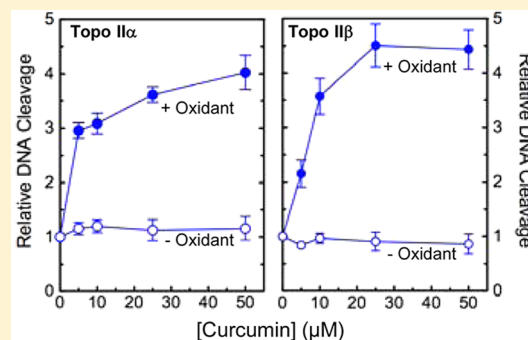


# Oxidative Metabolites of Curcumin Poison Human Type II Topoisomerases

Adam C. Ketron,<sup>†,||</sup> Odaine N. Gordon,<sup>||,‡</sup> Claus Schneider,<sup>\*,||,‡</sup> and Neil Osheroff<sup>\*,†,||,§</sup>

Departments of <sup>†</sup>Biochemistry, <sup>‡</sup>Pharmacology, and <sup>§</sup>Medicine (Hematology/Oncology) and <sup>||</sup>Vanderbilt Institute of Chemical Biology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, United States

**ABSTRACT:** The polyphenol curcumin is the principal flavor and color component of the spice turmeric. Beyond its culinary uses, curcumin is believed to positively impact human health and displays antioxidant, anti-inflammatory, antibacterial, and chemopreventive properties. It also is in clinical trials as an anticancer agent. In aqueous solution at physiological pH, curcumin undergoes spontaneous autoxidation that is enhanced by oxidizing agents. The reaction proceeds through a series of quinone methide and other reactive intermediates to form a final dioxxygenated bicyclopentadione product. Several naturally occurring polyphenols that can form quinones have been shown to act as topoisomerase II poisons (i.e., they increase levels of topoisomerase II-mediated DNA cleavage). Because several of these compounds have chemopreventive properties, we determined the effects of curcumin, its oxidative metabolites, and structurally related degradation products (vanillin, ferulic acid, and feruloylmethane) on the DNA cleavage activities of human topoisomerase II $\alpha$  and II $\beta$ . Intermediates in the curcumin oxidation pathway increased the level of DNA scission mediated by both enzymes  $\sim$ 4–5-fold. In contrast, curcumin and the bicyclopentadione, as well as vanillin, ferulic acid, and feruloylmethane, had no effect on DNA cleavage. As found for other quinone-based compounds, curcumin oxidation intermediates acted as redox-dependent (as opposed to interfacial) topoisomerase II poisons. Finally, under conditions that promote oxidation, the dietary spice turmeric enhanced topoisomerase II-mediated DNA cleavage. Thus, even within the more complex spice formulation, oxidized curcumin intermediates appear to function as topoisomerase II poisons.



**T**urmeric is a common spice that is used in curries and a variety of other Asian cuisines.<sup>1–3</sup> It is isolated from the rhizomes of *Curcuma longa*, which is an herbaceous perennial of the ginger family.<sup>3</sup>

Curcumin (Figure 1) is the principal flavor and color component of turmeric. Beyond its culinary uses, curcumin is believed to positively impact human health and commonly is used in traditional Chinese herbal medicine and Ayurvedic medicine.<sup>2,3</sup> The compound has antioxidant, anti-inflammatory, and antibacterial activities.<sup>4,5</sup> Furthermore, it appears to have chemopreventive properties against a variety of human malignancies and currently is in clinical trials as an anticancer agent.<sup>4–9</sup>

Curcumin has poor oral bioavailability and is unstable under physiological conditions.<sup>10–14</sup> Thus, it has been suggested that curcumin metabolites mediate at least some of the biological effects of the parent compound.<sup>15–18</sup> Several metabolic pathways have been proposed. Following oral administration, curcumin often is conjugated to form glucuronides or sulfates. Alternatively, if administered intraperitoneally, it can undergo reductive reactions to form a variety of hydrogenated curcuminoids. However, most studies have concluded that neither the conjugated nor the reduced products of curcumin are biologically active.<sup>18</sup>

Curcumin also can undergo spontaneous autoxidation in aqueous solutions at physiological pH (Figure 1).<sup>14</sup> This

reaction gives rise to novel products that have potential for biological activity. Autoxidation of curcumin is stimulated by peroxidases and oxidizing agents and is initiated by the abstraction of hydrogen from one of the two phenolic hydroxyl moieties.<sup>14</sup> Following this abstraction, the reaction is proposed to proceed through several unstable and reactive intermediates, including an electrophilic quinone methide radical.<sup>14</sup> The final product of curcumin autoxidation is a dioxxygenated bicyclopentadione.<sup>14</sup>

