Lag Phase during the Action of Phospholipase A₂ on Phosphatidylcholine Modified by Alkanols*

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The action of pig pancreatic phospholipase A₂ (EC 3.1.1.4) on phosphatidylcholine bilayer is studied under a variety of substrate modification conditions including the incorporation of long chain alcohols (hexanol and several isomeric octanols) into the bilayer. The rate of hydrolysis shows a biphasic dependence upon the concentration of the activating alcohol. The hexanol to lipid molar ratio in the bilayer is approximately 1.4:1 at the optimal alkanol concentration. The lag phase at the beginning of hydrolysis has been shown to depend upon the nature of the bilayer as modified by different alkanols and by intrinsic differences in the unilamellar vesicles (approximate diameter ~250 Å) compared to the multilamellar vesicles. The rate constant for the activation process responsible for the lag period is first order and does not depend upon the concentration of the enzyme, substrate, alkanol, and calcium. These and other experiments are interpreted in terms of a hypothesis that the pancreatic phospholipase interacts with the bilayer by a catalytic and a recognition site. The data suggest that the packing of the interface regulates the interaction of both the catalytic and the recognition site. It is postulated that the biphasic activation profile as a function of hexanol concentration may be a consequence of two-site interactions between the enzyme and the substrate interface.

The action of phospholipases on phospholipids is an intriguing phenomenon. The naturally occurring phospholipids are insoluble in water and they spontaneously form aggregate structures. The interface of these aggregates serves as the site of action of the lipolytic enzymes. Studies with monolayers, for example, have shown that the "quality of the interface" governs the enzyme substrate interaction (1). However, the interface of the bilayer formed by the natural phospholipids in water is not an ideal substrate unless the bilayer is modified, for example, by incorporation of long chain alcohols (2). These molecules apparently serve as spacers that permit insertion of the enzyme into the bilayer. The activity of the enzyme on the modified interface shows a biphasic dependence as a function of alkanol concentration. This suggests that the enzyme has two sites of interaction with the bilayer, a catalytic and a recognition site, both of which interact with the lipid bilayer. In this communication, we wish to report the effect of alkanols on the presteady state penetration phase of the action of the pig pancreatic phospholipase A₂ on the alkanol modified bilayer.

MATERIALS AND METHODS

Most of the methods and materials have been described previously (2). Phospholipase A₂ from pig pancreas was purchased from Sigma Chemical Co. The specific activity of this preparation was 1000 to 1100 μmol/min/mg of protein when assayed by the procedure described by de Haas et al. (3). The preparation was judged to be better than 95% pure by the following criteria (3): stability of the enzymic activity after heating to 80°C for 6 min, a procedure which inactivates nonspecific lipases; migration as a single band of M₉ = 14,000 during electrophoresis on 7.5% polyacrylamide gel at pH 8.5 in Tris-glycine buffer; the specific activity of ~1100 μmol/min/mg of protein where εᵣ = 13.0 for absorption of protein at 280 nm.

Pure phosphatidylcholine from egg yolk was used for all the kinetic studies described in this paper. The reaction mixture for the kinetic studies consisted of 10 ml of 20 mM calcium chloride, and 20 mM potassium chloride at 37°C. The multilamellar vesicles (MLV)² were prepared by hand shaking phosphatidylcholine in 140 mM KCl as described earlier (2). Unilamellar vesicles (ULV) were prepared by sonicating the MLV in a sealed tube in a bath-type sonicator under nitrogen (Heat Systems, Hicksville, NY). The final concentrations of all the components in the various experiments are given in the figure legends. Progress of hydrolysis was followed by titrating the released fatty acid with 1 mM 2-amino-2-methyl-1,3-propanediol by pH-stat titration (Radiometer model TTT-60 equipped with ABU-13, PHM-62, REC-61, TTA-60). The rates are given in arbitrary units of chart divisions/unit of time, where one division corresponds to 2 nmol of base added. In a typical experiment, the reaction mixture contained 280 μM (or 2.3 μmol) substrate, 42 μM hexanol for MLV (and 18 μM for ULV), and 250 ng of enzyme in a 10-ml reaction mixture. Under these conditions, the steady state rate of hydrolysis was linear for more than 10 min after the latency period. Typically, the rates of hydrolysis were eight chart divisions or 16 nmol/min. The combined drift due to the Titrimeter and the environmental factors was less than ±1 nmol/min.

