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Dietary Polyphenols as Topoisomerase II Poisons: B Ring and C Ring Substituents Determine the Mechanism of Enzyme-Mediated DNA Cleavage Enhancement

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Abstract

Dietary polyphenols are a diverse and complex group of compounds that are linked to human health. Many of their effects have been attributed to the ability to poison (i.e., enhance DNA cleavage by) topoisomerase II. Polyphenols act against the enzyme by at least two different mechanisms. Some compounds are traditional, redox-independent topoisomerase II poisons, interacting with the enzyme in a noncovalent manner. Conversely, others enhance DNA cleavage in a redox-dependent manner that requires covalent adduction to topoisomerase II. Unfortunately, the structural elements that dictate the mechanism by which polyphenols poison topoisomerase II have not been identified. To resolve this issue, the activities of two classes of polyphenols against human topoisomerase II α were examined. The first class was a catechin series, including (–)-epigallocatechin gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG), and (–)-epicatechin (EC). The second was a flavonol series, including myricetin, quercetin, and kaempferol. Compounds were categorized into four distinct groups: EGCG and EGC were redox-dependent topoisomerase II poisons, kaempferol and quercetin were traditional poisons, myricetin utilized both mechanisms, and ECG and EC displayed no significant activity. Based on these findings, a set of rules is proposed that predicts the mechanism of bioflavonoid action against topoisomerase II. The first rule centers on the B ring. While the C4'-OH is critical for the compound to act as a traditional poison, the addition of –OH groups at C3' and C5' increases the redox activity of the B ring and allows the compound to act as a redox-dependent poison. The second rule centers on the C ring. The structure of the C ring in the flavonols is aromatic, planar, and includes a C4-keto group that allows the formation of a proposed pseudo ring with the C5-OH. Disruption of these elements abrogates enzyme binding and precludes the ability to function as a traditional topoisomerase II poison.

Introduction

Dietary polyphenols (i.e., bioflavonoids) are a diverse and complex group of compounds that are found in a variety of fruits, vegetables, and plant leaves (1-6). It is believed that the consumption of bioflavonoids provides a number of health benefits to adults, including protection against cancer and cardiovascular disease (1-10). Despite these beneficial effects, the ingestion of dietary polyphenols during pregnancy has been linked to the development of specific types of infant leukemia that feature aberrations in the mixed lineage leukemia gene (*MLL*) at chromosomal band 11q23 (11-15).

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Green tea, which is one of the most commonly consumed beverages in the world, is a rich source of polyphenols (16-19). The most abundant bioflavonoids in green tea are catechins, primarily (–)-epigallocatechin gallate (EGCG)¹ and related compounds (16-19). In addition, flavonols and other classes of bioflavonoids also are present (19,20).

Because dietary polyphenols affect a number of cellular processes (16,21-26), the mechanistic basis for their physiological actions is not well-defined. However, several bioflavonoids are potent topoisomerase II poisons (14,27-31), and many of their cellular effects have been attributed, at least in part, to their actions against the type II enzymes (14,15,28,32-34).

Type II topoisomerases are ubiquitous enzymes that alter DNA under- and overwinding and remove knots and tangles from the genome (35-40). Vertebrates encode two closely related isoforms of the enzyme, topoisomerase II α and II β (37,38,40-45). Topoisomerase II α is essential for the survival of actively growing tissues (46-48) and is required for proper DNA replication and chromosome segregation (43,45). Topoisomerase II β is dispensable at the cellular level, but is required during development (49,50). To date, its physiological functions have not been well defined (44,51,52).

To maintain genomic integrity during DNA strand passage, type II topoisomerases form a covalent bond with the 5'-termini of the cleaved nucleic acid (53-55). This covalent enzyme-cleaved DNA intermediate is known as the cleavage complex. Despite the essential nature of topoisomerase II, conditions that increase the concentration of cleavage complexes generate permanent breaks in the genetic material (38,40,56-58). If these strand breaks overwhelm the cell, they induce death pathways (57).