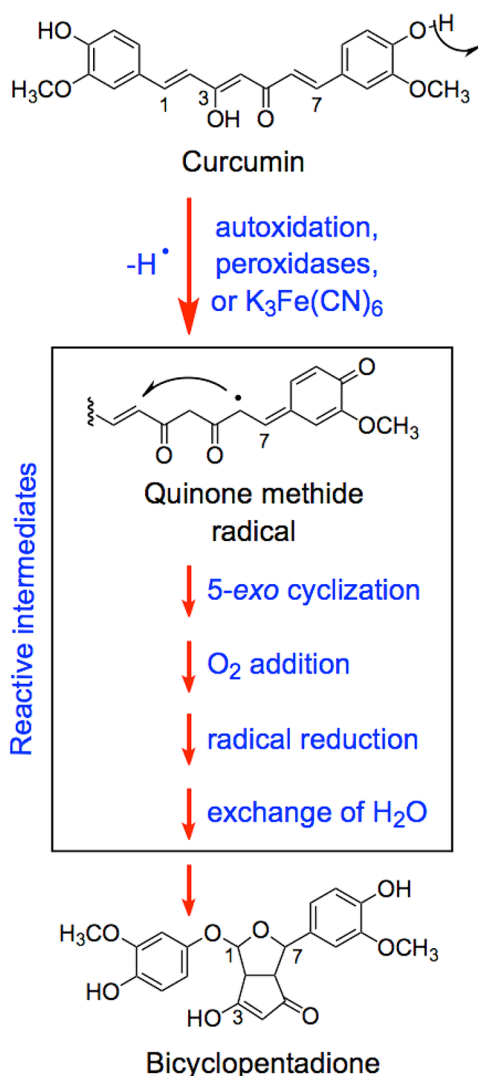
In addition to oxidation, the heptadienone chain of curcumin can be fragmented into vanillin, ferulic acid, feruloylmethane, and related compounds (see Figure 8).<sup>18</sup> These fragmentation products have been shown to display antioxidant and anti-inflammatory properties.<sup>18</sup> However, at physiological pH, degradation of the curcumin heptadienone chain appears to be a minor reaction.<sup>19</sup>

Several naturally occurring polyphenols that can form quinones display activity against human type II topoisomerases.<sup>20</sup> Many of these, including bioflavonoids such as myricetin (which is common in grapes, berries, and other fruits and vegetables) and catechols such as (–)-epigallocatechin gallate (EGCG, which is the active polyphenol in green tea),<sup>21–23</sup> are believed to have chemopreventive proper-

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**Figure 1.** Oxidative transformation of curcumin. Adapted from ref 14.

ties.<sup>24–26</sup> All of these compounds increase levels of DNA cleavage mediated by type II topoisomerases.<sup>21–23</sup>

Type II topoisomerases are enzymes that modulate DNA under- and overwinding and remove knots and tangles from chromosomes.<sup>20,27–30</sup> They function by passing an intact double helix through a transient break that they generate in another segment of DNA.<sup>20,27–30</sup> To maintain genomic integrity during the double-stranded DNA passage event, type II topoisomerases form a covalent intermediate between active site tyrosine residues and the newly generated DNA termini.<sup>20,27–30</sup> This requisite intermediate is known as the “cleavage complex”.<sup>20</sup> A number of widely used anticancer drugs, such as etoposide and doxorubicin, function by stabilizing cleavage complexes.<sup>20,27,29–31</sup> Thus, these “topoisomerase II poisons” convert type II topoisomerases into toxic enzymes that generate DNA strand breaks in treated cells.<sup>20,27,29–31</sup> Human cells express two closely related topoisomerase II isoforms,  $\alpha$  and  $\beta$ .<sup>20,27–30</sup> Both appear to be targets for anticancer drugs.<sup>20,27,29–31</sup>

Treatment of human cells with curcumin induces DNA cleavage complexes formed by topoisomerase II $\alpha$  and II $\beta$ .<sup>32</sup> The formation of the cleavage complex is prevented by the addition of an antioxidant, suggesting the importance of

oxidative pathways in curcumin activity against the type II enzymes.<sup>32</sup>

An earlier study found that curcumin could induce topoisomerase II-mediated DNA cleavage in vitro.<sup>33</sup> However, because curcumin can undergo oxidation and degradation in aqueous solution, it is not clear whether the parent compound or metabolites (or both) are the topoisomerase II-reactive species. Therefore, we tested the ability of curcumin to poison human type II topoisomerases under conditions in which the compound remains stable or undergoes oxidation. We also examined the activity of vanillin, ferulic acid, and feruloylmethane toward the human enzymes. Results indicate that oxidative metabolites of curcumin poison human topoisomerase II $\alpha$  and II $\beta$ . In contrast, neither the parent compound nor its fragmentation products displayed significant activity toward the human enzymes.

