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Studies on curcumin and curcuminoids. XXII: Curcumin as a reducing agent and as a radical scavenger

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Summary

The function of curcumin in the reduction of Fe^{3+} to Fe^{2+} and in oxygen radical reactions is discussed. The presence of the diketone moiety in the curcumin molecule seems to be essential both in redox reactions and in the scavenging of oxygen radicals.

Introduction

During the last 10–15 years the yellow compounds, curcumin, demethoxy- and bisdemethoxycurcumin, isolated from the plant *Curcuma longa* L. (Turmeric) (Zingiberaceae), have been attracting the interest of scientists. In the search for new compounds with biological activity, the curcuminoids have proved to be promising as they exhibit anti-inflammatory and antineoplastic properties. Moreover, they are readily obtained in large quantities and at low cost. Turmeric has been used in traditional medicine for more than 2000 years and its broad spectrum of biological activity is ascribed to the curcuminoid content. Toxicological studies indicate that the main constituent, curcumin, is non-toxic even at high dosage (Tønnesen, 1986). Several studies have been undertaken to evaluate the mechanism of the anti-inflammatory action of the curcuminoids (Kunchandy and Rao, 1989, 1990; Tønnesen, 1989a–d). The results obtained indicate that curcumin has a dual effect in oxygen radical reactions, i.e., it can act as a scavenger of hydroxyl radicals or catalyse the formation of hydroxyl radicals depending upon the experimental conditions (Tønnesen, 1989b; Kunchandy and Rao, 1990). Recent studies have also demonstrated that curcumin exerts an inhibitory effect on the growth of skin tumors in mice (Huang et al., 1988). The observed effect is partly ascribed to the radical scavenging properties of curcumin. It is not known which part of the curcumin molecule promotes the catalytic or inhibitory effects observed in the various model systems. Both the phenolic hydroxyl groups and the β-diketone moiety could be responsible for radical reactions. Curcumin seems to differ from other phenolic antioxidants in its profile of activities (Huang,

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personal communication). A better understanding of the mechanism of action of the curcumin molecule would facilitate the planning of structural modifications. The present paper discusses the function of curcumin in oxygen radical reactions. The discussion is partly based upon previous studies of the chemical properties of the curcuminoids. The importance of the β-diketone unit in the reduction of Fe^{3+} is demonstrated by the use of the curcumin derivative 5-hydroxy-1,7-diphenyl-1,4,6-heptatriene-3-one (Fig. 1).

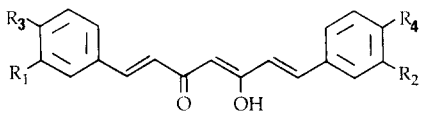

Fig. 1. Structure of curcumin and the derivative 5-hydroxy-1,7-diphenyl-1,4,6-heptatriene-3-one. $R_1 = R_2 = OCH_3$, $R_3 = R_4 = OH$: curcumin. $R_1 = R_2 = R_3 = R_4 = H$: curcumin derivative.

Materials and Methods

Throughout the experiments, the samples were protected from light.

Materials

Curcumin was synthesized according to the method described by Pabon (1964). 5-Hydroxy-1,7-diphenyl-1,4,6-heptatriene-3-one was a gift from S.-O. Lawesson, University of Århus, Denmark. $FeSO_4$ (iron(II) sulphate heptahydrate, > 98%), $FeCl_3$ (ferric chloride hexahydrate, 98%), EDTA (disodium salt dihydrate, > 99%) and 1,10-phenanthroline monohydrate (> 99%) were purchased from Aldrich-Chemie, Germany. 2-Deoxy-D-ribose, 2-thiobarbituric acid and trichloroacetic acid (100%) were obtained from Sigma, U.S.A. H_2O_2 (hydrogen peroxide 30%) was supplied by Norsk Medisinaldepot, Norway. Ferulic acid (> 98%) was obtained from Fluka AG, Switzerland.

Reduction of Fe^{3+} to Fe^{2+}

The reduction of Fe^{3+} to Fe^{2+} was measured via the 1,10-phenanthroline complexation method (Gutteridge, 1985). The following stock solutions were used: curcumin and curcumin derivative in methanol (each 4×10^{-4} M), $FeCl_3$, $FeSO_4$ and EDTA in water (each 2×10^{-3} M), 1,10-phenanthroline in methanol (15 mg/50 ml), and H_2O_2 30%. The various combinations tested are listed in Table 1. The samples were determined spectrophotometrically at 510 nm.

