α-TOCOPHEROL AND THE PERMEABILITY TO GLUCOSE AND CHROMATE OF UNSATURATED LIPOSOMES

A. T. DIPLOCK, J. A. LUCY, M. VERRINDER and A. ZIELENIEWSKI
Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine, University of London, 8 Hunter Street, London WC1N 1BP, England

Received 5 September 1977

1. Introduction

Much of the biological activity of vitamin E is mediated by its function as a lipid antioxidant: both as a scavenger for free radicals derived from polyunsaturated fatty acids in the gastrointestinal tract, and among the polyunsaturated fatty acyl moieties of membrane phospholipids. Tappel [1] has proposed that the prevention of lipid peroxidation in cells is the single, primary function of vitamin E and the recent finding that glutathione peroxidase is a selenoenzyme [2], which can utilise lipid peroxides as substrates [3], has given great impetus to this concept. The rate of metabolism of small quantities of α-[^{14}C]tocopherol has, however, been found to be unaffected by the quantity of dietary polyunsaturated fatty acid [4]. This indicates that the nutritional relationship between α-tocopherol and dietary polyunsaturated fatty acids is considerably more complex than is implied by the antioxidant hypothesis. We have therefore proposed that a structural relationship between vitamin E and polyunsaturated fatty acyl residues of membrane phospholipids may be important [5] because molecular model-building indicates that interactions may occur between the phytyl side-chain of α-tocopherol and acyl chains of polyunsaturated phospholipids, particularly those containing arachidonic acid. We have also suggested that interactions of this kind might result in changes in membrane permeability and stability.

Phospholipid liposomes provide a useful model system with which to investigate the latter part of this hypothesis. In the present experiments, liposomes were prepared from phospholipids that varied in their content of arachidonic acid residues, and the effects of the presence or absence of α-tocopherol on permeability of the liposomes to D-[^{14}C]glucose and to chromate ions were studied. Our observations indicate that α-tocopherol decreases the permeability of liposomes prepared from phospholipids containing a relatively high proportion of arachidonyl residues.

2. Materials and methods

Preparations of egg phosphatidylcholine, containing differing proportions of arachidonic acid residues, were supplied by Lipid Products Ltd (Redhill). Phosphatidic acid was from Sigma (London) Chemical Company, cholesterol from BDH (Poole, Dorset) and DL-α-tocopherol from Roche Products Ltd (London). Stock solutions, prepared in chloroform:methanol (2:1, v/v), were divided into small portions suitable for one experiment and sealed under N_2 in glass ampoules. The molecular weight of egg phosphatidylcholine was taken as 762 (i.e., that of 1-palmitoyl-2-linoleylglycerylphosphorylcholine). Stock solutions of phosphatidylcholine, phosphatidic acid and α-tocopherol were mixed in the proportions specified, and the solvent was removed by evaporation under N_2 in a rotary evaporator at 70°C. 2.5 ml 300 mM solution in Hepes buffer (pH 7.4) of D-[^{14}C]glucose (Radiochemical Centre Ltd, Amersham), or 2.5 ml 1 M K_2CrO_4 solution in Hepes buffer (pH 7.4) containing 75 mM KCl and 75 mM NaCl, was added to the dried lipids (total approx. 8 mg), under an atmosphere of N_2. The mixture was shaken at room temperature on a Vortex mixer for 5–10 min to yield a milky-white

suspension, free from oily droplets. Sonication was not employed in order to minimise peroxidation of the polyunsaturated phospholipids [6].

Liposome suspension, 1.5 ml, was applied to a 20 cm column of Sephadex G-50 from Pharmacia (Uppsala) and eluted with Hepes buffer, pH 7.4. The liposome band moved as a pale opalescent region; it was possible to collect the entire liposome fraction in 2.5 ml eluant. Untrapped D-[^{14}C]glucose, or chromate, remained quantitatively on the column.

Duplicate 1 ml samples of the eluant were placed in dialysis bags (1.5 cm diameter) and dialysed at 30°C against 7.5 ml pre-warmed Hepes buffer in 15 ml scintillation counting vials, while shaking in a water-bath. At 15 min intervals the dialysis bag was removed, carefully blotted dry with papertissue, and placed in a fresh vial containing 7.5 ml Hepes buffer at 30°C. In experiments with D-[^{14}C]glucose, 1 ml samples of diffusate were added to 10 ml scintillant fluid. Radioactivity was counted in a Packard liquid scintillation counter; samples were continuously monitored for quenching by the external standard ratio technique but quenching was only rarely encountered. In experiments with chromate, the ion was assayed in 5 ml diffusate using the diphenylcarbazide reagent [7]. Peroxide measurements [8] on liposomes made from the same lipids, and incubated in the same way, as those used in the experiments showed no detectable amounts of malondialdehyde.