## EXPERIMENTAL PROCEDURES

**Enzymes and Materials.** Human topoisomerase II $\alpha$ , topoisomerase II $\beta$ , and the mutant topoisomerase II $\alpha^{C392A/C405A}$  were expressed in *Saccharomyces cerevisiae*<sup>34</sup> and purified as described previously.<sup>35,36</sup> Negatively supercoiled pBR322 DNA was prepared from *Escherichia coli* using a Plasmid Mega Kit (Qiagen) as described by the manufacturer. Curcumin and 4',4''-dimethylcurcumin were synthesized as described previously.<sup>14</sup> The bicyclopentadione oxidative product of curcumin was isolated from autooxidation reactions by high-performance liquid chromatography. Potassium ferricyanide [ $K_3Fe(CN)_6$ ] was obtained from Acros and was stored at  $-20^{\circ}C$  as a 50 mM stock solution in water. Turmeric was obtained from Spice Islands Trading Co. and was stored at  $-20^{\circ}C$  as a 37.5 mg/mL stock solution in 100% dimethyl sulfoxide (DMSO). Vanillin, ferulic acid, and feruloylmethane were obtained from Sigma. All other chemicals were analytical reagent grade. Unless stated otherwise, curcumin and its derivatives were stored at  $-20^{\circ}C$  as 20 mM stock solutions in 100% DMSO.

**Plasmid DNA Cleavage.** DNA cleavage reactions were conducted using the procedure of Fortune and Osheroff.<sup>37</sup> Topoisomerase II DNA cleavage assays contained 220 nM human topoisomerase II $\alpha$ , topoisomerase II $\beta$ , or mutant topoisomerase II $\alpha^{C392A/C405A}$  and 10 nM negatively supercoiled pBR322 in a total of 20  $\mu$ L of 10 mM Tris-HCl (pH 7.9), 5 mM  $MgCl_2$ , 100 mM KCl, 0.1 mM EDTA, and 2.5% (v/v) glycerol. Assay buffer contained  $\sim 2 \mu$ M residual dithiothreitol (DTT) that was carried over from the topoisomerase II storage buffer. Unless stated otherwise, reaction mixtures were incubated at  $37^{\circ}C$  for 6 min, and enzyme–DNA cleavage complexes were trapped by the addition of 2  $\mu$ L of 5% sodium dodecyl sulfate (SDS) followed by 2  $\mu$ L of 250 mM EDTA (pH 8.0). Proteinase K (2  $\mu$ L of a 0.8 mg/mL solution) was added, and samples were incubated at  $45^{\circ}C$  for 30 min to digest the enzyme. Samples were mixed with 2  $\mu$ L of 60% sucrose in 10 mM Tris-HCl (pH 7.9), 0.5% bromophenol blue, and 0.5% xylene cyanol FF, heated at  $45^{\circ}C$  for 5 min, and subjected to electrophoresis in 1% agarose gels in 40 mM Tris-acetate (pH 8.3) and 2 mM EDTA containing 0.5  $\mu$ g/mL ethidium bromide. DNA bands were visualized with long-range ultraviolet light and quantified using an Alpha Innotech digital imaging system. DNA cleavage was monitored by the conversion of supercoiled plasmid DNA to linear molecules.

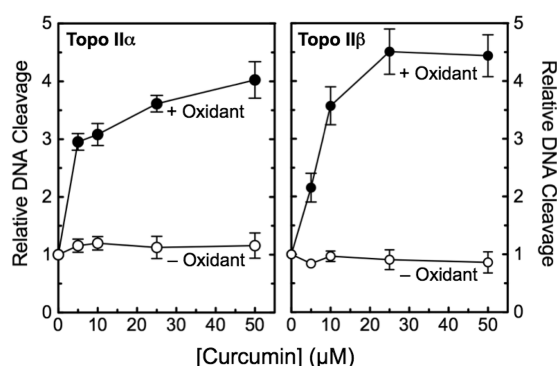
Assays were carried out in the absence or presence of 0–50  $\mu$ M curcumin or derivatives (oxidation or degradation) in the

absence or presence of 0–50  $\mu\text{M}$   $\text{K}_3\text{Fe}(\text{CN})_6$ . Unless stated otherwise, curcumin or a derivative was always the last component added to reaction mixtures. In some cases, assays were conducted in the presence of 250  $\mu\text{M}$  DTT, which was added either before or after topoisomerase II-mediated DNA cleavage complexes had been established.

