NOTE

Solubilization of Progesterone and its Derivatives into Gemini Surfactant Solutions

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Abstract: The solubilization of poorly water-soluble progesterone derivatives into micelles of a gemini surfactant was investigated in an aqueous medium. The aqueous solubility at different temperatures was determined spectroscopically using an ultraviolet visible light spectrophotometer. Thermodynamic parameters for the solubilization were calculated under the basis of the solubility change against temperature. The solubility of progesterone was quite low and remained constant below the critical micelle concentration (cmc) of the surfactant. On the other hand, the solubility increased considerably with increasing surfactant concentration above the cmc. It was suggested that the solubilizes were located in the vicinity of polar regions of micelles.

Key words: progesterone, gemini surfactant, solubilization, micelle

1 Introduction

The solubility of drugs is one of important physicochemical properties for drug delivery systems. In general, drugs with poor water solubility have a low drug absorption rate, which results in poor bioavailability. The solubility also greatly influences the ability to deliver drugs to target tissues and organs and achieve the desired drug concentration¹⁾.

Progesterone is an endogenous steroid sex hormone that plays a multifunctional role, especially in the female reproductive system. Its deficiency, especially in female menopause, causes various acute or chronic gynecological disorders such as headache, hot flashes, dizziness, and vaginal dryness^{2,3)}. Therefore, progesterone replacement therapy is performed to relieve such symptoms. Oral administration of progesterone and its derivatives entails many obstacles, such as low bioavailability due to the low water solubility (10 µg/mL)⁴⁾, short half-life due to the hepatic first-pass effect⁵⁾, and enzymatic degradation in the digestive tract⁶⁾.

One of the approaches for improving the water solubility of hydrophobic drugs is the use of surfactant aqueous solutions. There are a few reports on the solubilization of progesterone in typical ionic surfactant solutions, such as sodium dodecyl sulfate (SDS) and bile acids⁷⁻⁹⁾, and in non-ionic surfactant solutions¹⁰⁾. Commonly, nonionic surfactant

solutions exhibit a much lower critical micelle concentration (cmc) and are therefore used as efficient solubilizers in various fields of industry. We have investigated the solubilization of poorly soluble substances using gemini surfactant solutions^{11,12)}. Gemini surfactants are a dimer of typical surfactants in which the surfactant molecules are linked by a spacer. In comparison to the corresponding monomer surfactants, gemini surfactants have excellent properties, such as much smaller cmc and superior surface activity^{13,14)}. In this regard, gemini surfactants have the unique property that the value of cmc is comparable to that of nonionic surfactants despite them having a low molecular weight and a charge in the hydrophilic group. Therefore, gemini surfactants are expected to be useful and functional solubilizers.

In the present study, 14-6-14,2Br⁻ gemini surfactant, which exhibits the simplest chemical structure, has relative ease of synthesis among gemini surfactants, and has been examined extensively by many researchers, was used as a model solubilizer of progesterone. In addition to progesterone, we also utilized some of its derivatives, 17 α -hydroxyprogesterone and medroxyprogesterone, as solubilizes. The derivatives are used in the clinical field and have only a few structural differences from progesterone. Their use was expected to provide useful information on the relationship between structure and solubilization efficacy. Mea-

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surement for solubilization was done in the temperature range of 288.2 to 308.2 K to evaluate the thermodynamic transfer of the solubilizates from the bulk to the 14-6-14,2Br⁻ micelle. The solubilization site of progesterone and the derivatives in the micellar aggregate was investigated by employing absorption spectra.

2 Experimental

2.1 Materials

Hexanediyl-1,6-bis(dimethyltetradecylammonium bromide) (abbr. 14-6-14,2Br⁻) was synthesized via a previously reported procedure¹⁵. The crude crystal obtained was purified by recrystallization three times from acetone/ethanol mixed solvents. The identification was checked by ¹H NMR, ¹³C NMR, and elemental analysis, as mentioned in a previous paper¹⁶. Progesterone (abbrev. PROG, >97%) was purchased from Sigma-Aldrich (MO, USA). 17 α -hydroxyprogesterone caproate (17-OHPC, >98%), medroxyprogesterone (MP, >98%), and 1-octanol (>99.5%) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). *n*-dodecane (99%) was obtained from Nacalai Tesque (Kyoto, Japan). These reagents were used as received. The water utilized in the whole experiments was from a Direct-Q UV3 water purification system (Merck, Darmstadt, Germany) with a resistivity of 18 M Ω ·cm.

