Metal-requiring and Non-Metal-requiring Catalysts in the Autoxidation of Methyl Linoleate[1]

M. MORITA, M. TANAKA, Y. TAKAYAMA, and Y. YAMAMOTO, Department of Food and Nutrition, Nihon Women's University, 2-8-1, Mejirodai, Bunkyo-ku, Tokyo, Japan 112

ABSTRACT

During the autoxidation of methyl linoleate, peroxide-containing substances are formed which, when added to unoxidized methyl linoleate, will catalyze oxygen uptake. Materials active only in the presence of added metal ions (MCs) were not inactivated during aerobic thin-layer chromatography on silicic acid or alumina but were selectively inactivated by treatment with triphenylphosphine. Catalysts not requiring added metal ions for activity (NCs) are not affected by triphenylphosphine, but the catalytic activity is lost during aerobic thin-layer chromatography. Autoxidized methyl linoleate was separated into four peroxide-containing fractions by elution from a silicic acid column with hexane-diethyl ether mixtures. Each fraction was found to contain both MCs and NCs.

INTRODUCTION

As previously reported (1), there are two types of catalytic materials formed in autoxidizing methyl linoleate. The first type promotes oxygen uptake in the absence of added transition metal ions and will be referred to hereafter as "non-metal-requiring catalysts" (NCs). The second type, or "methal-requiring catalysts" (MCs) are active only in the presence of added metal ions. In the present investigation, autoxidized methyl linoleate was chromatographically separated into four fractions which were tested for the presence of these two types of catalysts.

EXPERIMENTAL PROCEDURES

Methyl linoleate was oxidized by shaking in the presence of air at 26 C until a peroxide content of 800-1,000 meq/liter was attained. The autoxidized linoleate (150 ml) was diluted with two volumes of hexane and applied to a column (4 cm in diameter and 40 cm long) packed with 250 g silicic acid, partially inactivated by the addition of 50 ml water, slurried in hexane. The silicic acid was a chromatographic grade material obtained from Kanto Chemical Co., Ltd. (Tokyo, Japan). Four fractions containing hydroperoxides were eluted with hexane-diethyl ether mixtures and analyzed for hydroperoxide content and ultraviolet absorption (Table I).

Preparative Thin-Layer Chromatography

Separations were not achieved by this technique. The sole purpose of the procedure was to destroy or inactivate the catalysts which promoted oxygen uptake in the absence of added metal ions. Fractions 1 and 2 were chromatographed on 2 mm layers of Kieselgel G with benzene-ethyl acetate (95:5 v/v), and fractions 3 and 4 were chromatographed on 2 mm layers of alumina (neutral type T, Merck, Darmstadt, West Germany) developed with benzene-ethyl acetate (85:15 v/v). Samples were located by the transcription method previously described (2) and eluted with ethyl acetate.

Reduction of Hydroperoxides

An equivalent amount of triphenylphosphine (ϕ_3P), 10-20 mM in benzene, was added to aliquots of each column fraction at room temperature. After 10 min, an excess of hydrogen peroxide in 2-propanol was added, the resultant mixture washed with water, and the solvent removed under vacuum.

Oxygen Uptake Determinations

Pure methyl linoleate, prepared by passing the crude ester in hexane through a silicic acid column under nitrogen, was used as the substrate for oxidation studies. The substrate, 1 ml, and a peroxide sample, 100 μeq, were placed in a Warburg manometric flask, and the uptake of oxygen was measured in the presence or absence of cupric stearate (added as a benzene solution) at 30 C. When a sample had been pretreated by thin-layer chromatography (TLC) or with ϕ_3P, the quantity to be added was determined by the peroxide content prior to either treatment.

RESULTS AND DISCUSSION

In the absence of added metal ions, aliquots of each of the four untreated column fractions promoted oxygen uptake by methyl linoleate, whereas comparable aliquots subjected to TLC had little effect (Fig. 1). When cupric stearate (0 to 2×10^{-4}M) was added, the catalytic activity of both the untreated samples and those subjected to TLC was enhanced. Aliquots of each of the four column fractions treated with ϕ_3P promoted oxygen uptake by methyl linoleate without added metal ions. A facile explanation may be formulated by postulating as follows: The untreated fractions each contained both MCs and NCs. TLC selectively destroyed or inactivated the NCs while treatment with ϕ_3P selectively inactivated the MCs.