## RESULTS AND DISCUSSION

### Oxidative Metabolites of Curcumin Enhance DNA Cleavage Mediated by Human Type II Topoisomerases.

Although curcumin increases levels of DNA cleavage mediated by topoisomerase II $\alpha$  and II $\beta$  in cultured human cells,<sup>32</sup> the ability of the compound to affect enzyme activity in purified systems has not been well characterized. Therefore, the effects of the phytochemical on the human type II enzymes were determined. As seen in Figure 2, curcumin displayed no activity

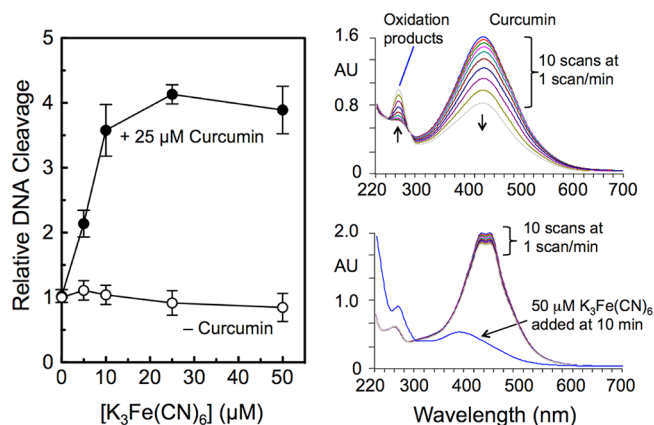


**Figure 2.** Enhancement of topoisomerase II-mediated DNA cleavage by curcumin in the presence of oxidant. The effects of curcumin on the cleavage of negatively supercoiled plasmid DNA by human topoisomerase II $\alpha$  (left) and topoisomerase II $\beta$  (right) were determined in the absence (○, –Oxidant) or presence (●, +Oxidant) of 50  $\mu\text{M}$   $\text{K}_3\text{Fe}(\text{CN})_6$ . Error bars represent standard deviations for three independent experiments.

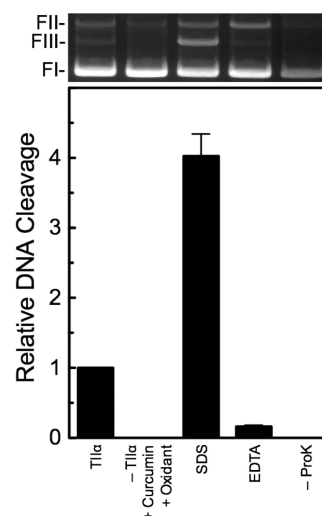
toward either topoisomerase II $\alpha$  or II $\beta$ . However, in the presence of an oxidizing agent, such as potassium ferricyanide [ $\text{K}_3\text{Fe}(\text{CN})_6$ ], curcumin became a potent topoisomerase II poison. We observed between 4- and 5-fold DNA cleavage enhancement with human topoisomerase II $\alpha$  and II $\beta$ , respectively. The activation of curcumin required stoichiometric concentrations of  $\text{K}_3\text{Fe}(\text{CN})_6$ , and the oxidant had no effect on topoisomerase II-mediated DNA cleavage in the absence of the phytochemical (Figure 3, left).

Curcumin undergoes rapid oxidation in aqueous solutions (Figure 3, top right).<sup>14</sup> However, the compound was much more stable in the buffer used for topoisomerase II-mediated DNA cleavage assays, and little oxidation was observed over the 6 min course of the reaction (Figure 3, bottom right). The increase in curcumin stability was due largely to the  $\text{MgCl}_2$  that was included in the assay buffer (data not shown). Complete oxidation of curcumin was observed in assay mixtures shortly after addition of  $\text{K}_3\text{Fe}(\text{CN})_6$  (Figure 3, bottom right).

Several control reactions with human topoisomerase II $\alpha$  were performed to ensure that the DNA cleavage enhancement observed with oxidized curcumin was mediated by the type II enzyme (Figure 4). No DNA scission was seen in the presence of curcumin and  $\text{K}_3\text{Fe}(\text{CN})_6$  when the type II enzyme was omitted from reaction mixtures. Furthermore, topoisomerase II $\alpha$ -mediated DNA cleavage induced by oxidized curcumin was



**Figure 3.** Effects of  $\text{K}_3\text{Fe}(\text{CN})_6$  on curcumin oxidation and the DNA cleavage activity of human topoisomerase II $\alpha$ . The left panel shows the effects of  $\text{K}_3\text{Fe}(\text{CN})_6$  on the cleavage of negatively supercoiled plasmid DNA by human topoisomerase II $\alpha$  determined in the absence (○) or presence (●) of 25  $\mu\text{M}$  curcumin. Error bars represent standard deviations for three independent experiments. The right panels show ultraviolet–visible spectroscopic analysis of the loss of curcumin (maximal wavelength at 430 nm) and the appearance of oxidized products (peak at 263 nm) in Tris-HCl buffer (pH 7.9) (top) and in topoisomerase II assay buffer (pH 7.9) (bottom). Scans were obtained at a frequency of one per minute.  $\text{K}_3\text{Fe}(\text{CN})_6$  was added to the reaction mixture in topoisomerase II assay buffer at 10 min.