TABLE 1

Composition of the samples tested in the study of the reduction of Fe^{3+} to Fe^{2+}

Sample no.	Curcumin (0.25 ml)	Curcumin derivative (0.34 ml)	$FeCl_3$ (0.5 ml)	$FeSO_4$ (0.5 ml)	EDTA (0.5 ml)	MeOH (0.5 ml)	H_2O (ad 10 ml)	MeOH (ad 10 ml)	Phenanthroline (0.5 ml)	H_2O (0.5 ml)	A_{510nm}
1A	x		x		x		x		x		0.060
1B	x		x		x	x	x				0.059
1C	x		x				x		x		0.150
1D	x		x			x	x				0.083
1E	x		x		x			x	x		0.076
1F	x		x		x			x			0.083
1G	x		x					x	x	x	0.203
1H	x		x					x		x	0.173
2A		x	x		x			x	x		0.002
2B		x	x		x			x			0.000
2C		x	x					x	x		0.072
2D		x	x					x			0.043
2E				x				x	x		0.169
2F				x	x			x	x		0.063

A_{510nm} refers to the absorbance measured according to the phenanthroline complex method. The values have not been corrected for differences in absorbance at 510 nm in media with and without EDTA. Results are the average of at least three parallels.

TABLE 2

Composition of the samples tested in the study of the generation of hydroxyl radical from hydrogen peroxide

Sample no.	Curcumin (ad 10 ml)	Deoxyribose (0.5 ml)	$FeCl_3$ (0.5 ml)	EDTA (0.5 ml)	Buffer (ad 10 ml)	H_2O_2 (20 μl)	Buffer (1 ml)	Buffer (0.5 ml)	H_2O (ad 10 ml)	A_{532nm}
3A		x	x	x	x	x				0.088
3B	x	x	x	x		x				0.121
3C		x			x	x				0.047
3D	x	x				x	x			0.060
3E	x	x	x			x		x		0.085
3F		x	x			x			x	0.243
3G		x	x	x	x					0.018
3H	x	x	x	x						0.018
3I		x	x						x	0.021
3J	x	x	x					x		0.021

A_{532nm} corresponds to the absorbance measured according to the deoxyribose method. Results are the average of at least three parallels.

Production of hydroxyl radical

The production of hydroxyl radical was maintained by following the degradation of deoxyribose (Halliwell and Gutteridge, 1985). Stock solutions were the following: A saturated solution of curcumin in phosphoric acid buffer, pH 7.2, was prepared by suspending 5 mg curcumin in 100 ml buffer followed by gentle tumbling for 1 h. After centrifugation, a given volume of the supernatant was added to the reaction mixture. 2-Deoxy-D-ribose in water (5×10^{-3} M), 2-thiobarbituric acid (1%) in 0.05 M NaOH, trichloroacetic acid

