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Notes

Interaction of curcumin with glutathione

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Summary

Curcumin interacts with glutathione spontaneously and more rapidly in the presence of glutathione *S*-transferase. The interaction may involve Michael-type addition reaction between the α,β-unsaturated chromophore of curcumin and the nucleophilic glutathione.

Our earlier studies showed that curcumin is a potent scavenger of reactive oxygen free radicals, such as superoxide anion and hydroxyl radicals (Elizabeth and Rao, 1989, 1990). It has recently been reported that curcumin decreases glutathione levels in freshly isolated hepatocytes (Donatus et al., 1990). This will result in the depletion of cellular defense against oxidant stress, which will be contrary to the antioxidant properties of curcumin. However, curcumin is an α,β-unsaturated carbonyl compound which can undergo Michael-type addition reaction with nucleophilic substances. Glutathione is an important biological nucleophile because of its electron-rich sulfhydryl group. In the present study, we have shown that curcumin reacts directly with glutathione in a non-enzymatic manner and more rapidly in the presence of the enzyme glutathione *S*-transferase.

Curcumin, glutathione, and 5,5′-dithiobis(2-nitrobenzoic acid) were obtained from Sigma Chemical Co. All other reagents were of analytical grade. Curcumin was dissolved in the appropriate amount of dioxane and diluted with distilled water or buffer. The final concentration of dioxane in all the solutions was less than 0.5%. Curcumin solutions were prepared freshly before use.

Interaction of curcumin with glutathione was studied by measuring the absorption spectra of curcumin solution after the addition of various concentrations of glutathione in distilled water. Interaction at various pH values was studied by taking a solution of curcumin (250 μM) and glutathione (200 μM) in phosphate buffer (0.1 M) of various pH values. After incubation for 5 min, the absorbance of curcumin was measured at 436 nm. The difference in absorbance in the presence and absence of glutathione was plotted (Fig. 2).

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Interaction in the presence of glutathione *S*-transferase: a hepatic post-mitochondrial fraction from male albino mice was obtained and assayed for the activity of glutathione *S*-transferase using dinitrochlorobenzene as substrate (Gorden, 1986). To a reaction mixture containing curcumin (200 μM) and mouse liver post-mitochondrial fraction (0.5 ml) was added glutathione (120 μM) in a final volume of 3 ml in 0.1 M phosphate buffer pH 6.5. The absorbance was recorded at 436 nm for a period of 5 min. A control experiment was carried out without glutathione.

The glutathione level was determined by the Ellman method using 5,5′-dithiobis(2-nitrobenzoic acid) (Ellman, 1959).

Fig. 1 demonstrates the absorption spectra of curcumin in the presence of glutathione. Curve A corresponds to the absorbance of curcumin in the absence of glutathione. Two absorption maxima were observed at 436 and 360 nm. Addition of glutathione (curves 1–9) resulted in a decrease in absorption and the effect was dose dependent. The decrease in absorption indicates that glutathione undergoes reaction in the chromophore region of curcumin. Such a reaction takes place in the Michael-type addition where a nucleophile adds to the double bond. The interaction was studied at various pH values using phosphate buffer (Fig. 2). At pH 6.5 and 7.4, no appreciable interaction was observed. However, at alkaline pH, the interaction was maximum. Michael addition reactions are catalysed at alkaline pH values. Diethylmaleate, an α,β-unsaturated carbonyl compound, reacts with glutathione in a more rapid manner at alkaline pH (Boyland and Chasseaud, 1967). Thus, the interaction between curcumin and glutathione is similar to that of diethylmaleate and glutathione.

The enzyme glutathione *S*-transferase catalyses the reaction between glutathione and α,β-unsaturated carbonyl compounds and other electrophiles (Chasseaud, 1979). Hence, the interaction was studied in the presence of glutathione *S*-transferase with a mouse liver post-mitochondrial fraction being used as the enzyme source. (Fig. 3). The absorbance of curcumin decreased rapidly in the presence of the enzyme as compared to the control where the reaction was non-