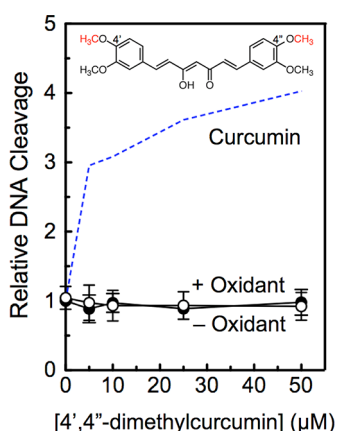
**Figure 4.** DNA cleavage induced by oxidized curcumin is reversible and protein-linked. Assay mixtures contained enzyme in the absence of curcumin or oxidant (TII $\alpha$ ), 50  $\mu\text{M}$  curcumin, and 50  $\mu\text{M}$   $\text{K}_3\text{Fe}(\text{CN})_6$  in the absence of enzyme (–TII $\alpha$  + Curcumin + Oxidant) or complete reaction mixtures treated with SDS prior to the addition of EDTA (SDS). To determine whether DNA cleavage induced by oxidized curcumin was reversible, reaction mixtures were incubated with EDTA prior to trapping cleavage complexes with SDS (EDTA). To determine whether DNA cleavage induced by oxidized curcumin was protein-linked, proteinase K treatment was omitted (–ProK). Error bars represent standard deviations for three independent experiments. A representative agarose gel is shown at the top. DNA lanes correspond to the bars shown in the graph. The positions of negatively supercoiled (form I, FI), nicked (form II, FII), and linear (form III, FIII) plasmid DNA are indicated.

reversed when the active site  $\text{Mg}^{2+}$  ions were chelated with EDTA prior to trapping cleavage complexes with SDS. This reversibility is not consistent with an enzyme-independent



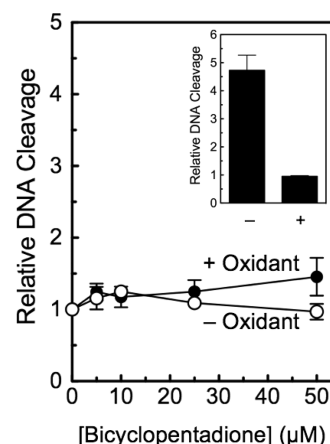
reaction. Finally, cleaved plasmid products were covalently linked to topoisomerase II. In the absence of proteinase K, the linear DNA band disappeared and was replaced by a band that remained at the origin of the gel (not shown). These results demonstrate that the DNA scission observed in the presence of curcumin and  $K_3Fe(CN)_6$  is mediated by the type II enzyme.

The findings described above provide strong evidence that oxidized metabolites of curcumin, rather than the parent compound, are responsible for the enhancement of topoisomerase II-mediated DNA cleavage. As further evidence supporting this conclusion, the ability of 4',4''-dimethylcurcumin to poison human topoisomerase II $\alpha$  was determined. Because the methyl groups protect the 4'- and 4''-hydroxyl moieties from hydrogen abstraction, the compound undergoes oxidation rates that are >1000-fold slower than that of curcumin.<sup>14</sup> As seen in Figure 5, no enhancement of topoisomerase II-mediated DNA cleavage was observed in the absence or presence of  $K_3Fe(CN)_6$ .



**Figure 5.** Effects of 4',4''-dimethylcurcumin on topoisomerase II-mediated DNA cleavage. The effects of 4',4''-dimethylcurcumin (structure at the top, 4'- and 4''-methyl groups colored red) on the cleavage of negatively supercoiled plasmid DNA by human topoisomerase II $\alpha$  were determined in the absence (O, -Oxidant) or presence (●, +Oxidant) of 50  $\mu M$   $K_3Fe(CN)_6$ . Data for oxidized curcumin (dashed line from Figure 2) are included for comparison. Error bars represent standard deviations for three independent experiments.

Upon treatment with an oxidizing agent, curcumin is rapidly converted to a stable bicyclopentadione (Figures 1 and 3). En route to this ultimate oxidation product, the parent compound moves through a series of reactive quinone methide intermediates (Figure 1).<sup>14</sup> As discussed below, a number of quinone-based compounds have been shown to poison type II topoisomerases.<sup>38–41</sup> Therefore, two experiments were carried out to determine whether the quinone methide intermediates (as opposed to the final bicyclopentadione) are the more likely compounds that poison topoisomerase II in the presence of an oxidant. In the first experiment, curcumin was incubated with  $K_3Fe(CN)_6$  for 10 min prior to its addition to a topoisomerase II $\alpha$ –DNA cleavage reaction mixture. Under these conditions, the majority of the parent phytochemical was converted to the bicyclopentadione (Figure 3, bottom right). As seen in Figure 6 (inset), no enhancement of DNA scission was observed. In the second experiment, purified bicyclopentadione was added to DNA cleavage assays in the absence or presence of  $K_3Fe(CN)_6$  (Figure 6). Virtually no enhancement of topoisomerase II $\alpha$ -mediated DNA cleavage was observed under either condition.