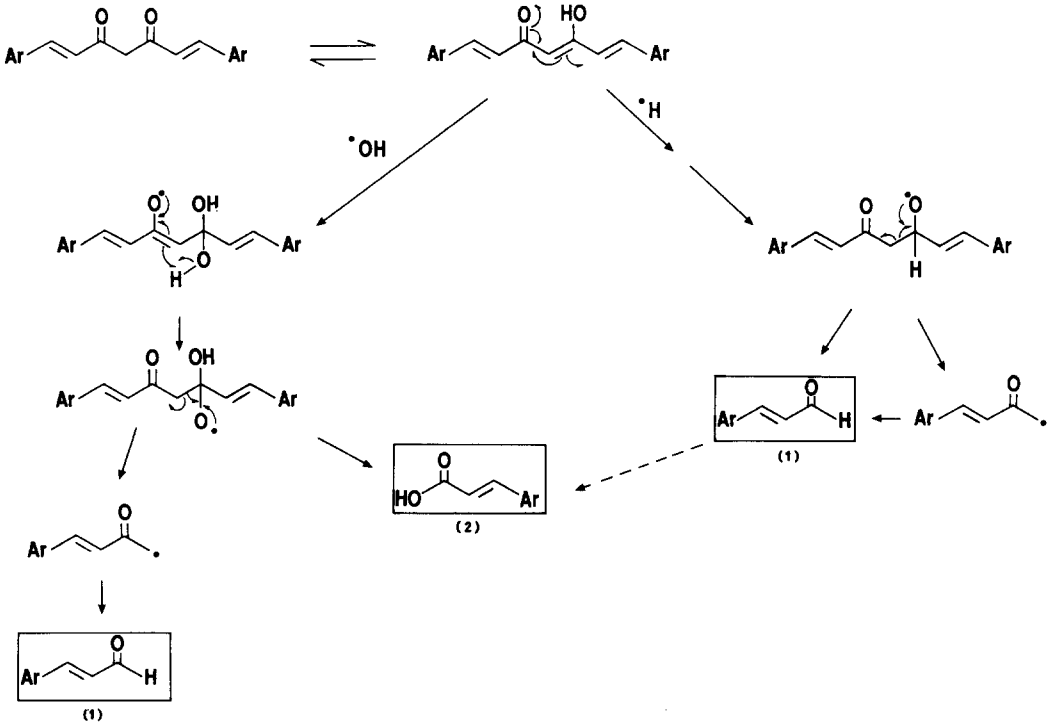

Fig. 2. Postulated reaction pathway for the formation of the photodecomposition products ferulic aldehyde (1) and ferulic acid (2).

in water (2.8%), $FeCl_3$, $FeSO_4$ and EDTA in water (each 2×10^{-3} M), and H_2O_2 30%.

The various combinations tested are detailed in Table 2. The samples were incubated for 10 min at 20 °C. To 1.0 ml sample were added 1.0 ml 2-thiobarbituric acid solution and 1.0 ml trichloroacetic acid solution. The mixture was heated at 100 °C for 20 min, cooled to 25 °C and assayed spectrophotometrically at 532 nm.

Spectrophotometer and scanning conditions

Interactions between curcumin and Fe^{2+} or Fe^{3+} were studied by scanning the solutions of curcumin (1×10^{-5} M) from 200 to 600 nm after the addition of iron salts in the concentration range 1×10^{-6}–1×10^{-4} M. The spectra were compared to those obtained after addition of EDTA (1×10^{-5} M) to the solutions.

The spectrophotometer was a Shimadzu UV-260 UV-Visible recording spectrophotometer.

Stock solutions of curcumin in methanol (4×10^{-3} M) and iron salts in water (2×10^{-3} M) were used. The samples were further diluted in water.

Chromatographic conditions

The TLC analyses were carried out on silica gel HF_{254} (Merck) plates. The mobile phase consisted of chloroform/ethanol (25:1). For the HPLC analysis, a Spectra Physics SP 8700 model was used. Other apparatus and conditions were: injector, Shimadzu Sil-6A Auto Injector; detector, Shimadzu SPD-6AV UV-Vis spectrophotometric detector; detection wavelength, 350 nm; integrator, Shimadzu C-R3A. The HPLC column was a gift from Whatman, U.S.A. (Whatman Amino partisphere, 12 μm). The mobile phase was ethanol/water (96:4).

