Lipid-Oxidation Catalyses by Substances in Water on Lipid-Water Interface[1]

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ABSTRACT

Methyl linoleate and water containing heme, ferrous sulfate, cysteine, or ascorbic acid were shaken, and the oxidation was measured by means of a Warburg manometer. In spite of a small area of the interface between the lipid and water, oxidation was greatly accelerated by these catalysts. The catalyses occurred limitedly in the interface region, except for cysteine. Triphenylphosphine added to the lipid phase inhibited the catalyses completely. A small amount of Tween 20 (0.2%) almost completely inhibited these catalyses, with the exception of that by cysteine. It is suggested that trace amounts of some active peroxides react with the catalysts and supply radicals on the interface.

INTRODUCTION

From the point of view of the food chemist, promotion of lipid oxidation by catalysts in the aqueous phase, e.g., heme, water-soluble salts of transition metals, thiols, and reductones, is more important than promotion of lipid oxidation by such lipid-soluble catalysts as heavy metal soaps. Although a number of studies on lipid-water systems have been presented, of which references 1-3 are typical, the complexity of the heterogeneous system has resulted in relatively little knowledge regarding the mechanism of catalysis. The present paper deals with the promotion of lipid oxidation at the lipid-water interface and offers an explanation of the mechanism.

EXPERIMENTAL PROCEDURES

Materials

Methyl linoleate purified by passage through a silicic acid column was used as the substrate for the oxygen uptake studies (4). Autoxidized methyl linoleate was prepared as described previously, and the hydroperoxide groups were reduced by treatment with triphenylphosphine, ϕ_3P (5). The samples of pure and autoxidized methyl linoleate were stored under carbon dioxide at low temperature until needed. A hematin solution was prepared by dissolving hemin in a minimum volume of 1/1,000 N sodium hydroxide and then diluting to a final concentration of 1×10^{-5}M. The pH of the solution was nearly neutral. Aqueous solutions of L-cysteine (10^{-3}M), CySH, and ascorbic acid (10^{-3}M), ASA, were freshly prepared before use. The free form of CySH was used, and the solution of ASA was neutralized with sodium bicarbonate (10^{-3}M, final).

Methods

Oxygen uptake was determined manometrically at 30 C on a Warburg apparatus. Methyl linoleate, usually 1 ml, and 0.5 ml of an aqueous solution of catalyst were shaken for 30-60 min prior to beginning measurements of oxygen uptake. Lipid-soluble materials such as autoxidized linoleate, Tween 20, decyl alcohol, or triphenylphosphine were added to the lipid phase and their concentration expressed on the basis of the volume of lipid phase.

RESULTS AND DISCUSSION

In preliminary experiments, aqueous solutions of heme, ferrous sulfate, cysteine, and ascorbic acid were found to promote the uptake of oxygen by methyl linoleate in the absence of any agents capable of emulsifying or dissolving both lipid and aqueous phases. Figure 1 shows the results of an experiment in which we sought to determine whether the catalytic activity was expressed only at the interface or occurred by permeation of some active species through the interface into the lipid phase. The relative volumes of the lipid and aqueous phases were varied, 1.0, 1.5, and 2.0 ml, while maintaining the total volume, 3.0 ml, constant. With the manometric flasks used in these experiments, the interfacial surface areas at rest were essentially constant, with values observed in all flasks at the various ratios of lipid: water utilized being within ± 2% of the average value, 8.29 cm^2.

Figure 1a shows that the rate of oxygen uptake was nearly proportional to the volume of the lipid in the case of cysteine. The rates of oxygen uptake were essentially independent of lipid volume with heme (Fig. 1b), ferrous sulfate (Fig. 1c), or ascorbic acid (Fig. 1d) as the catalyst. It was, therefore, concluded that the actions of the catalysts, except cysteine, are limited to the interfacial region, and cysteine acts not only at the interface but also in the lipid phase.

Table I illustrates an experiment where the addition of ϕ_3P (0.2%) prior to incubation effectively inhibited the

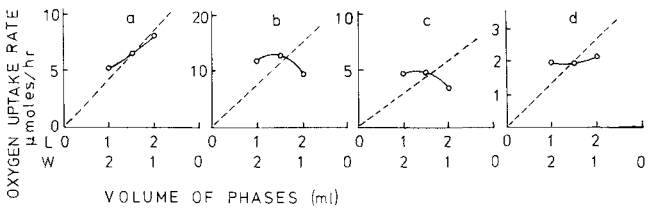

FIG. 1. Effect of change in phase volumes on autoxidation of methyl linoleate. Catalysts in the aqueous phase: (a) CySH (L-cysteine), (b) heme, (c) $FeSO_4$ and (d) ASA (ascorbic acid). Phases: L = methyl linoleate; W = water. Broken lines indicate proportional relation of oxidation rates to lipid volumes.

