Radiolysis of 2,6-di-*tert*-butyl-4-methylphenol (ionol) in a lipid membrane in the presence of oxygen

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Radiation-induced destruction of the ionol molecules in liposomes built of L-α-lecithin depends on the ionol concentration in the lipids and on the concentration of the lipid in the dispersion.

Ionol (2,6-di-*tert*-butyl-4-methylphenol) has been used to study the action of a flux of active particles on the liposomes built of egg yolk lecithin (L-α-phosphatidylcholine). Decomposition of ionol, which is a radical acceptor, incorporated in the lipid membrane of liposomes, has been investigated. The ionol present in a lipid membrane (ionol is readily soluble in hydrocarbons and lipids but is insoluble in water; according to direct experimental measurements, its concentration in water does not exceed 2×10^{-5} mol dm^{-3}) competes with the membrane lecithin for trapping the active species resulting from radiolysis. From the amount of ionol consumed, one can estimate the flux of active particles falling on the membrane. In our experiments, the concentration of ionol in the lipid membrane varied, while the lipid concentration in the aqueous phase remained constant. The following lipid concentrations were used: 2.4 and 4.8 mg ml^{-1}. The radiation yield of the destruction of ionol was calculated using a standard procedure from the experimental dependences of the consumption of ionol on the absorbed dose of radiation at low radiation doses.

The general approach to the preparation of aqueous dispersions of liposomes is described in a monograph.[1] A 10.0±0.5% ethanolic solution of the 'lecithin standard' (egg yolk lecithin[2]) was used. To remove the alcohol, the lecithin was kept in a flask of a known weight on a rotary evaporator at 25–30 °C until a constant weight was attained. The quantity of lecithin obtained was determined as the difference between the weights of the filled and empty flasks. An ethanolic solution of ionol was then introduced by an analytical syringe into the flask containing lecithin, so that the final concentration of ionol in the lecithin varied from 0.2×10^{-4} to 2.5×10^{-4} mol dm^{-3}. The resulting mixture was thoroughly stirred and dried on a rotary evaporator at 26–30 °C to a constant weight. After that, distilled water was added to the dried mixture of lecithin with ionol in such a way that the lipid concentration in water was either 24 or 48 mg ml^{-1}. The resulting suspension was sonicated (the absorbed ultrasound power varied from 1.8 to 2.4 W cm^{-3}), centrifuged for 15 min at 625 g and diluted to the required concentration with distilled water in a measuring flask. The average size of liposomes in the suspension was determined from the modified Rayleigh equation[3,4] using turbidity spectra[5] recorded on a Specord M40 spectrophotometer at wavelengths ranging from 440 to 900 nm. Using the Angstrom relation $D = const\cdot\lambda^{-n}$, where D is the optical density of the liposome dispersion at the wavelength λ and n is the exponent, and experimental $D(\lambda)$ values, the n and $const$ values were calculated. The resulting values were used to calculate the average radius of liposomes from the Rayleigh equation.

Table 1 Dependence of the radiation yield of the destruction of ionol on the initial concentration of ionol in a lipid membrane for two concentrations of lecithin in an aqueous suspension.

$C_{lecithin}$/ mg ml^{-1}	C_{ionol}/mol per g of lipid	G/molec per 100 eV	R_{av}/nm
4.8	2.5×10^{-4}	1.3±0.02	56
4.8	2.4×10^{-4}	1.4±0.01	48
4.8	2.0×10^{-4}	1.7±0.03	42
4.8	1.2×10^{-4}	1.1±0.03	40
4.8	8.3×10^{-5}	1.0±0.04	45
4.8	3.4×10^{-5}	0.4±0.06	49
4.8	2.1×10^{-5}	0.5±0.01	43
2.4	2.3×10^{-4}	1.5±0.09	38
2.4	2.0×10^{-4}	1.5±0.02	39
2.4	1.9×10^{-4}	1.1±0.02	50
2.4	1.0×10^{-4}	0.8±0.02	49
2.4	3.6×10^{-5}	0.5±0.03	52

The consumption of ionol and the formation of radiolysis products were monitored by HPLC (Millichrom chromatograph, 64×4 mm column with Silasorb C_{18}, acetonitrile–ethanol mixture as eluent). The chromatograph permitted recording the UV spectra of the products during the separation. The concentration of ionol in the samples of the liposome suspension was determined by comparing the heights of the chromatographic peaks for the solution under analysis and for the standard solution.

The liposome suspensions were irradiated using an RC-100M cobalt setup. Aqueous suspensions of liposomes were irradiated under aerated conditions (air was bubbled through the cell) at a dose rate of 110 Gy min^{-1}.

Dispersions of L-α-lecithin liposomes with a concentration of 2.4 or 4.8 mg ml^{-1} containing 2.1×10^{-5} to 2.5×10^{-4} mol g^{-1} of ionol per gram of the lipid were exposed to radiation. The average radius of the liposome vesicles was 47±9 nm (Table 1). During irradiation, air was permanently bubbled through the suspensions.

