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Studies on curcumin and curcuminoids. XV. Catalytic effect of demethoxy- and bisdemethoxycurcumin on the peroxidation of linoleic acid by 15-lipoxygenase

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Curcumin and the curcuminoids belong to the group of diarylheptanoids. The rhizomes of *Curcuma longa* L. (turmeric) contain 3–5% of curcumin and two related compounds; demethoxy- and bisdemethoxycurcumin. The rhizomes of *Curcuma longa* L. are reported to have anti-inflammatory activity, which is ascribed to the content of the curcuminoids (Mukopadhyay et al., 1982; Rao et al., 1982; Deodhar et al., 1980; Satoskar et al., 1986; Yegnanarayan et al., 1976; Srimal and Dhawan, 1973; Arora et al., 1971; Sharma and Chandra, 1987; Ghatak and Basu, 1972).

Recent studies in animals indicate that the anti-inflammatory activity of pure curcumin is less than the activity observed when a mixture of the 3 curcuminoids equal to what is isolated from plant material is used (Srimal and Dhawan, personal communication). This might indicate a synergistic effect of the curcuminoids in vivo.

One of the postulated action mechanisms for non-steroidal anti-inflammatory drugs (NSAID's) is the blocking of the lipoxygenase (LO) pathway or the cyclo-oxygenase pathway (Palmer and Salmon, 1985; Paulus et al., 1987). Due to the simplicity of the method, soybean lipoxygenase has been widely used for the primary screening of LO inhibitors (Alcaraz and Ferrandiz, 1987).

In a previous work (Tønnesen, 1989), the possible inhibitory effect of pure, synthetic curcumin on 15-lipoxygenase was investigated by use of soybean lipoxygenase. Linoleic acid was used as substrate.

The results obtained showed that curcumin had a significant catalytic effect on the peroxidation of linoleic acid under the experimental conditions given. The catalytic effect was proportional to the curcumin concentration. Curcumin, however, is shown to have inhibitory effect on mammalian 5-lipoxygenase and cyclo-oxygenase (Flynn et al., 1986). The results reported by Flynn et al. were obtained using commercially available curcumin. Commercially obtained curcumin is found to contain about 6% demethoxycurcumin and about 0.3% bisdemethoxycurcumin (Tønnesen, unpublished work).

For further studies on the mechanism of the anti-inflammatory action of curcumin, a possible inhibitory effect of the other curcumin analogs and of different qualities of commercially ob-

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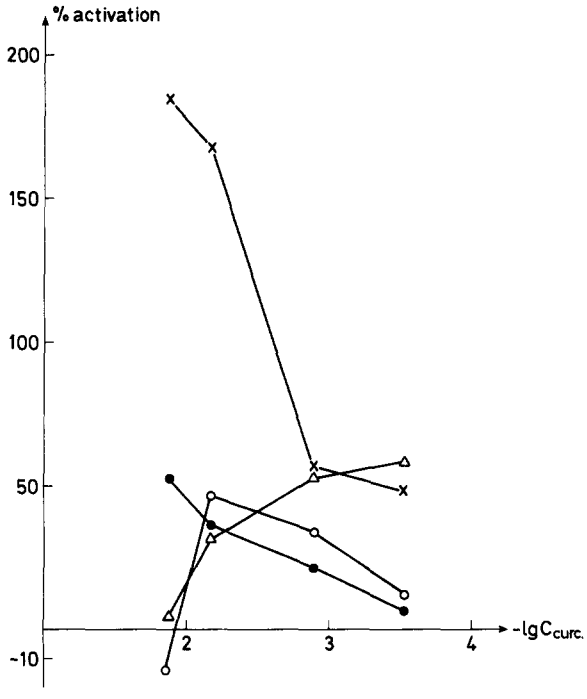

Fig. 1. Catalytic effect on the peroxidation of linoleic acid by addition of curcuminoids to the 15-lipoxygenase/linoleic acid system. (●———●), Curcumin (synthetic); (△———△), demethoxycurcumin (synthetic); (○———○), bisdemethoxycurcumin (synthetic); (×———×), curcumin (commercially obtained, Chr. Hansen Lab.).

tained curcumin on 15-lipoxygenase was investigated. Pure demethoxy- and bisdemethoxycurcumin were synthesized after the method of Pabon (Pabon, 1964). Commercially obtained curcumin was provided by Christian Hansen's Laboratories, Denmark, and by Fluka, Switzerland.