Agents that increase topoisomerase II-mediated DNA cleavage are called topoisomerase II poisons (38,40,59-62). A number of widely prescribed and highly successful anticancer drugs target the type II enzyme (38,40,60,63-66). However, topoisomerase II-active agents also have been associated with the development of leukemias that involve the *MLL* gene (58,67-70).

Other than DNA lesions (71-75), topoisomerase II poisons can be categorized into two broad classes. Members of the first group act by a "traditional," redox-independent mechanism. These compounds interact with topoisomerase II at the protein-DNA interface (in the vicinity of the active site tyrosine) in a non-covalent manner (38,40,60-62). Redox-independent topoisomerase II poisons include etoposide (76), as well as several other anticancer drugs. Because the actions of these compounds against topoisomerase II do not depend on redox chemistry, they are unaffected by reducing agents (76). In addition, these compounds induce similar levels of enzyme-mediated DNA scission whether they are added to the binary topoisomerase II-DNA complex or are incubated with the enzyme prior to the addition of the nucleic acid substrate (76).

Topoisomerase II poisons in the second class act in a redox-dependent manner (40,76-82) and form covalent adducts with the enzyme at amino acid residues distal to the active site (79). The best-characterized members of this group are quinones, such as 1,4-benzoquinone and polychlorinated biphenyl (PCB) metabolites (76-81). Because the actions of these compounds depend on redox chemistry, their ability to enhance topoisomerase II-mediated DNA cleavage is abrogated by the presence of reducing agents such as DTT (76,79,83,84). Furthermore, redox-dependent poisons increase DNA cleavage when they are added to the enzyme-DNA complex, but inhibit topoisomerase II activity when incubated with the protein prior to the addition of DNA (31,76,79,83,84).

¹Abbreviations: EGCG, (–)-epigallocatechin gallate; EGC, (–)-epigallocatechin; ECG, (–)-epicatechin gallate; EC, (–)-epicatechin; DTT, dithiothreitol.

Because many bioflavonoids are capable of undergoing redox chemistry (including complex oxidation reactions) (16,21,85-89), their mechanism of action against topoisomerase II, *a priori*, is not obvious. For example, while genistein (an isoflavone) acts exclusively as a traditional topoisomerase II poison (30), EGCG (a catechin) poisons the enzyme in a redox-dependent manner (31).

Due to the high consumption of dietary polyphenols and proposed relationships between their effects on human health and the ability to enhance topoisomerase II-mediated DNA cleavage, it is important to understand the mechanism by which they poison the type II enzyme. Therefore, the present study was undertaken to define the structural elements in bioflavonoids that control the mechanistic basis for their actions against topoisomerase II. A further goal was to establish rules that have the potential to predict whether a given bioflavonoid acts as a traditional (redox-independent) or redox-dependent topoisomerase II poison.

Results strongly suggest that the ability of bioflavonoids to act as redox-dependent poisons depends on the multiplicity of –OH groups on the B ring. Furthermore, specific C ring characteristics are required for these compounds to bind topoisomerase II at the enzyme-DNA interface and to act as traditional poisons. However, they do not affect the ability to function as redox-dependent poisons.

Experimental Procedures

Enzymes and Materials

Recombinant wild-type human topoisomerase II α was expressed in *Saccharomyces cerevisiae* and purified as described previously (90-92). Negatively supercoiled pBR322 DNA was prepared from *Escherichia coli* using a Plasmid Mega Kit (Qiagen) as described by the manufacturer. (–)-Epigallocatechin gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG), (–)-epicatechin (EC), myricetin, quercetin, and kaempferol were purchased from LKT. 1,4-Benzoquinone and etoposide were obtained from Sigma. All compounds were prepared as 20 mM stock solutions in 100% DMSO and stored at –20 °C.

