Interaction of Nitrosylruthenium with Glycine and Alanine in Seawater

—Studies on nitrosylruthenium complex-formations with glycine and alanine by a chelating resin—

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RuNO–Chelex 100 resin, [R·RuNO(OH)(OH₂)] was prepared by the reaction of RuNO-chloro complex with Chelex 100 resin, and it is expected that aqua and hydroxo groups contained in RuNO-Chelex 100 resin act as adsorption sites for glycinate or alaninate anion by ligand-exchange reaction. The equilibrium constants, \( K_{eq} \), of 1:1 complex formation reactions of RuNO-Chelex 100 resin with glycine and alanine were found to be 96.3±5.76 and 82.6±2.69, respectively. The inhibitive effect of glycine and alanine on adsorption of \([\text{RuO}_2(\text{NO}_2)(\text{NO}_3)(\text{H}_2\text{O})\text{NO}]^-\) to anion exchange resin increased in the order of glycine>alanine. It was observed that the inhibitive effect was dominated by \( K_{eq} \) of RuNO-Chelex 100 resin with glycine and alanine.

Key Words: complex formation, nitrosylruthenium-glycinate, nitrosylruthenium-alaninate, chelating resin

1. Introduction

Radioruthenium is one of the predominant fission products found in the fuel-reprocessing wastes. The process solutions are known to contain radioruthenium as nitrosylruthenium (RuNO) compounds. Most of the nitrosylruthenium compounds tend to exist as colloidal forms or particles in the aquatic environment, and one of their representative chemical formula has been shown as diaquatrihydroxonitrosylruthenium, \([\text{Ru(OH)}_3(\text{H}_2\text{O})_2\text{NO}]^0\). Many organic compounds of potential complexing ability are known to occur in seawater, for example: amino acids, proteins, glycolic acid, fulvic and humic acids. Belot, et al. suggested that some nitrosylruthenium-organic complexes might be formed through interactions of nitrosylruthenium hydroxide with organic compounds in seawater. Suzuki and Ikeda, and Kimura and Honda studied the effect of organic matters on the adsorption of cobalt by using the ion exchangers and the chelating resin, respectively. They reported that the interfering effect of amino acids on the adsorption of Co was dominated by the chelate formation constant of amino acids with Co²⁺. The studies of the complex formation of RuNO compounds with amino acids in seawater is important for the assessment of the concentration of \(^{106}\text{Ru}\) in marine organic debris or sediments. In the previous papers it was reported that two new RuNO-glycinato and alaninato complexes, \( K([\text{Ru(gly}_3]_2(\text{OH})_2\text{NO}] \) and \( K([\text{Ru(ala)}_2(\text{OH})_2\text{NO}] \), were prepared, and characterized that these two complexes were both stable as an anionic species in aquatic environment by isotachophoretic analysis. This paper describes the equilibrium constants in the formation reaction of monoglycinato- and monoalaninato complexes of RuNO-chelating resin, together with the effects of glycine and alanine on adsorption of \([\text{RuO}_2(\text{NO}_2)(\text{NO}_3)(\text{H}_2\text{O})\text{NO}]^-\) to anion exchange resin.

2. Experimental

2.1 Materials

2.1.1 Radionuclides

\(^{106}\text{Ru}\): Radioruthenium used was obtained
from the Radiochemical Centre, Amersham, as a form of carrier-free $^{106}$Ru chlorocomplex in about 8N hydrochloric acid. The stock solutions of [RuCl$_3$(H$_2$O)$_2$NO] and (RuO-Ru) nitrate complex, [RuO$_2$(NO)$_2$NO$_3$]O were prepared in accordance with the methods reported previously. The concentration of radioactivity and carrier of the stock solutions of (RuCl$_3$(H$_2$O)$_2$NO) and (RuO$_2$(NO)$_2$NO$_3$)O were adjusted to be $1.85 \times 10^3$ Bq/ml ($5 \times 10^{-3}$ Ci/ml) and $4.4 \times 10^{-4}$ mol/l in 0.2 M CH$_3$COO$^-$Na 0.2 M CH$_3$COOH buffer solution of pH 4.62, and $2.03 \times 10^2$ Bq/ml ($5.5 \times 10^{-3}$ Ci/ml) and 4.0 $\times 10^{-3}$ mol/l in seawater, respectively.

$^{14}$C: (1-14C)Glycine (specific activity 2.14 GBq/mmole (60 mCi/mmol)) and L-(1-14C)Alanine (specific activity 1.92 GBq/mmole (50 mCi/mmol)) used in this experiment were both obtained from the Radiochemical Centre, Amersham. The concentration of radioactivity and carrier of both the stock solutions were adjusted to be 3.3 $\times 10^3$ Bq/ml ($0.1 \mu$Ci/ml) and 0.1 mol/l, respectively.