2.2 Methods

14-6-14,2Br⁻ solutions suspended in the solubilizates, which were contained in glass vials and agitated with a rotor, were kept under thermostat controlled conditions to within ± 0.1 K at 288.2, 298.2, and 308.2 K for 24–48 h to attain complete solubilization equilibrium. Prior to the measurement, the suspensions were filtrated through a membrane filter with a pore size of 0.1 μ m (Millipore MILLEX VV) to remove extra undissolved materials. The solubilizate concentrations in the surfactant solutions were determined spectrophotometrically with a UV/VIS spectrophotometer (V-530, Jasco, Tokyo, Japan). The points in the figures are mean $\pm$ S.D. The set of measurements was repeated at least three times to ensure repeatability.

3 Results and Discussion

3.1 Solubility of the steroids in 14-6-14,2Br⁻ solutions

The chemical structures of the steroids used here are shown in Fig. 1. 17-OHPC and MP exhibit small differences in structure from PROG: a caproic acid ester at C-17 for 17-OHPC and a hydroxy at C-17 and methyl group at C-6 for MP. In 1-octanol, the steroids have significant UV absorbances at 240 nm. Figure 2 shows plots of the absorbances against steroid concentration. The change in absorbance

Fig. 1 Chemical structures of (A) PROG, (B) 17-OHPC, and (C) MP.

Fig. 2 Plots of absorbance of PROG, 17-OHPC, and MP in 1-octanol against concentration.

for all of the steroids indicates good linearity with respect to concentration: $r^2 = 0.999$ for PROG, 0.998 for 17-OHPC, and 0.988 for MP. Thus, the molar extinction coefficients (ϵ) at 240 nm, which are based on the slope in Fig. 2, are 1.69×10^4 , 1.59×10^4 , and 1.01×10^4 mol⁻¹ L cm⁻¹ for PROG, 17-OHPC, and MP, respectively. The ϵ value of PROG agrees well with the reference value (1.56×10^4 mol⁻¹ L cm⁻¹)¹⁷.

The solubilizate concentrations in the 14-6-14,2Br⁻ surfactant solutions were determined by UV spectroscopy

Fig. 3 Solubility changes of PROG, 17-OHPC, and MP with 14-6-14,2Br[−] concentration at 298.2 K. The inset shows the enlarged view below the cmc.

using the ε values. The solubilities of the steroids at 298.2 K are plotted against 14-6-14,2Br[−] concentration in Fig. 3. The critical micelle concentration (cmc) of 14-6-14,2Br[−] is 0.161 mmol L^{−1} at 298.2 K⁽¹⁶⁾. The solubilize concentrations below the cmc remain almost constant (see the inset in Fig. 3). The solubility in water at 298.2 K of PROG, 17-OHPC, and MP was found to be 0.0104, 0.000988, and 0.0186 mmol L^{−1}, respectively. These values support the poor solubility of these compounds in water and imply their strength of hydrophobicity: 17-OHPC > PROG > MP. An increment of surfactant concentration above the cmc considerably increases the steroid solubility. Furthermore, the solubility varies linearly with the surfactant concentration. The difference in solubility change from below to above the cmc is attributed to the transfer of the solubilizes from the aqueous bulk to the interior of micelles^(18,19). The solubility of PROG in 5 mmol L^{−1} surfactant solution is the highest among the steroids used here. This suggests that important factors for the solubilization to micelles include not only the hydrophobicity of the solubilizes but also the van der Waals interaction and hydrogen bonding between the steroids and the surfactants constituting micelles. The solubility plots at the different temperatures of 288.2 and 308.2 K are shown in Figs. S1–3 in the Supplementary Material.