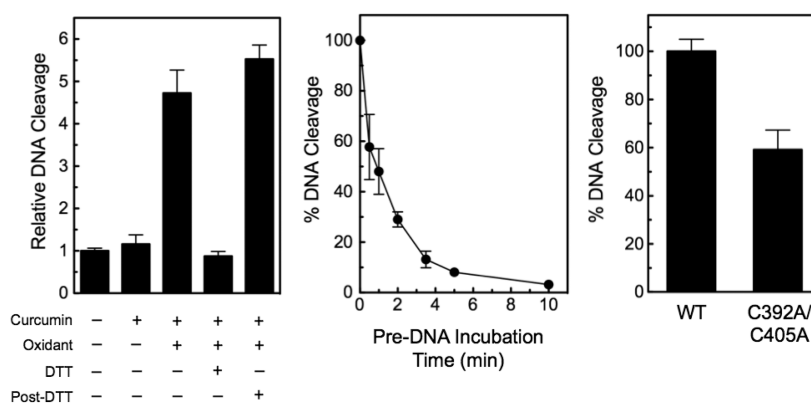


**Figure 6.** Effects of bicyclopentadione on topoisomerase II-mediated DNA cleavage. The effects of bicyclopentadione (structure shown in Figure 1) on the cleavage of negatively supercoiled plasmid DNA by human topoisomerase II $\alpha$  were determined in the absence (O, -Oxidant) or presence (●, +Oxidant) of 50  $\mu M$   $K_3Fe(CN)_6$ . The inset shows data for the experiment in which curcumin was incubated in the absence (–) or presence (+) of  $K_3Fe(CN)_6$  for 10 min before being added to topoisomerase II $\alpha$ –DNA cleavage assay mixtures that contained 50  $\mu M$   $K_3Fe(CN)_6$ . Error bars represent standard deviations for three independent experiments.

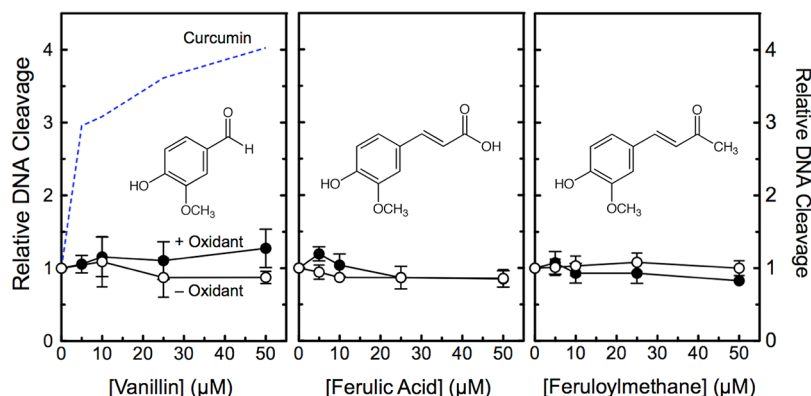
Thus, it appears that the quinone methide metabolites of curcumin are the chemical species that poison topoisomerase II.

**Oxidative Metabolites of Curcumin Are Redox-Dependent Topoisomerase II Poisons.** Compounds that poison type II topoisomerases can be categorized into two broad classes: interfacial and redox-dependent. Members of the first group interact with topoisomerase II at the protein–DNA interface (in the vicinity of the active site tyrosine) in a noncovalent manner.<sup>20,31,42</sup> Interfacial topoisomerase II poisons include several anticancer drugs (e.g., etoposide) and dietary bioflavonoids (e.g., genistein) and display a number of characteristic properties.<sup>20,23,38,39,41,43,44</sup> Primarily, because their actions against topoisomerase II do not depend on redox chemistry, they are unaffected by the presence of reducing agents. Furthermore, these compounds induce similar levels of enzyme-mediated DNA scission regardless of whether they are added directly to the binary topoisomerase II–DNA complex or are incubated with the enzyme prior to the addition of the nucleic acid substrate.<sup>20,38</sup>

