Reduced Hydrolytic Lability of Epoprostenol in the Presence of Cationic Micelles

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Abstract □ The rapid hydrolysis of epoprostenol to 6-keto-prostaglandin $F_{1\alpha}$ is hydronium ion-catalyzed even at pH 10 or higher. In the presence of 1.0% hexadecyltrimethylammonium chloride, the rate was reduced significantly (1580-fold at pH 2 and 283-fold at a physiological pH). This decrease in the hydrolysis rate is attributed to two causes: favorable partitioning of epoprostenol in the micellar phase and electrostatic repulsion between the hydronium ion and the cationic micellar surface.

Keyphrases □ Prostaglandins—epoprostenol, reduced hydrolytic lability in the presence of cationic micelles □ Micelles—reduction of hydrolytic lability of epoprostenol □ Epoprostenol—reduced hydrolytic lability in the presence of cationic micelles

The antiplatelet (anti- and deaggregatory) and vasodilator actions of epoprostenol (I) has brought about not only some potential clinical applications but also "a direct impact on research dealing with the mode of antiaggregatory cyclic AMP activity" (1). Being a vinyl ether, however, I is extremely unstable in aqueous media; half-life ($t_{1/2}$) of its hydrolysis is only several minutes at a physiological pH and 25° (2). Subsequently it was found that the compound is unusually reactive even as a vinyl ether and that the hydronium ion-catalyzed hydrolytic lability of the carboxylate anion is nearly two orders of magnitude greater than that of the free acid or methyl ester (Fig. 1) (3). The instability of this endogenous hormone-like substance has hampered the progress of clinical applications and in the basic research area. The present study reports that in the presence of cationic micelles the hydrolysis rate can be retarded by a factor of 1.5×10^3 at pH 2 and 10^2 at a physiological pH.

EXPERIMENTAL

A UV spectrophotometric[1] procedure was used to follow the hydrolysis of I (2). Essentially, the absorbance change at 235 or 240 nm was monitored continuously immediately after the sodium salt of I (~4 mg) was dissolved in a non-UV absorbing buffer solution (~3 ml) of 0.5 *M* ionic strength in a cell. When the spectral change was scanned continuously, an isosbestic point was observed at 256 nm throughout a kinetic run over the pH range studied. Hexadecyltrimethylammonium chloride[2], was used as received.

RESULTS AND DISCUSSION

Negative micellar catalysis has not been subject to numerous applications in the area of drug formulation development, although stabilization of a drug compound can be achieved in aqueous media (4). This is surprising since micelle-catalyzed organic reactions in aqueous media have been studied extensively as model systems of enzymatic reactions.

Aqueous solutions containing a surface-active agent form molecular aggregates known as micelles when the total concentration reaches a certain value, which is referred to as the critical micellar concentration (CMC). The CMC of hexadecyltrimethylammonium chloride $[(CH_3)_3N^+(CH_2)_{15}CH_3 \cdot Cl^-]$ is $\sim 1.3 \times 10^{-3} M$(5), and hence in a 1.0% $(3.12 \times 10^{-2} M)$ solution (in which the stability of I was investigated), most of the surfactant molecules are engaged in micelles. Since the polarity of the micellar phase can be much lower than that of the bulk aqueous phase, I free acid is expected to partition favorably into the micellar phase. However, as pH increases the apparent fractional concentration of I found inside the micellar phase will decrease because the dynamic equilibrium becomes more favorable for the anion to exist in an aqueous environment.