TABLE I

Effect of Triphenylphosphine on Autoxidation in the Presence of Catalysts

Catalyst	Rate of oxygen uptake (μmol/hr)	
	Control	ϕ_3P[a] (0.2%)
Heme	6.41	-0.06
$FeSO_4$	0.41	0.06
CySH (L-cysteine)	0.76	0.04
ASA (ascorbic acid)	0.31	0.04
NC[b]	1.46	1.72

[a] ϕ_3P = triphenylphosphine.

[b] NC (non-metal-requiring catalyst) was contained in methyl linoleate; see text.

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TABLE II

Effect of Tween 20 on the Rate of Autoxidation of Methyl Linoleate with Catalysts

	Rate of oxygen uptake (μmol/hr)					
	Substrate					
	Pure methyl linoleate					Autoxidized methyl linoleate[a]
	Catalyst[b]					
Additive (%)	No catalyst	Heme	$FeSO_4$	CySH	ASA	No catalyst
Tween 20						
0	0	4.88	1.10	2.33	2.97	1.93
0.1	0.05	0.26	0.21	0.83	-0.12	
0.2	-0.06	0.17				1.95
0.5	0.03	0.19	0.25	0.72	-0.20	
1	-0.13	0.20	0.31	1.04	-0.06	
Decyl alcohol[c]						
0		5.31	1.60	4.55	0.71	
0.2		5.90	2.44	2.34	0.80	

[a]The sample was diluted with pure methyl linoleate so as to be at a 100 μeq level of peroxide.

[b]CySH = L-cysteine, ASA = ascorbic acid.

[c]The experiment for decyl alcohol was a different run from the other experiments in the table.

catalytic effects of heme, ferrous sulfate, cysteine, and ascorbic acid. Since ϕ_3P is capable of reducing hydroperoxides at room temperature (5), the prooxidants must act through free-radical production from preformed hydroperoxides. Materials thought to be C–O–O–C type peroxides are present in autoxidized methyl linoleate and are capable of promoting lipid oxidation in the absence of added transition metal ions (6). A sample of autoxidized methyl linoleate treated with ϕ_3P to reduce hydroperoxides and then with hydrogen peroxide to destroy the excess ϕ_3P was utilized as a source of such materials. Thermal homolysis of the C–O–O–C type peroxides to produce alkoxy free radicals promotes lipid oxidation in the absence of, and independent of, added metal ions. Addition of ϕ_3P to this incubation mixture did not effect oxygen uptake, suggesting that ϕ_3P does not function as an effective free-radical scavenger.

An attempt was made to crudely evaluate the relative polarity of the peroxides, which are the source of free radicals at the interface. Amphiphilic materials were added to attempt to displace the peroxides from the water-lipid interface. Tween 80 had been previously shown to have this effect in studies with a heme catalyst (3). A preliminary experiment showed that the minimum concentration of Tween 20 necessary for the inhibition was 0.001%. Tween 20 neither promoted nor inhibited the autoxidation of methyl linoleate in the absence of aqueous solutions of catalysts (Table II). Decyl alcohol (0.2%) did not have an inhibitory effect on the prooxidant effect of the materials tested (Table II). The polarity of decyl alcohol seems to be similar to that of the linoleate monohydroperoxide by the criteria of R_f during thin layer chromatography on silicic acid. Since the peroxides giving rise to new free radicals are able to successfully compete for position at the lipid-water interface with decyl alcohol, but not Tween 20, it is suggested that they are more polar than linoleate monohydroperoxide.

A hypothesis is presented to attempt to explain why both transition metal ions and reducing agents can promote lipid oxidation via reactions at the interface. An electron or a hydrogen atom is supplied by a catalyst molecule in the aqueous phase to the OH group of a hydroperoxide molecule in the lipid phase. Contact and therefore interaction can only occur at the lipid-water interface. The resultant cleavage of the –O–O– bond produces an alkoxy free radical in the lipid phase.

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