The Hydrolytic Reactivity of Homoprostacyclin: Implications for the Physiological Control of Bleeding

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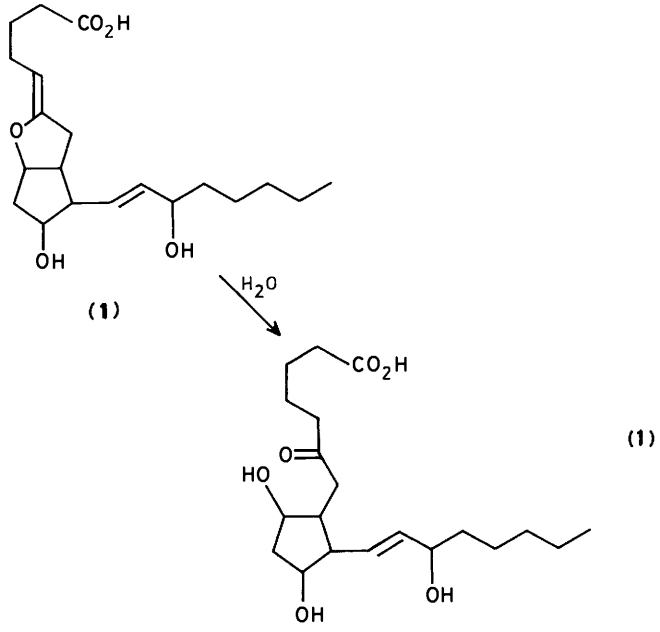
Rates of hydrolysis of the vinyl ether group of homoprostacyclin (**2**), which differs from prostacyclin only in that it has four instead of three carbon atoms in the chain joining this group to a carboxy function, were measured in H_2O solution over the pH range 1—9 and in D_2O solution in $D_2PO_4^-$–DPO_4^{2-} buffers. The rate profile so obtained shows that homoprostacyclin is eight times more reactive in its carboxylate than in its carboxylic acid form, and an isotope effect on the accelerated reaction indicates that this rate increase is the result of intramolecular catalysis by the carboxylic acid group. This eight-fold acceleration is significantly less than the 100-fold increase produced by intramolecular catalysis in the case of prostacyclin itself, and that suggests that natural evolution has given prostacyclin the correct lifetime it needs to be effective in the physiological mechanism for the control of bleeding by adjusting the length of the carbon chain joining its vinyl ether and carboxy groups.

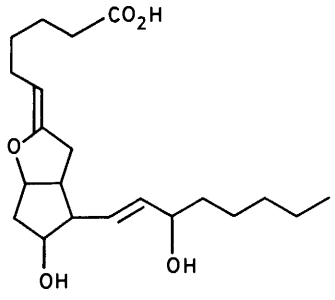
Prostacyclin (**1**) is a naturally occurring bioregulator with remarkable anti-blood-clotting properties; it is the most potent inhibitor of blood platelet aggregation so far known.[1] This gives it tremendous potential for biomedical applications. Unfortunately, however, prostacyclin is also very unstable: its lifetime at physiological pH values is only 3 min. We have shown recently that this instability comes from hydrolysis of prostacyclin's vinyl ether functional group [reaction (1)], accelerated 100-fold through intramolecular general acid catalysis by the molecule's carboxy function.[2]



(1)

This instability limits the biomedical usefulness of prostacyclin severely, but it appears also to play a beneficial role in a physiological mechanism for the control of bleeding.[3] Prostacyclin is produced in the endothelial lining of blood vessels and is released into the blood, where it is in homeostatic balance with thromboxane, a potent platelet-aggregating factor. When a blood vessel is injured or broken, manufacture of prostacyclin at the site of the lesion is impaired, and rapid hydrolysis of the remaining prostacyclin quickly lowers its concentration in that region to a level where it can no longer offset the effects of thromboxane. Blood platelet aggregation, which is the first step in clot formation, then takes place, and that stems the flow of blood from the broken vein or artery. If the lifetime of prostacyclin at physiological pH values were significantly greater than 3 min, clotting would occur more slowly and significantly more blood would be lost.

It is interesting to speculate that natural evolution has given prostacyclin an optimum hydrolysis lifetime by adjusting the efficiency of the intramolecular catalysis which governs the hydrolytic reactivity of this molecule. This could have been achieved through regulation of the length of the carbon chain which joins prostacyclin's vinyl ether and carboxy groups, for, as is well known, the efficacy of intramolecular catalysis is a sensitive function of the size of the ring formed when catalyst and reaction site come together.[4] In order to test this hypothesis, we have examined the hydrolytic reactivity of homoprostacyclin (**2**), a molecule which is similar to prostacyclin in all respects except that it has one carbon atom more in the chain joining these two functional groups.



