experiments: KLG CAA. Performed the experiments: KLG. Analyzed the data: KLG CAA. Contributed reagents/materials/analysis tools: KLG CAA. Wrote the paper: KLG.

References