DNA Cleavage Mediated by Human Topoisomerase II α

DNA cleavage reactions were performed using the procedure of Fortune and Osheroff (93). Assay mixtures contained 220 nM human topoisomerase II α , 5 nM negatively supercoiled pBR322 DNA, and 0–500 μ M EGCG, EGC, ECG, or EC in 20 μ L of DNA cleavage buffer [10 mM Tris-HCl, pH 7.9, 5 mM MgCl₂, 100 mM KCl, 0.1 mM EDTA, and 2.5% (v/v) glycerol]. DNA cleavage mixtures were incubated for 6 min at 37 °C. In some cases, 0–10 min time courses for DNA cleavage were monitored with 100 μ M myricetin, quercetin, or kaempferol. Enzyme-DNA cleavage intermediates were trapped by adding 2 μ L of 5% SDS followed by 1 μ L of 375 mM EDTA, pH 8.0. Proteinase K was added (2 μ L of a 0.8 mg/mL solution), and reaction mixtures were incubated for 30 min at 45 °C to digest topoisomerase II. Samples were mixed with 2 μ L of 60% sucrose in 10 mM Tris-HCl, pH 7.9, 0.5% bromophenol blue, and 0.5% xylene cyanol FF, heated for 2 min at 45 °C, and subjected to electrophoresis in 1% agarose gels in 40 mM Tris-acetate, pH 8.3, and 2 mM EDTA containing 0.5 μ g/mL ethidium bromide. DNA cleavage was monitored by the conversion of negatively supercoiled plasmid DNA to linear molecules. DNA bands were visualized by ultraviolet light and quantified using an Alpha Innotech digital imaging system.

To examine the effects of a reducing agent on the actions of catechins against topoisomerase II α , 500 μ M EGCG or EGC (or 25 μ M 1,4-benzoquinone or 50 μ M etoposide as controls) were incubated with 1 mM DTT for 5 min prior to their addition to DNA cleavage reactions.

Alternatively, DTT was added to reaction mixtures for 5 min following a 6 min DNA cleavage reaction.

To examine the effects of a reducing agent on the actions of flavonols against topoisomerase II α , 100 μ M myricetin, quercetin, or kaempferol were incubated in the absence or presence of 1 mM DTT for 5 min prior to initiating DNA cleavage reactions. Reactions were monitored for 0–20 min.

To examine the effects of flavonols on topoisomerase II α in the absence of DNA, 100 μ M myricetin, quercetin, or kaempferol was incubated with 220 nM enzyme for 0–15 min at 37 °C in 15 μ L of DNA cleavage buffer. Cleavage was initiated by adding 5 nM negatively supercoiled pBR322 DNA (in 5 μ L of cleavage buffer) to the reaction mixture. In some cases, flavonols (100 μ M) were treated with 1 mM DTT for 5 min prior to their incubation with topoisomerase II α .

To determine the ability of EC to compete with quercetin for the type II enzyme, DNA cleavage reactions containing 220 nM topoisomerase II α and 5 nM negatively supercoiled pBR322 DNA were performed in the presence of 100 μ M quercetin and 0–1000 μ M EC. Competition was determined by the loss of quercetin-induced DNA scission.

Results

Mechanism of Green Tea Catechins as Topoisomerase II Poisons

Catechins are the most abundant class of biologically active polyphenols in green tea (brewed from the leaves of *Camellia sinensis*) (16,21). EGCG represents the major catechin (~40–60% of total polyphenols), followed by EGC and ECG (~15–20% each) and EC (~5%) (23,94). Structures of these compounds are shown in Figure 1.

A recent study demonstrated that EGCG poisons human type II topoisomerases in a redox-dependent manner (31). Based on the analysis of oxidation products, it was suggested that the redox activity of EGCG is centered primarily in the B ring (16,21,85–89). However, earlier studies implied that the gallate ring (D ring) also has the potential to undergo redox chemistry (95–97). Therefore, the effects of three related catechins, EGC, ECG, and EC, on the DNA cleavage activity of human topoisomerase II α were compared to those of EGCG. EGC is identical to EGCG, except it lacks the D ring. ECG is identical to EGCG, except that it contains two –OH groups rather than three on its B ring. Finally, EC lacks both the D ring and the third –OH group on its B ring.