RESULTS

A lag at the beginning of the hydrolysis of phosphatidylcholine in liposomes by phospholipases can be followed by pH-stat titration. Since in the system under investigation, the lag periods are about 5 min, the substrate in the form of multilamellar vesicles (MLV), calcium ions, hexanol, and the enzyme can be used to start the reaction. A typical titration curve is presented in Fig. 1. The automatic addition of the base is begun soon after the reaction is started by the addition of the enzyme (250 ng). A steady state, zero order rate of hydrolysis is achieved in about 15 min. During that time, approximately 5 to 8% of the total substrate present in the medium is hydrolyzed. If the hydrolysis is allowed to continue, about 51% of the total available substrate is hydrolyzed before equilibrium is attained.

The process leading to the steady state, zero order rates

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appears to follow a first order kinetics. The following experiments are based on the suggestion that a slow activation or penetration step (or both) precedes establishment of the catalytic cycle of the enzyme in the lipid bilayer. This slow step may be represented as:

\[ \text{inactive enzyme} \xrightarrow{k} \text{active enzyme} + S \xrightarrow{} E \cdot S \rightarrow E + P \]

where \( k \) is the rate constant for the slow activation step. If \( E_i \) and \( E_a \) are the concentrations of the inactive and the active enzyme, respectively, the total enzyme concentration, \( E_T = E_i + E_a \). Thus,

\[ E_i = E_T e^{-kt} \]

or

\[ E_a = E_T - E_T e^{-kt} \]

Since the concentration of the active enzyme is proportional to the rate of addition of the base, that is \( E_a = a \cdot (dB/dt) \), and \( E_T = aB_m \) where \( a \) is the proportionality constant, and \( B_m \) is the rate of addition of the base per unit time. The total volume of the base added up to any given time is given by

\[ a \int dB/dt \cdot dt = B_a \cdot \Delta t = \int (E_T - E_T e^{-kt}) dt = aB_m \cdot t + \frac{E_T}{k} e^{-kt} + C \]

When \( B = t = 0, C = -(E_T/k) \).

The extrapolated portion of the linear steady state rate region (when all the enzyme is activated) would be a straight line given by \( B \cdot a = E_T t - (E_T/k) \). When \( B = 0 \), the intercept of this line on the abscissa gives the latency period, \( t_l \). Therefore,

\[ t_l = \frac{1}{k} \]

Similarly the half time for activation,

\[ t_{1/2} = \frac{0.693}{k} \]

therefore

\[ t_l = \frac{t_{1/2}}{0.693} \]

This relationship holds if and only if all the enzyme is in the inactive form at \( t = 0 \).

Equation 1 describes the activation profile of the type shown in Fig. 1. A plot of \( \log[aB_m \cdot t - B_a \cdot C] \) versus \( t \) would be linear with a slope of \( -k = 0.693/t_{1/2} \). The term in the square brackets, \( A \), can be obtained directly from the titration profiles as shown in Fig. 1a. A plot of \( \log[A] \) versus \( t \) is shown in Fig. 1b. As expected, the plot is linear. An excellent fit with a correlation coefficient of better than 0.97 is obtained for over 80% of the range. The half-time for activation and the time constant for activation described in this paper were obtained from plots of the type shown in Fig. 1b, whereas the latency period and the steady state rate of hydrolysis were obtained directly from the reaction progress curves of the type shown in Fig. 1a.

Plots of the type shown in Fig. 1b show that the latency is a consequence of a first order activation process. The half-time for this process appears to be about 3% min. To better understand this activation process, we studied the effect of the enzyme, substrate, calcium, and the hexanol concentration on the half-time for the activation and the zero order reaction rates for the pork pancreatic phospholipase A2-catalyzed hydrolysis of egg lecithin liposomes.

**Effect of Hexanol Concentration Upon the Rates of Hydrolysis and Activation**—Hexanol promotes the hydrolysis of phospholipid bilayers by several phospholipases, presumably by modifying the packing of the substrate in the bilayers. The reaction progress curves for the pancreatic phospholipase A2-catalyzed hydrolysis of multilamellar vesicles were obtained at several hexanol concentrations while the concentration of other components was kept constant. The kinetic parameters obtained from these curves for the multilamellar and unilamellar vesicles as a function of hexanol are presented in Fig. 2. The steady state rate of hydrolysis of the substrate is...
accelerated more than 100-fold for MLV at the optimal hexanol concentration of 42 mM. However, the half-time for the activation does not appear to change significantly between 25 to 55 mM hexanol. A slight increase in the half-time of activation may, however, be noted at higher hexanol concentrations. At less than 25 mM hexanol, the half-time values could not be determined accurately because, not only the rate of hydrolysis, but also the extent of hydrolysis is considerably smaller than that at higher concentrations of hexanol. This suggests that the half-time for activation depends neither upon the steady state rates of hydrolysis of the substrate nor upon the hexanol concentration.