2.1.2 Seawater
Artificial seawater was prepared according to the receipt of Lyman and Fleming. The chlorinity of the seawater is 19.00%. The seawater was filtered by using membrane filters of 0.45 $\mu$m pore size prior to use. The pH of the seawater was adjusted to be 8.1 $\pm$ 0.2 with sodium carbonate solution.

Sodium perchlorate solution at the ionic strength of 0.67 which is approximately the same as in seawater of 35%. Salinity was used for the batch equilibrium test in order to calculate the equilibrium constants in the formation reaction of monoglycinato- and monoalaninato complexes of RuNO-chelating resin.

2.1.3 Ion exchange resin
RuNO-Chelox 100 resin: Chelox 100 chelating resin (Bio-Rad Laboratories of Richmond, California) of 100-200 mesh size was used. RuNO complex of Chelox 100 resin was prepared by the following procedure. An RuNO ion solution which was prepared by dissolving 1.37 g of diaquatrichloronitrosylruthenium, [RuCl$_3$(H$_2$O)$_2$NO] into 200 ml of 0.2 M sodium acetate-acetic acid buffer (pH=4.63) was mixed with 10 g of sodium form Chelex 100 resin. The resulting RuNO complex resin was washed with deionized water until the washed solution became free from RuNO ion, sucked off to dewater, and stored in a sealed bottle in a refrigerator. The ruthenium content in the resin was determined by measuring the gamma activity of $^{106}$Ru-$^{106}$Rh adsorbed on Chelex 100 resin, and expressed in terms of $F$(mmol/g of dry RuNO resin). The ruthenium content ($F$) was found to be 0.326 mmol/g dry resin. The saturated adsorption capacity was estimated to be 1.7 mmol for RuNO ion per 1 g of oven dried resin.

Anion exchange resin: Dowex 1 x 4 Cl form of 100-200 mesh size was used.

2.2 Experimental procedures
2.2.1 Procedure for adsorption equilibria
Adsorption equilibria were investigated by batch operation. A weighed amount of dry RuNO resin, ca. 0.5 g was shaken with 50 ml of sodium perchlorate solution of [1-14C]glycine or L-[1-14C]alanine containing a carrier at a constant temperature of 20°C for 24 hr, which was sufficient to attain approximate equilibrium. Carrier concentrations in the sodium perchlorate solutions were adjusted to be $(10^{-2} \sim 4 \times 10^{-2})$M. After equilibrium, the concentrations of glycine and alanine in the supernatant and in the resin phase, $Q$(mmol/g) were determined by measuring the beta activities of $^{14}$C-glycine and $^{14}$C-alanine.

2.2.2 Adsorption of (Ru-O-Ru) nitrate complex, $[^{106}$RuO$_2$(NO)$_2$NO$_3$]O on anion exchange resin
The batch technique was used in this adsorption test. A weighed amount of dry Dowex 1 x 4 Cl form resin, ca. 0.25 g was shaken with 25 ml of a seawater solution of (Ru-O-Ru) nitrate complex for 1 hr at 25°C $\pm$1°C. After centrifuging, 1 ml of the supernatant was analyzed by gross gamma counting. The results are expressed in terms of the distribution coefficient ($K_d$). The $K_d$ is represented by the following equation.

$$K_d = \frac{C_0 - C}{C} \cdot \frac{V}{G}$$

where $C_0$ is the activity in the seawater solution before equilibrium (cpm), $C$ is the activity in the supernatant after equilibrium (cpm), $V$ is the volume of the seawater solution (ml), $G$ is
the dry weight of the resin introduced into the systems (g).

2.2.3 Measurement of activity

The beta activity of $^{14}$C was measured using PACKARD TRICARB Model 3380 liquid scintillation spectrometer. The gamma activity of $^{106}$Ru-$^{106}$Rh was measured using well gamma system Model TDC-6U manufactured by Aloka Co. Ltd.

3. Results and Discussion

3.1 Nature of RuNO chelating resin

Since Chelex 100 chelating resin used in this experiment has a three-dimensional network structure containing an iminodiacetic acid moiety as a chelating group, the coordination site of the Chelex 100 resin behaves as a terdentate ligand to RuNO ion, resulting in a more stable chelate than that of bidentate aminocarboxylic acid ligands. As the result, RuNO ion is so tightly bound to the resin that it is not easily released from resin even in the presence of a large excess of amino acids and sodium ions in seawater. The formation reaction of the RuNO chelating resin may be expressed as a following equation, and the structure may be given as formula (I).

RuNO-Chelex 100 resin as shown in formula (I) may have been leaving two coordination sites, one is aqua and the other is hydroxo groups. The sites are expected to act as adsorption sites for bidentate anionic ligands, such as glycinate and alaninate anions by ligand-exchange reaction as suggested by Siegel and Degens14f in seawater.

