Vol. 80, No. 4, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1978 Pages 704-708

A QUANTITATIVE RELATIONSHIP BETWEEN PERMEABILITY AND THE DEGREE OF PEROXIDATION IN UFASOME MEMBRANES

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Received December 9,1977

SUMMARY: Lipoxygenase was used to induce release of sequestered glucose from vesicles made from linoleic acid. Small amounts of peroxide (up to 4-5%) did not produce any significant efflux of glucose, but peroxidation above this level led to a rapid increase in the leakage rate. The maximum rate of glucose efflux was proportional to the rate of peroxidation of the fatty acids in the vesicle membranes.

INTRODUCTION

Peroxidation of membrane lipids in vivo has been implicated in many forms of tissue breakdown (1). It was usually assumed that an increase in membrane permeability resulted from the peroxidation, because both were often seen to occur together (2, 3). However it has not proved possible to establish a quantitative relationship between the degree of peroxidation and leakiness of natural membranes because the techniques available for measurement of peroxidation and permeability are neither fast nor reliable enough for the purpose (3, 4, 5). Even studies with the relatively simple phospholipid liposomes have proved inconclusive (6, 7).

In this study, the permeability of the membranes of ufasomes (8, 9) was measured as a function of the degree of peroxidation of their constituent fatty acids. The chemical simplicity of ufasomes means that both these variables could be measured reliably.

METHODS

Oleic and linoleic acid ufasomes with sequestered glucose were prepared as described previously (8). After removal of untrapped glucose by dialysis, the preparations were kept in ice to ensure that no peroxidation or glucose efflux took place. All measurements of peroxidation and permeability were completed within 5 hours of removal of the samples from dialysis.

Peroxidation was induced with soy bean lipoxygenase, EC 1.13.11.12 (Sigma Chemical Co., St. Louis, Mo.). Formation of linoleate peroxides was

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followed continuously at 234 nm in a Pye Unicam SP1800 spectrophotometer at 22°C. A molar extinction coefficient of 28,000 litre $mole^{-1}cm^{-1}$ was used to calculate the hydroperoxide concentration (10). The specific activity of the enzyme was measured before each experiment (11).

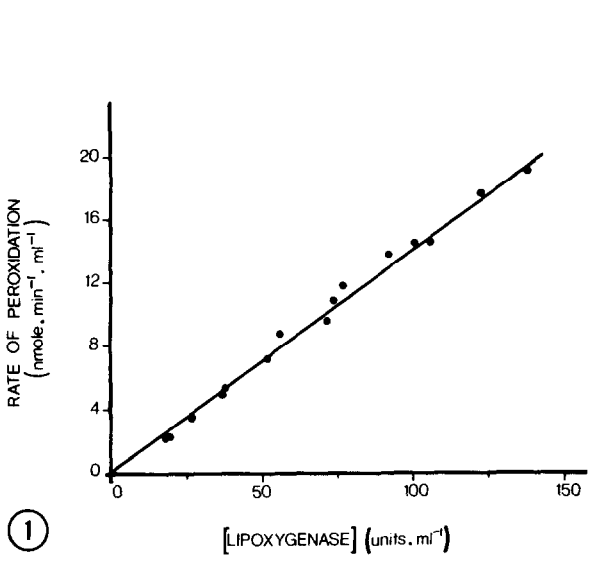
The concentration of glucose in solution was measured as an increase in NADPH absorbance at 340 nm using the linked reactions of hexokinase (EC 2.7.1.1) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) (8).

RESULTS AND DISCUSSION

The rate of peroxidation of linoleic acid ufasomes was directly proportional to the concentration of lipoxygenase (figure 1). Inactivated lipoxygenase was ineffective. The kinetics of glucose release from the vesicles under different conditions are shown in figure 2. Detergent addition (curve A) solublised the membranes, releasing all trapped glucose. Increasing amounts of lipoxygenase (curves C and B) produced increasing rates of glucose release after a lag period, the duration of which was inversely proportional to the lipoxygenase concentration. However, even the highest concentration of lipoxygenase used did not release all of the trapped glucose. This agrees with the finding that peroxidation of linoleic acid by the enzyme does not go to completion (12).

