Surface Chemical Study on Base-Catalyzed Degradation (Isomerization) of Prostaglandin A₂

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Abstract: Base-catalyzed degradation (isomerization) of prostaglandin A₂ (PGA₂) was measured at pH 12 and 60°C, and the effect of micellization of PGA₂ on the degradation was investigated. The rate of degradation was determined by measuring the rate of appearance of PGB₂ with a spectrophotometer. The apparent rate constant, kₐₚₚ, for degradation of free PGA₂ was approximately constant below the critical micelle concentration (cmc), while kₐₚₚ was decreased by the micellization of PGA₂. This is considered to be due to the electrostatic repulsion between OH⁻ and negatively charged PGA₂ micelles. The value of kₐₚₚ of PGA₂ above the cmc was slightly increased by the addition of sodium chloride as an electrolyte. The apparent rate, v, for degradation of PGA₂ above the cmc could be explained in terms of the sum of the two degradation rates, v₁ and vₘ, in the bulk and micellar phases, respectively: the increase in v by the addition of NaCl was due to the decrease in v₁ and the increase in vₘ. For PGA₂-nonionic surfactant mixed micellar systems, kₐₚₚ was decreased as the mole fraction of PGA₂ increased. These phenomena could be explained by the micellar surface potential in conformity with the Gouy-Chapman theory.

Key words: prostaglandin A₂, degradation, kinetics, micelle, surface potential

1 Introduction

Prostaglandins (PGs) are extremely potent, ubiquitous compound with a variety of physiological and pharmacological actions. However, PGs are also known to be unstable: for instance prostaglandin E₂ (PGE₂) is undergone a conversion into PGA₂ and PGB₂ by dehydration and isomerization reactions, where PGB₂ is the final decomposition product. PGE₂ has high pharmacological efficacy, namely an uterine muscle contraction, a peripheral blood vessel extension, a bronchus muscle slackness, a secretion suppression of gastric juice and so on, while PGA₂ and PGB₂ have a reduced and no pharmacological potencies, respectively. The application of PGs in a number of areas has been severely hampered by their apparent instability in solution. The instability of PGEs limits the development of dosage formulas. Hirayama et al. reported the improvement of instability of PGE₂ and PGA₂ by the inclusion complexation with methylated-cycloextrins. Kinetics of dehydration and isomerization of PGE₁ and PGE₂ have been studied. However, any kinetic analysis on the degradation of PGs in the presence of surfactant and the effect of micellization on the degradation of PGs have not yet been studied.

In this investigation, a kinetic study on the base-catalyzed degradation of PGA₂ was carried out in the absence and presence of PGA₂ micelles or mixed micelles of PGA₂ with nonionic surfactant, and the effect of micellization of PGA₂ on the degradation reaction of PGA₂ to PGB₂ was...
discussed taking account of the critical micelle concentration\textsuperscript{13} and the micellar surface potential\textsuperscript{14} of PGA\textsubscript{2}, before we investigate the consecutive degradation reaction \(\text{PGE}_2\rightarrow\text{PGA}_2\rightarrow\text{PGB}_2\).

2 Experimental

2.1 Materials

PGA\textsubscript{2} of 99\% purity was purchased from Sigma Chemical Company. Heptaethyleneglycol dodecyl ether (HED) as a nonionic surfactant was the same as that used for a previous study\textsuperscript{14}. Pinacyanol chloride was purchased from Sigma Chemical Company. All other chemicals were commercial products of reagent grade.

PGA\textsubscript{2}-HED mixed micelles with various mole fraction of PGA\textsubscript{2} were prepared as previously described\textsuperscript{14}. On the basis of the previous result\textsuperscript{14}, all PGA\textsubscript{2} molecules are considered to form mixed micelles with HED under this experimental condition.