As shown in Fig. 1, the lag period in the hydrolysis depends significantly upon the state of the bilayer. The multilamellar vesicles show a lag period of 3 min, whereas the lag period was less than 30 s when sonicated unilamellar vesicles were used as substrate. Furthermore, as shown in Fig. 2, the optimal activation of the hydrolysis of the unilamellar vesicles was obtained at 18 mM hexanol as compared to 42 mM required for the optimal hydrolysis of the multilamellar vesicles. These two types of vesicles also show significant difference in the shape of the hexanol activation profiles. Thus, for the multilamellar vesicles, the activation profile is biphasic with a sharp rise and fall. By contrast, the activation profile for the sonicated unilamellar vesicles is monotonic and broad. The optimal concentrations of hexanol (42 and 18 mM for MLV and ULV, respectively) are approximately proportional to its partition coefficients in bilayer/water for the two systems (48 and 90 for MLV and ULV, respectively (4)). This suggests that the hexanol to lipid molar ratio in the bilayer at the optimal hexanol concentrations is approximately 1.5 for multilamellar and 1.35 for the unilamellar vesicles.

**Effect of the Enzyme, Substrate, and Calcium Concentration**—The effect of varying the concentrations of the components required for the hydrolysis of lecithin by phospholipase A2 is shown in Fig. 3. The effect on the rate constant of activation and on the rate of hydrolysis of the substrate under steady state conditions is also shown in Fig. 3. In those experiments, the concentration of one of the components was varied, while the concentrations of all other components were kept constant at the optimal values. All the components were added within the first 15 s of the reaction. The hydrolysis was initiated by the addition of the enzyme unless otherwise stated. Fig. 3a shows that the rate of hydrolysis of the multilamellar vesicles depends upon the concentration of the substrate, whereas the rate constant for the activation step does not change over a 50-fold concentration range of the substrate. The substrate concentration dependence seems to obey Michaelis–Menten kinetics, with $K_m = 83 \pm 18 \mu M$ and $V_{max} = 63 \pm 5.2 \mu M/\min/mg$ of protein.

Fig. 3b shows a hyperbolic relationship between the steady state rate and the concentration of the substrate in the form of unilamellar vesicles. The latency period at all of the substrate concentrations in ULV was less than 30 s. Values of $K_m$ and $V_{max}$ for the ULV are $121 = 17 \mu M$ and $88 = 5 \mu M/\min/mg$ of protein. $K_m$ and $V_{max}$ for MLV and ULV are almost identical. Since the multilamellar vesicles are osmotically shocked, the $K_m$ values imply that the amount of the substrate interface available to the enzyme in MLV and ULV is similar (4).

Fig. 3c shows that the rate of hydrolysis increases linearly with the enzyme concentration, whereas the half-time for activation is independent of the enzyme concentration. The slope of the log[E] versus log(rate) plot (not shown) is 1.05, suggesting that the aggregation of the enzyme may not be involved in the catalytic process.

Fig. 3d shows that the rate of hydrolysis of the substrate also depends upon the calcium concentration, while the rate constant for the activation step is independent of the calcium concentration in the medium.

The data presented in Fig. 3, a to d thus demonstrate that the activation step is independent of the enzyme, substrate, and calcium concentrations. These different species are, however, required in the hydrolysis step.

**Effect of the Isomers of 1-Octanol on the Activation Parameters**—The experiments described in the preceding section suggest that the activation process responsible for the lag in the hydrolysis of phosphatidylcholine is a pure first order process. The half-time for this activation does not depend upon the concentration of any of the components required for the reaction. A lack of latency during hydrolysis of unilamellar vesicles and a slight change in the half-time as a function of hexanol concentration (Fig. 2) suggest that the activation process may depend upon the packing of the substrate in the lipid bilayer. This was investigated further. Besides hexanol, a variety of other alkanols can perturb the lipid bilayer and thus activate the phospholipase catalyzed hydrolysis. Of the various alcohols tested, octanols are most effective in perturbing the lipid bilayer. Hydrolysis of phosphatidylcholine in MLV by pancreatic phospholipase A2 was also found to be stimulated by several isomeric octanols. Activation profiles similar to those shown in Fig. 1 were obtained by adding the octanols at the concentrations required for a maximal rate of hydrolysis (5). The activation parameters obtained in the presence of optimal activating concentrations of the isomeric octanols are presented in Table I. The data show that both the steady state rate and the half-time for the activation are significantly affected by the position of the hydroxyl group in octanol. With increasing branching (compare octan-1-, 2-, 3-, and 4-ols), one notices a significant decrease in the half-time of activation.

