THE EFFECT OF ULTRASONIC IRRADIATION ON THE CHEMICAL STRUCTURE OF EGG LECITHIN

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SUMMARY

Prolonged exposure of egg lecithin dispersions to ultrasound of low intensity and short exposure to ultrasound of high intensity lead to appreciable chemical degradation even in the presence of N_2 although oxidation of the unsaturated hydrocarbon chains is then prevented. The main degradation products are lysolecithin, fatty acids, glycerylphosphorylcholine, phosphorylcholine and choline. The amount of lysolecithin depends on the intensity of ultrasonic cavitation. Some of the physical properties of egg lecithin sonicated under conditions which eliminate the possibility of chemical degradation are studied and compared with results of other workers.

Sonicated aqueous dispersions of phospholipids have gained importance because of their use in model membrane, lipoprotein and enzyme studies. The numerous reports on the effects of ultrasonication have been mainly concerned with changes in the size and shape of the phospholipid aggregates. In the majority of cases little attention has been paid to the possibility of chemical modification and degradation of the phospholipid induced by intense ultrasonic irradiation. It has been reported (1) that the unsaturated hydrocarbon chains are oxidized when lecithin dispersions are sonicated in the presence of oxygen. There are other possible modes of decomposition, such as the hydrolysis of covalent bonds, which have not been considered. Many investigators concerned with the physical properties of sonicated lecithin dispersions have ignored the necessity of checking the purity of the lecithin after sonication. No attempt has been made to investigate the effect of impurities on the physical properties of sonicated lecithin dispersions. This work describes the effect of sonication on the chemical structure of egg lecithin dispersed in aqueous solution, and some of the physical properties of egg lecithin dispersions sonicated under conditions which eliminate chemical degradation.

MATERIALS AND METHODS

Egg lecithin and dispersions of it in H_2O or buffer (0.1 M NaCl, 0.01 M TRIS p_H 8.1, 0.02% sodium azide) were prepared as described elsewhere (2). Sonication was carried out with a Kerry Vibrason System 150 (Kerry Ultrasonics Ltd., Hitchin, U.K.) at low power (nominal frequency 20 KHz, input power 135 Watt). The power supply was tuned to maximum cavitation and with this instrumental setting the power output was measured as the sonic pressure generated in a 100 ml beaker (filled with 75 ml H_2O, tip of the soniprobe 1 cm from bottom): 0.31 Newton/m^2. The volumes of the dispersions were either 2 or 10 ml, and sonication was performed in a 5 ml glass tube (diameter 1.3 cm, length 7 cm) and a 10 ml glass tube (diameter 1.6 cm, length 10.5 cm) respectively with the tip of the soniprobe (diameter 0.95 cm, length 7 cm) immersed to half height of the dispersion. The glass tube was surrounded by ice-H_2O and flushed with N_2. Ti released from the tip of the soniprobe was removed by centrifugation. Thin layer chromatography was carried out on silicagel H or G plates (20 x 20 cm) using chloroform-methanol-7 M ammonia (230:90:15, by vol) and cyclohexane-ether-ethanol-formic acid (73:24.5:0.5:2, by vol) respectively. The sonicated egg lecithin dispersions were freeze dried, the residue dissolved in chloroform-methanol (1:1, by vol) and 0.3-1 mg of it applied. Lysolecithin and other degradation products were separated on silicagel H or G and determined quantitatively either as described by Skidmore and Entenman (3) or by Barrett et al. (4). Glycerylphosphorylcholine, phosphorylcholine and choline were separated by descending paper chromatography (on Whatman 54 SFC) using ethanol-aqueous ammonia (spec. gravity 0.91) - H_2O (6:3:1, by vol) (5). Spots containing P or choline were detected according to Hanes and Isherwood (6) and Marchbanks (7) respectively.
Analytical gel filtration on Sepharose, analytical ultracentrifugation, electron microscopy and density measurements used to characterize the physical properties of sonicated egg lecithin dispersions are described elsewhere (2).

RESULTS AND DISCUSSION

Ultrasonic irradiation of egg lecithin caused two forms of chemical changes:

1. oxidation of unsaturated hydrocarbon chains, and
2. cleavage of covalent bonds.

Experiments concerning the extent of oxidation as a function of sonication time confirmed previous work (1). The protective effect of an N_2 - atmosphere was increased by bubbling N_2 through the dispersion before sonication or by adding 2.6 di-tert.-butyl-p-cresol.