The maximum rate of glucose efflux from linoleic acid ufasomes was proportional to the concentration of lipoxygenase (figure 3). The figure also shows that the enzyme was unable to induce leakage from oleic acid ufasomes. Lipoxygenase fails to peroxidise monoenoic fatty acids. Treatment of oleic or linoleic acid ufasomes with inactivated lipoxygenase or with bovine serum albumin at similar concentrations to the active enzyme failed to release trapped glucose. Thus, in the above systems, increases in membrane permeability were strictly dependent on the peroxidation of the constituent fatty acids.

When the kinetics of glucose leakage and the extent of peroxidation in linoleic acid ufasomes were followed simultaneously, (figure 4), it became evident that there was a significant accumulation of peroxide before glucose was released to any great extent. Little glucose leaked out when less than 5% of the membrane fatty acid was peroxidised. However, beyond that point



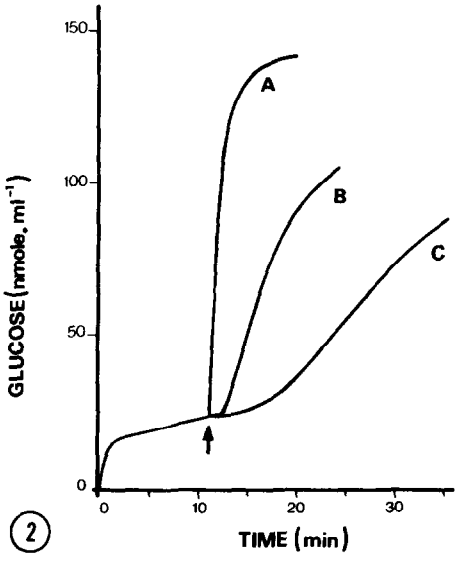


Figure 1: Rate of peroxidation of linoleic acid ufasomes as a function of lipoxygenase concentration. Between 0.05 and 0.25 ml of 0.025 mg/ml enzyme solution was added to 2.7 ml Tris pH 8 (Sigma). The reaction was started by adding 0.05 ml of ufasome preparation containing 0.5 mg fatty acid. Rates were calculated from recorder traces of A_{234} with time.

Figure 2: Typical glucose assays traced from chart recorder. The reaction was started at t = 0 by addition of 0.05 ml of a ufasome preparation containing 0.5 mg linoleic acid to a cuvette containing the reaction mixture described previously (8). The initial increase (to t = 12 min) represents the glucose remaining outside the ufasomes. Curve A shows glucose release after addition of 0.1 ml of 10% Triton X-100 (Calbiochem). Curves B and C show the kinetics of glucose release after addition of 170 and 56 units of lipoxygenase/ml assay respectively. Both detergent and enzyme additions were made at the arrow.

the amount of glucose released increased rapidly with peroxidation. Figure 5 shows that the rate of glucose release increases after an accumulation of 4-5% peroxide, reaching a maximum after about 10% peroxidation. This final rate was proportional to the concentration of the enzyme, as shown in figure 3.

The main effect of peroxidation on the ufasome membranes is likely to be a disturbance of the normal bilayer arrangement of fatty acid molecules (9). Introduction of a bulky hydrophilic group would distort the hydrophobic membrane interior, allowing an easier passage of water soluble

