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

Hanne Hjorth Tønnesen

Institute of Pharmacy, University of Oslo, Oslo (Norway)

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The rhizome of *Curcuma longa* Linn. (turmeric) is widely used in indigenous systems of medicine for the treatment of various inflammatory conditions. The anti-inflammatory activity is ascribed to curcumin, the main coloured compound present in the rhizomes (Mukhopadhyay 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). The mechanism of action for curcumin as a non-steroidal anti-inflammatory drug (NSAID) is still unknown. The symptomatic relief for patients with inflammatory diseases on NSAID therapy is partly recognised to be by inhibition of the cyclo-oxygenase pathway or the lipoxygenase (LO) pathway (Palmer and Salmon, 1985; Paulus et al., 1987). In vitro studies of various NSAIDs have shown a marked inhibition of lipoxygenase or cyclo-oxygenase enzymes, but there are few reports of corresponding in vivo activity (Palmer and Salmon, 1985; Paulus et al., 1987). The preparation and assay of 5- and 12-lipoxygenase inhibition is time-consuming (Kingston, 1981). Due to the simplicity of this method and since plant and mammalian lipoxygenases have biochemical properties in common, soybean lipoxygenase has been widely used for the primary screening of LO inhibitors (Alcaraz and Ferrandiz, 1987). Most of the results obtained are in good agreement with the results obtained using animal LO (Kingston, 1981; Alcaraz and Ferrandiz, 1987), although contradictory inhibitory effects are observed for certain anti-inflammatory drugs (Chang et al., 1984).

Curcumin is shown to have an inhibitory effect on mammalian 5-lipoxygenase and cyclo-oxygenase (Flynn et al., 1986). For further studies on the mechanism of anti-inflammatory action of curcumin, a possible inhibitory effect on 15-lipoxygenase was investigated by use of soybean lipoxygenase. Linoleic acid was used as substrate. This fatty acid is cheap and easy to handle compared to arachidonic acid, and it is not affected by cyclo-oxygenase (Hanche-Olsen, 1988).

Lipoxygenase from soybeans has a pH optimum of 9, and acts on linoleic acid producing 13,L-hydroperoxide and a small amount of 9,D-hydroperoxide (Halliwell and Gutteridge, 1985). This can be measured spectrophotometrically by an increase in absorbance at 234 nm. However,

Correspondence: H.H. Tønnesen, Institute of Pharmacy, University of Oslo, P.O. Box 1068, Blindern, 0316 Oslo 3, Norway.

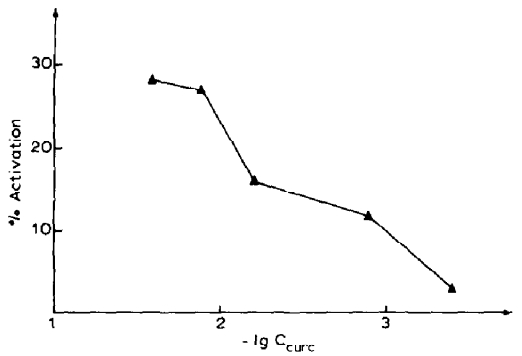

Fig. 1. Catalytic effect on the peroxidation of linoleic acid by addition of curcumin to the 15-lipoxygenase/linoleic acid system.

curcumin undergoes hydrolytic degradation at pH 9 (Tønnesen and Karlsen, 1985), and a 0.05 M phosphoric acid buffer at pH 7.2 was used as reaction medium. No pure curcumin could be obtained from commercial sources, and curcumin was synthesized after the method of Pabon (Pabon, 1964). Curcumin was dissolved in ethanol previous to mixing with the reaction medium containing linoleic acid. The lipoxygenase was added directly to this mixture in the cuvette, and the reaction was followed for 90 s. The reaction was performed with different concentrations of curcumin and also with various concentrations of substrate, and the results are given in Figs. 1 and 2. Curcumin has a significant catalytic effect on the peroxidation of linoleic acid in the concentration range 0.0005–0.027 mg/ml (Fig. 1). The Lineweaver–Burke plots indicate an uncompetitive activation mechanism (Fig. 2).

The results obtained in this experiment show that curcumin differs markedly in the activity towards different preparations of lipoxygenases, e.g. cellular 5-lipoxygenase (Flynn et al., 1986). The results reported on 5-lipoxygenase were obtained using commercially available curcumin, which normally contains a mixture of 3 curcuminoids. The catalytic effect on peroxidation of linoleic acid was therefore confirmed by use of commercially obtained curcumin (Fluka). Although linoleic acid is stable compared to arachidonic acid, polyunsaturated fatty acids are easily peroxidized by non-enzymatic methods (Halliwell and Gutteridge, 1985). Products can be formed by attack of species such as hydroxyl radicals (Brooks and Day, 1985). Results obtained in other studies on curcumin indicate that this compound is likely to be involved in hydroxyl radical reactions (Tønnesen, 1989). Fortunately, products formed by enzyme-catalysed reactions are highly stereospecific, while products from non-enzymatic reactions usually are not. To assume whether the catalytic effect on the peroxidation of linoleic acid is due to the activation of 15-lipoxygenase by curcumin or it should be ascribed to a radical reaction, the stereochemical purity of the products should be investigated. However, the observed catalytic effect of curcumin is not necessarily in contradiction with the observed anti-inflammatory effect of the compound. Speculations are made that drugs that selectively inhibit 15-lipoxygenase actually enhance rather than inhibit the formation of inflammatory arachidonic acid metabolites (Chang et al., 1984). If this should be the case, curcumin might have anti-inflammatory properties due to its catalytic effect on the 15-lipoxygenase mechanism.

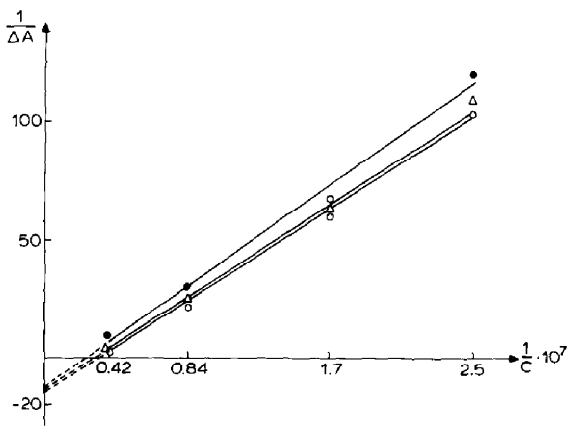

Fig. 2. Lineweaver–Burke plot of the inverse of the molar substrate concentration vs the inverse of the change in absorbance at 234 nm from $t = 30$ s to $t = 90$ s. (●●●●), Sample without curcumin ($y = 5.21 \cdot 10^{-6}x - 15.61$, $r = 0.9946$); (Δ-Δ-Δ-Δ-Δ), curcumin concentration 0.0067 mg/ml ($y = 4.89 \cdot 10^{-6}x - 16.27$, $r = 0.9977$) (O-O-O-O-O), curcumin concentration 0.013 mg/ml ($y = 4.7 \cdot 10^{-6}x - 17.00$, $r = 0.9968$).

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