As seen in Figure 2, EGC enhanced DNA cleavage mediated by topoisomerase II α nearly as well as EGCG. This finding suggests that the D ring makes only a small contribution to the activity of the parent compound against the type II enzyme. It is notable that the concentration of EGCG in plasma and salivary samples is estimated to be as high as 4 and 48 μ M, respectively, following the consumption of ~3 cups of green tea (22,98). Significant topoisomerase II-DNA cleavage enhancement was observed in this range. Unfortunately, comparable cellular data are not available for EGC.

In contrast to EGC, ECG and EC displayed little ability to enhance DNA cleavage mediated by human topoisomerase II α (Figure 2). This result provides strong evidence that the third –OH moiety on the B ring is critical to the activity of these catechins against the type II enzyme. Once again, the presence of the D ring (compare ECG to EC) contributed little to the activity against topoisomerase II α .

Since EGCG acts as a redox-dependent topoisomerase II poison, experiments were performed to determine whether EGC functions in a similar manner. Consequently, the effects of the reducing agent, DTT, on the activity of EGC were determined (Figure 3, left panel). Prior to the addition of the catechin or other agents to the topoisomerase II α -DNA complex, compounds were incubated with 1 mM DTT for 5 min. While this treatment had no significant effect on the enzyme alone or on the actions of etoposide (a traditional poison), it markedly decreased the ability of EGC to enhance DNA cleavage. Levels of scission dropped >90% as compared to reactions in the absence of the reducing agent. Similar results were seen for the redox-dependent topoisomerase II poisons, 1,4-benzoquinone and EGCG. These data provide strong evidence that EGC acts in a redox-dependent manner.

A common feature of redox-dependent topoisomerase II poisons is that they form covalent adducts with the enzyme (76-81). Since the maintenance of this covalent interaction is independent of the redox state of the poison, DTT has no effect on DNA cleavage after adducts are formed (76,79,83,84). Similar to 1,4-benzoquinone and EGCG, once cleavage complexes were formed in the presence of EGC, DTT did not diminish the efficacy of the catechin (Figure 3, right panel). This finding suggests that EGC acts by forming covalent adducts with topoisomerase II α .

Finally, as found for other redox-dependent topoisomerase II poisons (76,79,83,84,99), EGC inactivated enzyme function when it was incubated with the protein prior to the addition of DNA (not shown). Taken together, these data indicate that EGC enhances DNA cleavage mediated by human topoisomerase II α in a redox-dependent manner similar to that of EGCG.

Hydroxyl Groups on the B Ring Determine the Mechanism by which Flavonols Poison Topoisomerase II α

A number of bioflavonoids, including flavonols, flavones, and isoflavones, have been shown to act as topoisomerase II poisons (14,27-31). However, genistein is the only flavonoid whose mechanism of action against human topoisomerase II has been characterized in detail (30). This compound, which has only a single -OH group on its B ring, was shown to poison topoisomerase II α and β in a redox-independent manner (30). On the basis of this finding, it was assumed that flavonols, flavones, and isoflavones all function by a similar redox-independent mechanism. However, the fact that many bioflavonoids contain multiple -OH groups on their B rings suggests that some of these compounds may have a redox-dependent component to their mechanism of action against topoisomerase II.

Therefore, to address this possibility, the mechanistic basis for the actions of three closely related flavonols, myricetin, quercetin, and kaempferol, against human topoisomerase II α was determined. These compounds are identical except that they contain three, two, or one -OH groups on their B rings, respectively (Figure 4). Furthermore, they share common A and B ring elements with the catechins discussed above (see Figure 1).

Consistent with earlier reports (28-30), myricetin, quercetin, and kaempferol all enhanced DNA scission mediated by topoisomerase II α (Figure 5). However, the cleavage time course for myricetin differed significantly from those of the other two flavonols. Typical of assays that include redox-independent poisons, topoisomerase II α established rapid (≤ 15 s) DNA cleavage-ligation equilibria in reactions that contained 100 μ M quercetin or kaempferol. In contrast, levels of DNA cleavage increased at a much slower rate in reactions that contained 100 μ M myricetin. In fact, scission was still increasing at 10 min. As shown previously for EGCG (31), this slow enhancement of DNA cleavage is suggestive of a redox-dependent topoisomerase II poison with low reactivity towards the protein.