3.2 Thermodynamic parameters for micellar solubilization

The mole fractions of the solubilizes in the aqueous phase (X_R^W) and the aggregate phase (X_R^A) are given by Eqs. 1 and 2, respectively:

$$X_R^W = \frac{[R]}{55.5 + [R] + \text{cmc}} \quad (1)$$

Table 1 Thermodynamic parameters (in kJ mol^{−1}) of the steroids for the transfer from the aqueous bulk to the surfactant micelle at 298.2 K.

steroids	ΔG^0	ΔH^0	$-T\Delta S^0$
PROG	−32.0	−13.8	−18.3
17-OHPC	−38.7	10.6	−49.3
MP	−31.2	−4.7	−26.4

$$X_R^A = \frac{[R]_t - [R]}{(C - \text{cmc}) + ([R]_t - [R])} \quad (2)$$

where the subscript R refers to the solubilize and the superscripts W and A refer to the aqueous phase (water) and the aggregate phase, respectively. $[R]$ is the value of solubilize concentration (in mol/L) in the aqueous medium, which is obtained as the mean value of the solubilize concentrations below the cmc. $[R]_t$ is the total equivalent concentration of the solubilizes in the surfactant solution, and C is the total surfactant concentration. The chemical potential (μ) of solubilizes in each phase (W and A) is expressed as follows at temperature T and pressure P :

$$\mu_R^W = \mu_R^{\theta,W}(T, P) + RT \ln X_R^W \quad (3)$$

$$\mu_R^A = \mu_R^{\theta,A}(T, P + \Delta P) + RT \ln X_R^A \quad (4)$$

where μ^{θ} is the standard chemical potential at infinite dilution (W), μ^{θ} is the standard chemical potential (A) based on a symmetrically standardized state, and ΔP is the difference in pressure between the W and A phases. Under the solubilization equilibrium, the following equation is obtained from Eqs. 3 and 4.

$$\Delta G^0 = RT \ln \frac{X_R^W}{X_R^A} \quad (5)$$

ΔG^0 means the Gibbs energy change for transfer of the solubilize molecules from phase W to phase A. This is calculated according to the partition equilibrium, where the aggregate including the solubilizes is regarded as a separate phase^(12,18,20). The changes of enthalpy (ΔH^0) and entropy (ΔS^0) for the transfer can be evaluated by the following equations.

$$\Delta H^0 = \left[\frac{\partial(\Delta G^0/T)}{\partial(1/T)} \right]_P \quad (6)$$

$$-T\Delta S^0 = \Delta G^0 - \Delta H^0 \quad (7)$$

The variation of $\Delta G^0/T$ with respect to $1/T$ is fitted by linear regression to provide the ΔH^0 value. The thermodynamic parameters of ΔG^0 , ΔH^0 , and $-T\Delta S^0$ are listed in Table 1 and Tables S1–3. The ΔG^0 values of the solubilizes increase with increasing temperature (Tables S1–3). In addition, it was found that the $-T\Delta S^0$ term contributes more greatly to the negative value of ΔG^0 in the whole system. Thus, the solubilization of the steroids used here to the surfactant micelle is entropy-driven under the basis of

Table 2 Solubilization power for the 14-6-14,2Br⁻/steroids/water systems.

	PROG	17-OHPC	MP
288.2 K	0.119	0.0928	0.0904
298.8 K	0.140	0.109	0.0967
308.2 K	0.152	0.115	0.109

release of the water molecule surrounding the steroids. As seen in **Table 1**, PROG and MP indicate similar ΔG^0 values, whereas 17-OHPC has a lower value. This may be caused by a hydrophobic interaction between the hydrocarbon chains of caproic acid and 14-6-14,2Br⁻ micelles, which is supported by a small and positive ΔH^0 value.