Thus, if one assumes that the alcohols are localized in the bilayer with their hydroxyl group towards the aqueous phase, it would appear that the alcohols with a large cross-sectional...
area such as octan-3-ol and 2-ethylhexanol are probably most effective in lowering the half-time for activation. This implies that the alcohol exerts its effect on the half-time of activation by modifying the packing at the interface. This is also consistent with the observed low (less than 20 s) half-time of activation in sonicated unilamellar vesicles. The optimal activation concentrations of the various alcohols are proportional to the activating concentrations of these alcohols required for the bee venom phospholipase A2-catalyzed hydrolysis. As discussed elsewhere (5), this trend corresponds to the intrinsic perturbing ability of these isomeric octanols as reflected in their ability to modulate the order-disorder transition in lipid bilayers.

**Effect of Preincubation and the Sequence of Addition of Calcium and Hexanol**—The phospholipase A2-catalyzed reaction requires several components, and it is of interest to examine the effect of the sequence of their addition on the reaction progress curve. In the experiments described thus far, all the components were added within the first 15 to 20 s of the reaction. This may mean that the contribution of any or all of these components to the activation process is simultaneous. No lag period is observed with other phospholipases which also require calcium and hexanol for optimal activity, and so it is reasonable to assume that the lag is not due to a slow hexanol- or calcium-induced change (or both) in the lipid bilayer. Since latency is probably due to a change in the state of the enzyme, the effect of preincubating the enzyme with calcium + hexanol, calcium + lipid, lipid + hexanol, and all of these components with the products of hydrolysis was investigated. The results are presented in Table II. In each case, the reaction was started with one component, while all the other ingredients required for the reaction were preincubated. In all cases, the steady state rate of hydrolysis does not seem to depend upon the sequence of addition. Experiments I and II outlined in Table II show that the half-time of activation does not change whether the enzyme is preincubated with calcium alone or with lipid + calcium. Addition of hexanol at various times after preincubation of MLV + calcium + enzyme shows that the half-time for activation and the steady state rates of hydrolysis do not change significantly. Preincubation of the enzyme in the absence of calcium, however, yields different results. As shown in Experiment II, preincubation of the enzyme with lipid + hexanol reduces the half-time of activation and it is immeasurably small after about 10 min of preincubation in the absence of calcium. This experiment demonstrates that calcium is not required for the activation step, and it suggests that the calcium binding step (if any) follows the slow activation step.

Latency in the hydrolysis of the substrate could also be due to the activation of the enzyme by the products of hydrolysis at low concentrations. This hypothesis was tested by Experiment IV in Table II. By pretreating the liposomes in the presence of 40 mM hexanol, 20 mM CaCl2 at pH 8.0 with 10 ng of phospholipase A2 (bovine venom, about 60 nmol of phosphatidylcholine was hydrolyzed in 15 min. The amount of the products thus formed is the same as that formed in 3 to 5 half-times of activation by 250 ng of the pancreatic enzyme under identical conditions. To this mixture was then added 250 ng of pancreatic phospholipase A2. An increase in the rate of hydrolysis was observed with a half-time of activation of about 4 min and a latency period of 5 to 8 min. Since both of the enzymes form the same products, this observation shows that the products of hydrolysis do not cause the activation of the enzyme.

**DISCUSSION**

The data presented in this paper show that the action of the pancreatic phospholipase A2 on phosphatidylcholine multibilayer is potentiated by 1-hexanol. Like its activating effect on the action of other phospholipases (2), the hexanol dependence for the pancreatic enzyme is also biphasic. The most significant feature of the pancreatic phospholipase is that the progress curve for its action shows a lag period preceding the steady state phase. This phenomenon appears to be characteristic of the pancreatic enzyme since under similar conditions, we have not seen the lag phase in the reaction progress curve of phospholipase A2 from the venoms of honey bee, Russell's viper, and *Naja Naja* (4, 5). This difference alone rules out the mixing diffusion of the enzyme to the bilayer (or both) as the slow steps responsible for the lag period. In the multilamellar vesicles, a lag phase could arise at the non-saturating substrate concentrations because hydrolysis of the outer lamellae would allow the passage of the enzyme to the