(2)

Results

Rate Profile.—Rates of hydrolysis of the vinyl ether group of homoprostacyclin were determined over the pH range 1—9 by making measurements in perchloric acid solutions and a number of different buffers. All determinations were performed in wholly aqueous media at 25 °C, and ionic strength was held constant at 0.10M. The measurements in perchloric acid solutions covered the concentration range 0.0007—0.02M; these data are summarized in Table S1.†

† Tables S1—S3 are available as Supplementary Publication No. SUP56710 (10 pp.). For details of Supplementary Publications see Instructions for Authors (*J. Chem. Soc., Perkin Trans. 2*, 1988, Issue No. 1).

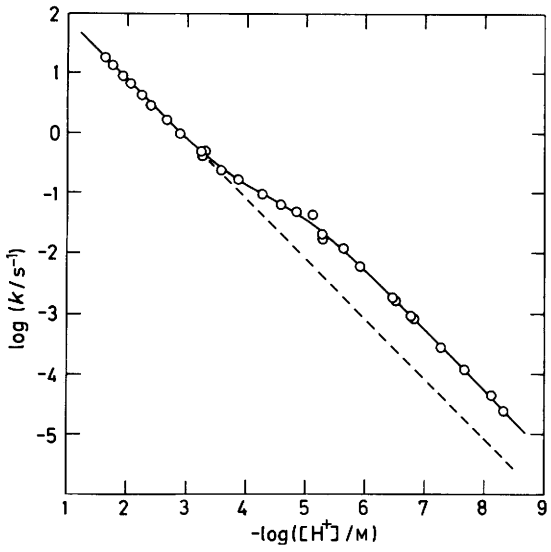


Figure 1. Rate profile for the hydrolysis of homoprostacyclin in aqueous solution at 25 °C, ionic strength 0.10M

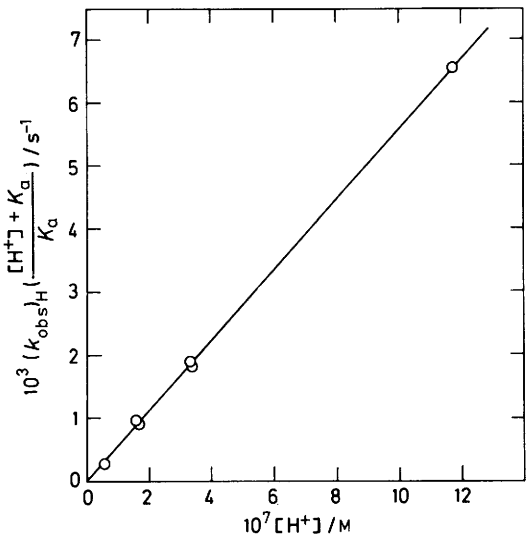


Figure 2. Relationship between $[H^+]$ and modified buffer-independent rate constants for the hydrolysis of homoprostacyclin in aqueous $H_2PO_4^-$–HPO_4^{2-} buffer solutions at 25 °C, ionic strength 0.10M

For the measurements in buffers, series of solutions of constant buffer ratio but changing buffer concentration were used. Six buffer acids [formic, acetic, cacodylic, dihydrogen phosphate ion, monohydrogen t-butylphosphonate ion, and tris(hydroxymethyl)methylammonium ion] were employed; these data are summarized in Table S2.*

Marked buffer catalysis was observed in all the buffer solutions. The data were therefore extrapolated to zero buffer concentration by linear least-squares analysis, and the observed oxonium ion catalytic coefficients so obtained, together with the rate constants measured in perchloric acid solutions, were used to construct the rate profile shown in Figure 1.† The combined data were also fitted to a rate law [equation (2)] based upon a

$$(k_{obs})_{H^+} = \frac{k_{H^+}[H^+]^2 + k'_{H^+}K_a[H^+]}{[H^+] + K_a} \quad (2)$$

$$PH \underset{}{\overset{K_a}{\rightleftharpoons}} P^- + H^+$$

k_{H^+} H^+ k'_{H^+} H^+

hydrolysis product (3)

kinetic scheme (3) which allows for hydrolysis of the substrate in both the carboxylic acid, *P*H, and carboxylate ion, P^-, forms. The data fit this scheme well. Least-squares analysis gave $k_{H^+} = (7.32 \pm 0.05) \times 10^2$ l mol^{-1} s^{-1}, $k'_{H^+} = (6.04 \pm 1.31) \times 10^3$ l mol^{-1} s^{-1}, and $K_a = (1.16 \pm 0.42) \times 10^{-5}$ mol l^{-1}. The latter is the acid dissociation constant of the carboxy group of homoprostacyclin in aqueous solution at ionic strength 0.10M. This may be converted, with the aid of estimated activity coefficients,[5] into a zero-ionic strength acidity constant which corresponds to $pK_a = 5.13 \pm 0.16$; that is a reasonable value for a carboxylic acid of this structure.