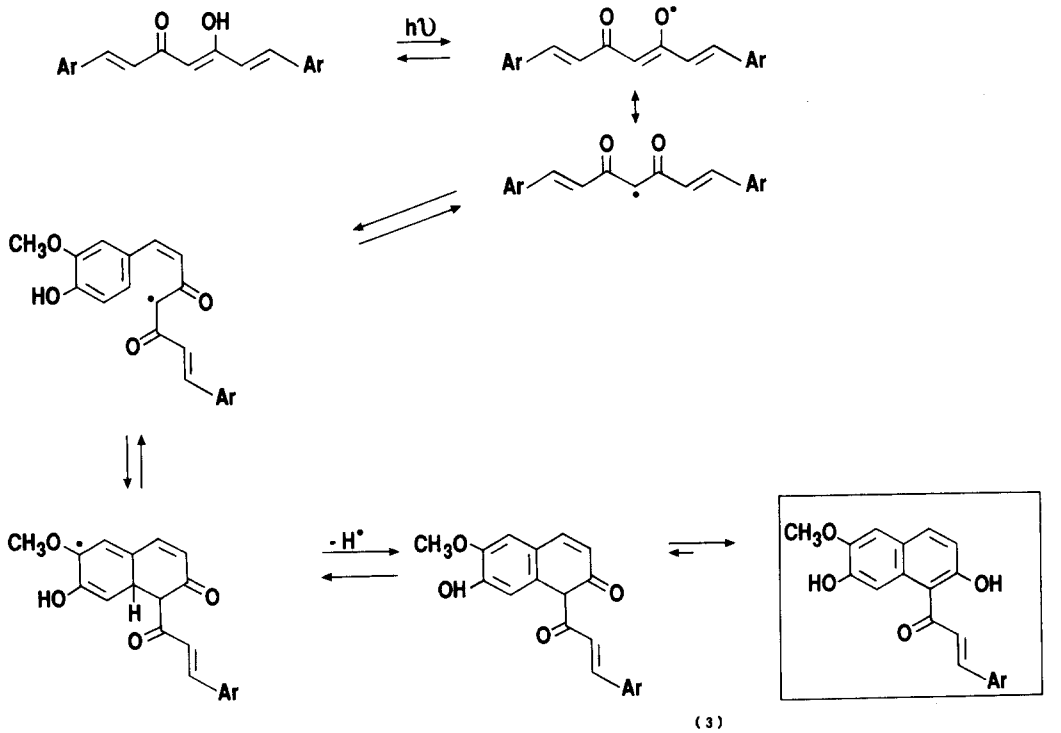

Fig. 3. Postulated reaction pathway for the formation of the cyclization product of curcumin (3). The reaction can reverse to make curcumin available for more products to form.

Results and Discussion

The photochemical degradation of curcumin has been studied previously (Tønnesen et al., 1986). Under the influence of light, curcumin was shown to act as a sensitizer of oxygen radicals and to undergo a self-sensitized decomposition. The main photochemical degradation products of curcumin were isolated and identified as compounds **1–6** (Figs 2–4) (Tønnesen et al., 1986). The formation of these products involves reactions between curcumin and various oxygen radicals. However, by evaluation of the reaction pathways leading to the photochemical degradation products, information can also be obtained about the reactions between curcumin and oxygen radicals in general.

The postulated pathways leading to the photodecomposition products identified previously are given in Figs 2–4. Despite much thought we are unable to suggest reasonable alternative mechanisms involving the curcumin phenolic groups. Since the non-phenolic curcumin derivative shows similar redox reactions (see below), it is likely that the β-diketone moiety is involved both in the scavenging of the hydroxyl radical (Fig. 2) and in redox reactions (Fig. 3).

The interactions between curcumin or the curcumin derivative and Fe^{3+} or Fe^{2+} were studied spectrophotometrically. An interaction between curcumin and Fe^{2+} was observed by an increase in the absorbance at 500 nm and a decrease in the absorbance at 428 nm as illustrated in Fig. 5. No further changes in the spectra were observed when the concentration of Fe^{2+} exceeded that of curcumin, indicating that a 1 : 1 complex between curcumin and Fe^{2+} had been formed. When EDTA was added to the samples, the spectra obtained were identical to that of curcumin measured in the absence of iron, indicating that curcumin has a lower affinity for Fe^{2+} than EDTA. When the concentration of Fe^{2+} was greater than that of EDTA, curcumin complexed with the excess iron.

Interactions between curcumin (1×10^{-5} M) and Fe^{3+} were demonstrated by an increase in the absorbance at 500 nm in the concentration range 1×10^{-6}–1×10^{-5} M Fe^{3+} (Fig. 6). There was no corresponding decrease in the absorbance at 428 nm. In the concentration range 1×10^{-5}–1