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**Table I**

| Alkanol  | Concentration | Steady state rate | $t_{1/2}$ |
|---------|---------------|-------------------|----------|
| Octanol-1-ol | 7.8            | 38                | 5.2      |
| Octan-2-ol    | 10.5           | 75                | 4.5      |
| Octan-3-ol    | 12             | 60                | 2.6      |
| Octan-4-ol    | 14             | 85                | 3.1      |
| Oct-1-en-3-ol | 12             | 55                | 4        |
| 2-Ethylhexan-1-ol | 6           | 75                | 1.4      |
| Hexan 1 ol    | 42             | 60                | 3.3      |

**Table II**

| Sequence of addition | Half-time | Latency | Steady state rate |
|----------------------|-----------|---------|-------------------|
| I. L + Ca + E at $t = 0$ and hexanol at $t = 0$ min | 3.5 | 7.7 | 70 |
| II. E + Ca at $t = 0$ and hexanol + lipid at $t = 60$ min | 3.5 | 5.5 | 75 |
| III. L + E + hexanol at $t = 0$ and calcium at $t = 0$ min | 3.8 | 6.8 | 80 |
| IV. L + H + Ca + PLA2 (BV) at $t = 0$ and E at $t = 20$ min | $3.8 \pm 1$ | $-5.8$ | 60 |

$^a$ $t_{1/2}$, half-time could not be estimated.
inner lamellae as the reaction progresses (4). Such a latency would disappear when the substrate concentration is several times above the \( K_m \) or when the liposomes are osmotically shocked before the addition of the enzyme (or both). However, a lag period is observed even when both of these conditions are satisfied. Moreover, the \( t_{1/2} \) values remain constant over a 25-fold change in the substrate concentration. Both the \( K_m \) and \( V_{max} \) for the unilamellar and the multilamellar vesicles are similar, whereas only the MLV show measurable lag period. These observations rule out all the mechanisms of latency that involve a change in the concentration of the available substrate as the reaction progresses. An activating effect of the products of hydrolysis is also ruled out since no change in the \( t_{1/2} \) values was observed when the substrate was preincubated with the bee venom phospholipase \( A_2 \) to produce the products of hydrolysis in situ. Similarly, the latency could not arise from a slow step in the catalytic cycle since the turnover number of the enzyme is \( >18,000 \) min for both the unilamellar and multilamellar vesicles, whereas the latency is observed only with the later form of the substrate.

The lag period thus appears to be due to a slow penetration or activation of the enzyme in the bilayer (or both). Data presented in this paper demonstrate: that this activation process follows first order kinetics; that it is independent of the substrate, calcium, and the enzyme concentration; that it does not require calcium; that it is only slightly dependent upon the hexanol concentration; and that it is quite sensitive to the nature of the bilayer interface as modified by the incorporation of the branched chain octanols or as in the small unilamellar vesicles. All these observations suggest that the activation/penetration step underlying the latency is a purely first order process. This process does not seem to occur before the interaction of the enzyme with the bilayer since the halftime for activation depends so critically upon the nature of the bilayer interface. This implies that the process underlying the latency involves a spontaneous activation of the enzyme either during or after binding to the bilayer interface (or both).

Verger et al. (6) have observed a similar lag period in the progress curve for the hydrolysis of dinonanoylphosphatidylcholine monolayer films at air/water interfaces in a "zero order" trough. The results presented in this communication show remarkable similarities between the monolayer (zero order trough) and the hexanol-modified bilayer system. Analogous to the biphasic dependence of the rate of hydrolysis on the hexanol concentration, the rate of hydrolysis in a monolayer depends upon its surface pressure, where the high surface pressure side in the monolayer system corresponds to the low alcohol concentration side in the bilayer system. Although Verger et al. (6) did not report the rate constant for the activation, the values of half-time of activation calculated from their data is about 3\% min which is in accord with the \( t_{1/2} \) values of 3 to 3\% min observed under a variety of conditions in this communication. The calcium and the enzyme concentrations do not seem to have any effect on the kinetics of the activation process in either of the systems.

