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ENZYMATIC OXIDATION OF QUINOLPHOSPHATE IN THE PRESENCE OF LYOTROPIC LIQUID CRYSTALS

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Abstract Enzymatic oxidation of quinolphosphate and hydrazobenzene in the presence of lecithin is discussed. To understand the dependence of the oxidation rate on the lecithin concentration, orientation of the reagents on the liposome surface and transfer of the enzyme from one vesicle to another on their collision should be taken into account.

The ability of phospholipids to aggregate in solution, to give rise to multiple vesicle structures, and to solubilize proteins to create a specific microenvironment allows to use such phospholipids as a model for examining the effect of the medium on the enzymic activity.

Consideration is given to the catalyzed by horseradish peroxidase (HRP) oxidation of 2,3-dimethyl-1,4-naphthoquinol-1-dimethylphosphate (QP) and hydrazobenzene (HB) with hydrogen peroxide in the presence of a lyotropic liquid crystal made of lecithin (Lec) which is organized in lyposomes. Egg lecithin, HRP, QP synthesized as described elsewhere[1] and HB recrystallized from heptane were used. Liposome dispersions were obtained by ultrasound treatment of water solutions. The distribution of the reagents and the kinetics of the reaction has been studied by the means of fluorescence and UV-spectroscopy. The liposomes were observed by electron microscopy.

The oxidation rate constant for QP (k_{QP}) was found to decrease with the concentration of Lec (Fig. 1) and to be independent of those of [QP] and [HRP]. The oxidation rate constant for HB (k_{HB}) exceeds k_{QP} by a factor of 100 or so therefore the reaction can be also observed in the absence of HRP (Fig. 2). In the case of nonenzymic oxidation the rate constant is independent of HB concentration as it varies fourfold. The addition of Lec leads to a rapid increase in the oxidation rate constant by ca. 40 times, and in the Lec concentration range discussed does not reach the plateau. In this case the rate constant is reproducible from one series of liposome samples to another.

The dependence of k_{HB} (in the presence of HRP) on [Lec] is shown in Fig. 3. All the curves have maxima whose positions do not depend on the concentrations of either HB or HRP but depend on the series of liposome samples. It is important that the maximal rate constants at a given HB concentrations change little from one series of liposomes samples to another but the positions of the maxima differ markedly. The reaction order with respect to HB in the presence of Lec drops from 1 to 0.25 whereas that with respect to HRP is always equal to unity.

The results obtained can be explained as follows. HRP is probably localized on the liposome surface, its activity remaining practically intact. The fluorescence spectra suggest that at equilibrium QP is distributed between the polar aqueous and nonpolar Lec phases[2]. HB appears to behave in the same way. Due to the fact that the substrates molecules contain nonpolar fragments they are likely localized mainly on the liposome surface. The charge distribution in the molecule and

merely geometrical considerations are in favour that HB polar group is oriented towards the aqueous phase and is accessible to H_2O_2 and HRP. The QP reaction group is localized in the nonpolar Lec phase that is why the substrate oxidation proceeds with difficulty. Because of this the Lec addition increases the HB oxidation and decreases QP oxidation.

The following explanation can be given for the dependence of enzymic HB oxidation on [Lec]. The enzyme is predominantly localized on the liposomes and as number of HRP molecules is much lower than the number of liposomes each liposome on average contains not more than one HRP molecule. Let us estimate the characteristic time during which the HRP molecule is retained by the liposome (τ_R). At equilibrium the number of HRP molecules passing into aqueous phase per unit time (n_o) is equal to that of the molecules adsorbing by the liposomes (n_i). The latter value is equal to the number of the encounters between HRP molecules present in the aqueous phase and the liposomes. Since in the Lec concentration range the concentration of the liposomes exceeds that of the HRP by a few orders of magnitude and, besides, the major portion of the HRP is adsorbed by the liposomes, the value of $n_o = n_i$ is much smaller than the number of encounters between the liposomes. Therefore one can suppose that the transfer of the enzyme from one liposome to the other occurs through the collision of the liposomes rather than an aqueous phase. It follows then that τ_R is inversely proportional to liposome concentration.