Cupric stearate inhibited the catalytic effect of ϕ_3P treated aliquots on oxygen uptake. It is well known that

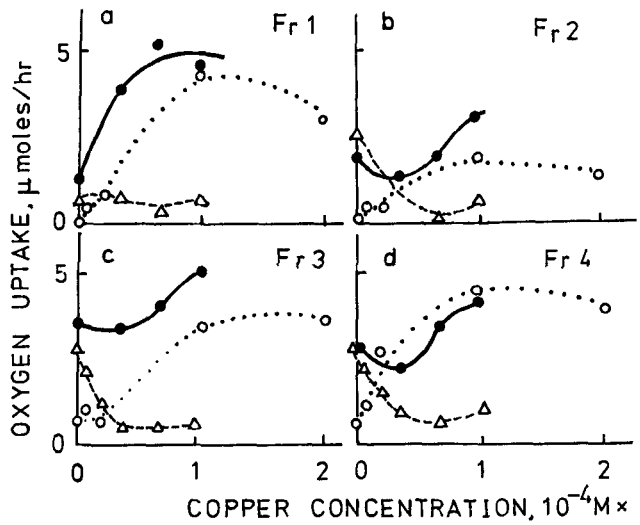

FIG. 1. Dependence of oxidation rates on copper concentration. Curves: ●——● for intact samples; ○·····○ for thin-layer chromatographic treated samples; △- - -△ for ϕ_3P-treated samples.

[1]Presented at the 4th International Symposium on Metal Catalyzed Lipid Oxidation, London, April 1975.

TABLE I

Data on Peroxide Fractions

Fraction number	Elution condition: Hexane-ethyl ether	Elution condition: ml	Rf value[a]	Yield: Weight (g)	Yield: Peroxide (mmol)	Yield: (%)	Weight of 1 mol peroxide (g)	UV absorption: λ_{max} (nm)	UV absorption: OD for 1M peroxide
1	80:20[b]	3,000	0.53	12.4	39.54	(72.7)	314	233	2.77×10^4
2	70:30[c]	1,500	0.36	1.76	5.78	(10.6)	304	232	9.14×10^3
3	50:50	3,000	0.21	2.34	8.08	(14.9)	288	232	8.72×10^3
4	0:100	1,000	0.07	0.32	0.99	(1.8)	323	232	8.28×10^3

[a]On Kieselgel G, thin layer with hexane-ethyl ether (2:1).
[b]Eluates which contained the monohydroperoxides were combined as fraction 1.
[c]After the elution of the monohydroperoxides was over, eluates were successively collected as fraction 2. The eluates by the 30% ether solvent were also combined to fraction 2.

under certain conditions copper ions act as good free-radical scavengers and may, therefore, exert an antioxidative effect (3-5). At cupric stearate concentrations greater than 1×10^{-4}M, the rate of oxygen uptake in the presence of either the untreated or chromatographed materials reached plateau. The rate of initiation resulting from the interaction of metal ions with MCs and the rate of termination reactions resulting from metal ion scavenging of free radicals are both expected to increase with increasing metal ion concentration. A balance between these positive and negative effects is visualized as producing the plateau observed at the higher metal ion concentrations.

Column fraction 1, which consisted mainly of the monohydroperoxide, was relatively free of NC activity. It is well known that hydroperoxides react with transition metal ions to generate free radicals (6). Triphenylphosphine quantitatively reduces hydroperoxides at room temperature but does not reduce C–O–O–C type peroxides such as dialkylperoxides (7-9). Since the catalytic activity of the NCs can be destroyed under more drastic conditions of reduction, it has been suggested (1) that the MCs are hydroperoxides and the NCs contain the C–O–O–C group. The NCs were essentially completely destroyed or inactivated on the TLC plates under aerobic conditions, whereas hydroperoxides and MCs are known to be unaffected by this treatment. Presumably during the thin-layer chromatographic procedure, a degradative reaction, probably oxidative in nature, occurred while the material was thinly distributed on the surface of the adsorbent.

Hydroperoxides do not readily undergo thermal homolysis at room temperature, whereas thermal homolysis of the C–O–O–C type peroxides procedes rapidly (10). The C–O–O–C type peroxides therefore contribute to free-radical generation in the absence of added metal ions by a reaction which is relatively insensitive to metal ions or reducing agents.

ACKNOWLEDGMENTS

The authors wish to thank Prof. M. Fujimaki (University of Tokyo) for his interest and continuing encouragement.

REFERENCES

1. Morita, M., and M. Fujimaki, Agric. Biol. Chem. 37:1213 (1973).
2. Morita, M., and M. Fujimaki, J. Agric. Food Chem. 21:860 (1973).
3. Kharasch, M.S., and A. Fono, J. Org. Chem. 24:72 (1959).
4. Kharasch, M.S., and A. Fono, Ibid. 24:606 (1959).
5. Kochi, J.K., in "Free Radicals," Vol. I, Edited by J.K. Kochi, John Wiley & Sons Inc., New York, NY, 1973, pp. 595-598.
6. Hiatt, R., in "Organic Peroxides," Vol. II, Edited by D. Swern, John Wiley & Sons Inc., New York, NY, 1971, pp. 102-105.
7. Mair, R.D., and R.T. Hall, Ibid., pp. 538-540.
8. Mair, R.D., and R.T. Hall, Ibid., pp. 601-609.
9. Dulog, L., and K.H. Burg, Z. Anal. Chem. 203:184 (1964).
10. Hiatt, R., T. Mill, and F.R. Mayo, J. Org. Chem. 33:1416 (1968).

[Received April 14, 1975]