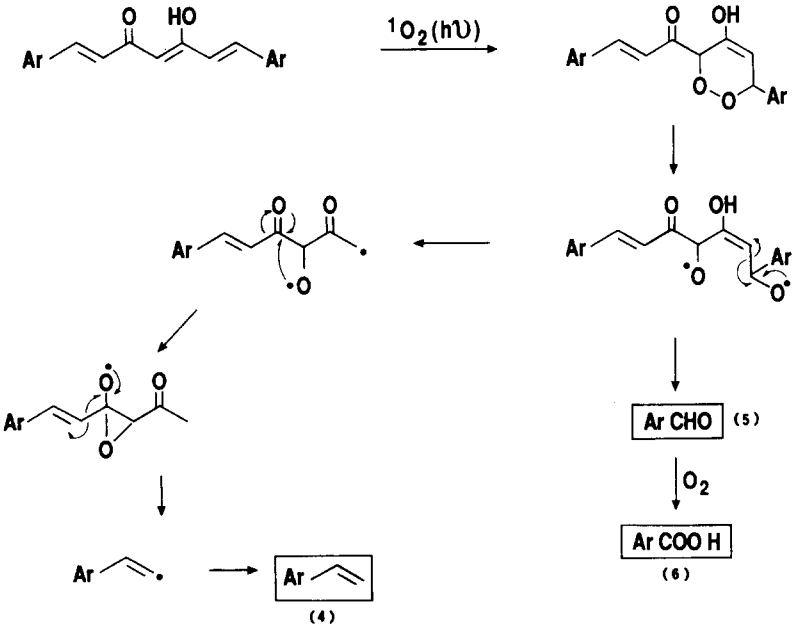

Fig. 4. Postulated reaction pathway for the formation of the photodecomposition products 4-vinylguaiacol (**4**), vanillin (**5**) and vanillic acid (**6**).

$\times 10^{-4}$ M Fe^{3+}, an increase in the absorbance at 520 nm accompanied by a decrease in the absorbance at 428 nm could be detected. A further increase in the iron concentration led to a bathochromic shift in the curcumin spectrum. In the presence of EDTA no complex between curcumin and Fe^{3+} could be detected unless the concentration of Fe^{3+} exceeded that of EDTA.

The interactions between the curcumin derivative 5-hydroxy-1,7-diphenyl-1,4,6-heptatriene-3-one and Fe^{2+} or Fe^{3+} were observed as changes in absorption spectra as illustrated in Figs 7 and 8. In the presence of EDTA no shift in the absorption spectra or increase in the absorbance above 450 nm could be detected, indicating that the curcumin derivative has a lower affinity for Fe^{2+} and Fe^{3+} than EDTA.

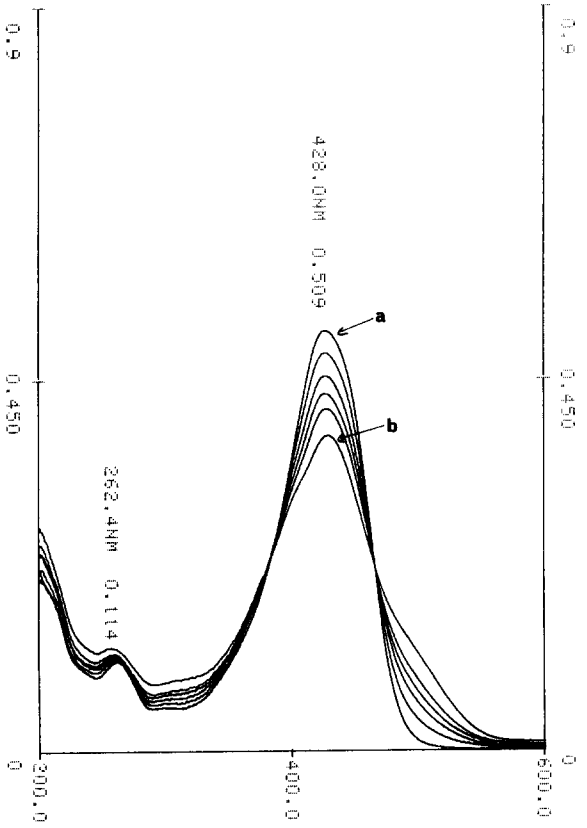

Fig. 5. Absorption spectra of curcumin (1×10^{-5} M) after addition of Fe^{2+} in the concentration range 1×10^{-6}–1×10^{-5} M. (a) 1×10^{-6} M Fe^{2+}, (b) 1×10^{-5} M Fe^{2+}.