Topoisomerase II poisons in the second class (redox-dependent poisons) form covalent adducts with the enzyme at amino acid residues distal to the active site.<sup>36,45</sup> The best-characterized members of this group are quinones, such as 1,4-benzoquinone and polychlorinated biphenyl (PCB) metabolites.<sup>38,40,41</sup> In contrast to interfacial topoisomerase II poisons, the ability of redox-dependent poisons to form topoisomerase II adducts (and, consequently, increase enzyme-mediated DNA cleavage) requires them to be in an oxidized form. Thus, DNA cleavage enhancement is blocked by the presence of reducing agents.<sup>20,23,38,39,41,43,44</sup> However, once final protein adducts are formed, their oxidation state appears to be irrelevant. As a result, if reducing agents are added to assay mixtures after DNA cleavage–ligation equilibria have been established in the presence of a quinone-based poison, they are unable to reverse the cleavage enhancement.<sup>20,38,39,41</sup> Finally, while redox-dependent poisons enhance topoisomerase II-mediated DNA cleavage when they are added to the enzyme–DNA complex,



**Figure 7.** Oxidized curcumin intermediates act as redox-dependent topoisomerase II poisons. The left panel shows the effects of DTT on the enhancement of topoisomerase II $\alpha$ -mediated DNA cleavage by oxidized curcumin intermediates. Reaction mixtures contained DNA and human topoisomerase II $\alpha$  in the absence or presence of 50  $\mu$ M curcumin, 50  $\mu$ M  $K_3Fe(CN)_6$ , or 250  $\mu$ M DTT. In some reaction mixtures, DTT was added after the enzyme–DNA cleavage complexes had been established (Post-DTT). The middle panel shows the effects of oxidized curcumin intermediates on topoisomerase II $\alpha$  activity when compounds were incubated with the enzyme prior to the addition of DNA. Human topoisomerase II $\alpha$  was incubated with a combination of 50  $\mu$ M curcumin and 50  $\mu$ M  $K_3Fe(CN)_6$  for 0–10 min prior to the addition of DNA to initiate 6 min cleavage reactions. The right panel shows the effects of 50  $\mu$ M oxidized curcumin intermediates on the enhancement of DNA cleavage by human wild-type topoisomerase II $\alpha$  (WT) and mutant quinone-resistant topoisomerase II $\alpha^{C392A/C405A}$  (C392A/C405A). Error bars for all three panels represent standard deviations for three independent experiments.



**Figure 8.** Effects of curcumin degradation products on topoisomerase II-mediated DNA cleavage. The effects of vanillin (left), ferulic acid (middle), or feruloylmethane (right) on the cleavage of negatively supercoiled plasmid DNA by human topoisomerase II $\alpha$  were determined in the absence (O, –Oxidant) or presence (●, +Oxidant) of 50  $\mu$ M  $K_3Fe(CN)_6$ . Data for curcumin (dashed line from Figure 2) are included in the left panel for comparison. Error bars represent standard deviations for three independent experiments.

they inactivate topoisomerase II when incubated with the protein prior to the addition of DNA.<sup>20,38,39,41</sup>

Curcumin requires an oxidant in order for it to increase the extent of topoisomerase II-mediated DNA cleavage. Furthermore, many of the proposed metabolites in the oxidation pathway of the compound contain quinones.<sup>14</sup> Thus, it seems likely that the active metabolites of curcumin function as redox-dependent (as opposed to interfacial) topoisomerase II poisons. Four approaches were utilized to address this hypothesis.

In the first, a 5-fold molar excess of DTT over curcumin and  $K_3Fe(CN)_6$  was added to assay mixtures prior to the start of the reactions. As expected (considering that curcumin requires oxidation for activation), no enhancement of topoisomerase II $\alpha$ -mediated DNA cleavage was observed (Figure 7, left).

In the second, DTT was added to reaction mixtures after cleavage complexes had been established. As seen in Figure 7 (left), levels of DNA scission remained high. As discussed above, this finding suggests that the oxidized metabolites of curcumin form covalent topoisomerase II adducts.

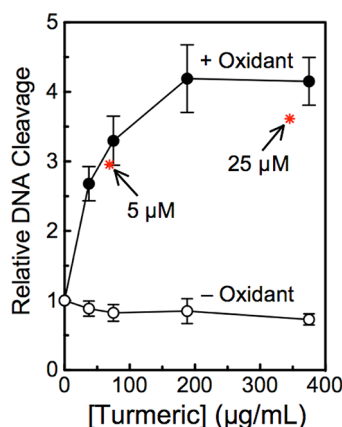
In the third, curcumin was incubated with human topoisomerase II $\alpha$  in the presence of  $K_3Fe(CN)_6$  prior to the addition of DNA (Figure 7, middle). As predicted for redox-dependent poisons, enzyme activity fell to nearly zero with a  $t_{1/2}$  of 0.9 min. In contrast, the activity of topoisomerase II $\alpha$  was considerably more stable when the enzyme was incubated with either curcumin ( $t_{1/2}$  = 10.4 min) or  $K_3Fe(CN)_6$  ( $t_{1/2}$  > 10 min) alone prior to the addition of DNA (not shown).