Thus, to further investigate the mechanism of action of myricetin, a longer DNA cleavage time course was performed in the absence or presence of 1 mM DTT (Figure 6). Similar levels of DNA cleavage (~3-fold enhancement) were observed for the first ~3 min of both reactions. However, starting at this point, the two time courses diverged. While cleavage complexes continued to accumulate up to 20 min in the absence of DTT (additional cleavage was not observed at longer times), they plateaued in the presence of the reducing agent. The resulting DNA cleavage enhancement at 20 min was ~7- and ~3-fold in the absence and presence of DTT, respectively. These data suggest that myricetin has both redox-dependent and redox-independent components to its mechanism of action against topoisomerase II α . While the flavonol appears to act primarily as a traditional topoisomerase II poison early in the time course, the slower redox-dependent mechanism dominates with time.

Different results were observed with quercetin and kaempferol (Figure 6, right panel). Levels of DNA scission mediated by topoisomerase II α in the presence of the two compounds were unaffected by 1 mM DTT, and remained high following a 20 min cleavage reaction. This finding provides strong evidence that quercetin and kaempferol poison topoisomerase II α in a redox-independent manner.

To further characterize the mechanistic basis for the actions of flavonols against human topoisomerase II α , myricetin, quercetin, and kaempferol were incubated with the protein prior to the addition of DNA (Figure 7, left panel). As predicted for a redox-independent poison, kaempferol did not inhibit enzyme activity. In contrast, myricetin inactivated topoisomerase II α within 15 min. Results with quercetin were intermediate to those of the other two flavonols. This result was unexpected based on the DNA cleavage results in Figure 6 (right panel). However, quercetin is a strong antioxidant and is known to undergo redox chemistry in vitro (95). Therefore, while the compound may have some redox-dependent inhibitory effects on the activity of topoisomerase II α , its ability to poison the enzyme appears to utilize the traditional, redox-independent mechanism exclusively.

Finally, to determine whether the ability of quercetin and myricetin to inhibit the human enzyme requires redox chemistry, the flavonols were incubated with 1 mM DTT before their addition to topoisomerase II α . As seen in Figure 7 (right panel), the reducing agent reversed the inhibitory effects of quercetin and myricetin. This result confirms that the inhibition of enzyme activity prior to the addition of DNA results from a redox-dependent process.

Structure of the C Ring Precludes Catechins from Acting as Traditional Topoisomerase II Poisons

Although quercetin and EC differ solely in their C rings, only the flavonol enhances DNA cleavage mediated by human topoisomerase II α . Since quercetin and EC have identical A and B rings, the dramatic difference between the ability of the two compounds to increase DNA scission must be related to their C rings. Therefore, it is proposed that EC is unable to act as a traditional poison because the structure of its C ring precludes binding to the noncovalent drug interaction domain on topoisomerase II α . To this point, the aromatic C ring of quercetin is planar, and the C4-keto group has been reported to form a pseudo ring with the C5-OH moiety of the A ring (100). Both of these attributes have been proposed to contribute to the binding of bioflavonoids to topoisomerase II (30,100). In contrast, the non-aromatic C ring of EC is nonplanar, and the catechin lacks the C4 ketone necessary to establish the pseudo ring.

To test the above hypothesis, the ability of EC to displace quercetin (a traditional poison) from topoisomerase II α was determined. A DNA cleavage assay that included 100 μ M quercetin was utilized to monitor the competition. As seen in Figure 8, no inhibition of quercetin-enhanced DNA cleavage by the human enzyme was observed at EC concentrations as high as 1 mM. This finding supports the hypothesis that the structure of the catechin C ring prevents

it from binding to the drug interaction domain on topoisomerase II and hence does not allow it to act as a traditional poison.

