Distribution of Pyridylazophenols and Their Metal Chelates between Two Phases Separated from a Micellar Solution of Octyl-β-D-thioglucoside in the Presence of Polyethylene Glycol

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Aqueous micellar solutions of octyl-β-D-thioglucoside (OTG) were separated into two phases upon addition of polyethylene glycol 6000 (PEG). In this polymer-induced phase separation, one phase was an aqueous phase in which PEG was retained; the other was a surfactant-rich phase into which pyridylazophenols and their three metal chelates (nickel(II), zinc(II), and cadmium(II)) were extracted. Their distribution constants were highly dependent upon the PEG concentration and the nature of the metal chelate. This observation provides a possibility of controlling the extraction selectivity in polymer-induced phase separation.

Keywords: Distribution, metal chelate, octyl-β-D-thioglucoside, polyethylene glycol, phase separation

We previously reported a new polymer-induced phase separation in a micellar solution of octyl-β-D-thioglucoside (OTG) for the isolation of hydrophobic membrane proteins. A micellar solution of OTG separates into two phases below room temperature and even at 0°C, upon the addition of polyethylene glycol 6000 (PEG). One of the phases is an aqueous phase in which PEG is retained; the other is an OTG-rich phase. In the present study, the distribution equilibria of metal chelates with pyridylazophenols were investigated in order to elucidate the properties of the OTG-rich phase as an extracting solvent. The distribution constants were compared with those in the two-phase system of an aqueous polyoxyethylene(7.5)-nonylphenyl ether (PONPE-7.5) solution separated above the cloud-point temperature (temperature-induced phase separation).

Experimental

Reagents

Octyl-β-D-thioglucoside (OTG) was obtained from Dojindo Laboratory (Kumamoto Japan). Polyethylene glycol 6000 (molecular weight: 7500, PEG) and 8-hydroxyquinoline (8Q) were supplied from Wako Chemicals (Tokyo, Japan). Standard solutions of nickel(II), zinc(II) and cadmium(II) were prepared by dissolving their nitrates in 0.01 M nitric acid and then standardizing by EDTA titration. 2-(2-Pyridylazo)phenol (PAP) and 2-(2-pyridylazo)-5-methylphenol (PAP-5Me) were prepared in our previous study. Buffer solutions were prepared by mixing an aqueous HNO₃ solution (0.1 M) or an aqueous NaOH solution (0.1 M) with a 0.1 M aqueous solution of appropriate buffer components. The buffer solutions were 2-(N-morpholino)ethanesulfonic acid (MES), 3-cyclohexylaminopropionate sulfonic acid (CAPS), acetic acid, and tris(hydroxymethyl)aminomethane (Tris).

Apparatus

A Tomy high-speed microcentrifuge (MCX-150) was used for rapid phase separation. A Hitachi spectrophotometer 320 with 1 cm quartz cells was used for measuring the absorbance in both the aqueous and surfactant-rich phases. All of the experiments were carried out in a water bath thermostated at 298±0.1 K with a Yamato Coolnics instrument (CTR220/CTE220). A Shimadzu GVM-1000P inductively coupled plasma atomic emission spectrometer was used for the determinations of OTG.

Procedure

Prescribed amounts of OTG, a chelating reagent, a metal ion (if necessary), NaNO₃, PEG and a buffer component were placed into a centrifuge tube (1.5 cm³). The entire mixture was diluted to 1.00 cm³. The thus-prepared turbid solution was kept at 298±0.5 K for 1 h and then centrifuged at 10000 rpm for 5 min. The two phases were taken separately. A 500-mm³ portion of the aqueous phase was then taken and submitted to pH measurements. To the aqueous phase, 50 mm³ of the CAPS buffer solution was added for controlling the pH at 12.5. Finally, 50 mm³ of a 1% (w/v) Brij 35 solution and 100 mm² of water were added. In the extraction of metal chelates, 100 mm³ of an appropriate buffer solu-
tion, 50 mm$^3$ of an aqueous solution of PAP-5Me, 50 mm$^3$ of the 1%(w/v) Brij 35 and 100 mm$^3$ of water were added to 500 mm$^3$ of the aqueous phase. The buffer solution was chosen so as to satisfy the pH value for the determination of the respective metal chelates given in Table 1. Finally, the absorbance was measured at the wavelength in Table 1.