In the fourth, the ability of oxidized curcumin to stimulate DNA cleavage mediated by topoisomerase II $\alpha^{C392A/C405A}$  was determined. A previous study established that quinones can adduct human topoisomerase II $\alpha$  at Cys392 and Cys405 and that topoisomerase II $\alpha^{C392A/C405A}$  is partially (~2-fold) resistant to redox poisons, such as benzoquinone and PCB quinones, but not to interfacial poisons.<sup>36</sup> As seen in Figure 7 (right), the ability of oxidized curcumin to increase the level of DNA cleavage mediated by topoisomerase II $\alpha^{C392A/C405A}$  was ~60% of that of the wild-type enzyme.

Taken together, these findings provide strong evidence that the oxidized intermediates of curcumin can be classified as redox-dependent topoisomerase II poisons.

**Degradation Products of Curcumin Do Not Poison Topoisomerase II.** Under the conditions of the DNA cleavage assays, no significant degradation of curcumin to vanillin, ferulic acid, or feruloylmethane was observed (not shown). However, because some of these compounds display biological activity,<sup>18</sup> the effects of vanillin, ferulic acid, and feruloylmethane on topoisomerase II $\alpha$ -mediated DNA cleavage were determined. As seen in Figure 8, none of the compounds increased levels of DNA scission in the absence or presence of K<sub>3</sub>Fe(CN)<sub>6</sub>. Therefore, curcumin fragmentation products do not appear to be interfacial or redox-dependent topoisomerase II poisons.

**Oxidized Turmeric Is a Topoisomerase II Poison.** Because curcumin generally is ingested in the form of turmeric,<sup>2,3</sup> the powdered spice was dissolved and assessed for its ability to stimulate DNA cleavage mediated by human topoisomerase II $\alpha$  (Figure 9). In the absence of an oxidant, the



**Figure 9.** Effects of turmeric on topoisomerase II-mediated DNA cleavage. The effects of turmeric on the cleavage of negatively supercoiled plasmid DNA by human topoisomerase II $\alpha$  were determined in the absence (○, -Oxidant) or presence (●, +Oxidant) of 50  $\mu$ M K<sub>3</sub>Fe(CN)<sub>6</sub>. The turmeric stock solution was determined to contain ~2.7% curcumin. On the basis of this concentration, DNA cleavage results for 5 and 25  $\mu$ M oxidized curcumin intermediates (asterisks) are overlaid at the associated turmeric concentrations for comparison. Error bars represent standard deviations for three independent experiments.

spice had no significant effect on enzyme-mediated DNA scission. However, when K<sub>3</sub>Fe(CN)<sub>6</sub> was included in reaction mixtures, turmeric stimulated DNA cleavage >4-fold at concentrations of 200–400  $\mu$ g/mL. As determined by high-performance liquid chromatography, the turmeric solution contained ~2.7% curcumin. On the basis of this value, DNA cleavage data for 5 and 25  $\mu$ M curcumin [with K<sub>3</sub>Fe(CN)<sub>6</sub>] were overlaid for comparison (asterisks). As seen in Figure 9, there is a strong correlation between the activities of turmeric and curcumin. Therefore, even within the more complex spice formulation, oxidized curcumin appears to function as a topoisomerase II poison.

**Conclusions.** Oxidized metabolites of curcumin, even in a solution of turmeric, are redox-dependent poisons of human type II topoisomerases. In contrast, degradation products of the parent compound do not affect topoisomerase II-mediated DNA cleavage.

Curcumin displays a number of medically relevant biological properties, including antioxidant, anti-inflammatory, antibacterial, and chemopreventive activities.<sup>4–6,9</sup> The compound also is in cancer chemotherapy trials.<sup>7,8</sup> A number of chemopreventive

natural products (including genistein and EGCG) and several highly successful anticancer drugs (including etoposide and doxorubicin) are potent topoisomerase II poisons.<sup>20–23,27,29–31,43</sup> Coupled with the findings that (1) curcumin enhances DNA cleavage mediated by topoisomerase II $\alpha$  and II $\beta$  in cultured human cells and (2) cleavage enhancement is abrogated by antioxidant treatment,<sup>32</sup> the findings described above suggest that at least some of the anticancer activities of curcumin may be mediated by the effects of its oxidized metabolites on the type II topoisomerases.

## AUTHOR INFORMATION

### Corresponding Author

\*Correspondence regarding topoisomerase II and topoisomerase II poisons should be addressed to N.O.: telephone, (615) 322-4338; e-mail, neil.osheroff@vanderbilt.edu. Correspondence regarding curcumin chemistry should be addressed to C.S.: telephone, (615) 343-9539; e-mail, claus.schneider@vanderbilt.edu.

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### Notes

The authors declare no competing financial interest.

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