Discussion

Dietary polyphenols are a diverse and complex group of compounds. A variety of health-promoting and leukemogenic properties have been attributed to them, and they display a multifaceted array of cellular activities (1-15). Although specific links between polyphenol function and human health are widely debated, some of the cytotoxic, genotoxic, and leukemogenic effects of these bioflavonoids appear to be related to their ability to poison topoisomerase II (14,15,28,32-34).

Even against this singular enzyme target, the activities of polyphenols have been difficult to understand. For example, the isoflavone, genistein, acts as a traditional, redox-independent topoisomerase II poison, and interacts with the enzyme in a noncovalent manner (30). In marked contrast, the catechin, EGCG, enhances enzyme-mediated DNA cleavage in a redox-dependent manner that requires covalent adduction to topoisomerase II (31). Despite the myriad of available compounds, the structural elements that dictate the mechanism by which polyphenols poison topoisomerase II have not been identified.

In order to resolve this fundamental issue, the activities of two classes of polyphenols against human topoisomerase II α were examined. The first class was a series of catechins that included EGCG, EGC, ECG, and EC. The second was a series of flavonols that included myricetin, quercetin, and kaempferol. Compounds were categorized into four distinct groups: EGCG and EGC were redox-dependent topoisomerase II poisons, kaempferol and quercetin were traditional poisons, myricetin utilized both mechanisms, and ECG and EC displayed no significant activity against the human type II enzyme.

Based on these results, a set of rules is proposed that predicts the mechanism of bioflavonoid action against the type II enzyme. These rules are consistent with all data published to date, and are shown in Figure 9.

The first rule relates the B ring to bioflavonoid mechanism and states that the number of –OH groups on the ring determines the potential for a bioflavonoid to act against topoisomerase II in a redox-independent or -dependent manner. This rule has two postulates. 1) Assuming that the B ring has a phenolic structure, the C4'–OH is critical for the compound to act as a traditional, redox-independent, topoisomerase II poison (28-30). 2) The addition of –OH groups at both the C3' and C5' positions (presumably other positions also are possible) increases the redox activity of the B ring (86,87,95,97) and allows the compound to act as a redox-dependent topoisomerase II poison. This explains why genistein, kaempferol, and quercetin act as traditional poisons, EGCG and EGC act in a redox-dependent manner, and myricetin is able to employ both mechanisms.

The second rule relates the C ring to bioflavonoid mechanism and states that structural elements associated with this ring determine the ability of polyphenols to bind to the drug interaction domain (used by traditional poisons) on topoisomerase II. The C ring in the flavonols is aromatic, planar, and includes the C4-keto group that allows the formation of a proposed pseudo ring with the C5–OH (100). The rule postulates that disruption of these properties [by the loss of the C2–C3 double bond or the C4-keto group in the catechins, or by the loss of the 5–OH group (30)] abrogates binding to human topoisomerase II α . Because EGCG and EGC contain the catechin C ring, they are unable to act as traditional topoisomerase II poisons and function exclusively as redox-dependent poisons.² Furthermore, since ECG and EC lack the requisite third –OH group on their B rings that would allow them to function as redox-dependent poisons, they show virtually no activity against topoisomerase II α .

In summary, polyphenols are an important class of dietary compounds that include a number of topoisomerase II poisons. Despite the impact of polyphenols on human health, the mechanistic basis for their actions against the type II enzyme has been poorly understood. The present study establishes a set of rules that for the first time relate the individual structural elements in catechins and bioflavonoids to the mechanism by which they enhance topoisomerase II-mediated DNA cleavage.

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²The potential effects of the gallate D ring on topoisomerase II binding are not known. However, since EGC and EC lack the gallate ring, the presence of the catechin C ring in itself precludes enzyme binding.

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(-)-Epigallocatechin Gallate (EGCG)

(-)-Epicatechin Gallate (ECG)

(-)-Epigallocatechin (EGC)

(-)-Epicatechin (EC)

Figure 1.
Structure of EGCG and related catechins.

Figure 2.

