THE EFFECT OF CALCIUM ON PHOSPHOLIPID PEROXIDATION

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Received November 29,1976

SUMMARY

The effect of calcium ions on the peroxidation of ox-brain phospholipid liposomes in different free-radical catalysing systems has been assessed. Using thiobarbituric acid-reactivity (TBA) as a measure of lipid peroxidation, calcium ions both inhibited and enhanced peroxidation in the different systems.

Changing the composition of the ox-brain phospholipid liposome with synthetic non TBA-reactive phosphatidylcholine, significantly altered its susceptibility to peroxidation both in the presence and absence of calcium ions.

The results are discussed with reference to the possibility that calcium ions induce conformational changes in membrane phospholipids. Susceptibility to peroxidation is then influenced by a complex interrelationship between the qualitative lipid composition of the membrane, the pro-oxidant catalyst and the presence of calcium or other active ions.

INTRODUCTION

Lipid peroxidation in isolated tissue homogenates, cells and organelles is a complex event known to involve both enzymic and non-enzymic catalytic mechanisms.

Transition metal ions are important components of non-enzymic tissue peroxidation. These in combination with compounds such as

ascorbic acid become extremely effective redox-coupled free-radical generating systems (1, 2, 3). Relatively few metals, which undergo a change in valency involving a single electron transfer, can catalyse a rapid rate of oxidation in unsaturated lipids (4). Non-variable valence state metals such as calcium, magnesium and zinc which cannot take part in redox-coupled homolysis have also been shown to influence lipid peroxidation in cell and organelle preparations (5, 6, 7).

The present communication was prompted by a finding in this laboratory that the in vitro susceptibility to peroxidation of human erythrocyte membrane lipids following incubation with hydrogen peroxide, can be prevented by the addition of calcium ions. This finding has been further investigated using ox-brain phospholipid liposomes peroxidised in different free-radical catalysing systems, before and after the addition of calcium ions. The resulting peroxidation was measured as TBA-reactive compounds and expressed as malonyldialdehyde (MDA). Liposomes prepared from ox-brain phospholipids and varying amounts of non TBA-reactive phospholipid were also incubated with calcium ions. The addition of both calcium and non TBA-reactive phospholipid significantly altered the susceptibility of ox-brain phospholipids to peroxidise.

MATERIAL AND METHODS

Chemicals were obtained from BDH Ltd. Ferrous and ferric ammonium sulphate, cuprous and cupric chloride, ascorbic acid and calcium chloride, were of 'Analar' grade and hydrogen peroxide of 'Aristar' grade.

Dialuric acid and L-3 dipalmitoyl phosphatidylcholine were obtained from Koch-Light Ltd. The solutions containing ferrous ions, cuprous ions and dialuric acid were prepared in deionised

water purged with oxygen-free nitrogen and used within 2 mins of preparation.

Ox-brain phospholipids prepared by a Folch extraction (8) were rapidly extracted from fresh brain. Oxyhaemoglobin from normal human erythrocytes was freed from protective enzymes (mainly catalase) using DEAE Cellulose DE-52 (9). Ox-brain phospholipid liposomes containing 5 mg/ml lipid in 0.15 M NaCl pH 7.4 were prepared by vigorous vortex mixing for 5 min (10). Liposomes with a varied lipid composition were prepared by adding 10 mg of ox-brain phospholipid dissolved in chloroform to glass tubes containing differing amounts of synthetic phosphatidylcholine in chloroform. The lipid mixtures were dried under nitrogen, 2 ml of 0.15M NaCl pH 7.4 added and the samples sonicated for 1 min (Rapids 50 with 3 mm probe, Ultrasonics Ltd.). The tubes were flushed with nitrogen and allowed to stand for 1 hour at $4^{\circ}C$ before use.