The most significant and probably the only difference between the behavior of an expanded monolayer and that of the hexanol-modified bilayer is in the dependence of the half-time of activation upon the surface pressure in the monolayer and a lack of such effect on the hexanol concentration in the bilayer. The steady state, zero order rate of hydrolysis in the bilayer shows a biphasic dependence upon the hexanol concentration. Similarly, in a monolayer, the rate of hydrolysis shows a biphasic dependence upon the surface pressure (7, 8). Thus, the packing of monolayer at 10 dynes/cm is optimal for the hydrolysis of dinonanoylphosphatidylcholine; a 42 mM hexanol concentration in the aqueous phase is optimal for the hydrolysis of the egg lecithin bilayer. The hexanol to lipid ratio is about 1.5 in the membrane phase at this concentration based on the lipid/water partition coefficient of 48 (6). Interestingly, the lag period in a monolayer increases more than 2-fold both at the lower and the higher surface pressures. By contrast, the \( t_{1/2} \) values for bilayers change only slightly with the hexanol concentrations even though the initial rates change more than 100-fold. This difference can be rationalized in terms of a separation between the molecules at the interface. This implies that the peak activating concentration of hexanol in the bilayer brings about an optimal separation of the phospholipid molecules, and that this separation is comparable to the separation between the lipid molecules in the monolayer at the optimal surface pressure. Indeed, if one assumes that the areas of hexanol and phosphatidylcholine are additive, the average area per molecule of phospholipid at the peak activating concentration of hexanol in multilamellar vesicles would be approximately 94 \( \AA^2 \) if the area of hexanol is 18 \( \AA^2 \). The actual value would be somewhat smaller depending upon the mode of packing and the orientation of the two species. The separation between the dinonanoyl lecithin molecules in a monolayer at the optimal pressure of 12 dynes/cm is 86 \( \AA^2 \) (10). Thus, it appears that a separation of 90 \( \AA^2 \) is required for the optimal activity of pancreatic phospholipase \( A_2 \) on a planar organized interface.

A requirement for the optimal separation of molecules at the interface could be rationalized by a hypothesis that the phospholipase molecule makes contact with two sites on the phospholipid interface. Such a contact would require an optimal separation between the molecules at the interface so that the two sites can orient and align with each other. These sites have been postulated as a catalytic and a recognition site on the enzyme (1). Penetration of the recognition site has been postulated as the slow step causing the lag period (6, 11, 12). By changing the surface pressure or by altering the alkanol concentration at the interface, the catalytic site can be aligned with the adjacent lipid molecule which acts as a substrate. By changing the average area per molecule at the interface the catalytic activity would pass through a maximum, thus accounting for a biphasic activation profile both in the monolayer and the bilayer.

In this model, the lag period would be a consequence of the slow step, the penetration of the recognition site into the interface. This process would require an optimal packing or lateral compressibility of the interface (or both). In a monolayer, both below and above the optimal surface pressure, the lag period increases. If the latency is due to a simple penetration of the recognition site, the lag period will increase monotonically with the surface pressure as the lateral compressibility decreases. This would imply that the recognition site requires an optimal orientation of the molecules at the interface, and that the orientation of the molecules at the interface changes as a function of the surface pressure.

In the alkanol-modified bilayer, the surface pressure is not expected to change significantly with the hexanol concentration. This is because the bilayer arrangement is retained following the incorporation of hexanol (13-16) and the stability of the bilayer requires high interfacial pressure (16). In dependence of the \( t_{1/2} \) values on the hexanol concentration also suggests that the factors governing penetration of the recognition site are not significantly altered by the incorporation of hexanol in the bilayer. These packing factors are, however, favorably altered by the introduction of the branched chain alcohols or in the unilamellar vesicles. This would imply that under certain conditions, the recognition site may be spontaneously and freely inserted into the interface. The quality of interface for such a facile penetration...
appears to require a loose packing. To a limited extent, it is
achieved by the introduction of 2-ethylhexane-1-ol. It is al-
much completely achieved in the unilamellar vesicles where
no measurable latency is observed. However, it appears that
yet another factor is operating in the hydrolysis of ULV, since
no measurable latency is observed and the hexanol concen-
tration dependence is monotonic. Such behavior could result if
somehow the recognition site does not penetrate the bilayer
for the interaction of the catalytic site. Geometrical factors
arising from a large radius of curvature of ULV could conceiv-
ably lead to the interaction of only one of the sites with the
interface. Measurement of the rate of hydrolysis would man-
ifest only the encounters of the catalytic site. Relative contri-
bution of the geometric and the packing factors in regulating
the interaction of recognition and catalytic sites with the
bilayer interface would require not only an understanding of
the packing in the bilayer but also a knowledge of the topog-
raphy of the enzyme.

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