Isotope Effects.—The kinetic isotope effect on hydrolysis of homoprostacyclin in the carboxylate form, k'_{H^+}/k'_{D^+}, was determined from rate measurements made in H_2O and D_2O solutions of phosphate buffers ($L_2PO_4^-/LPO_4^{2-}$). In these buffer solutions, the carboxylic acid group of homoprostacyclin was largely, but not quite completely, ionized. Allowance was made for this by using the rate law of equation (2) in a rearranged form (4). The factor $([H^+] + K_a)/K_a$, which modifies $(k_{obs})_{H^+}$ in this expression and represents the reciprocal of the fraction of acid ionized, ranged from 1.01 to 1.10 for the H_2O solutions used.

$$(k_{obs})_{H^+}\left(\frac{[H^+] + K_a}{K_a}\right) = k_{mod} = \frac{k_{H^+}[H^+]^2}{K_a} + k'_{H^+}[H^+] \quad (4)$$

Figure 2 shows that rate constants modified in this way are accurately proportional to the first power of $[H^+]$, which indicates that the quadratic term of equation (4) makes a negligible contribution under the conditions employed. This conclusion is corroborated by the results of a quadratic least-squares analysis $k_{mod} = -(0.53 \pm 3.98) \times 10^{-6} + (5.64 \pm 0.22) \times 10^3[H^+] - (0.68 \pm 1.62)[H^+]^2$, which provide a coefficient for the quadratic term the uncertainty of which exceeds its value; this analysis also shows that the constant term is zero as well, as required by equation (4). A linear least-squares analysis is therefore adequate, and that gives $k_{mod} = (0.88 \pm 1.89) \times 10^{-5} + (5.55 \pm 0.04) \times 10^3[H^+]$. The coefficient of the linear term so obtained, which is equal to k'_{H^+}, is in good agreement with the linear term of the quadratic fit, and either of these results provides a more precise estimate of k'_{H^+} than the value obtained from the analysis of the whole rate profile, $k'_{H^+} = (6.04 \pm 1.31) \times 10^3$ l mol^{-1} s^{-1}.

A corresponding treatment of data determined in D_2O solution (Table S3)* requires knowledge of the acid dissociation constant of homoprostacyclin in D_2O, $K_a(D_2O)$. This has not been measured, but the factor $\{[D^+] + K_a(D_2O)\}/K_a(D_2O)$ is not very sensitive to the value of $K_a(D_2O)$ at the concentrations of D^+ used; an adequate estimate may be made by assuming that the isotope effect on this acid dissociation constant is the same as that for acetic acid,[6] the pK_a value of which is similar to

* See footnote on p. 1083.

† Values of $[H^+]$ in the buffer solutions needed for this purpose were obtained by calculation using literature pK_a values of the buffer acids and activity coefficients recommeded by Bates.[5]

Table. Comparison of reaction parameters[a]

Parameter	Homoprostacyclin	Prostacyclin[b]	(*Z*)-6,9-Epoxynon-5-enoic acid[c]
pK_a	5.1	5.0	4.9
k_{H^+}/l mol^{-1} s^{-1}	732	439	745
k'_{H^+}/l mol^{-1} s^{-1}	6 040	43 600	60 900
k'_{H^+}/k_{H^+}	8	99	82

[a] In aqueous solution at 25 °C; the pK_a values refer to zero ionic strength and the rate constants refer to ionic strength 0.10M. [b] Ref. 2*a*. [c] Ref. 2*b*.

that of the carboxy group of prostacyclin. This produces values of $\{[D^+] + K_a(D_2O)\}/K_a(D_2O)$ in the range 1.01—1.03. Linear-least squares analysis then gives $k_{mod} = (1.31 \pm 1.55) \times 10^{-5} + (2.65 \pm 0.25) \times 10^3[D^+]$, which leads to the isotope effect $k'_{H^+}/k'_{D^+} = 2.10 \pm 0.20$.

