Nitrate Reductase Activity and the Speciation of Selenium at the Mouth of Chesapeake Bay*

Kazufumi Takayanagi†, George T.F. Wong† and Margaret J. Filardo‡†

Abstract: Nitrate reductase activity and the concentrations of selenite and selenate were monitored for six months at the mouth of Chesapeake Bay (USA). A positive correlation was found between nitrate reductase activity and the concentration of selenite, suggesting that selenite may be formed in coastal waters and nitrate reductase may be involved in the process.

1. Introduction
Organisms play an important role in controlling the chemical speciation of a number of elements in the marine environment (Stumm and Morgan, 1981). Selenium is one of these bio-active elements. Its concentration ranges from undetectable to 2.5 nM in the oceans and its distribution is similar to those of nutrients. Although selenate, the oxidized species, is the thermodynamically stable species of selenium and should be the only detectable species in seawater at a pH of 8.1 and pE of 12.5 (Sillen, 1961), the existence of selenite, the reduced species, in seawater has been extensively reported (Sugimura et al., 1976; Suzuki et al., 1980; Measures and Burton, 1980; Measures et al., 1980, 1983, 1984; Takayanagi and Wong, 1983, 1985; Cutter and Bruland, 1984; van der Sloot et al., 1985). A possible mechanism for the production of the reduced species is via the biologically mediated enzymatic reduction of selenate.

Tsunogai and Sase (1969) have shown that iodide, the thermodynamically unstable, reduced species of dissolved iodine in oxic seawater, may be produced by the reduction of iodate in the presence of the enzyme nitrate reductase (NR) and the co-enzyme NADH or NADPH. The standard electrode potentials at pH 7 and 25°C for the iodate/iodide, nitrate/nitrite and NADP+/NADPH couples are 0.67, 0.42 and -0.33 V, respectively (Table 1). Therefore, during the oxidation of NADPH to NADP+, enough energy may become available for the reduction of nitrate to nitrite and iodate to iodide. In this situation, NR acts as the vehicle for transferring energy to the nitrogen and iodine systems. Because the standard electrode potential of the selenate/selenite couple is 0.45 V at pH 7 and 25°C (Table 1), the reduction of selenate to selenite is energetically possible by the combination of NADPH or NADH and NR.

Our field results of NR activity, selenate and selenite from Chesapeake Bay will be presented in this short contribution.

2. Materials and methods
2.1. Sampling
Surface water samples were periodically collected at the mouth of Chesapeake Bay, using a polyethylene bucket, from January to July of 1982. The samples were kept in a 10 l polyethylene container and immediately transported to the laboratory where they were analyzed for NR activity, chlorophyll a, total selenium and selenite. NR activity was determined on the same day while filtered samples for total selenium and selenite were kept refrigerated in polyethylene bottles until they were analyzed (usually in less than a week). Chlorophyll a was determined fluorometrically after it had been extracted with 90% acetone from cells retained on a glass fiber filter which was stored frozen for a few days.

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Table 1. Equilibrium constants and electrode potentials for iodine, selenium, nitrogen and NADP redox couples.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>log $K$</th>
<th>log $K^{(a)}$</th>
<th>Eh(V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/6 IO$_3^-$+H$^+$+e$^-$=1/6 I$^-$/H$_2$O</td>
<td>18.35</td>
<td>11.35</td>
<td>0.67</td>
</tr>
<tr>
<td>1/2 SeO$_3^{2-}$+3/2 H$^+$+e$^-$=1/2 HSeO$_3^-$+1/2 H$_2$O</td>
<td>18.15</td>
<td>7.65</td>
<td>0.45</td>
</tr>
<tr>
<td>1/2 NO$_3^-$+H$^+$+e$^-$=1/2 NO$_2^-$+1/2 H$_2$O</td>
<td>14.15</td>
<td>7.15</td>
<td>0.42</td>
</tr>
<tr>
<td>1/2 (NADP)$^+$+1/2 H$^+$+e$^-$=1/2 (NADPH)</td>
<td>-2.0</td>
<td>-5.5</td>
<td>-0.33</td>
</tr>
</tbody>
</table>

$^{(a)}$ Log $K'$ is the equilibrium constant for the redox reaction in natural water, pH=7.0 at 25°C, and mathematically expressed as $\log K' = \log K - (nH/2) \log K_w$, where $nH$ is the number of moles of protons exchanged per mole of electrons and $K_w$ is the dissociation constant of water (Stumm and Morgan, 1981).

Table 2. The concentration of selenium and NR activity in the waters collected from the mouth of Chesapeake Bay.