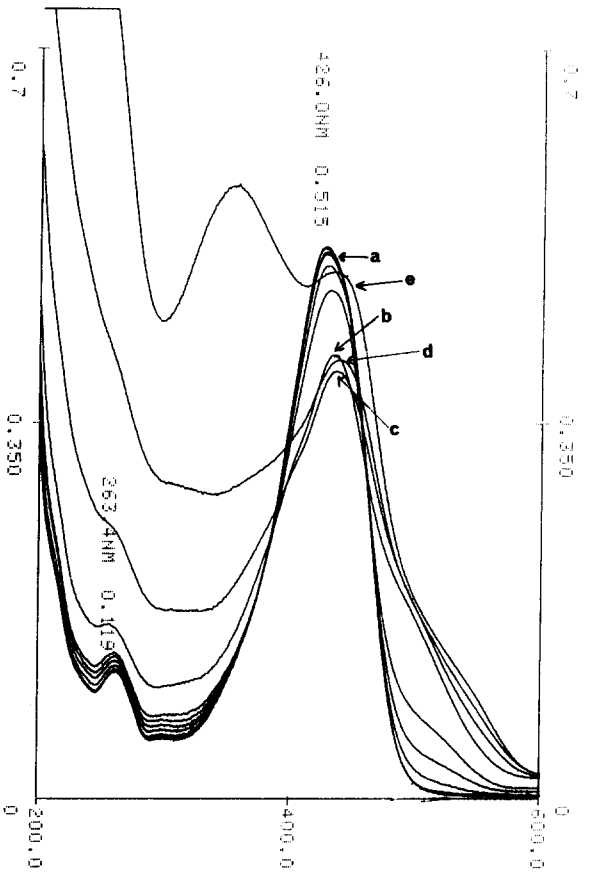

Fig. 6. Absorption spectra of curcumin (1×10^{-5} M) after addition of Fe^{3+} in the concentration range 1×10^{-6}–2×10^{-4} M. (a) 1×10^{-6}–4×10^{-6} M Fe^{3+}, (b) 2×10^{-5} M Fe^{3+}, (c) 5×10^{-5} M Fe^{3+}, (d) 1×10^{-4} M Fe^{3+}, (e) 2×10^{-4} M Fe^{3+}.

The reduction of Fe^{3+} to Fe^{2+} can be determined by the increase in absorbance at 510 nm (phenanthroline-complex method). The results are given in Table 1. As demonstrated above, the complexes formed between curcumin or the curcumin derivative and iron also absorb at 510 nm. Samples containing phenanthroline were therefore compared to similar solutions without phenanthroline as reference solutions, instead of using solutions without curcumin or the curcumin derivative in the blank cell. Curcumin forms a 1:1 complex with Fe^{2+}. In the presence of phenanthroline it is possible to obtain a mixture of complexes. To evaluate this possibility, the affinity of curcumin for Fe^{3+} originally present in

the samples and for Fe^{2+} formed in the redox reaction will have to be studied and compared to the complex formation between phenanthroline and Fe^{2+}. In the samples studied, the total concentration of iron was 10-fold greater than that of curcumin. The increase in absorbance at 510 nm was taken as a direct measure of the complex formation between Fe^{2+} and phenanthroline.

Due to the low water solubility of the curcumin derivative, the reduction of iron in the presence of this compound could only be carried out in methanol.