The kinetic data suggest that HB and the product of its oxidation, i.e., azobenzene, are largely adsorbed by the liposomes, the characteristic time of HB

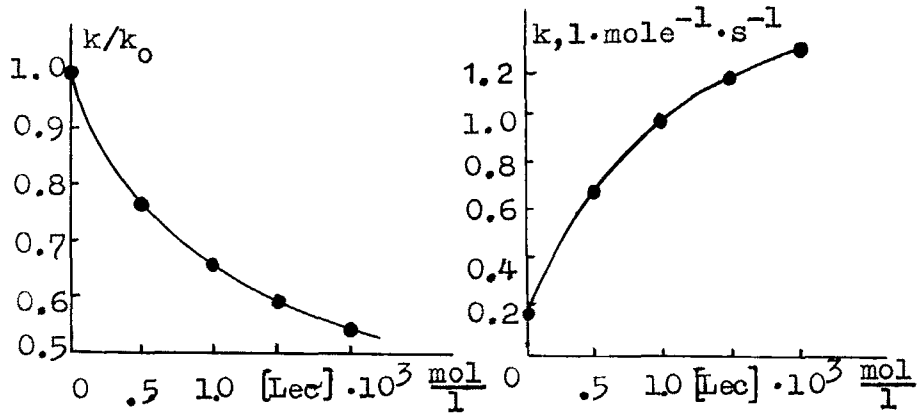

FIGURE 1 Rate constant (k_{QP}) for QP oxidation with hydrogen peroxide, catalyzed by HRP versus Lec concentration at 22°C ([QP] = 0.5÷2.10^{-4} mole/l); k_o - is the oxidation rate constant in the absence of Lec.

FIGURE 2 Rate constant (k_{HB}) for HB oxidation with hydrogen peroxide versus Lec concentration at 22°C ([HB] = 0.4÷1.6·10^{-4} mole/l).

exchange between the aqueous phase and the liposomes being greater than that of the reaction. Thus in the course of the reaction the HRP molecule converts during the time τ_K all HB molecules adsorbed by a given liposome after which the reaction is limited by a slower process of desorption of the resulting azobenzene and its substitution by the HB molecules. Therefore, if $\tau_R > \tau_K$, the enzyme for some time remains without "job". The increase of liposome concentration gives rise to a lower τ_R value thereby shortening the "jobless" time of enzyme and raising the observed rate constant. The reaction rate has a peak value at $\tau_R \simeq \tau_K$ and then it begins to drop slowly due to the dilution effects. Consequently, the position of the maximum

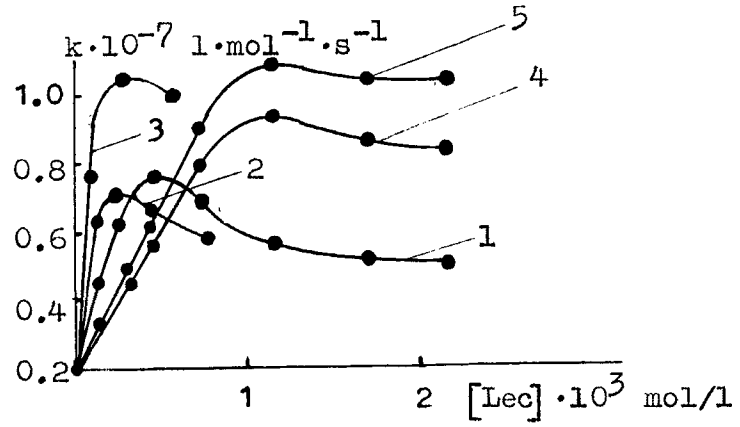

FIGURE 3 Rate constant (k_{HB}) for HB oxidation with hydrogen peroxide catalyzed by HRP versus Lec concentration at 22°C: 1,2 - [HB] = $1.63 \cdot 10^{-4}$ mole/l, 3,4 - [HB] = $0.82 \cdot 10^{-4}$ mole/l; 5 - [HB] = $0.41 \cdot 10^{-4}$ mole/l. Curves 1 and 4 (also curves 3 and 5) were obtained with different series of liposome samples.

(Fig. 3) depends on the concentration of the liposomes, which in turn is related to Lec concentration and liposome size. As the liposome dimensions are difficult to control because of their sensitivity to preparing conditions the positions of the maxima change from one series of liposome samples to another. The reaction order according to Michaelis-Menten mechanism and above discussed reasons is expected to have a tendency to decrease as the Lec concentration grows.

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