The curcuminoids were dissolved in ethanol and mixed with the reaction medium containing linoleic acid. Soybean lipoxygenase was then added. The reaction was followed by measuring an increase in absorbance at 234 nm for 90 s as described previously (Tønnesen, 1989). Different concentrations of the curcuminoids and of linoleic acid (substrate) were investigated, and the results are given in Figs. 1 and 2.

Demethoxycurcumin has a significant catalytic effect on the peroxidation of linoleic acid in the concentration range 0.0133–0.0003 mg/ml. The catalytic effect increases with decreasing concentrations of demethoxycurcumin.

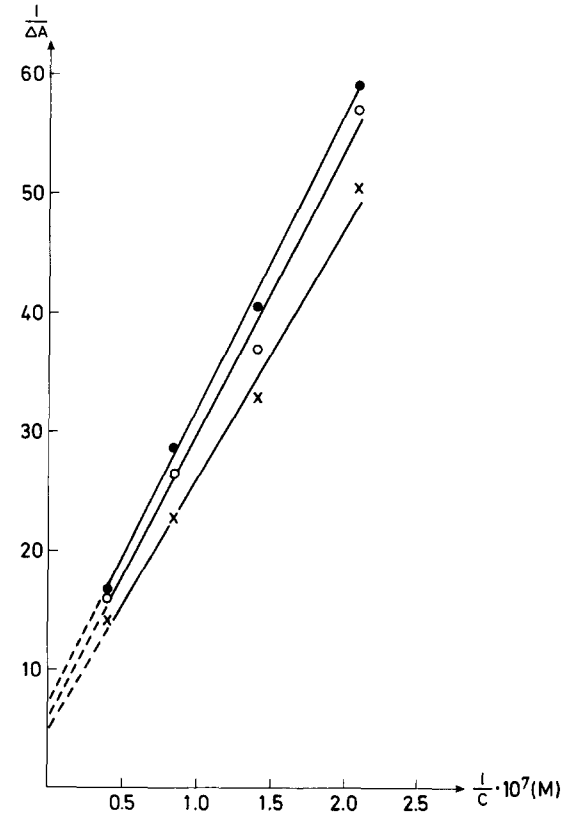

Fig. 2. Lineweaver–Burke plot of the inverse of the molar substrate concentration vs the inverse of the change in absorbance at 234 nm from $t = 30$ s to $t = 90$ s. (●———●), Sample without curcumin ($y = 2.49 \cdot 10^{-6}\ x + 6.77$, $r = 0.9988$); (○———○), demethoxycurcumin concentration 0.00133 mg/ml ($y = 2.39 \cdot 10^{-6}\ x + 5.68$, $r = 0.9965$); (×———×), bisdemethoxycurcumin concentration 0.00133 mg/ml ($y = 2.13 \cdot 10^{-6}\ x + 4.71$, $r = 0.9968$).

Bisdemethoxycurcumin in a concentration of 0.0133 mg/ml inhibits the peroxidation of linoleic acid. The experiment was performed with two different batches of synthetic bisdemethoxycurcumin.

At lower concentrations (0.0067–0.0003 mg/ml), bisdemethoxycurcumin has a catalytic effect on the peroxidation reaction. This effect is proportional with the concentration of bisdemethoxycurcumin in the samples.

The Lineweaver–Burke plots of demethoxy- and bisdemethoxycurcumin indicate an uncompetitive activation mechanism (Fig. 2). This is in agreement with what is obtained with pure curcumin (Tønnesen, 1989).

The two qualities of commercially obtained curcumin increase the peroxidation rate with a factor of about 180 compared to a sample without the addition of curcuminoids.

The results obtained in this experiment show that the 3 naturally occurring curcumin analogs have a catalytic effect on the peroxidation of linoleic acid by soybean lipoxygenase. When a mixture of the curcuminoids is used (commercially available curcumin products) the catalytic effect seems to be synergistic. If the anti-inflammatory properties of the curcuminoids are related to their catalytic effect on the 15-lipoxygenase mechanism, the synergistic effect of the curcuminoids could be a possible explanation for the difference in activity observed when pure curcumin and commercially obtained curcumin are used in animals.

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