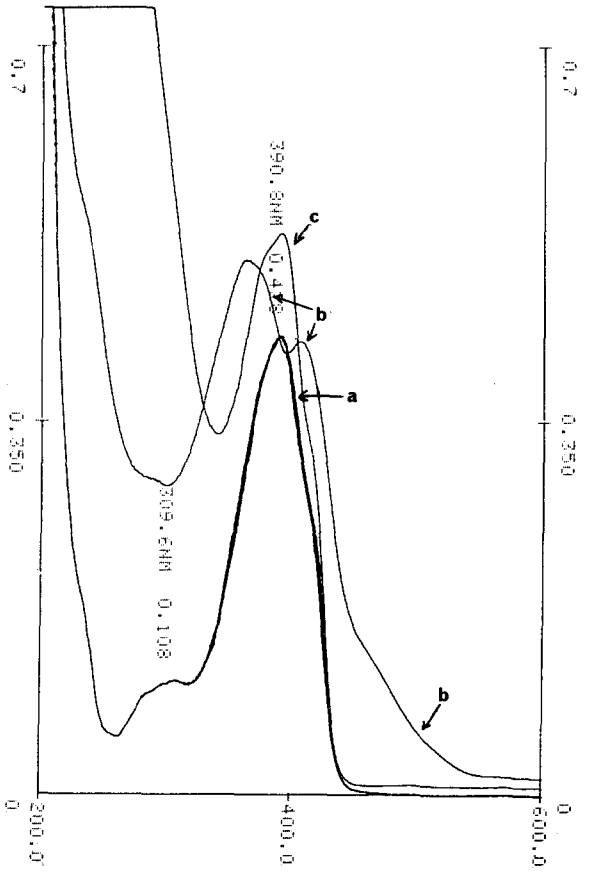

Fig. 7. (a) Absorption spectrum of the curcumin derivative (1×10^{-5} M) in methanol. (b) Absorption spectrum of the curcumin derivative (1×10^{-5} M) in methanol after addition of $FeCl_3$ (1×10^{-4} M). (c) Absorption spectrum of the curcumin derivative (1×10^{-5} M) in methanol after addition of $FeCl_3$ (1×10^{-4} M) and EDTA (1×10^{-4} M).

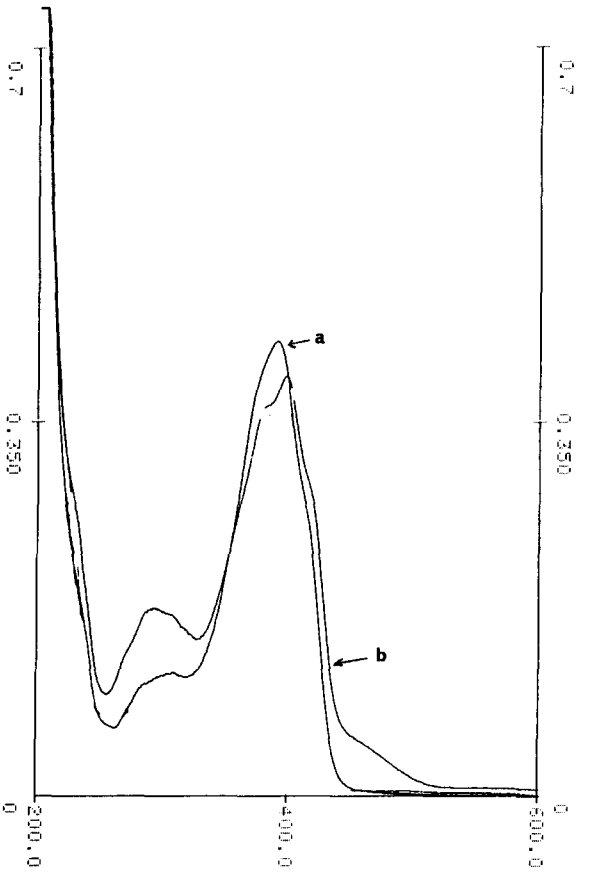

Fig. 8. Absorption spectrum of the curcumin derivative (1×10^{-5} M) in methanol (a) compared to that obtained after addition of $FeSO_4$ (1×10^{-4} M) (b).

Reduction of Fe^{3+} to Fe^{2+} could not be detected in the presence of EDTA in any of the samples (samples 1A/1B, 1E/1F and 2A/2B; Table 1). The absorbance measured at a given concentration of Fe^{2+} is reduced by approx. 60% in the presence of EDTA. This is illustrated in Table 1 (samples 2E and 2F). To compare the formation of Fe^{2+} in media with and without EDTA, the absorbance measured after addition of EDTA should be multiplied by 1.6.

Formation of Fe^{2+} could be detected in the absence of EDTA (Table 1: samples 1C/1D, 1G/1H and 2C/2D) in all the samples. HPLC analysis of the samples demonstrates that curcumin decomposes during the reaction. The first

step in the redox reaction might be the formation of the cyclization product of curcumin (Fig. 3). However, the cyclization product was not detectable by HPLC. The chromatographic conditions used will allow specific detection of curcumin and the cyclization product. A solution of curcumin in isopropanol, irradiated at 400–510 nm for 2 h, was used as reference. Under these conditions, the cyclization product of curcumin is the main decomposition product formed (Tønnesen et al., 1986). In the presence of EDTA the curcumin concentration is maintained throughout the experiment, while no decomposition occurs.

