Reactivity of plasmalogens: kinetics of acid-catalyzed hydrolysis

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ABSTRACT The acid-catalyzed hydrolysis of the α,β-unsaturated ether group of two plasmalogens, lysophosphatidal choline and lysophosphatidal ethanolamine, and several model compounds (isobutyl vinyl ether, 1-butenyl ethyl ether, and dihydropyran) was studied by determining the true second-order rate constants. The results indicate that the chemical reactivity of the substituted vinyl ether group in plasmalogens is not appreciably affected by the presence of a bulky substituent on the β-carbon. Activation energies, enthalpies, and entropies were also determined (from measurements of the rate constants at different temperatures).

SUPPLEMENTARY KEY WORDS vinyl ether · lysophosphatidal choline · lysophosphatidal ethanolamine · energy of activation · enthalpy of activation · entropy of activation

THE MECHANISM of the acid-catalyzed hydrolysis of α,β-unsaturated ethers has been the subject of several recent studies (1–4). These indicate that the process involves several steps:

$$CH_2{=}CH{-}OR + H^+ \underset{}{\overset{\text{slow}}{\rightleftharpoons}} CH_3{-}\overset{+}{C}H{-}OR \quad (1)$$

$$CH_3{-}\overset{+}{C}H{-}OR + H_2O \xrightarrow{\text{very fast}} CH_3{-}\underset{\underset{OH}{|}}{CH}{-}OR + H^+ \quad (2)$$

$$CH_3{-}\underset{\underset{OH}{|}}{CH}{-}OR \overset{\text{fast}}{\rightleftharpoons} CH_3CHO + ROH \quad (3)$$

where the rate-determining step is the addition of a proton to the unsaturated ether group to form a carbonium ion (reaction 1). The exact nature of the transition state is still under discussion. With substituted vinyl ethers (having a hydrogen on the β-carbon replaced with a methyl or ethyl group), the rates of hydrolysis are much slower than with the corresponding unsubstituted vinyl ether (1).

Substituted α,β-unsaturated ethers occur naturally principally as plasmalogens, a class of phospholipids that is found in highest concentration in brain and heart. In these phospholipids, designated as phosphatidal ethanolamine and phosphatidal choline, the β-substituent of the unsaturated ether is an alkyl chain of 14 or 16 carbon atoms rather than one or two. The question may be asked whether the reactivity of the unsaturated center is appreciably changed by the presence of such a bulky substituent. We believe that measurement of the hydrolysis constants provides the answer.

Since native plasmalogens contain two hydrocarbon chains and are insoluble in aqueous media, we have used compounds derived from them by removal of the acyl-ester chain, namely lysophosphatidal choline and lysophosphatidal ethanolamine. These substances could be dissolved in 80% dioxane, the medium used by Jones and Wood (1) to study the mechanism of vinyl ether hydrolysis.

MATERIALS AND METHODS

Lysophosphatidal Choline

The crystalline substance was prepared by alkaline hydrolysis of bovine heart lecithin as recently described (5). The sample had 5.93% P, a molar ratio of alde-

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hydogenic lipid to P of 0.86, and no detectable acyl-ester groups. For analytical methods, see (5).

Lysophosphatidal Ethanolamine

The method of preparation of this compound was very similar to that of lysophosphatidal choline. The starting material was a homogeneous preparation of ethanolamine phosphatide from bovine brain white matter. After alkaline hydrolysis and neutralization, the product was purified by chromatography on silicic acid (Unisil; correct fraction eluted with chloroform–methanol, 1:3) and recrystallized from absolute ethanol. The sample had 6.49% P, a molar ratio of aldehydogenic lipid to P of 0.87, and no detectable acyl-ester groups.

Vinyl Ethers

Isobutyl vinyl ether and dihydropyran were obtained as commercial products and redistilled. Preparations of 1-butenyl ethyl ether were synthesized and partially resolved according to Norton, Gottfried, and Rapport (6). Infrared absorption indicated that the predominantly *cis* fraction was 85% *cis*–15% *trans* whereas the predominantly *trans* fraction was 72% *trans*–28% *cis*.

Kinetic Studies

Vinyl ethers have an intense UV absorption band below 200 nm, which is replaced, as hydrolysis proceeds, by a much weaker absorption at 280 nm due to the formation of aldehydes (3). The UV absorption of a vinyl ether is sufficiently strong to permit measurement of its disappearance at wavelengths detectable with conventional equipment. With isobutyl vinyl ether, lysophosphatidal choline, and lysophosphatidal ethanolamine, measurements were made at 226 nm; with dihydropyran, 236 nm was used. Absorption of the plasmalogens at 226 nm was proportional to concentration over the range tested (0–6 mM). The measurements were made using a glass-stoppered silica cell of 1 cm light path and a Cary recording spectrophotometer, model 14 (Applied Physics Corp., Monrovia, Calif.). The cell compartment was held at constant temperature by circulating water from a constant-temperature bath.

The reaction medium contained 0.80 ml of *p*-dioxane (Matheson, Coleman & Bell, spectral grade), 0.19 ml of water, and 0.01 ml of 1.00 N perchloric acid. Synthetic vinyl ethers were studied at an initial concentration of 50 mM whereas the two phosphatides were at 6 mM.

