Phytochemistry, 1969, Vol. 8, pp. 1675 to 1677. Pergamon Press. Printed in England

SHORT COMMUNICATION

A NOTE ON LIPOXYGENASE

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(*Received* 19 *August* 1968)

Abstract—Cauliflower tissue possesses a lipoxygenase which does not conform well with Michaelis-Menten kinetics.

THE ENZYME lipoxygenase catalyses the oxidation, by molecular oxygen, of fatty acids having a *cis, cis*-1,4-pentadiene system to form hydroperoxides; intermediates of this reaction catalyse the oxidation of carotenoid pigments and polyphenols. Similar oxidations may be catalysed by haemoproteins and other lipid peroxidation catalysts, although in these cases a wider range of unsaturated fatty acids react.[1]

The distribution of the enzyme is confined to some higher plants, occasional reports of animal sources probably being due to confusion with non-enzymic oxidations. It has been stated generally that the sources for the enzyme are legumes, some cereal grains and oil seeds,[2] although more recently this list has been extended to include other plants such as apple.[4] However, during an investigation of carotenoids in cauliflower buds,[3] it became clear that an enzyme-catalysed oxidation of these pigments was occurring immediately after disruption of the tissue. The primary cause of this carotenoid destruction has been shown to be a lipoxygenase which has been partially purified.

The partially purified cauliflower enzyme was shown manometrically to oxidize linoleate at pH 7·0 and effect an increase in the extinction of a linoleate-containing incubation mixture (pH 9·0) at 232·5 nm which could be coupled with the destruction of carotenoid. Oleate would not act as a substrate in these reactions.

The antioxidant, *n*-propyl gallate (5×10^{-3} M), partially inhibited the oxidation of linoleate and linoleate-coupled carotenoid oxidation.[5] Cysteine (5×10^{-3} M) activated crude preparations but was without effect on the partially purified enzyme. The K_m of the enzyme, estimated by the spectrophotometric technique, was found to be 5×10^{-5} M. However, at substrate concentrations above approximately 10^{-4} M, the rate of reaction was higher than that expected from the normal reciprocal plot (see Fig. 1). Using a simple incubation mixture, spectrophotometric estimations are not feasible at very high substrate concentrations owing to the insolubility of linoleate. However, manometric estimations at high substrate concentrations (above about 2×10^{-3} M) gave a K_m at about 2×10^{-3} M and a reciprocal plot deviating only at low concentrations from linearity.

[1] A. L. TAPPEL, in *The Enzymes* (edited by P. D. BOYER, H. LARDY and K. MYRBÄCK), 2nd ed., Vol. 5, p. 275 (1963).
[2] P. K. STUMPF, in *Plant Biochemistry* (edited by J. BONNER and J. E. VARNER), p. 322 (1965).
[3] S. BROWN and J. PREBBLE, *Biochem. J.* **100**, 54 (1966).
[4] L. S. C. WOOLTORTON, J. D. JONES and A. C. HULME, *Nature* **207**, 999 (1965).
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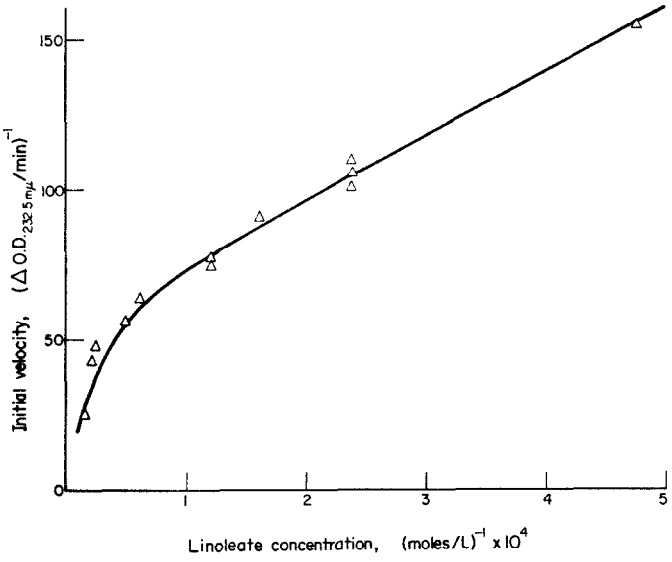