Another isotope effect, that on catalysis of the hydrolysis of homoprostacyclin in the carboxylate form by the external acid $H_2PO_4^-$, $k'(H_2PO_4^-)/k'(D_2PO_4^-)$, may also be obtained from these phosphate buffer experiments. The buffer-catalysed portion of the rate constants observed in these solutions represents catalysis by $H_2PO_4^-$ and, at least in principle, by H_3PO_4 as well.[7] That leads to the rate law shown as equation (5), in

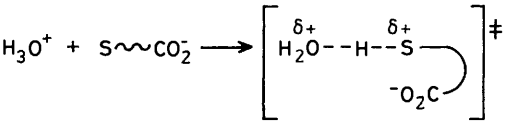
$$\frac{\Delta(k_{obs})_{buff}}{\Delta[H_2PO_4^-]}\left(\frac{[H^+] + K_a}{K_a}\right) = (k_{mod})_{buff} = \frac{k(H_3PO_4)}{K_1K_a}[H^+]^2 + \left(\frac{k(H_2PO_4^-)}{K_a} + \frac{k'(H_3PO_4)}{K_1}\right)[H^+] + k'(H_2PO_4^-) \quad (5)$$

which K_1 is the first acid dissociation constant of H_3PO_4, and unprimed rate constants refer to hydrolysis of the carboxylic acid form of homoprostacyclin and primed rate constants, the carboxylate form. Quadratic least-squares analysis gives a result, $(k_{mod})_{buff} = (1.02 \pm 0.08) \times 10^{-1} + (7.16 \pm 4.21) \times 10^4[H^+] - (1.87 \pm 3.16) \times 10^{10}[H^+]^2$, which again shows that the quadratic term makes a negligible contribution; this was also the case with prostacyclin itself.[2a] A linear least-squares treatment is therefore adequate, and that gives $(k_{mod})_{buff} = (1.06 \pm 0.04) \times 10^{-1} + (4.70 \pm 0.72) \times 10^4[H^+]$. The coefficient of $[H^+]$ in this expression represents two parallel reactions which depend upon $[H^+]$ in the same way and the rate constants of which therefore cannot be separated by this treatment. The intercept, however, refers to a single process and gives $k'(H_2PO_4^-) = (1.06 \pm 0.04) \times 10^{-1}$ l mol^{-1} s^{-1}. A similar treatment of the D_2O data leads to $k'(D_2PO_4^-) = (1.69 \pm 0.07) \times 10^{-2}$ l mol^{-1} s^{-1}, and combination of the H_2O and D_2O results provides the isotope effect $k'(H_2PO_4^-)/k'(D_2PO_4^-) = 6.25 \pm 0.35$. This is a reasonable value for a process involving rate-determining proton transfer from an undissociated acid to the β-carbon atom of a vinyl ether group, and it is consistent with $k'(H_2PO_4^-)/k'(D_2PO_4^-) = 7.0 \pm 1.8$ determined for prostacyclin itself.[2a]

Discussion

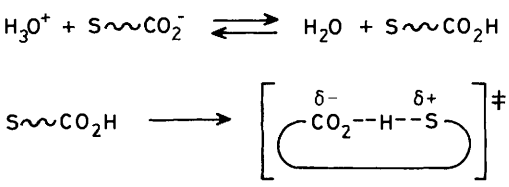
The rate profile in Figure 1 shows that homoprostacyclin, like prostacyclin itself,[2a] is more reactive in the carboxylate than in the carboxylic acid form. This increased reactivity may be evaluated quantitatively as $k'_{H^+}/k_{H^+} = 8$, which is considerably less than the factor of 10^2 obtained for prostacyclin.[2a]

In the case of prostacyclin, solvent isotope effects served to distinguish between two alternative mechanistic explanations of this increased reactivity. One of these explanations involved a stabilizing electrostatic interaction between the carboxylate group and the positive charge being generated on the substrate in the reaction's rate-determining transition state [reaction (6)], while the other postulated protonation of the carboxylate



(6)

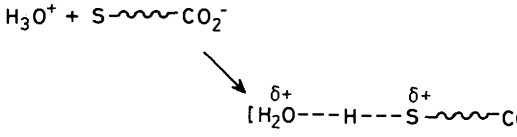
group to give an intramolecular general acid catalyst which would utilize the well known advantage of intra- over intermolecular processes [reaction (7)]. A choice between these two



(7)

alternatives can be made on similar grounds in the present case.