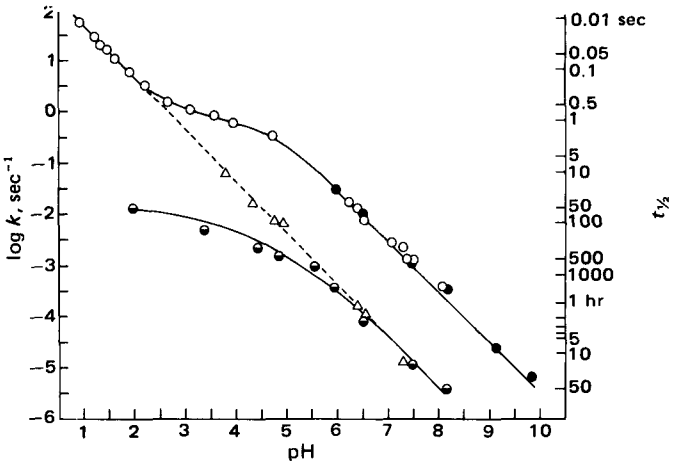
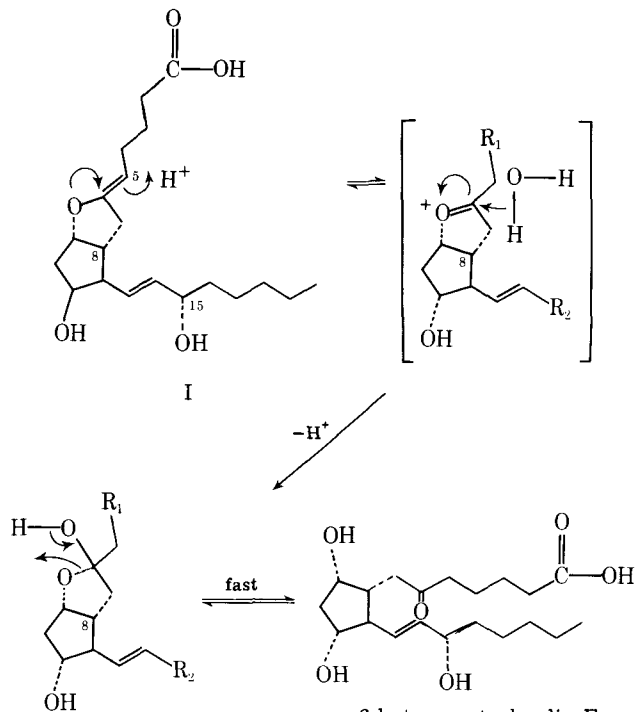


Figure 1—*pH dependence of the hydrolysis rate of I (closed circles from Ref. 2 and open circles from Ref. 3), I methyl ester (triangles from Ref. 3), and in the presence of 1.0% hexadecyltrimethylammonium chloride (half-circles), all at 25°. The former two are the rate constants at zero buffer concentration, and the last one was at 0.5* M *ionic strength.*

From this pH-dependence of the partitioning behavior of I and from



Scheme I—Reaction pathway of I hydrolysis.

[1] Zeiss Model DMR-21 Spectrophotometer
[2] Eastman Kodak Co., Rochester, N.Y.

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the general belief that the thermodynamic activities of water (reactant) and hydronium ion (catalyst) can be extremely low inside the micellar phase, one would expect the pH-profile of log k_{obs} shown in Fig. 1 (half-filled circles). Note that the overall rate-determining step in the hydrolysis of I is the protonation at C-5, which makes the Δ^5 bond polarized and susceptible to the subsequent water attack (Scheme I) (3). That is, if an intrinsic reactivity is assigned to the free acid present inside the micellar phase, regardless of the pH of the bulk phase, then the pH-profile of the observed rate constant should resemble that of the apparent partition coefficient. This analysis is consistent with what was observed.

In the presence of 1.0% hexadecyltrimethylammonium chloride, the apparent hydrolysis rate at pH below 3 is $\sim 1.5 \times 10^3$ fold slower than in the absence of the surfactant (Fig. 1). At neutral pH values, however, such a comparison can be made only after taking the general acid catalysis by buffer components into consideration. At pH 7.45, for instance, if the data shown in Fig. 1 are compared, only a 62-fold decrease in the rate is obtained. However, the hydrolysis rate shown in Fig. 1 in the absence of the surfactant is the rate extrapolated to zero buffer concentration, whereas that in the presence of the surfactant was obtained in a 0.165 *M* phosphate buffer of 0.50 *M* ionic strength. In an identical buffer system without the surfactant, it was found previously that the hydrolysis is ~4.56 faster than at zero buffer concentration (2). The net effect of 1.0% hexadecyltrimethylammonium chloride is, therefore, a reduction of the hydrolysis rate of ~280-fold; $t_{1/2}$ from 3.5 min to 16.5 hr.