2.2 Method

2.2.1 Measurement of Degradation Rate of PGA\textsubscript{2}

The degradation (isomerization) of PGA\textsubscript{2} was spectrometrically monitored by measuring the increased absorbance of PGB\textsubscript{2} at 278 nm. The molar absorptivity of PGB\textsubscript{2} (\(\varepsilon_{278}\)) was \(2.05 \times 10^4\) M\(^{-1}\) cm\(^{-1}\). PGA\textsubscript{2}, which has a \(\lambda_{max}\) at 210 nm, does not interfere the measurement of absorbance of PGB\textsubscript{2} at 278 nm. When micelles were existed in the reaction systems, the absorbance of PGB\textsubscript{2} was measured after micelles were destroyed by dilution. The structural formulas of PGA\textsubscript{2} and PGB\textsubscript{2} and their isomerization reactions are schematically shown in Chart 1. PGA\textsubscript{2} was dissolved in a pH 12, ionic strength 0.093 M phosphate buffer (Na\textsubscript{2}HPO\textsubscript{4}-NaOH) at 60°C. The initial concentrations of PGA\textsubscript{2} in the solutions were \(8 \times 10^{-5}\), \(2 \times 10^{-4}\) and \(4 \times 10^{-4}\) M as a concentration below the cmc and 0.1 M as a concentration above the cmc.

2.2.2 Measurement of cmc of PGA\textsubscript{2}

The cmc of PGA\textsubscript{2} at pH 12 and 60°C was determined by the surface tension measurement\textsuperscript{13,15} with a Du Noüy tensiometer. The cmc of PGA\textsubscript{2} in the presence of various concentrations of NaCl was determined by the pinacyanol chloride method\textsuperscript{16} to economize high-priced PGA\textsubscript{2}.

When the isomerization of PGA\textsubscript{2} has proceeded, the micelles composed of PGA\textsubscript{2} alone change to the mixed micelles composed of PGA\textsubscript{2} and PGB\textsubscript{2}. PGA\textsubscript{2} and PGB\textsubscript{2} are fortunately alike in the value of cmc\textsuperscript{19}. Thus, we assume that the change in cmc of PGA\textsubscript{2} during the isomerization reaction is negligible and that PGA\textsubscript{2} monomer is not supplied with micellar PGA\textsubscript{2}:PGB\textsubscript{2} monomer derived from the isomerization of PGA\textsubscript{2} monomer takes the place of PGA\textsubscript{2} monomer even if the monomer state of PGA\textsubscript{2} has undergone the faster isomerization than the micellar PGA\textsubscript{2}.

2.2.3 Estimation of Surface Potential of PGA\textsubscript{2} Micelles

The surface potential of PGA\textsubscript{2} micelles was determined as previously described\textsuperscript{14}. The surface potential of PGA\textsubscript{2} micelles in the presence of various concentrations of NaCl was obtained by the calculation according to the Gouy-Chapman theory\textsuperscript{14} to economize high-priced PGA\textsubscript{2}. The relationship between surface potential and surface charge density of PGB\textsubscript{2} micelles has already been shown\textsuperscript{14}.

When the isomerization of PGA\textsubscript{2} has proceeded, the micelles composed of PGA\textsubscript{2} alone change to the mixed micelles composed of PGA\textsubscript{2} and PGB\textsubscript{2}. We assume that the change in surface potential (\(\Delta \psi\)) of PGA\textsubscript{2} micelles during the isomerization is negligible, since PGA\textsubscript{2} and PGB\textsubscript{2} are alike in the value of \(\Delta \psi\).\textsuperscript{14}

3 Results

3.1 Degradation (Isomerization) of Free PGA\textsubscript{2} below the cmc

The degradation (isomerization) of free PGA\textsubscript{2} with an initial concentration below the cmc was measured, and the percentage of PGA\textsubscript{2} isomerized was plotted against time by closed circles in Fig.1.
The apparent rate constant for degradation (isomerization) of PGA₂, \( k_{\text{app}} \), is defined as follows:

\[
\ln(x_e-x) = \ln x_e - k_{\text{app}} t
\]

where \( x_e \) is the equilibrium value of isomerization reaction and \( x \) is the value of PGA₂ isomerized during time \( t \). For convenience, we used the percent value of isomerized PGA₂ based on the initial concentration of PGA₂. Plots of \( \ln(x_e-x) \) versus \( t \), calculated from the relationship between the percentage isomerized and time, were linear as shown by closed circles in Fig. 2, thus \( k_{\text{app}} \) was obtained from the slope of straight line.

The value of \( k_{\text{app}} \) for degradation of free PGA₂ was obtained as \( 1.258 \times 10^{-3} \text{s}^{-1} \). This is nearly consistent with the value of \( 1.218 \times 10^{-3} \text{s}^{-1} \) estimated from the results presented by Stehle and Oesterling\(^{17}\), indicating that the results obtained here is reasonable. The value of \( k_{\text{app}} \) of free PGA₂ with \( 8 \times 10^{-5} \text{M} \) or \( 4 \times 10^{-4} \text{M} \) was nearly equal to that with \( 2 \times 10^{-4} \text{M} \). It was found that \( k_{\text{app}} \) was approximately constant below the cmc of PGA₂.