The γ-irradiation resulted in a diminution of the concentration of ionol, which followed a linear dependence on the absorbed dose, at least until the concentration halved. The radiation yields for the decomposition of ionol were calculated from the linear sections of the ionol consumption plots; they are given in Table 1. It is noteworthy that our study is the first in which the radiation yields of the transformation of a radical acceptor implanted in a liposome membrane were directly determined.

It can be seen from Table 1 that the radiation yield of the ionol decomposition G(–PhOH) increases with an increase in its concentration. The largest G value attained was 1.7 molecules of ionol per 100 eV. Since the flux of radicals from the solution bulk depends only slightly on the concentration of liposomes,[6] both series of experimental results can be regarded, within experimental accuracy, as a unified set of data, because both series of experimental points fall satisfactorily on a common dependence. The resulting non-linear dependence implies the occurrence of a competition between ionol and lecithin for the radicals resulting from the radiolysis of the disperse system. Transformations of ionol caused by the direct action of radiation can be neglected, because its concentration was small (the electron fraction of ionol in the whole system was less than 0.0003, that of lecithin was less than 0.005).

To describe the experimental dependence of the radiation yield of ionol destruction on its initial concentration in the

lecithin membrane, two simplifying assumptions were made. Firstly, we assumed that ionol was uniformly distributed in the liposome membrane and that it reacted with only one sort of radical present in the system. Then we may consider two competing reactions:

$$R + PhOH \xrightarrow{K_1} \text{products}, \quad (1)$$

$$R + Lec \xrightarrow{K_2} \text{products}, \quad (2)$$

where PhOH is ionol, Lec is lecithin and K_1 and K_2 are the rate constants for the corresponding reactions.

Using the method of steady-state concentrations, we obtain

$$[R] = G_R^0 P/(K_1[PhOH] + K_2[Lec]) \quad (3)$$

and

$$G_{(-PhOH)}P = G_R^0 PK_1[PhOH]/(K_1[PhOH] + K_2[Lec]) \quad (4)$$

where G_R^0 is the overall yield of radicals participating in reactions (1) and (2), P is the dose rate and [PhOH] and [Lec] are the concentrations of ionol and lecithin, respectively.

A simple expression for the dependence of G_R on the lecithin and ionol concentrations follows,

$$1/G_{(-PhOH)} = (1/G_R^0)(1 + K_2[Lec]/K_1[PhOH]) \quad (5)$$

which is in good agreement with experimental data.

Solution of this equation for each series of lecithin concentrations gives the following average values of G_R^0 and K_2/K_1 for the two series:

$$G_R^0 = 1.7 \pm 0.8 \text{ molecule/100 eV}; \; K_2/K_1 = 0.05 \pm 0.02$$

Having found the parameters of equation (5), we can reconstruct the exact form of the dependence of the destruction of ionol with the assumption that the method of steady-state concentrations is valid.

This curve is described by the following differential equation

$$-d[PhOH]/dt = K_1[PhOH][R] \quad (6)$$

By substituting expression (5) into (6), we obtain the differential equation

$$(1 + K_2[Lec]/K_1[PhOH])\,d[PhOH] = G_R^0 P dt \quad (7)$$

Under the boundary condition $[PhOH] = [PhOH]_0$ and at $t = 0$, the solution of this equation is

$$G_R^0 Pt = ([PhOH]_0 - [PhOH]) + (K_2[Lec]/K_1)\ln([PhOH]_0/[PhOH]) \quad (8)$$

Within the parameters found from equation (5), equation (8) holds within the accuracy of determination of the ionol concentration and is described satisfactorily by a straight line up to a 50–60% degree of destruction of ionol; after that, the destruction of ionol is retarded.

It follows from the above K_2/K_1 value that under the assumptions made, ionol is almost 20 times more efficient as a radical acceptor than lecithin. This fact makes it possible to tentatively identify the radical R. Radiolysis of the disperse system under study yields OH and H_2O/O_2^- radicals, H atoms and hydrated electrons. The concentration of H atoms is very small; hydrated electrons do not react with lecithin;[7] besides, both of these species are efficiently trapped by oxygen. The rate constant for the reaction of OH with lecithin was reported[7] to be 5×10^8 dm^3 mol^{-1} s^{-1}.

Thus, it can be expected that the rate constant for the reaction of OH with ionol would be *ca.* 10^{10} dm^3 mol^{-1} s^{-1}, which is quite permissible in view of the fact that the rate of reaction of lecithin with OH radicals is limited by the hydration shell of lecithin. The HO_2/O_2^- radicals exhibit a low reactivity towards organic substances except for some radicals and compounds containing charged groups; for example, the rate constants for the reaction of HO_2/O_2 with molecules containing polar quaternary alkylammonium groups, similar to the choline group of lecithin, are *ca.* 10^7–10^8 dm^3 mol^{-1} s^{-1}.[8] It could hardly be expected that the rate constant for the reaction of HO_2/O_2^- with ionol would be an order of magnitude larger than that for the reaction with lecithin. Therefore, as a first approximation, we consider that the radicals R in our model are OH radicals.

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