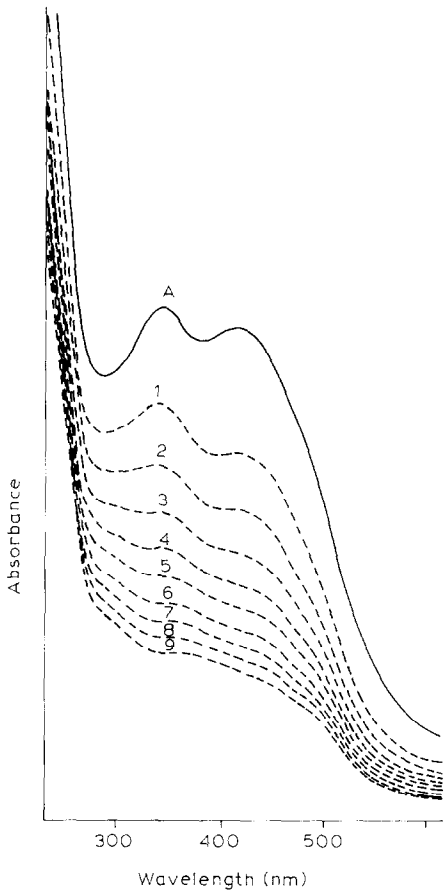
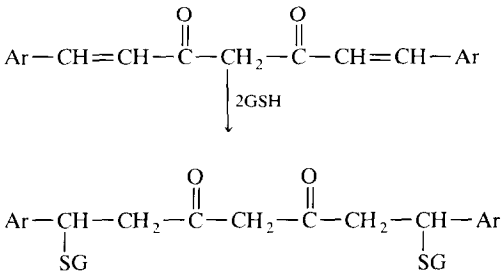


Fig. 1. Absorption spectra of curcumin (250 μM) in the presence of glutathione at various concentrations. Curve A, without glutathione; curves 1–9, with glutathione at concentrations of 15, 30, 45, 60, 75, 90, 105, 120 and 135 μM, respectively.

enzymatic. The experiment was specifically conducted at pH 6.5 in order to minimize the spontaneous non-enzymatic reaction. It has also been reported that aerobic oxidation of glutathione is at its minimum at acidic pH values (Boyland and Chasseaud, 1967). To confirm the occurrence of the enzyme-catalysed reaction, the amount of glutathione was measured after incubation with various concentrations of curcumin. The amount of free glutathione remaining after incubation decreased rapidly with increasing curcumin concentrations (data not shown).

Thus, the present study shows that curcumin can directly interact with glutathione. This reac-

tion is further catalysed by the enzyme glutathione *S*-transferase. Although the actual reaction product has not been isolated or characterized, the following reaction sequence can be proposed:



where Ar corresponds to 4-hydroxy-3-methoxyphenyl- and GSH is glutathione.

In the above reaction, curcumin acts as an electrophile and glutathione as a nucleophile to undergo Michael-type vinalogous addition at the double bond. Since glutathione is an important component of cellular defense against oxidant stress, its reaction with curcumin will result in the depletion of cellular defense. This increase in susceptibility of the cells to oxidant stress may be compensated to a certain extent by the ability of curcumin to scavenge oxygen free radicals, thereby inhibiting lipid peroxidation and other antioxidant properties.

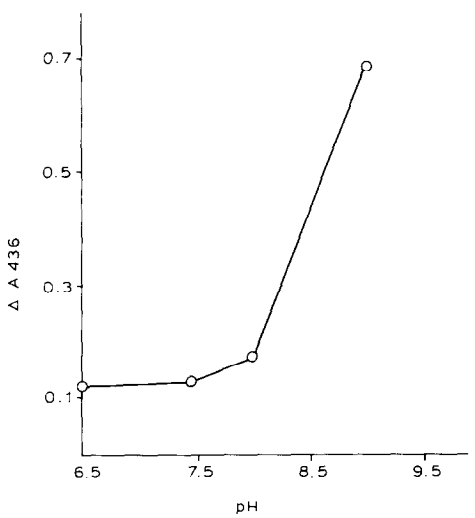


Fig. 2. Interaction of curcumin with glutathione at various pH values.

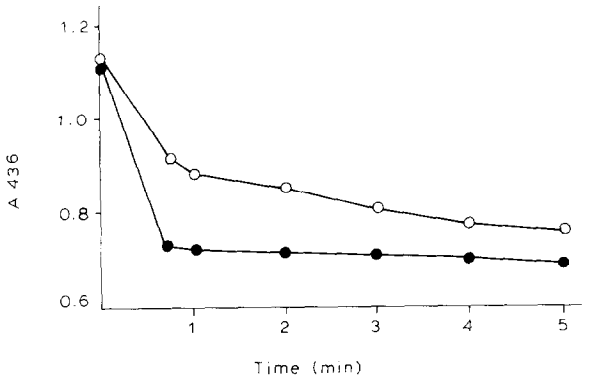


Fig. 3. Interaction of curcumin with glutathione in the presence of mouse liver glutathione *S*-transferase. (○———○) control, without glutathione *S*-transferase; (●———●) with glutathione *S*-transferase.

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