3. Results and discussion

Analysis of four different preparations of egg phosphatidylcholine used are given in table 1, from which it is seen that preparation D contained a particularly high proportion (8%) of arachidonic acid residues.

Studies on the permeability of the liposomes to glucose were undertaken with vesicles prepared from phosphatidylcholine (preparations A and C), phosphatidic acid and α-tocopherol, in molar proportion approx. 6:1:1. Liposomes containing α-tocopherol were slightly less permeable to glucose when phospholipid A (< 1% arachidonic acid residues) was used (fig.1a), and considerably less permeable when phospholipid B (5% arachidonic acid residues) was employed (fig.1b), by comparison with liposomes that were free from α-tocopherol.

Table 1
Fatty acid compositions of preparations of egg phosphatidylcholine

Fatty acid	Egg phosphatidylcholine			
	A	B	C	D
$C_{16:0}$ (Palmitic acid)	35	33	30	31
$C_{16:1}$ (Palmitoleic acid)	2	2	2	2
$C_{18:0}$ (Stearic acid)	12	10	15	15
$C_{18:1}$ (Oleic acid)	38	37	35	31
$C_{18:2}$ (Linoleic acid)	13	13	13	13
$C_{20:4}$ (Arachidonic acid)	< 1	3	5	8
$C_{22:0}$	< 1	2	< 1	< 1

Analyses were made by gas–liquid chromatography on the methylated derivatives of free fatty acids obtained following hydrolysis of the phospholipids. The figures given are the percentage content of each fatty acid and are the mean values of at least two analyses, which differed by less than ± 1%

In similar experiments with phospholipid D, the presence of α-tocopherol greatly decreased the permeability of liposomes to glucose (fig.1c), but with this phospholipid it was necessary to increase the proportion of α-tocopherol present (2:1.5:1). With