3.2 Adsorption of glycinate and alaninate anions on RuNO-Chelex 100 resin

The complex formation reaction of RuNO-Chelex 100 resin with amino acid can be schematically written as follows.

\[
[R \cdot RuNO(OH)(OH_2)] + H_{am} \xrightarrow{K_{eq}} [R \cdot RuNO_{(am)}] + 2H_2O
\]  

where \( [R \cdot RuNO(OH)(OH_2)] \), \( H_{am} \) and \( K_{eq} \) denote the RuNO-Chelex 100 resin, amino acid and equilibrium constant of the complex formation reaction, respectively. From the uptake, \( Q (\text{mmol/g}) \), of glycine and alanine by RuNO-Chelex 100 resin, the equilibrium constant, \( K_{eq} \), was calculated according to the method reported by Takeshita, et al.\(^{15}\) using the following equation from (3) to (6).

\[
C = -\frac{1}{K_{eq}} + Q_{eq} \left( \frac{C}{Q} \right) \quad (3)
\]

\[
X(R\cdot RuNO_{(am)}) = Q/Q_{eq} \quad (4)
\]

\[
X(R\cdot RuNO(OH)(OH_2)) = 1 - X(R\cdot RuNO_{(am)}) \quad (5)
\]

\[
K_{eq} = \frac{X(R\cdot RuNO_{(am)})}{X(R\cdot RuNO(OH)(OH_2))} \cdot \frac{1}{C} \quad (6)
\]

where the saturated adsorption capacity of the resin, the molar concentration of amino acid in the seawater phase, the mole fractions of adsorbed and unadsorbed sites in the resin are expressed as \( Q_{eq} \), \( C \), \( X(R\cdot RuNO_{(am)}) \), and \( X(R\cdot RuNO(OH)(OH_2)) \), respectively. The adsorption isotherms of glycinate and alaninate ions with the RuNO-Chelex 100 resin are shown in Fig. 1, where the resin of \( F=0.326 \) was used at 20°C and pH 8.1 in sodium perchlorate aqueous solution adjusted to ionic strength 0.67. The uptake of glycinate and alaninate ions increases with increase in the concentration of glycine and alanine in the aqueous phase, \( C \), approaching saturation at higher concentration. The plots

![Fig. 1 Adsorption isotherms of the RuNO-Chelex 100 resin (F=0.326) for glycine and alanine in sodium perchlorate solution (I= 0.67, pH=8.1) at 20°C.](image)
of \( C \) against \( C/Q \) gave a straight line in the range \( (10^{-2} - 4 \times 10^{-2}) M \) of the amino acid concentration in sodium perchlorate aqueous solution, as shown in Fig. 2. Thus \( Q_w \) can be obtained from the slope of the straight line, according to Eq. (3). \( X_{(R-RuNO_{\text{aq}_{10}})} \), \( X_{(R-RuNO(OH)(OH_2))} \) and \( K_{eq} \) can be obtained from Eq. (4), (5) and (6), respectively. The values of \( Q_w \) and \( K_{eq} \) are summarized in Table 1. The stoichiometric ratios, \( Q_w/F \) of glycine and alanine were 1.35 and 1.26, respectively, indicating that the greater part of the resin bound RuNO ions acted as adsorbable sites for glycine and alanine. Iminodiacetonitrosylruthenium chelate is not identified in CH\(_3\)COONa-CH\(_3\)COOH buffer solution, but the fact that the values of \( Q_w/F \) are nearly unity indicates the 1:1 complex formation by the interaction of glycinate or alaninate ion with the RuNO-Chelex 100 resin. The equilibrium constant, \( K_{eq} \) for glycinate complex of RuNO-Chelex 100 resin is slightly greater than that for alaninate complex of RuNO-Chelex 100 resin. The result coincides with the cases of the first transition metal series\(^{16}\). The equilibrium constants in the RuNO-monglycinato- and RuNO-monoalaninato complexes are significantly lower than those of monglycinato and monoalaninato complexes of heavy metal ions, such as Cd\(^{2+}\), Co\(^{3+}\), Cr\(^{3+}\), Mn\(^{2+}\), Ni\(^{2+}\), Pb\(^{2+}\) and Zn\(^{2+}\), assuming that the complex formation of RuNO ion with glycine and alanine is inferior to those of the other metal ions in seawater.