At time zero, the perchloric acid was added to a solution of vinyl ether in dioxane–water (at the specified temperature) in a 15 ml glass-stoppered centrifuge tube. After the reaction mixture had been thoroughly mixed for 15 sec, it was quickly transferred to the silica cuvette in the sample compartment of the recording spectrophotometer (base line previously set with a mixture of dioxane–water–perchloric acid). The recording was started at 1.00 min and continued for at least 20 min. Each experiment was done two to four times. The initial linear portion of the plot of $\log_{10}$ absorbance against time was used to establish k_1, the specific reaction rate constant. A true second-order rate constant k_2 was then calculated:

$$k_2 = k_1/[\text{Acid}]. \qquad (2)$$

Energies of activation, Ea, were obtained graphically from the Arrhenius equation,

$$\log k_2 = \frac{-\text{Ea}}{2.303R} \cdot \frac{1}{T} + \log A$$

by plotting $\log k_2$ against $1/T$. Enthalpies and entropies of activation ($\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$) were obtained graphically from the Eyring equation,

$$\log \frac{k_2}{T} = \frac{-\Delta H^{\ddagger}}{2.303R} \cdot \frac{1}{T} + \log \frac{R}{Nh} + \frac{\Delta S^{\ddagger}}{2.303R}$$

by plotting $\log k_2/T$ against $1/T$.

RESULTS

The values (Table 1) show that lysophosphatidal choline was hydrolyzed somewhat more rapidly than lysophos-

TABLE 1 Hydrolysis of Vinyl Ethers and Plasmalogens Catalyzed by $HClO_4$*

	Second-Order Rate Constants $10^4\ k_2$ (liter mole^{-1} sec^{-1})						
	25°C	30°C	37.5°C	50°C	Ea	$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$
					kcal/mole	*kcal/mole*	*cal/mole*
Dihydropyran	56		153	374	15.3	14.2	−20.4
Isobutyl vinyl ether	317		865	2230	15.1	14.3	−17.2
Lysophosphatidal choline	199	293	480		13.5	12.8	−23.4
Lysophosphatidal ethanolamine	136	197	351		12.8	12.1	−26.6

* The reaction medium contained 0.80 ml of *p*-dioxane, 0.19 ml of water, and 0.01 ml of 1.00 N $HClO_4$. Synthetic vinyl ethers were studied at an initial concentration of 50 mM; lysophosphatidal choline and lysophosphatidal ethanolamine at 6 mM.

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phatidal ethanolamine. Both of these compounds were hydrolyzed more rapidly than dihydropyran and less rapidly than isobutyl vinyl ether. The second-order rate constant found for hydrolysis of isobutyl vinyl ether at 25°C (317×10^{-4} liter mole^{-1} sec^{-1}) compares favorably with the value recorded by Jones and Wood (409×10^{-4} liter mole^{-1} sec^{-1}). The activation energies for the natural products, namely 13.5 and 12.8 kcal/mole, are similar to those of the model ethers (15.1 and 15.3 kcal/mole); these differences are so small as to preclude any inversions of relative rates of activity in the important temperature range of 10–50°C.

Rate constants showed that the *cis*-enriched fraction of 1-butenyl ethyl ether is much more rapidly hydrolyzed than the *trans*-enriched fraction. With the *cis*-enriched fraction the values at 37.5°C and 50°C were 80 and 162 ($\times 10^{-4}$ liter mole^{-1} sec^{-1}) and with the *trans*-enriched fraction, 17 and 39.

DISCUSSION

Kresge and Chiang (3) showed that the course of hydrolysis of vinyl ethers could be followed by making measurements on the shoulder of the low-wavelength band, and that identical first-order rate constants were obtained either by measuring the disappearance of reactant in this way or by measuring the appearance of product from the increase in absorbance at 280 nm. Because of the limited solubility of plasmalogen derivatives and the relatively weak absorption of the aldehyde products, measurement of the disappearance of reactant was the method of choice. Values of the second-order rate constant found with lysoplasmalogens show that these compounds are hydrolyzed at rates lying between those found for isobutyl vinyl ether and dihydropyran, and thus fully retain the reactivity associated with the α,β-unsaturated ether structure.

Jones and Wood (1) found that β-substitution of ethyl vinyl ether caused a decrease in the second-order rate constant to about one-sixth the value with the unsubstituted compound. Their recorded values at 25°C were 530×10^{-4} liter mole^{-1} sec^{-1} for ethyl vinyl ether (unsubstituted β-position), 92 for the 1-propenyl ethyl ether (monosubstituted β-position), 91 for 1-butenyl ethyl ether (monosubstituted β-position), and 8.7 for 1-isobutenyl ethyl ether (disubstitution of the β-position). Plasmalogens, which are mono substituents in the β-position, do not show a reduction in hydrolysis rate as great as would be suggested by Jones' and Wood's work on vinyl ethers with short β-substituents. A possible explanation may be found in the configuration of the double bond in plasmalogens, which is known to be *cis* (6, 7). The synthetic β-substituted vinyl ethers studied by Jones and Wood (1) probably contain a major fraction of the compound in the *trans* configuration. We may therefore expect the *cis* form to be hydrolyzed much more rapidly than the *trans* form. Studies with partially resolved preparations of 1-butenyl ethyl ether confirm this expectation, since the mixture containing 85% *cis*–15% *trans* was hydrolyzed four times faster at 37.5°C than the mixture containing 28% *cis*–72% *trans*. Plasmalogens also differ from the synthetic models in having a glycerol phosphoryl ester rather than a simple alkyl group as the second substituent of the oxygen. Whether this affects the reactivity of the unsaturated ether is not known.

The thermodynamic values for the model compounds and the plasmalogens (Table 1) are similar. The relatively low values for energy of activation and enthalpy of activation reflect the rapid rates of reaction. The large negative values of entropy of activation, indicating a relatively ordered transition state which has a definite configuration, have been interpreted to mean that the transition state has a high degree of carbonium ion character (2, 8).

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