### 3.2 Degradation (Isomerization) of PGA₂ above the cmc

To investigate the effect of micellization on the degradation (isomerization) of PGA₂, a solution of PGA₂ with an initial concentration of 0.1 M above the cmc was supplied for the measurement. The relationship between the percentage isomerized and time was shown by closed squares in Fig. 1. The plots of \( \ln(x_e-x) \) versus time were shown by closed squares in Fig. 2. The value of \( k_{\text{app}} \) for degradation of PGA₂ above the cmc was obtained as \( 5.919 \times 10^{-4} \text{s}^{-1} \). The value of \( k_{\text{app}} \) above the cmc was smaller than that below the cmc. The decrease in \( k_{\text{app}} \) above the cmc is considered to be due to the fact that the approach of OH⁻ to micellar PGA₂ is suppressed by the electrostatic repulsion between the negatively charged micellar surfaces and OH⁻.

Micellar catalyzed reactions have been known also in other systems\(^{18\text{-}20}\).

The \( k_{\text{app}} \) value of \( 5.919 \times 10^{-4} \text{s}^{-1} \) for PGA₂ above the cmc is approximately twofold larger than that of \( 3.045 \times 10^{-4} \text{s}^{-1} \) for PGA₂ included in methylated-β-cyclodextrins\(^{12}\). This is considered as follows: the reaction site of PGA₂ may be not shielded by the micellization of PGA₂, the moiety of five-membered ring in PGA₂ molecule is probably localized near the PGA₂ micellar surfaces; while the reaction site of PGA₂ may be shielded by the inclusion complexation with methylated-β-cyclodextrins.

### 3.3 Effect of Electrolyte on Degradation (Isomerization) of PGA₂ above the cmc

To ascertain that the depressed rate for degradation of micellar PGA₂ is due to the micellar surface potential, NaCl as an electrolyte was added to the reaction system, and an expected increase in the rate was observed. The plots of percentage isomerized vs. \( t \) and \( \ln(x_e-x) \) vs. \( t \) for degradation of PGA₂ with an initial concentration
Effect of Electrolyte (NaCl) on the Apparent Rate Constant for Isomerization of PGA₂
Initial concentration of PGA₂: 0.1 M.
Solid line: theoretical curve based on the Gouy-Chapman theory.

Relationship between Percentage of PGA₂ Isomerized and Time for PGA₂-HED Mixed Micellar Systems.
Mole fraction of PGA₂: ○, 0.2; △, 0.4; □, 0.7.
Initial concentration of PGA₂: 2 × 10⁻⁴ M.

Effect of Mole Fraction of PGA₂ on kₐₚₚ for PGA₂-HED Mixed Micellar Systems.

3.4 Effect of Mixed Micellization of PGA₂ with HED on Degradation (Isomerization) of PGA₂
The relationship between the percentage of PGA₂ isomerized and time for PGA₂-HED mixed micellar systems was shown in Fig.4, and the plots of ln (xe-x) vs. time were shown in Fig.5. The values of kₐₚₚ obtained from Fig.5 were shown in Fig.6 against the mole fraction of PGA₂. The apparent rate constant for degradation of PGA₂ decreased with increasing mole fraction of PGA₂.
4 Discussion

4.1 Derivation of Rate Constants for Degradation of PGA₂ above the cmc in the Bulk and Micellar Phases

PGA₂ is distributed between the micellar and bulk phases at PGA₂ concentrations above the cmc. The rate for degradation of PGA₂ is, therefore, considered to be a sum of two terms:

\[-d[PGA₂]/dt = k_{app}[PGA₂] = v = v₁ + vₘ (2)\]

where \(v₁\) and \(vₘ\) are the rates of degradation in the aqueous and micellar phases, respectively. Next, \(k_{app}\) can be represented as follows by using the value of partition ratio of PGA₂ in the aqueous phase, \(f\):

\[k_{app} = k_f f + k_m (1-f) (3)\]

where \(k_f\) and \(k_m\) are the first order rate constants of degradation in the aqueous and micellar phases, respectively, and the value of \((1-f)\) means the partition ratio of PGA₂ in the micellar phase. The value of \(f\) can be estimated from the cmc of PGA₂. It has been found⁴) that the degradation reaction of PGA₂ is apparent first order in hydroxide ion concentrations. Thus, \(k_{app}\) is described by:

\[k_{app} = k_{2app}[OH⁻] (4)\]

where \(k_{2app}\) is the second-order rate constant for the hydroxide ion-catalyzed reaction. Equation 4 can be rewritten as follows:

\[k_{2app}[OH⁻] = k_{2f}[OH⁻] + k_{2m} (1-f) [OH⁻]_m (5)\]

where \(k_{2f}\) and \(k_{2m}\) are the second order rate constants of degradation in the aqueous and micellar phases, respectively. Equation 5 simply becomes

\[k_{2app} = k_{2f} (C_{PGA₂} < \text{cmc}) (6)\]

since \(f = 1\) and \([OH⁻]_m = 0\).

The rates of degradation in the aqueous and micellar phases, \(v₁\) and \(vₘ\), are defined from Eq. 5 as

\[v₁ = k_{2f} f [OH⁻], \quad vₘ = k_{2m} (1-f) [OH⁻]_m (7)\]

where \(v₁\) and \(vₘ\) are related to \(v\) or \(k_{app}\) by

\[v₁ + vₘ = v = v/[PGA₂] = k_{app} (9)\]

[OH⁻]ₘ is expressed as

\[ [OH⁻]ₘ = [OH⁻] e^{\frac{-zFΔψ}{RT}} (10)\]

where \(z\) is the valency of OH⁻, \(Fₚ\) is the Faraday constant, \(Δψ\) is the surface potential at the micelle surface, \(T\) is the absolute temperature, and \(R\) is the gas constant. The relationship among \(k_{2m}\), \(k_{2app}\) and \(Δψ\) can be expressed as

\[k_{2m} = \frac{(k_{2app} - k_{2f})}{1-f} e^{\frac{zFₚΔψ}{RT}} (11)\]

4.2 Degradation Rate of PGA₂ in the Bulk and Micellar Phases

The apparent rate for degradation of PGA₂ shown in Fig. 3 can be explained in terms of the sum of \(v₁\) and \(vₘ\) according to the Eqs. 7 and 8. The value of \(k_{2f}\) is 0.1258 s⁻¹ M⁻¹ as observed below the cmc and calculated by Eqs. 4 and 6. The value of \(vₘ\) is calculated from Eq. 9 by subtracting

Fig. 7 Effect of Electrolyte (NaCl) on the Degradation Rate of PGA₂ (a), \(f\) (b), \(Δψ\) (c) and [OH⁻]ₘ (d).

(a): \(●, k_{app}; ○, vₘ\), ○, v₁
Initial concentration of PGA₂: 0.1 M.
the value of $v_f'$ from the value of $k_{app} (= v_f' + v_m')$. The values of $v_f'$ and $v_m'$ are shown by open and closed circles, respectively, in Fig. 7(a).

The values of $v_f'$ decreased with increasing concentration of NaCl. This is caused by the decrease in $f$ as shown in Fig. 7(b), which is related to the cmc of PGA2 in the presence of NaCl. The values of $v_m'$ increased with increasing concentration of NaCl. This is caused by the increase in partition ratio of PGA2 in the micellar phase, which is expressed by $1-f$. Furthermore, the increase in $v_m'$ is considered to be due to the increase in $[\text{OH}^-]_m$ which depends on the surface potential of the micelles. The surface potential of PGA2 micelle in the absence of NaCl was measured as $-17.6 \text{ mV}$ and that in the presence of NaCl was calculated from the Gouy-Chapman Equation\(^{14}\), and the values of $\Delta \phi$ were shown in Fig. 7(c). The values of $[\text{OH}^-]_m$ were calculated from Eq. 10 and were shown in Fig. 7(d). As shown in Fig. 7(a)−(d), the increase in $v_m'$ with increasing concentration of NaCl can be explained in terms of the decrease in $| - \Delta \phi |$ and the consequent increase in $[\text{OH}^-]_m$.