An isotope effect (k_{H^+}/k_{D^+} of 1.5 can be predicted for the intramolecular catalysis scheme (7).[2a] In the present case, however, a somewhat larger effect would be observed because the acceleration here amounts to only a factor of 8, and reaction through the mechanism (7) would be accompanied by a small but significant amount (12%) of vinyl ether hydrolysis through straightforward proton transfer to carbon from H_3O^+ with no help from the carboxylate group [reaction (8)]. The rate



(8)

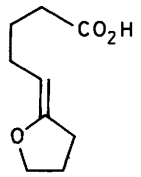
constant for such a process should be similar to that for hydrolysis of homoprostacyclin in the carboxylic acid form, k_{H^+} 732 l mol^{-1} s^{-1}, and this value may then be used in conjunction with a Marcus theory correlation of isotope effects with reaction rates made for a series of simple vinyl ethers[8] to provide the estimate $k_{H^+}/k_{D^+} = 3.8$ for this route. Combination of that with $k_{H^+}/k_{D^+} = 1.5$ in the appropriate proportion then leads to the prediction that $k_{H^+}/k_{D^+} = 1.7$ would be observed if intramolecular catalysis is the correct explanation for the eightfold increased reactivity.

A prediction of the isotope effect for the electrostatic stabilization scheme (6) may also be made from the Marcus theory correlation by using the rate constant for the accelerated reaction, $k'_{H^+} = 6\,040$ l mol^{-1} s^{-1}. This, fortuitously, produces an estimate, $k_{H^+}/k_{D^+} = 3.8$, which is the same as that made for the unassisted reaction (8),* and an adjustment for the simultaneous occurrence of some reaction by this additional route therefore need not be made. Thus, if electrostatic stabilization is the correct explanation of the reactivity increase, then $k_{H^+}/k_{D^+} = 3.8$ should be observed.

The measured isotope effect on hydrolysis of the vinyl ether group of homoprostacyclin in the carboxylate form (k'_{H^+}/k'_{D^+}) is 2.1 ± 0.1. This is in much better agreement with the prediction for intramolecular catalysis than that for electrostatic stabilization, and, as was the case for prostacyclin itself, intramolecular catalysis may therefore be assigned as the mechanism responsible for the increased reactivity of the carboxylate form.

* Both systems lie near the top of the isotope effect–rate correlation where k_{H^+}/k_{D^+} passes through a maximum value and is not strongly dependent upon k_{H^+}.

It was already noted that the increased reactivity found here for homoprostacyclin, $k'_{H^+}/k_{H^+} = 8$, is significantly less than the 100-fold factor determined for prostacyclin. This is the result of a reduced value of k'_{H^+} in the present case. As the data summarized in the Table show, k_{H^+} for homoprostacyclin is much the same as that for prostacyclin, and also as that for (*Z*)-6,9-epoxynon-5-enoic acid (**3**), a simple prostacyclin model



(**3**)

which mimics the hydrolytic reactivity of prostacyclin closely;[2b] the value of k'_{H^+} for homoprostacyclin, however, is considerably less than that for either of the other two substances. Increasing the length of the carbon chain which joins the vinyl ether and carboxy groups in these molecules has thus reduced the efficiency of intramolecular catalysis by lowering the rate of reaction of the carboxylate form.

As a result of this, homoprostacyclin's physiological lifetime is 30 min. Such a lifetime is too long to be effective in the control of bleeding, and our study therefore offers some support for the hypothesis that natural evolution has given prostacyclin the lifetime needed for efficient blood loss control by adjusting the length of the carbon chain joining its vinyl ether and carboxy groups.

Experimental

Materials.—Homoprostacyclin was obtained from the Ono Pharmaceutical Company. All other materials were best available commercial grades and were used as received. Solutions were prepared using deionized H_2O, purified further by distillation, or D_2O (Merck, Sharp, and Dohme; 99.8 atom % D) as received.

Kinetics.—Rate determinations were performed as for prostacyclin,[2a] by monitoring the decrease in absorbance of the strong vinyl ether band of homoprostacyclin at 200—210 nm. Measurements were made using either a Cary model 118 spectrometer or a Durrum-Gibson stopped-flow system. The kinetic data conformed well to the first-order rate law, and first-order rate constants were evaluated by standard methods.

Acknowledgements

We thank the Ono Pharmaceutical Company for a gift of prostacyclin. We are also grateful to the Natural Sciences and Engineering Research Council of Canada and the Donors of the Petroleum Research Fund, administered by the American Chemical Society, for financial support of this work.

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Received 10*th August* 1987; *Paper* 7/1474