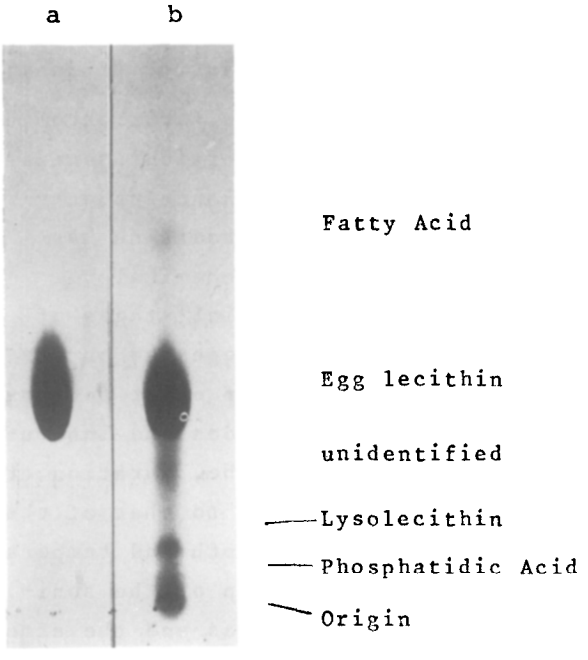

Figure 1.
Thin layer chromatogram on silicagel H; detection with conc. sulphuric acid saturated with $K_2Cr_2O_7$; lane a: egg lecithin before sonication, lane b: egg lecithin dispersed in 10 ml H_2O (1 % w/v) snicated for 200 min. under N_2.

Prolonged sonication caused appreciable chemical degradation even in the presence of N_2 (Fig. 1b) although oxidation of the unsaturated hydrocarbon chains was then negligible. The main degradation products formed were lysolecithin, fatty acids and more polar compounds which remained on the origin (Fig. 1b) and were separated by paper chromatography. Glycerylphosphorylcholine, phosphorylcholine and choline were identified by their R_F — values (0.69, 0.30, 0.75, respectively) and by using marker compounds; 3 - 4 further spots separated by paper chromatography were not identified. Small quantities of phosphatidic acid and traces of mono- and diglycerides were detected by thin layer chromatography on silicagel H and G respectively. A similar pattern of degradation products (Fig. 1b) was obtained when egg lecithin was sonicated with a Branson sonifier (Model B12, nominal frequency 20 KHz, input power 125 watt) at power level 5 - 6 under otherwise the same conditions as described for Fig. 1b.

The amount of lysolecithin and total degradation products (with $R_F <$ than that of egg lecithin) as a function of sonication time are shown in Fig. 2a and 2b. Short exposure to ultrasound of high intensity (2 ml sample, Fig. 2a) and prolonged exposure to ultrasound of low intensity (10 ml sample, Fig. 2b), both caused appreciable chemical degradation. The ratio of the sample volume to that of the soniprobe tip was crucial: while 1 min. exposure of 2 ml dispersions led to detectable decomposition, 10 ml dispersons gave degradation products only after 40 min. of sonication. 2 ml dispersions sonicated for 20 min. gave a thin layer chromatogram quantitatively similar to that of 10 ml dispersions sonicated for 160 min. In the presence of air, when oxidation of the hydrocarbon chain occurred, the extent of chemical degradation was about 2 - 3 times higher (Fig. 2a). The rate of chemical degradation seemed to be greatest during the initial stages of sonication. The extent of chemical degradation depended on the intensity of ultrasonic cavitation and probably on other factors which may affect the ultrasonic cavitation. Besides the instrumental settings (power output, tuning of the probe, duration etc.), the geometry of the tip of the soniprobe relative to that of the sample tube, the volume, concentration, liquid depth and temperature of the dispersion, the depth to which the tip of the soniprobe was immersed, the nature of the dissolved gas and the atmosphere surrounding the sample were all found to influence the effect of sonication. This dependence of sonication on a number of parameters which are difficult to control experimentally explains the rather large scatter of experimental points in Fig. 2 and the lack of reproducibility in the physical properties of sonicated dispersions, as is found from a comparison of published data (8 - 11) and from our own results (12).

In the light of the findings that under conditions of optimum cavitation even very short sonication times can cause significant chemical degradation, purity tests of the lecithin before and after sonication are imperative. Hence the possibility has to be considered that the results in ref. 8 - 11 where no such purity checks were carried out were obtained with contaminated egg lecithin. Since it has been shown that even small quantities of impurities can have a remarkable influence on the phase behaviour of lecithin - H_2O systems (13), a re-examination of the physical properties of egg lecithin carefully sonicated under conditions