Reduction of iron by curcumin in the presence of EDTA has previously been reported (Kunchandy and Rao, 1989). The discrepancy between the previous report and the present results might be explained on the basis of an important difference in the experimental conditions. In the previous article, curcumin was dissolved in 0.1% NaOH before further dilution in buffer, pH 7.4. Curcumin undergoes hydrolytic degradation in alkali, and 50% degradation is observed in less than 2 min in dilute solution (Tønnesen and Karlsen, 1985a,b). The stock solution of curcumin used by Kunchandy and Rao (1989) therefore probably consisted of a mixture of several products. The hydrolytic degradation products formed, e.g., the aldehyde vanillin, would probably cause the reduction of iron in the presence of EDTA.

The reduction of Fe^{3+} in the presence of the curcumin derivative clearly demonstrates that the reduction of iron is not dependent upon the presence of the phenolic hydroxyl groups in the curcumin molecule.

The formation of hydroxyl radicals is observed as an increase in the absorbance at 532 nm (deoxyribose method). The results are listed in Table 2. Water-miscible organic solvents in which curcumin and its derivatives are freely soluble are quenchers of the hydroxyl radical and must be avoided in these experiments (Cederbaum and Cohen, 1986). As the curcumin reagent, a saturated solution of curcumin in phosphoric acid buffer, pH 7.2, was used. The solubility of curcumin in this solvent is approx. 10^{-6} M (Tønnesen, 1989d). Due to the very low solubility of the curcumin derivative in the phosphoric acid buffer, it was impossible to measure any effects on the formation of hydroxyl radical due to this substance.

Curcumin appears to have a catalytic effect on the formation of hydroxyl radicals generated from H_2O_2 (Table 2: samples 3C/3D). This effect is slightly greater in the presence of $FeCl_3$-EDTA (Table 2: samples 3A/3B). This is in accordance with observations made previously on the catalytic effect on hyaluronic acid degradation (Tønnesen, 1989d). The catalytic effect might be due to trace amounts of iron present in the synthetic curcumin sample. The iron content of the curcumin has been found to be less than 0.1% (Tønnesen, unpublished results). The concentration of iron from curcumin in the solutions investigated in these experiments will amount to less than 10^{-8} M. This is unlikely to explain the observed catalytic effect of curcumin on the formation of hydroxyl radicals. The catalytic effect is more likely to be caused by other mechanisms. The redox potential of iron is strongly influenced by the ligand environment. The binding of redox metal ions to chelating agents can result in enhancement or inhibition of oxidative reactions (Saltman, 1989).

An inhibitory effect on the formation of hydroxyl radicals from H_2O_2 is observed when curcumin is added to a solution containing only $FeCl_3$ (Table 2: samples 3E/3F). In the absence of H_2O_2, curcumin does not act as a source of hydroxyl radicals in this system (Table 2: samples 3G/3H and 3I/3J). The concentration of Fe^{3+} is about 100-fold greater than that of curcumin in all the samples. By doubling the concentration of iron in samples 3E and 3F, a 50% increase in the absorbance at 532 nm is observed.

The inhibitory effect of curcumin on the formation of hydroxyl radicals in the presence of $FeCl_3$ can be ascribed to the inactivation or change in redox potential of Fe^{3+} as a result of chelation by curcumin, the reduction of Fe^{3+} to Fe^{2+} due to curcumin or a scavenging effect of curcumin on the hydroxyl radicals formed. In the reaction with H_2O_2, curcumin forms ferulic acid (identified by TLC). This is in accordance with previous results (Fig. 2), and indicates that the diketone unit of curcumin is the important part

of the molecule in the scavenging of hydroxyl radicals.

Conclusion

Complex formation between curcumin and iron and the reduction of Fe^{3+} to Fe^{2+} in the presence of curcumin are independent of the phenolic hydroxyl groups in the curcumin molecule. Curcumin exerts a catalytic effect on the formation of hydroxyl radicals from hydrogen peroxide and can also act as a scavenger of oxygen radicals present in the medium. This dual effect is comparable to the self-sensitization observed in the photochemical degradation of curcumin (Tønnesen et al., 1986). The diketone system of curcumin appears to be the part of the molecule involved in the scavenging of oxygen radicals.

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