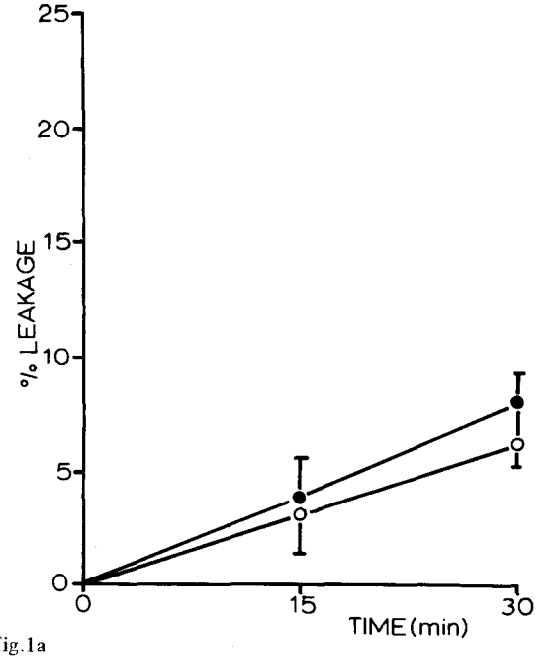


Fig.1a

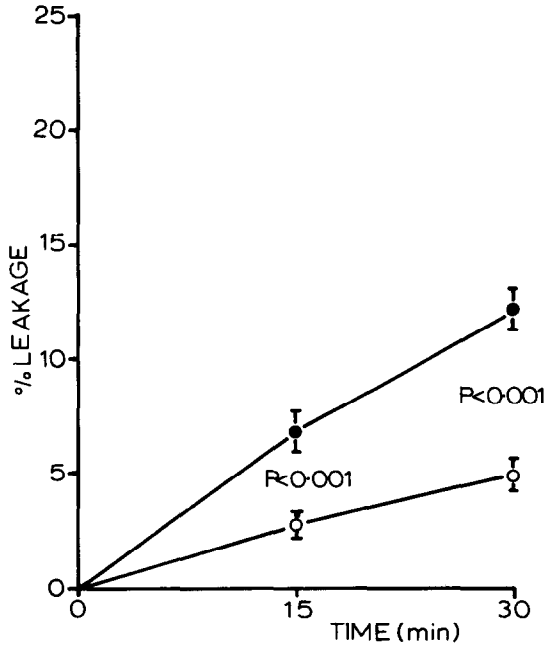


Fig.1b

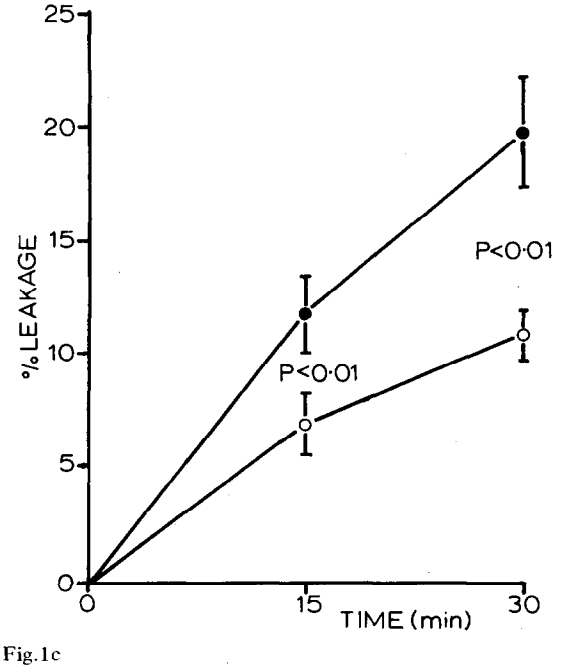


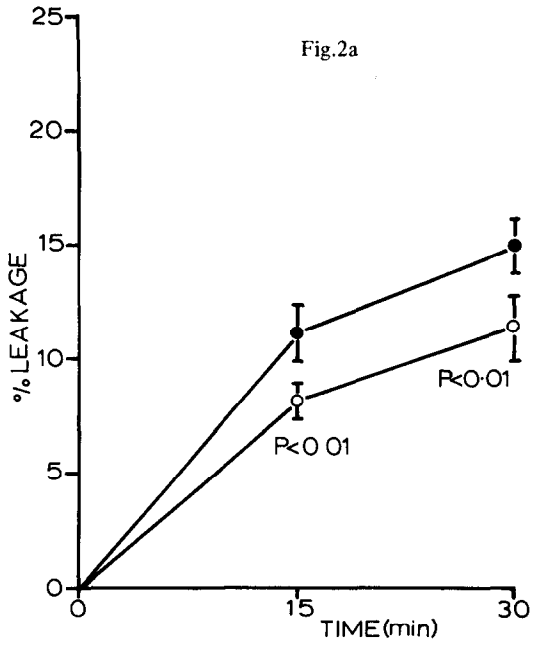
Fig.1c

the lower proportion of tocopherol that was used in experiments with preparations A and C, phospholipid D could not be satisfactorily dispersed, and optical microscopy showed the presence of large aggregates of lipid in the aqueous system. It is known that phospholipids form liposomes only when their fatty acyl chains are fluid. Dipalmitoylglycerylphosphorylcholine, for example, will not give liposomes below its transition temperature of 41°C [9]. The behaviour of preparation D in the presence of α-tocopherol at molar ratios of approx. 6:1 of phospholipid : α-tocopherol may thus indicate that the transition temperature of this relatively unsaturated phospholipid is increased by the presence of a small quantity of α-tocopherol.

Experiments were also undertaken with liposomes containing egg phosphatidylcholine (preparations B and C) in which the leakage of chromate ions was measured (fig.2). As with glucose, it was found that the presence of α-tocopherol decreased the permeability of the liposomes to chromate.

Although the membranes of retinal rod outer segments, which are highly unsaturated, contain approx. 10 mol% α-tocopherol [10], most biological membranes contain little α-tocopherol. In our studies, the proportion of α-tocopherol to phospholipid was therefore kept as low as possible. Initial experiments showed, however, that α-tocopherol had no effect on liposome permeability at a molar ratio of phospholipid : α-tocopherol of 20:1. The studies of fig.1(a), 1(b) and 2 were therefore undertaken with a ratio approx. 6:1. In these experiments the ability of α-tocopherol to decrease liposome permeability was greater with the phospholipid having the higher content of arachidonic acid residues. The largest effect of α-tocopherol in decreasing the permeability to glucose was observed with the most unsaturated

Fig.1. Effect of α-tocopherol on the leakage of D-[^{14}C] glucose. With phospholipid preparations A and C, the molar proportions were phosphatidylcholine 5.8, phosphatidic acid 1.5, D,L-α-tocopherol 1.0. With phospholipid D, the molar proportions were 2.0:1.5:1.0 (a) Phosphatidylcholine A. (b) Phosphatidylcholine C. (c) Phosphatidylcholine D. (-●-) Without α-tocopherol; (-○-) with α-tocopherol. Each point represents the mean value, ± SD of six permeability measurements derived from three separate preparations of liposomes.