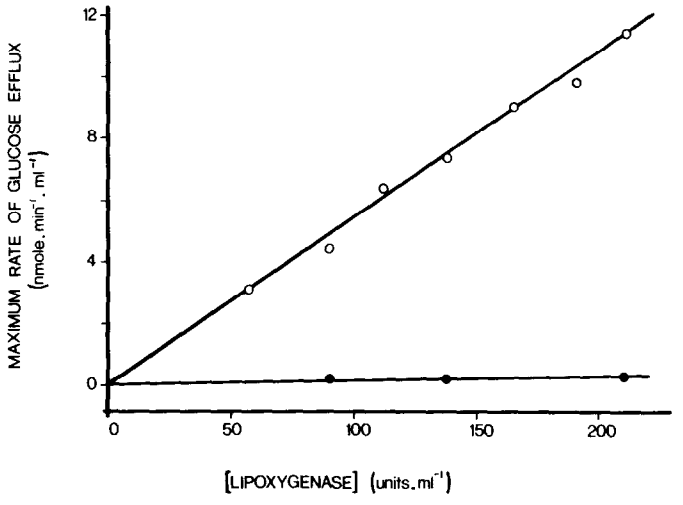


Figure 3: Effect of lipoxygenase concentration on maximum rate of glucose release from ufasomes. O linoleic, ● oleic acid ufasomes.

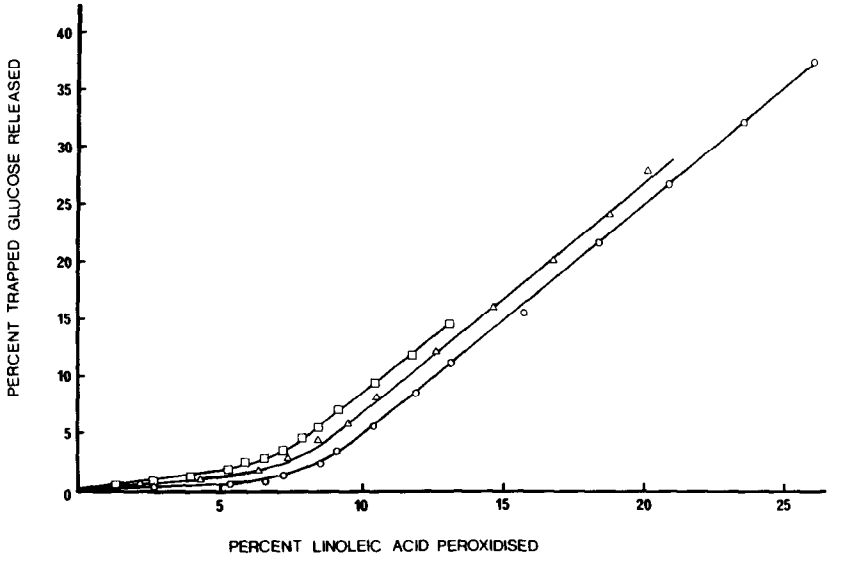


Figure 4: The fraction of trapped glucose released when the ufasome membranes reached different levels of peroxidation. Lipoxygenase levels were □ 111, △ 138, o 165 units/ml of assay.

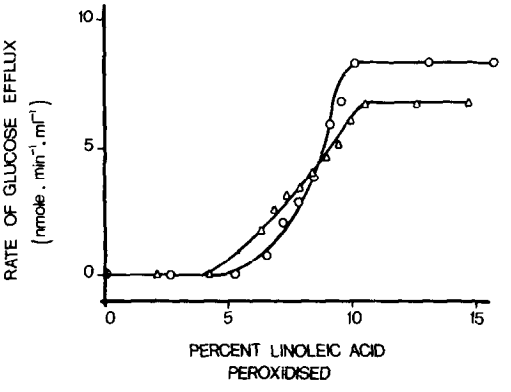


Figure 5: The effect of the degree of peroxidation on the rate of glucose efflux from ufasomes. Lipoxygenase concentrations were Δ 138, O 165 units/ml of assay.

molecules. Evidently the membranes can tolerate a considerable degree of peroxidation before a permeability change can be detected. It is interesting to note that the membranes do not disintegrate during peroxidation, since a limiting permeability is reached when 1 in 10 of the membrane fatty acid molecules is peroxidised.

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