FIG. 1. THE RELATION BETWEEN THE RECIPROCALS OF THE INITIAL VELOCITY AND SUBSTRATE CONCENTRATION FOR CAULIFLOWER LIPOXYGENASE AT pH 9·0 IN NH_4OH/NH_4Cl BUFFER. THE ENZYME WAS ASSAYED SPECTROPHOTOMETRICALLY.

In general then, the cauliflower enzyme has a K_m of 5×10^{-5} M measured at concentrations below approximately 10^{-4} M; at higher concentrations up to about 10^{-2} M the enzyme becomes progressively more activated. Above about 10^{-2} M the apparent K_m is of the order of 2×10^{-3} M. The system might be considered to show an allosteric activation of enzyme activity. An alternative but related possibility may nevertheless be considered. At pH 9·0, linoleate is soluble only at the lower concentrations used in these experiments, while at higher concentrations micelle formation occurs. It appears, in fact, more likely that enzyme activation could occur by adsorption of the enzyme on the micelles where the rate of oxidation might be very greatly increased either by a zip-like action of the enzyme on a sequence of suitably oriented substrate molecules or by a change in enzyme structure. From the u.v. spectra of solutions of linoleic acid over a range of concentrations at pH 9·0, it appears that micelle formation occurs at about a concentration of 10^{-4} M (cf. the values obtained by Allen[6] for Tris buffer preparations).

Similar experiments with a partially purified[7] but not crystallized soya bean lipoxygenase showed a similar but smaller activation above 10^{-4} M and a K_m of 2×10^{-5} M. Tappel, Boyer and Lundberg[8] and also Allen[6] obtained a similar result with the soya bean enzyme and noted a deviation from the expected rates of reaction above 3×10^{-4} M.

EXPERIMENTAL

The white bud tissue of commercial cauliflowers was ground with sand to a paste and reground after the addition of cold water (35 ml/100 g wet wt. tissue). The preparation was strained through muslin and centrifuged at 20,000 *g* for 20 min at 4°, the resulting pellet being ground in water and recentrifuged. Calcium phosphate gel[9] (150 ml/400 ml extract) was added to the combined supernatants which were then centrifuged

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[9] D. KEILIN and E. F. HARTREE, *Proc. R. Soc.* B **124**, 397 (1938).

(20,000 *g*, 20 min) and the pellet discarded. Cysteine hydrochloride (0·15 g/100 ml) was added and the pH adjusted to 7·5 with Na_2HPO_4. 25 g/100 ml $(NH_4)_2SO_4$ (Analar) was added slowly, the pH being maintained above 6·5. The precipitated protein was centrifuged and discarded. The $(NH_4)_2SO_4$ concentration was increased to 60 g/100 ml and the precipitated protein, containing the enzyme, was centrifuged and resuspended in 0·1 M potassium phosphate buffer, pH 7·0. The enzyme was assayed by measurement of the increase in extinction at 232·5 nm at 30° in a Beckman DB spectrophotometer using a 1 cm light path. The substrate was prepared by the method of Tappel[4] except that an ultrasonic vibrator was used to ensure an even preparation. The incubation mixture consisted of 0·5 ml of enzyme (pH 9·0[10]) and 2·5 ml NH_4OH/NH_4Cl-buffered linoleate. Manometric assays were carried out in pH 7·0 potassium phosphate buffer at 37°.

[10] G. R. Ames and T. A. King, *J. Sci. Fd Agric.* **17**, 301 (1966).

Acknowledgement—J. P. and S. W. thank the Science Research Council for financial support.