Finally, one should not attempt to interpret the coincidental overlap of the rate constants for the hydrolysis of methyl ester (triangles in Fig. 1) and I in the presence of the surfactant at pH above 6. As discussed previously (4), the latter is a function of the concentration of both substrate and the surfactant present in a given system.

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Determination of Tissue to Blood Partition Coefficients in Physiologically-Based Pharmacokinetic Studies

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Abstract □ The partition coefficient between tissue and blood used in physiologically-based pharmacokinetic modeling analysis was investigated using the concept of clearance. New equations were derived and compared with previously reported equations in constant intravenous infusion and bolus injection methods. The importance of differentiating arterial from venous blood is discussed.

Keyphrases □ Partition coefficient—tissue to blood, physiologically-based pharmacokinetics □ Pharmacokinetics—determination of tissue to blood partition coefficients in physiologically-based pharmacokinetic studies □ Blood sampling—differences between arterial and venous blood, physiologically-based pharmacokinetic studies

The formulation of a physiologically-based pharmacokinetic model requires an accurate determination of physiological parameters such as blood flow, organ volume, partition coefficient, and clearance (1). The estimation of the tissue to blood partition coefficients for a compound is of special interest to pharmacokineticists because it can be directly measured in the laboratory. Recently, Chen and Gross (2) pointed out different methods by which tissue to plasma partition coefficients can be determined under specific experimental conditions. The following equations were used in constant infusion and bolus injection studies, respectively:

$$R = \left(1 + \frac{K}{Q}\right)\frac{C_t^\infty}{C_p^\infty} \qquad \text{(Eq. 1)}$$

$$R = \frac{(Q + K)C_t^0}{(QC_p^0 + \alpha V_t C_t^0)} \qquad \text{(Eq. 2)}$$

where R is the partition coefficient of drug between organ tissue and plasma, K was defined as the first-order elimination rate constant [but was used as organ clearance in their calculations (2)], Q is the flow rate of plasma in the organ, C_t^∞ and C_p^∞ are the concentrations of drug in tissue and plasma at steady state, C_t^0 and C_p^0 are the concentrations of drug in tissue and plasma at time zero extrapolated from the terminal phase, α is the terminal rate constant, and V_t is the volume of the organ or tissue. The present study examined Eqs. 1 and 2 from the concept of physiological clearance and derived new equations for the determination of R in constant infusion and bolus injection studies. Flow and concentration in terms of blood were dealt with instead of plasma.

THEORETICAL

The concept of clearance and its applications are well defined in the pharmacokinetic literature (3–7). In an eliminating organ or tissue, it describes the volume of incoming blood completely cleared of drug by the organ per unit time. Conventionally, it is expressed as the organ clearance, CL_s, and is defined as:

$$CL_s = \frac{r}{C_i} \qquad \text{(Eq. 3)}$$

where r is the rate at which the drug is removed from the organ and C_i is the drug concentration in the incoming blood. However, it has been observed that CL_s might be dependent on the blood flow through the organ and the use of intrinsic clearance, CL_i, was proposed to correct for the influence of blood flow (3). CL_i is defined as:

$$CL_i = \frac{r}{C_0} \qquad \text{(Eq. 4)}$$

where C_0 is the effluent venous blood concentration which is in equilibrium with the eliminating organ. It measures the maximum capacity of the organ to eliminate the drug. An important relation obtained from the above clearance equation is:

$$r = CL_s C_i = CL_i C_0 \qquad \text{(Eq. 5)}$$

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