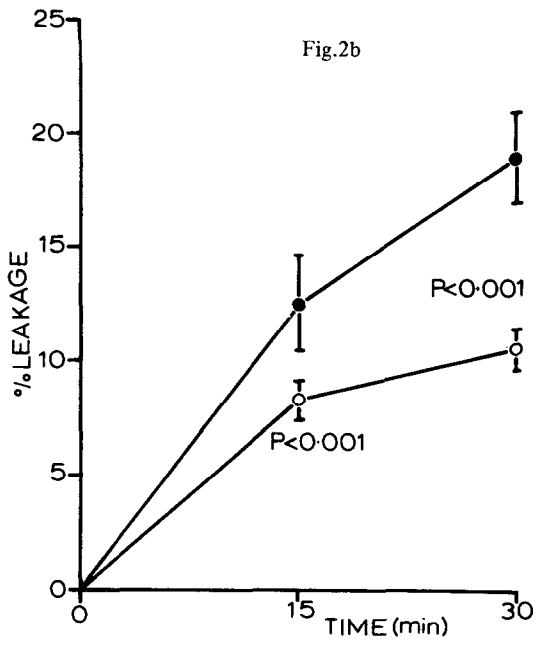


Fig.2. Effect of α-tocopherol on the leakage of chromate ions. The molar proportions used were phosphatidylcholine 5.8, phosphatidic acid 1.5, D,L-α-tocopherol 1.0. (a) Phosphatidylcholine B. (b) Phosphatidylcholine C. (-•-) Without α-tocopherol; (-○-) with α-tocopherol. Each point represents the means values, ± SD of six permeability measurements derived from three separate preparations of liposomes.

phospholipid, preparation D, fig.1(c), but with this preparation it was necessary to increase the proportion of α-tocopherol present as discussed above.

The liposomes used here contained no protein and they therefore have obvious deficiencies as a model for biological systems. Our experiments nevertheless indicate that α-tocopherol might be expected to modulate the structure and permeability in vivo of the phospholipid bilayer component of the membranes of cells and their intracellular organelles. The present observations, taken together with those reported previously on the ability of α-tocopherol to interact relatively specifically with polyunsaturated phospholipids at the air–water interface [11], are consistent with the hypothesis that vitamin E may have structural functions in biological membranes [5,12] as well as antioxidant properties.

Acknowledgement

This work was supported by a grant from the Science Research Council.

References

[1] Tappel, A. L. (1962) Vitam. Horm. 20, 493.
[2] Hoekstra, W. G. (1974) in: Trace Element Metabolism in Animals – 2 (Hockstra, W. G., Suttie, J. W., Ganther, H. E. and Mertz, W. eds) p. 61, University Park Press, Baltimore.
[3] Little, C. and O'Brien, P. J. (1968) Biochem. Biophys. Res. Commun. 31, 145.
[4] Green, J., Diplock, A. T., Bunyan, J., McHale, D. and Muthy, I. R. (1967) Brit. J. Nutr. 21, 69.
[5] Diplock, A. T. and Lucy, J. A. (1973) FEBS Lett. 29, 205.
[6] Klein, R. A. (1970) Biochim. Biophys. Acta 240, 486.
[7] Vogel, A. I. (1963) in: Textbook of Quantitative Inorganic Analysis, 3rd ed, p. 791, Longman, London.
[8] Dahle, L. K., Hill, E. G. and Holman, R. J. (1962) Arch. Biochem. Biophys. 98, 253.
[9] De Gier, J., Mandersloot, J. G. and Van Deenen, L. L. M. (1968) Biochim. Biophys. Acta 150, 666.
[10] Dilley, R. A. and McConnell, D. G. (1970) J. Membrane Biol. 2, 317.
[11] Maggio, B., Diplock, A. T. and Lucy, J. A. (1977) Biochem. J. 161, 111.
[12] Lucy, J. A. (1972) Annal. NY Acad. Sci. 203, 4.