Peroxidation was carried out on 0.5 ml of the lipid sample. To each test 0.1 ml of 100 mM calcium chloride was added and to the control 0.1 ml of 0.15M NaCl followed by 0.1 ml of the free-radical catalysing reagents to both tubes. Where necessary the total volume of each tube was brought to 0.8 ml by the addition of 0.15M NaCl. The tubes were capped and incubated for 2-hours at $37^{\circ}C$. Samples for UV irradiation were placed in plastic analyser cups and irradiated at room temperature for 2-hours at a distance of 8 cm from a 366 nm light source (Anderman Ltd.). After incubation or irradiation, 3 ml of 2.9 M HCl containing 1% sodium arsenite was added to each tube followed by centrifugation at 4,000 rpm for 10 min. 3 ml of supernatant was added to 1 ml 1% thiobarbituric acid in 0.05M NaOH. The tubes were heated in a boiling water bath for 15 min and the resulting chromogen read at 532 and 600 nm. Since copper ions cause some precipitation

TABLE 1

Peroxidation Catalyst	Control (100 %) mM MDA/mg PL ± SD	The Effect of 100 mM Ca^{++} on Lipid Peroxidation in Phospholipid Liposomes	
		% increase	% Inhibition
2mM Ferrous ions	10.3 ± 2.8	6	0
2mM Ferric ions	3.9 ± 0.3	0	10
2mM Cupric ions	4.6 ± 0.9	90	0
0.5 mM Cuprous ions	4.5 ± 1.0	0	37
10mM Ascorbic Acid	16.9 ± 1.1	0	62
10mM Dialuric Acid + 20 /uM Ferrous ions	3.7 ± 0.7	0	60
10 mg/ml Oxyhaemoglobin	2.4 ± 0.5	20	0
10 mg/ml Oxyhaemoglobin + 10 mM Hydrogen peroxide	4.4 ± 0.3	0	98
Ultra Violet light 366 nm	5.7 ± 0.4	70	0

of the TBA chromogen at acid pH, the tubes containing copper reagents were read at 540 nm after addition of 1 ml of 10 M NaOH. Malonyldialdehyde was calculated as nM MDA/mg phospholipid after subtracting absorbance at 600 from that at 532 nm.

RESULTS

Table 1 summarises the percentage mean increase or decrease in MDA formation (compared to the control (100 %)), brought about by addition of calcium ions to peroxidising ox-brain phospholipids. Reagent concentrations are expressed as initial values and not the final reaction concentration.

In all systems the tubes containing calcium ions showed visible evidence of phospholipid aggregation at the end of the incubation period. Calcium ions had no effect on the TBA assay procedure confirming that only phospholipid was involved.

The pro-oxidant effect of oxyhaemoglobin was enhanced by the addition of calcium ions. The results for peroxidation with hydrogen peroxide/oxyhaemoglobin were therefore calculated after subtraction of appropriate controls. Calcium ions significantly inhibited the peroxidation catalysed by hydrogen peroxide, confirming our previous observation with washed human erythrocytes.

In the second experiment increasing amounts of synthetic phosphatidylcholine (PC) were added to a constant weight of peroxidisable ox-brain substrate. The PC contained only saturated fatty acids and could not therefore contribute any TBA-reactive compounds to the final colorimetric measurement. It did however significantly alter the substrates susceptibility to peroxidiation. Ferrous ion catalysed peroxidation decreased with increasing concentrations of PC. The effect of calcium was to reverse

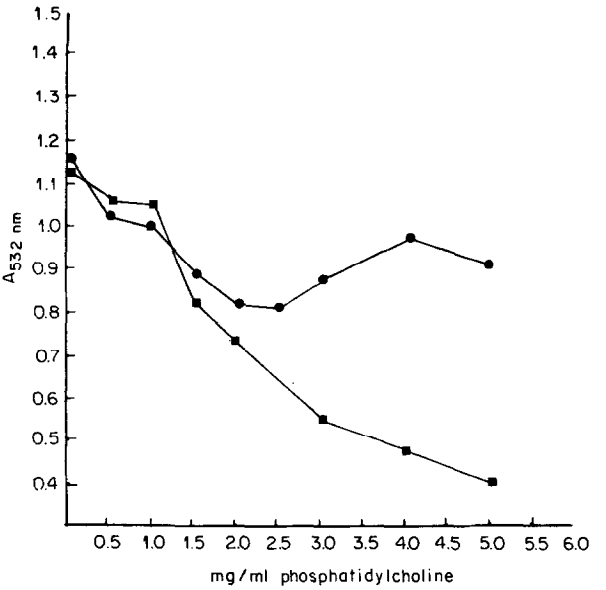

Figure 1.

