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ENZYMIC OXIDATION OF LINOLENATE BY *GINKGO* LEAVES: FRACTIONATION AND CHARACTERIZATION OF ACTIVE FRACTIONS

RANDOLPH T. MAJOR, JAY S. ROTH and A. STEPHEN KUENKLER

Departments of Chemistry and Biological Sciences, The University of Connecticut, Storrs, CT 06268, U.S.A.

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Key Word Index—*Ginkgo biloba*; Ginkgoaceae; enzymic oxidation of linolenate; *trans*-2-hexenal formation.

Abstract—An aqueous extract of defatted, macerated leaves of *Ginkgo biloba* L. catalyzed the oxidation of linolenate. Extracts prepared from quickly frozen *Ginkgo* leaves had almost the same activity as the extract from fresh leaves but no *trans*-2-hexenal was formed. The activity was surprisingly stable at 100° and to acids. However, at pH 12 a marked loss of activity was observed, particularly when the soln was heated, and Pronase, also destroyed most of the activity.

INTRODUCTION

IT HAS been shown that fresh leaves of *G. biloba* L. give an extract catalysing the oxidation in air of the linolenate present in the leaves. This extract does not appear to be the same as soyabean lipoxidase (lipoxygenase, E.C. 1.13.1.13);[1] the purpose of the present study was to characterize it further. The effects of quickly freezing *Ginkgo* leaves on the enzyme activity and in the formation of *trans*-2-hexenal were determined.

RESULTS

Factors affecting the catalytic effect of an aq extract of defatted, macerated Ginkgo *leaves*

The extract was prepared normally from fresh leaves of *G. biloba*, essentially by the method of Major and Thomas.[1] It was found to have maximum activity at 30° and pH 5 with a concentration of 5·7 mM linolenic acid (Table 1).

Effect of freezing. Ginkgo leaves which had been quickly frozen in liquid N_2 and stored at −37° for a period up to one year retained 90% of their activity. Extracts prepared from quickly frozen leaves were kept at 4° for several days without loss of activity as an oxidation catalyst.

Effect of heat. Ashing destroyed the activity but when extracts from frozen leaves were heated at 100° for 10 min and quickly cooled to 4°, they were found to have lost only 2% of their activity. Lipoxygenase is considerably less stable to heat.[2] When acidified to pH 1·0 and heated at 100° for 10 min, cooled and neutralized to pH 4·2, 30% of activity was retained. An unheated, acidified and neutralized control retained 85% activity. Another extract of frozen leaves was brought to pH 12, heated at 100° for 10 min and then neutralized to pH 4·2. This retained 13% of the original activity while the unheated control had retained 47% activity.

[1] MAJOR, R. T. and THOMAS, M. (1972) *Phytochemistry* **11**, 611.
[2] SVENSSON, S. G. and ERIKSSON, C. E. (1972) *Lebensm. Wiss. Technol.* **5**, 118.

Ginkgo leaves, picked in August in Connecticut, gave *trans*-2-hexenal when they were macerated in air and treated as described by Major and Thomas.[1] Although the quickly frozen *Ginkgo* leaves gave almost as active an extract as fresh leaves, no *trans*-2-hexenal was obtained from leaves which had been stored at $-37°$ for 6 months. This suggests that whatever is responsible for the initial oxidation of linolenate in *Ginkgo* leaves is much more stable to freezing than that responsible for the conversion of the oxidation product into *trans*-2-hexenal.

TABLE 1. THE EFFECT OF pH AND LINOLENATE CONCENTRATION ON OXYGEN UPTAKE BY AN Aq. EXTRACT OF DEFATTED, MACERATED *Ginkgo* LEAVES

	μl O_2 Uptake after 60 min at 20°		
pH	μl O_2	Conc. linolenate (mM)	μl O_2
3	18·5	0·7	10
4	40	1·4	28
5	53	2·7	53
6	7	5·7	95
7	6	11·4	89
		23·7	63

For the pH experiments the concentration of linolenate was 1·4 mM, 0·2 ml of extract was taken and 2 ml of 0·05 M citrate buffer of the indicated pH was used. No buffer was used in the concentration experiments but the pH was essentially 4·0 ± 0·3 throughout the experiment in all cases.

Gel-filtration of aq. Ginkgo *leaf extract*

In order to determine whether the oxidation was due to an enzyme or not, the extract was passed through a Sephadex G25 column at 4° and at pH 4·2 using 0·5 M citrate buffer.

It was found that the activity to oxidise linolenate was associated with the fractions with the highest protein content. Confirmation that an enzyme was involved came from the fact that the activity was destroyed by a protease from *Streptomyces griseus*. However, there is still the problem of the stability of the extract to extremes of pH and heat.

EXPERIMENTAL

Materials. The leaves of *Ginkgo biloba* were freshly picked from the tree before use except when quickly-frozen leaves were used. The linolenic acid used was obtained from the Hormel Institute, Austin, Minnesota. The gel-filtration column that was used was 32 cm, and with Sephadex G25, had a void vol. of 57·5 ml.[3] The protease from *S. griseus* was Pronase from the Calbiochem. Corp. 53702-B-grade-45000 proteolytic units g^{-1}.

Factors affecting the catalytic activity. The preparation of extract was essentially that described by Major and Thomas.[1] Activity was measured in the Warburg, normally with 2·0 ml soln of 3·0 mM linolenic acid, buffer and extract to 4·2 ml.

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[3] Blue Dextran 2000, Pharmacia Fine Chemicals, Piscataway, New Jersey.