### 3.3 Effect of glycine and alanine on adsorption of \((Ru-O-Ru)\)nitrate complex

It has been found by authors\(^{12}\) that \([RuO_2(NO_2)NO_3]_2O\) in aquatic systems was electrophoretically fractionated “anionic,” “neutral” and “cationic” species with aging, and about 75 percent of total Ru was present in the anionic fractions, assuming the \([RuO_2(NO_2)(NO_3)(H_2O)_2NO]^-\) as one of the possible chemical forms of anionic species. For this reason, the seawater solution of \([RuO_2(NO_3)NO_3]_2O\) was used in this adsorption test. The inhibitive effects of glycine and alanine on the adsorption reaction of \((Ru-O-Ru)\)nitrate complex, \([RuO_2(NO_3)NO_3]_2O\) to the anion exchange resin are shown in Table 2. The inhibitive effect of glycine and alanine on adsorption of RuNO-complex anion on anion exchange resin depends on the concentration of amino acids. The effect of glycine is greater than that of alanine. The order of the inhibitive effect of glycine and alanine on the adsorption of RuNO-complex anion coincided with the order of the equilibrium constant, \(K_{eq}\) of the formation of glycinate and alaninate. The values of \(Q_w \) and \(K_{eq} \) are summarized in Table 1. The stoichiometric ratios, \( Q_w/F \) of glycine and alanine were 1.35 and 1.26, respectively, indicating that the greater part of the resin bound RuNO ions acted as adsorbable sites for glycine and alanine. Iminodiacetonitrosylruthenium chelate is not identified in CH\(_3\)COONa-CH\(_3\)COOH buffer solution, but the fact that the values of \( Q_w/F \) are nearly unity indicates the 1:1 complex formation by the interaction of glycinate or alaninate ion with the RuNO-Chelex 100 resin. The equilibrium constant, \( K_{eq} \) for glycinate complex of RuNO-Chelex 100 resin is slightly greater than that for alaninate complex of RuNO-Chelex 100 resin. The result coincides with the cases of the first transition metal series\(^{16}\). The equilibrium constants in the RuNO-monglycinato- and RuNO-monoalaninato complexes are significantly lower than those of monglycinato and monoalaninato complexes of heavy metal ions, such as Cd\(^{2+}\), Co\(^{3+}\), Cr\(^{3+}\), Mn\(^{2+}\), Ni\(^{2+}\), Pb\(^{2+}\) and Zn\(^{2+}\), assuming that the complex formation of RuNO ion with glycine and alanine is inferior to those of the other metal ions in seawater.

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### Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Glycine</th>
<th>Alanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>( Q_w ) (mmol/g)</td>
<td>0.44±0.06</td>
<td>0.41±0.04</td>
</tr>
<tr>
<td>( Q_w/F )</td>
<td>1.35±0.18</td>
<td>1.26±0.12</td>
</tr>
<tr>
<td>( K_{eq} ) (l/mol)</td>
<td>96.3±5.76</td>
<td>82.6±2.69</td>
</tr>
<tr>
<td>( \log K_{eq} )</td>
<td>1.98±0.07</td>
<td>1.91±0.03</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Concentration of amino acid [M]</th>
<th>Glycine</th>
<th>Alanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5×10(^{-3})</td>
<td>44.9±4.1</td>
<td>48.2±4.4</td>
</tr>
<tr>
<td>5.0×10(^{-3})</td>
<td>36.2±3.4</td>
<td>47.0±4.3</td>
</tr>
<tr>
<td>7.5×10(^{-3})</td>
<td>32.2±2.3</td>
<td>33.1±2.8</td>
</tr>
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reaction of RuNO-glycinate and alaninate complexes. This fact indicates that the stability of \([\text{RuO}_2(\text{NO}_2)(\text{gly})\text{NO}]^-\) presumably formed by the ligand substitution reaction of \([\text{RuO}_2(\text{NO}_2)(\text{gly})(\text{H}_2\text{O})\text{NO}]^-\) with glycine is greater than that of \([\text{RuO}_2(\text{NO}_2)(\text{ala})\text{NO}]^-\).

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要　旨

海水を中心におけるニトロシルルテニウムとグリシンおよびアラニンとの相互作用

——キレート樹脂によるニトロシルルテニウムのグリシンおよびアラニン錯体生成に関する研究——

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RuNO-クロロ錯体とキレックス100樹脂との反応によりRuNO-キレックス100樹脂, \([\text{R}-\text{RuNO}-(\text{OH})(\text{OH}_2)]\)を調製した。この樹脂中にある配位水ならびにヒドロキシ基と glycinate または alaninate アニオンとの配位子交換反応により生ずる RuNO-グリシンまたは RuNO-アラニンの 1 ： 1 型錯体について, 生成平衡定数を測定したところ, その値はそれぞれ, 96.3±5.76 および 82.6±2.69であった。また \([\text{RuO}_2(\text{NO}_2)(\text{NO}_2)(\text{H}_2\text{O})\text{NO}]^-\)の陰イオン交換樹脂への吸着におよぼすグリシンおよびアラニンの阻害効果の大きさは, グリシン＞アラニンとなり, RuNO-グリシンおよび RuNO-アラニンの 1 ： 1 型錯体生成平衡定数の大きさの順に一致することが分かった。