Effects of catechin derivatives on double-stranded DNA cleavage mediated by human topoisomerase II α . Cleavage reactions were performed in the presence of 0–500 μ M EGCG (closed circles), EGC (open circles), ECG (closed squares), or EC; (open squares) are shown. Levels of cleavage are relative to those in the absence of compounds (set to 1.0). Error bars represent standard deviations for three independent experiments.

Figure 3.

Effects of DTT on the ability of EGC to enhance DNA cleavage mediated by human topoisomerase II α . DNA cleavage was performed in the absence of compound (hTII α) or in the presence of 500 μ M EGC, 500 μ M EGCG, 25 μ M 1,4-benzoquinone (BQ), or 50 μ M etoposide (Etop). Left panel: Compounds were incubated in the absence (–DTT; closed bars) or presence (+DTT; open bars) of 1 mM DTT for 5 min prior (i.e., Pre) to their addition to the topoisomerase II-DNA mixture. Right panel: Compounds were incubated in the absence (–DTT; closed bars) or presence (+DTT; open bars) of 1 mM DTT for 5 min after (i.e., Post) the formation of topoisomerase II-DNA cleavage complexes. Error bars represent standard deviations for three independent experiments.

Myricetin **Quercetin** **Kaempferol**

Figure 4.
Structure of myricetin and related flavonols.

Figure 5.

Time dependence of flavonol-induced stimulation of topoisomerase II-mediated DNA cleavage when incubated with the enzyme-DNA complex. Data for DNA cleavage mediated by topoisomerase II α in the presence of 100 μ M myricetin (closed circles), quercetin (open circles), or kaempferol (closed squares) are shown. Levels of cleavage are relative to those in the absence of compounds (set to 1.0). Error bars represent standard deviations for three independent experiments.

Figure 6.

Effects of DTT on the ability of flavonols to enhance DNA cleavage mediated by human topoisomerase II α . Left panel: Myricetin (100 μ M) was incubated in the absence (-DTT; closed circle) or presence (+DTT; open circles) of 1 mM DTT for 5 min prior to its addition to topoisomerase II-DNA mixtures. A 20-min time course for myricetin-induced DNA cleavage is shown. Right panel: Myricetin (M), quercetin (Q), or kaempferol (K) (100 μ M) was incubated in the absence (-DTT; closed bars) or presence (+DTT; open bars) of 1 mM DTT for 5 min prior to its addition to topoisomerase II-DNA mixtures. DNA cleavage was quantified after 20 min. Control reactions contained DNA and human topoisomerase II α in the absence of compounds (hTII α). Error bars represent standard deviations for three independent experiments.

Figure 7.

Flavonol-induced inhibition of topoisomerase II-mediated DNA cleavage when incubated with the enzyme prior to the addition of DNA. Left panel: Human topoisomerase II α was treated with 100 μ M myricetin (closed circles), quercetin (open circles), or kaempferol (closed squares) for 0–15 min prior to the addition of DNA to reaction mixtures. Levels of cleavage were relative to those when compounds were added to the enzyme-DNA mixture (set to 100%). Right panel: Myricetin (M), quercetin (Q), or kaempferol (K) (100 μ M) was incubated with 1 mM DTT for 5 min prior to its addition to topoisomerase II. DNA was added, and cleavage was quantified after 15 min. Error bars represent standard deviations for three independent experiments.

Figure 8.

Ability of EC to compete with quercetin for human topoisomerase II α . Effects of EC on the ability of quercetin to enhance enzyme-mediated DNA cleavage are shown. DNA cleavage reactions were performed in the presence of 100 μ M quercetin and 0–1000 μ M EC. Competition was quantified by the loss of quercetin-induced linear DNA molecules. Levels of cleavage are relative to those in the absence of compounds (set to 1.0). Control reactions contained 1 mM EC in the absence of quercetin (EC Only). Error bars represent standard deviations for three independent experiments.

Figure 9.

Rules for polyphenols as topoisomerase II poisons. Myricetin is used as the model compound. Structural features required for actions as a traditional, redox-independent topoisomerase II poison are highlighted in yellow. Structural features required for actions as a redox-dependent topoisomerase II poison are highlighted in blue. Details of the rules are described in the text.