The concentration of the chelating reagent and the metal ion in the aqueous phase was calculated based on the material balance and the volume of the aqueous phase. The distribution ratio ($D$) was defined as the ratio of the total concentration of the chelating reagent (or metal chelate) in the surfactant-rich phase to that in the aqueous phase. The latter was calculated from the material balance and the volume of the surfactant-rich phase. The volumes of the respective phases are given in Table 2.

### Results and Discussion

#### Phase separation in an aqueous mixture of OTG and PEG and in a micellar solution of PONPE-7.5

As was pointed out in our previous study, the phase diagrams for aqueous mixtures of OTG and PEG (Fig. 1) were different from that of an aqueous micellar solution of PONPE-7.5 (Fig. 2). Figure 1 for OTG shown plots of the critical temperatures vs. the PEG concentration. The respective curves in Figs. 1 and 2 separate two regions: one is a homogeneous micellar solution; the other is a two-phase region. The latter was calculated from the material balance and the volume of the surfactant-rich phase. The volumes of the respective phases are given in Table 2.

#### Distribution equilibria of chelating reagents and their chelates

A plot of the logarithmic distribution ratio ($\log D$) for PAP (HL) as a function of the pH value for the determination of the respective metal chelates given in Table 1. Finally, the absorbance was measured at the wavelength in Table 1.

The concentration of the chelating reagent and the metal ion in the aqueous phase was calculated based on the material balance and the volume of the aqueous phase. The distribution ratio ($D$) was defined as the ratio of the total concentration of the chelating reagent (or metal chelate) in the surfactant-rich phase to that in the aqueous phase. The latter was calculated from the material balance and the volume of the surfactant-rich phase. The volumes of the respective phases are given in Table 2.

### Table 1: Wavelength ($\lambda$) and molar absorptivity ($\varepsilon$) for chelating compounds under the optimum pH condition in an aqueous solution of 2.00%(w/v) OTG

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\lambda$/nm</th>
<th>$\varepsilon$/M$^{-1}$ cm$^{-1}$</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>8Q</td>
<td>355</td>
<td>2700</td>
<td>12.5</td>
</tr>
<tr>
<td>PAP</td>
<td>480</td>
<td>10200</td>
<td>12.5</td>
</tr>
<tr>
<td>PAP-5Me</td>
<td>490</td>
<td>13100</td>
<td>12.5</td>
</tr>
<tr>
<td>[Ni(pap-5Me)$_2$]</td>
<td>550</td>
<td>23400</td>
<td>6.5</td>
</tr>
<tr>
<td>[Zn(pap-5Me)$_2$]</td>
<td>535</td>
<td>25000</td>
<td>7.0</td>
</tr>
<tr>
<td>[Cd(pap-5Me)$_2$]</td>
<td>535</td>
<td>24800</td>
<td>7.0</td>
</tr>
</tbody>
</table>

### Table 2: Volumes of aqueous (AP) and surfactant-rich (SRP) phases formed from an 1.00 cm$^3$ aqueous mixture of 2.00%(w/v) OTG and PEG at 298 K

<table>
<thead>
<tr>
<th>PEG concentration, % (w/v)</th>
<th>Phase volume/cm$^3$</th>
<th>AP</th>
<th>SRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.928±0.001</td>
<td>0.0726±0.0012</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>0.969±0.002</td>
<td>0.0313±0.0015</td>
<td></td>
</tr>
<tr>
<td>20.0</td>
<td>0.975±0.001</td>
<td>0.0251±0.0009</td>
<td></td>
</tr>
</tbody>
</table>

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Fig. 1 Phase separation temperature of a 2.00%(w/v) aqueous OTG solution as a function of the PEG concentration. A two-phase region (aqueous and surfactant-rich phases) is below the curve, while a homogeneous region (micellar solution) is above the curve.

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Fig. 2 Phase-separation temperature of an aqueous PONPE-7.5 solution. A two-phase region is above the curve, while a homogeneous region is below the curve.
protonated form ($H_2U$). Thus, the distribution ratio can be expressed by Eq. (1):

$$D = \frac{K_{d_1} + K_{d_2} K_a[H^+]}{1 + K_a[H^+]},$$

where $K_a$ is the acid dissociation constant of $HL$ and $K_{d_2}$ is the distribution constant of the negatively charged species ($L^-$), as defined by

$$K_a = \frac{[H^+][L^-]}{[HL]},$$

$$K_{d_1} = \frac{[HL]}{[HL]}$$

and

$$K_{d_2} = \frac{[L^-]}{[L^-]}.$$

The acid dissociation constant and the distribution constants of PAP-5Me were calculated through a least-squares fit of the model described by Eq. (1) to the data given in Fig. 3. These constants are listed in Tables 3 and 4 along with those of other chelating reagents.