■ The effect of increasing amounts of synthetic phosphatidylcholine on the peroxidation of ox-brain phospholipids (5mg/ml) catalysed by 2mM ferrous ions.

● Addition of 100 mM Ca^{++} to the samples.

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this trend (fig.1). Ascorbic acid catalysed peroxidation (fig.2) was enhanced by the addition of low concentrations of PC. Higher concentrations of PC only slightly reduced susceptibility to peroxidation. Calcium ions were inhibitory and this paralleled the effect of PC on peroxidation.

DISCUSSION

The mechanisms involved in cell and organelle peroxidation are extremely complex involving a balance between naturally occurring antioxidants and pro-oxidants. The term antioxidant in this context is taken to include a less well defined variable,

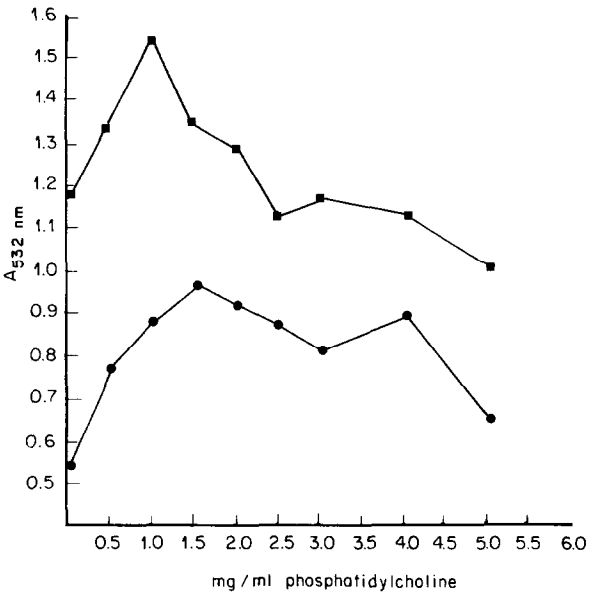

Figure 2.

■ The effect of increasing amounts of synthetic phosphatidylcholine on the peroxidation of ox-brain phospholipids (5mg/ml) catalysed by 10 mM ascorbic acid.

● Addition of 100 mM Ca^{++} to the samples.

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that of structural integrity of the cell or organelle. Studies with erythrocyte membranes indicate that the qualitative lipid composition of the membrane influences its susceptibility to peroxidation (11, 12). This change is not simply an increase in polyunsaturated fatty acids providing more substrate for MDA formation, but involves lipids which do not readily form a TBA-reactive chromogen.

Dialuric acid required the presence of micromolar amounts of iron, similarly hydrogen peroxide required oxyhaemoglobin for use with the TBA reaction (10).

Increasing the percentage composition of phosphatidylcholine in the ox-brain phospholipid liposomes significantly altered their susceptibility to peroxidation. Phosphatidylcholine can behave as an antioxidant; a property which may depend on its ability to form tightly bound ligands around catalytic metal ions (2). This could explain the inhibitory effect of an increasing PC concentration with ferrous ion catalysed peroxidation. Calcium might then act by displacing ferrous ions, so increasing peroxidation. Alternatively phosphatidylcholine may influence the membrane behaviour of other phospholipids by changes related to the orientation of its polar group (13).

Calcium ions have been shown to induce changes in membrane lipids of both intact cells and model bilayers. They are thought to play an important role in membrane flexibility, ionic permeability and structural conformation (14). The inhibitory effect of calcium ions on peroxidising liver microsomes was noted by Wills in 1969 (6) who suggested they acted by altering membrane phospholipid configuration. Calcium ions are also important in cell fusion (15) and they may facilitate this process by inducing conformational changes in membrane phospholipids (16).

The results show that peroxidation of cell membrane lipids, in addition to many other factors, involves a complex interrelationship between the qualitative nature of the lipid in the peroxidisable substrate, the pro-oxidant catalyst and the presence of calcium or other active ions in the system.

ACKNOWLEDGEMENT

The author wishes to thank the Whittington Hospital Management Committee for facilities to carry out this work, and Professor J.A.Lucy for his helpful advice.

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