3.3 Solubilization efficacy

The increase in solubility of poorly water-soluble compounds depends strongly on the hydrophobic room in the aqueous medium. In the case of the use of surfactants, the room is constructed by micelle formation. Thus, low cmc is a critical factor for efficacy of solubilization. Herein, we evaluated the efficacy using the solubilization power (SP) at the maximum additive concentration, which is defined as follows²¹⁾:

$$SP = \frac{d[R]_t}{d(C - cmc)} \quad (8)$$

The SP index means the number of dissolved molecules in the solvent per surfactant molecule comprised in micelles. All of the SP indices increase with increase in temperature (see **Table 2**). At the same temperature, the solubilization efficacy of PROG in 14-6-14,2Br⁻ solution is highest among the present steroids. This trend is different from that of the aforementioned hydrophobicity strength (17-OHPC > PROG > MP). Considering the thermodynamic parameters, 17-OHPC should transfer most easily to the interior of micelles. Thus, it is likely that there are other factors of the transfer, such as interaction between the polar region of micelles and the hydrophilic groups of steroids and spatial bulkiness of steroids.

The SP value for the dodecyltrimethylammonium chloride (DDAC)/sudan red B/water system at 298.2 K was reported to be 2.20×10^{-3} ²¹⁾. Although DDAC is not simply the corresponding monomer of 14-6-14,2Br⁻ gemini surfactant, it was found that the gemini surfactant can solubilize progesterone approximately 50 times more efficiently than the monomer surfactant. In a previous study, the SP index was evaluated for 14-6-14,2Br⁻/*n*-alkylbenzene/water systems: 1.00 for ethylbenzene, 0.76 for propylbenzene, 0.30 for butylbenzene, and 0.25 for pentylbenzene at 298.2 K¹¹⁾. The present systems provide lower SP values. This may be caused by the hydrophilic groups of the steroids, such as the hydroxy and carbonyl groups. In the case of *n*-alkylbenzenes, elongation of the alkyl chain length

Fig. 4 Absorption spectra of PROG in water, in 14-6-14,2Br⁻ aqueous solutions below the cmc (0.001 mmol L⁻¹) and above the cmc (0.5 mmol L⁻¹), and in *n*-dodecane at 298.2 K. The concentrations of PROG were saturated in each solvent or solution.

results in promotion of transfer to the micelle core but in degeneration of solubilization efficacy due to the limited space of hydrophobic micelle core. On the contrary, the steroids are considered to be more difficult to move into the micelle interior because of the hydrophilic groups and the bulkiness of steroid backbones.

3.4 UV absorption spectra

Absorption spectra of PROG in water, 14-6-14,2Br⁻ solutions, and *n*-dodecane at 298.2 K are shown in **Fig. 4**. The spectra of PROG in water and 0.001 mmol L⁻¹ 14-6-14,2Br⁻ solution, where micelles are not formed, have a peak centered at 249.5 nm. On the other hand, the distinct peak of the 0.5 mmol L⁻¹ solution (above the cmc) appears at 244.5 nm. In *n*-dodecane, the wavelength of the peak is exactly 235 nm. The peak wavelength in the absorption spectrum of a molecule depends greatly on the dielectric constant of the environment in which the molecule is located. The dielectric constant for water is 78.3 and that for *n*-dodecane is 2.0 at 298.2 K²²⁾. Therefore, the shift of spectra reveals that most of the PROG molecules move from the aqueous bulk to the inner part of surfactant micelles. However, the peak center in 0.5 mmol L⁻¹ solution did not reach 235 nm. The spectra of *n*-alkylbenzenes in 14-6-14,2Br⁻ solutions above the cmc nearly coincide with those in *n*-dodecane in terms of wavelength¹¹⁾. Therefore, it is suggested that the solubilized PROG molecule concentrates in the micellar polar region rather than the core.

4 Conclusion

The aqueous solubility of the sex hormones PROG, 17-OHPC, and MP was elucidated in a micellar solution of gemini surfactant. In the presence of the micelles in the aqueous medium, it is possible for the steroids to dissolve to a greater extent in comparison with the solubility in water and the solution below the cmc. The transfer of solubilizes from the aqueous bulk to the inner part of micelles is entropy-driven for all of the systems. The UV spectra suggest the possibility that the sterols concentrate at the polar region of surfactant micelles due to the hydrophilic groups in steroids and the spatial bulkiness of the skeleton. Determining the location will necessitate further work employing other spectroscopies as well as NMR.

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Supporting Information

This material is available free of charge via the Internet at <http://dx.doi.org/jos.69.10.5650/jos.ess20007>

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