**Distribution equilibria of metal chelates**

Figure 3 shows the distribution curves of three metal chelates with PAP-5Me as a function of the pH. The distribution ratio of a divalent metal ion ($M^{2+}$) with PAP-5Me can be expressed by (the distribution of the charged chelate ($ML^+$) is negligible):

$$D = \frac{K_0 \beta_1 [L^-]^2}{1 + \beta_1 [L^-] + \beta_2 [L^-]^2}.$$

Here, $K_0$ is the distribution constant of $ML_2$; $\beta_1$ and $\beta_2$ are the overall stability constants, as defined by

$$K_0 = [ML_2]_o/[ML_2],$$

$$\beta_1 = [ML^+]/[M^{2+}] [L^-]$$

and

$$\beta_2 = [ML_2]/[M^{2+}] [L^-]^2.$$

A plot of log $D$ vs. log $[L^-]$ for the zinc(II) chelate with PAP is shown in Fig. 4. The concentration of $L^-$ in the aqueous phase was computed according to the following equation, which was derived from the material balance of PAP:

$$\Sigma [L] = (\nu_s [L]_S + \nu_w [L])/(\nu_s + \nu_w).$$

Here, $\nu_s$ and $\nu_w$ are the volume of the surfactant-rich and aqueous phases, $\Sigma [L]_S$ is the total concentration of PAP in the entire solution ($\nu_s + \nu_w$) and $\Sigma [L]$ and $\Sigma [L]$ are the total concentrations of PAP in the surfactant-rich and aqueous phases.

The distribution constant and the two stability constants for the zinc(II) chelate were calculated through a
least-squares fit of the model described by Eq. (3) to the data in Fig. 4. The stability constants for other chelates were also determined in the same manner as described above, and are summarized in Tables 3 and 4.

Effect of the PEG concentration on the distribution constant

Figure 5 shows a correlation of the distribution constants with the PEG concentration. It can be seen that the distribution constants for the three chelates are not only dependent upon the nature of nonionic surfactants, but are also highly dependent upon the PEG concentration. The distribution constants of PAP-5Me and its zinc(II) and cadmium(II) chelates increase with an increase in the PEG concentration, while that of the nickel(II) chelate decreases with the PEG concentration. These findings are probably due to the decrease in the concentration of OTG in the aqueous phase with increasing PEG concentration. Indeed, it was 0.40%(w/v) in the presence of 2%(w/v) PEG, 0.35%(w/v) in 5%(w/v) PEG and 0.32%(w/v) in PEG concentration above 12%(w/v). These concentrations are still higher than the critical micelle concentration of OTG, 9 mM (=0.28%(w/v)). These data support the idea that the distribution constants are regulated by OTG micelles retained in the aqueous phase. However, the log $K_D$ values are also dependent upon the nature of the solute. Apparently, the effect on the value of the nickel(II) chelate was different from those on others.

Hence, for explaining the change in the log $K_D$ values, we must take into account another effect of PEG. It is well known that PEG induces a precipitation or crystallization of proteins in aqueous solutions. The effect of PEG can be explained in terms of an elimination of hydrated water molecules from the protein surface. Such an elimination could also work on water molecules around the metal chelates, depending on the extent of chelate hydration.

Figure 5 Logarithmic distribution constants of PAP-5Me and its metal chelates as a function of PEG concentration.

Table 4 also lists the distribution constants in the temperature-induced phase separation in which aqueous PONPE-7.5 solutions were employed. The magnitudes and orders of the distribution constants in the polymer-induced phase separation are almost the same as those in the temperature-induced phase separation. This observation suggests that the OTG-rich phase has almost the same solvent properties as those of the PONPE-7.5-rich phase. However, the distribution constants varied with the change in the PEG concentration. This would provide a means for controlling the extraction selectivity. Additionally, the polymer-induced phase separation can be applied to the extraction of termo-labile compounds, because the phase separates even at 0°C. Further work is under way for applying these phenomena to practical problems.

References


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