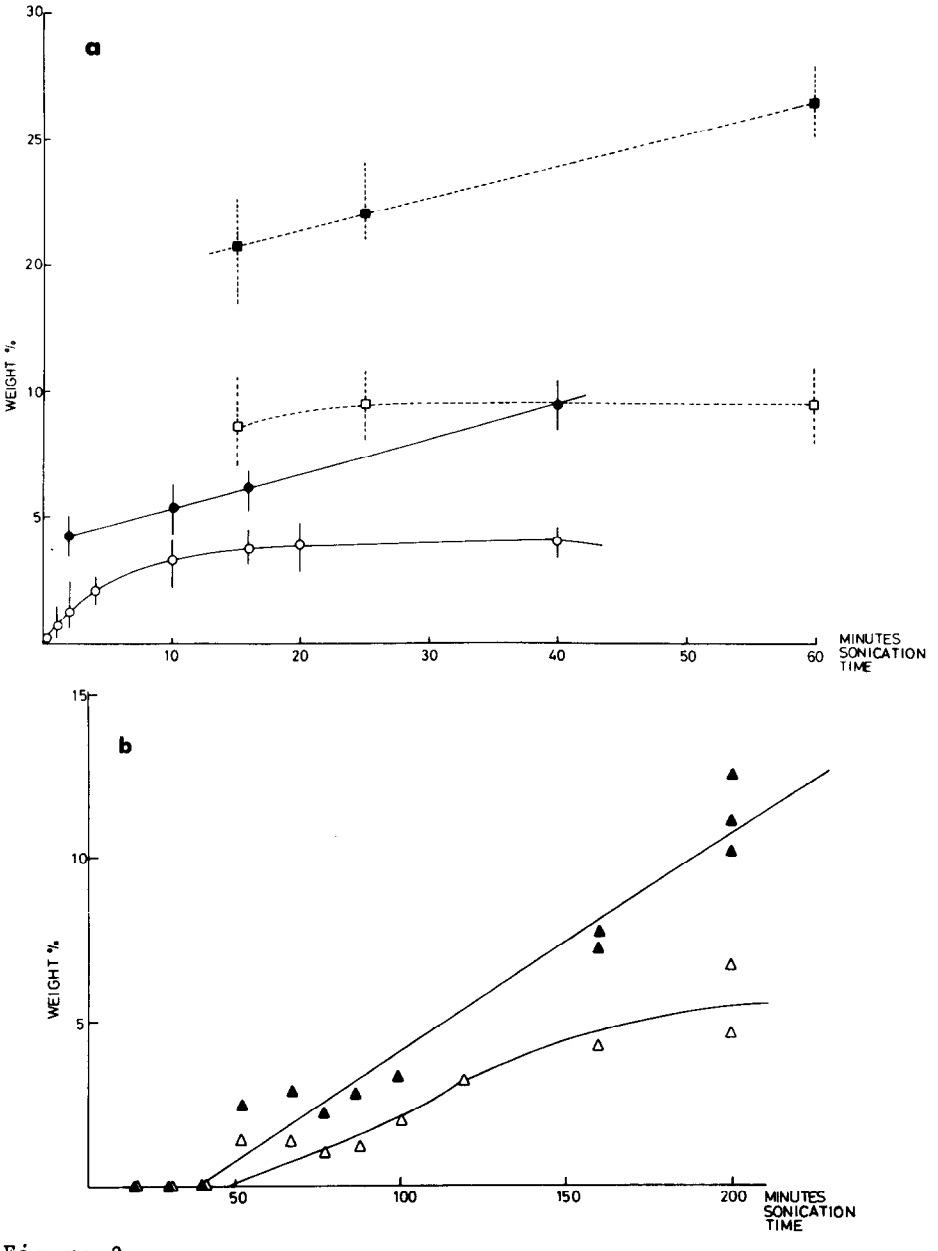

Figure 2.

Degradation of egg lecithin as a function of sonication time.

a. 2 ml of 1% (w/v) egg lecithin dispersions sonicated under N_2: O - O weight % lysolecithin formed, ● - ● weight % total decomposition products with R_F < than that of egg lecithin, dashed curve: sonication in air.

b. 10 ml of 1% (w/v) egg lecithin dispersions sonicated under N_2: △-△ weight % lysolecithin formed, ▲-▲ weight % total decomposition products with R_F < than that of egg lecithin There was no difference between the effect of sonication on egg lecithin dispersed in H_2O and in buffer.

which eliminated the possibility of chemical degradation was required. Egg lecithin dispersions free of degradation products were analysed on Sepharose 2B and consisted of unbroken large aggregates and of an inhomogeneous fraction of small vesicles. The latter was collected and the purity of the lecithin examined by thin layer chromatography: the fact that no degradation products were present means that the presence of impurities is not a pre-requisite for the formation of the small vesicles by sonication. Some of the physical properties of these vesicles are compared with those of egg lecithin vesicles prepared by sonication for 2.5 hours as described by Huang (11) (Table 1). No details of the purity of lecithin after sonication were given by this author. Table 1 shows that although there are significant differences in the homogeneity, stability, partial specific volume and hydration, the vesicle shape and average size (weight)

Table 1.

Physical Properties	This Work	Ref. 11
diameter	190-400 Å, average 230Å	250Å
weight	2-10 x 10^6, average: 3 x 10^6	2 x 10^6 daltons
shape	spherical	spherical
homogeneity	inhomogeneous *	homogeneous
stability with respect to aggregation	unstable *	stable
partial specific volume	0.9839 $\pm$ 0.0003 cm^3/g	0.9814 $\pm$ 0.0004 cm^3/g(14)
hydration in g/g dry lecithin	1.07	0.64

* the homogeneity and stability were increased by increasing the intensity of sonication or by incorporating lysolecithin (12). Lecithin particles containing degradation products had a negative ζ- potential and, unlike pure lecithin dispersions, could be precipitated by cations: $Ca^{++} > Na^{+} > K^{+}$.

are similar in both preparations. These results demonstrate that prolonged sonication cannot be used as a means of producing pure egg lecithin vesicles of uniform diameter whose surface area can readily be calculated from analytical data.

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