Next, the theoretical values of $k_{app}$ based on the value of $\Delta \phi$ shown in Fig. 7(c) were calculated by using the Gouy-Chapman theory and Eqs. 7−10, and the theoretical curve was shown by solid line in Fig. 3. The experimental values were nearly in agreement with the theoretical curve, although the theoretical values were slightly larger than the experimental values. The error is considered to be arisen from the values of $\Delta \phi$ and $f$: namely, the value of $\Delta \phi$ for PGB2 is not strictly the same as that for PGA2, and the micellar surface potential of PGA2 or PGB2 may be not exactly obeyed the Gouy-Chapman theory, thereby $[\text{OH}^-]_m$ is not sufficiently increased as shown in Fig. 7(d) when the concentration of NaCl is increased; the cmc of PGB2 is slightly smaller\(^{13}\) than that of PGA2, thereby $f$ may be slightly smaller than the values shown in Fig. 7(b).

4·3 Effect of PGA2-HED Mixed Micelles on Degradation of PGA2

The apparent rate constant $k_{app}$ for degradation of PGA2 in the PGA2-HED mixed micellar state decreased with increasing mole fraction of PGA2, as shown in Fig. 6. To explain this phenomenon, the surface potentials of PGA2-HED mixed micelles were shown in Fig. 8(a), and the values of $[\text{OH}^-]_m$ were calculated by using Eq. 10 were shown in Fig. 8(b).

The value of $| - \Delta \phi |$ is increased with increasing mole fraction of PGA2 and the value of $[\text{OH}^-]_m$ is consequently decreased due to the electrostatic repulsion between $\text{OH}^-$ and negatively charged micellar surfaces, thereby $k_{app}$ is decreased with increasing mole fraction of PGA2 as shown in Fig. 6.

5 Conclusion

The apparent rate for the base-catalyzed degradation of PGA2 was decreased by the micellization of PGA2 or mixed micellization of PGA2 with HED. These results can be explained in terms of the electrostatic effect at the micellar
surfaces: the decrease in the degradation rate in the micellar phase is due to the approach of OH\textsuperscript{-} to PGA\textsubscript{2} in the micellar phase being suppressed by the electrostatic repulsion between negatively charged micellar surfaces and OH\textsuperscript{-}, which is in conformity with the Gouy-Chapman theory.

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References

[報文] 蟻光検出逆相 HPLC とエレクトロスプレーイオン化マススペクトロメトリーによるホスファチジルコリン 2-アンスリルウレタン誘導体の分子種分析

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ホスファチジルコリン (PC) の分子種を分離定量する高感度分析法を確立した。大豆、サバ筋肉、サンマ筋肉およびサケ卵から分離した PC をホスホリパーゼ C で活性分解し、生成したジアシルグリセリンを 2-アンスリルウレタン誘導体に変換した。これを蛻光検出器を装備した逆相 HPLC と HPLC に直結したエレクトロスプレーイオン化マススペクトロメトリー (ESI/MS) により分析した。蛻光検出ではフェムトモルレベルで各分子種が明瞭に分離された。正イオンモードの HPLC/ESI/MS 分析では強い発射分子イオン [M+Na]+ が得られ、分子種の同定に有効であった。これらの結果から、蛻光検出 HPLC と ESI/MS を併用した手法は天然由来 PC の高感度分子種分析法として有用であることが認められた。

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[報文] プロスタグランジン A2 の塩基触媒性分解反応（異性化）に関する界面化学的研究

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プロスタグランジン A2 (PGA2) からプロスタグランジン B2 (PGB2) への塩基触媒性分解反応（異性化反応）を pH 12, 温度 60℃において測定し、分解に及ぼす PGA2 のミセル化の影響について検討した。PGA2 の分解速度は、分解に伴って生成する PGB2 の吸光度を測定することにより求めた。PGA2 の分解に対するみかけの速度定数 (kapp) は PGA2 の界面ミセル濃度 (cmc) 以下ではほぼ一定であったが、cmc 以上では PGA2 のミセル化によって kapp は低下した。これは、負に荷電した PGA2 ミセルと OH⁻ との間の静電的な反応によるものと考えられた。cmc 以上の PGA2 に対する kapp の値は、電解質としての塩化ナトリウム (NaCl) を添加することによってわずかに増加した。PGA2 のみかけの分解速度 v はcmc 以上ではパルク相とミセル相のそれぞれ 2 つの分解速度 v1 と v2 の和で表すことができた。すなわち、NaCl の添加による v の増加は v1 の減少と v2 の増加に起因することができた。PGA2−ノニオン性界面活性剤混合ミセル系においては、混合ミセル中の PGA2 のモル分率が高くなるにつれて kapp は減少した。これらの現象は Gouy-Chapman の理論によるミセル表面電位で説明することができた。

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