<table>
<thead>
<tr>
<th>Date</th>
<th>Salinity ($%$)</th>
<th>NR Activity (μmol/hr/l)</th>
<th>Chl $a$ (mg/m$^2$)</th>
<th>Specific NR Activity$^{(a)}$</th>
<th>Selenium (nM)</th>
<th>Selenite Selenate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>Selenite</td>
</tr>
<tr>
<td>1.</td>
<td>82-1-22</td>
<td>24.3</td>
<td>0.18</td>
<td>N.D$^{(b)}$</td>
<td>-----</td>
<td>0.52</td>
</tr>
<tr>
<td>2.</td>
<td>82-2-04</td>
<td>24.8</td>
<td>0.12</td>
<td>24.4</td>
<td>4.9×10$^{-3}$</td>
<td>0.48</td>
</tr>
<tr>
<td>3.</td>
<td>82-2-23</td>
<td>20.6</td>
<td>0.19</td>
<td>24.5</td>
<td>7.8×10$^{-3}$</td>
<td>0.58</td>
</tr>
<tr>
<td>4.</td>
<td>82-2-3-06</td>
<td>19.4</td>
<td>0.53</td>
<td>12.0</td>
<td>44.2×10$^{-3}$</td>
<td>0.53</td>
</tr>
<tr>
<td>5.</td>
<td>82-3-23</td>
<td>17.6</td>
<td>0.10</td>
<td>16.0</td>
<td>6.3×10$^{-3}$</td>
<td>0.51</td>
</tr>
<tr>
<td>6.</td>
<td>82-4-09</td>
<td>19.7</td>
<td>0.71</td>
<td>55.7</td>
<td>12.7×10$^{-3}$</td>
<td>1.05</td>
</tr>
<tr>
<td>7.</td>
<td>82-4-29</td>
<td>21.5</td>
<td>0.06</td>
<td>13.1</td>
<td>4.6×10$^{-3}$</td>
<td>0.37</td>
</tr>
<tr>
<td>8.</td>
<td>82-5-19</td>
<td>18.5</td>
<td>0.10</td>
<td>12.5</td>
<td>8.0×10$^{-3}$</td>
<td>0.37</td>
</tr>
<tr>
<td>9.</td>
<td>82-6-03</td>
<td>21.9</td>
<td>U.D$^{(c)}$</td>
<td>ND</td>
<td>-----</td>
<td>0.41</td>
</tr>
<tr>
<td>10.</td>
<td>82-6-18</td>
<td>17.8</td>
<td>1.62</td>
<td>20.6</td>
<td>78.6×10$^{-3}$</td>
<td>0.68</td>
</tr>
<tr>
<td>11.</td>
<td>82-7-08</td>
<td>20.2</td>
<td>N.D</td>
<td>N.D</td>
<td>-----</td>
<td>0.53</td>
</tr>
</tbody>
</table>

$^{(a)}$ Specific NR activity in mole NO$_2^-$ produced/μg of Chl $a$/hr.

$^{(b)}$ Not determined, thus no corresponding specific NR activities.

$^{(c)}$ Undetectable.

(Yentsch and Menzel, 1963; Strickland and Parsons, 1972). Salinity was determined with a Guildline Instruments Model 8400 salinometer.

2.2. Enzyme extraction and the determination of NR activity

Four liters of a sample were filtered through 47 mm diameter Gelman A/E glass fiber filters. The enzyme was extracted from the filters and the filtrate was used for subsequent total selenium and selenite analyses. The filters were ground immediately for 2 min with an electrically driven Teflon-glass tissue homogenizer at 4°C in a mixture of 1 ml of 10,000 ppm polyvinylpyrrolidone and 2 ml of 250 ppm dithiothreitol. The suspension of homogenized cells and the filters were then centrifuged for 10 min at 2,500 rpm. NR activity in the supernatant was determined by the method of Eppley et al. (1969). NR activity was expressed as the amount of nitrite produced from added nitrate during the incubation period. The analytical precision was estimated to be ±0.02 μmol NO$_2^-$/hr/l.

2.3. Determination of selenium

The concentrations of total selenium and selenite in the filtrate were determined fluorometrically (Takayanagi and Wong, 1983). The analytical precision was ±0.03 nmol/l for total selenium and for selenate using 400 ml of water. The concentration of selenate was calculated as the difference between these two concentrations.

3. Results

Results of salinity, NR activity, and the concentrations of chlorophyll $a$, total selenium, selenite and selenate are presented in Table 2. Temporal variations of NR activity, selenite and selenate are also shown in Fig. 1. NR activity varied considerably during this period of time. An exceptionally high value of 1.62 μmol/hr/l was observed on 18 June. The remaining data
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points ranged from undetectable to 0.71 μmol/hr/l. These values are similar to those reported previously for estuarine and coastal environments (Holmes et al., 1967; Harrison, 1973; Kristiansen, 1983). Three sharp maxima in NR activity were observed on 8 March, 9 April and 18 June (Fig. 1).

The concentrations of total selenium, selenite and selenate were all exceptionally high on 9 April with concentrations of 1.05, 0.59 and 0.46 nM, respectively (Table 2). Aside from the data on 9 April, the concentration of total selenium ranged from 0.37 to 0.68 nM with a mean of 0.51±0.09 nM (±18%). The corresponding variations in the concentrations of selenite and selenate were larger. Selenite ranged from 0.14 to 0.38 nM with a mean of 0.26±0.08 nM (±31%) while selenate varied from 0.15 to 0.34 nM with a mean of 0.25±0.07 nM (±28%). The selenite to selenate concentration ratio varied from 0.50 to 2.53. The temporal variation of the concentration of selenite was similar to that of NR activity with three concentration maxima observed on 8 March, 9 April and 18 June. High concentrations of selenite were also observed on the latter two dates (Fig. 1). The highest concentration of chlorophyll a, 55.7 mg/m³, was observed on 9 April. On the other dates, the concentration ranged from 12 to 24.5 mg/m³ (Table 2).

4. Discussion

Between January and July, salinity varied from 17.6 to 24.8% with a mean of 19.7±2.4% (±12%). These variations did not follow a simple seasonal trend and might have been the combined results of the temporal variations in the input of fresh water to Chesapeake Bay and the tidal stage at which each sample was collected. This magnitude of variations in salinity was similar to that of total selenium (0.51 nM±18%), but much smaller than those of selenite (0.26 nM±31%) or selenate (0.25 nM±28%). It is evident that salinity did not correlate well with total selenium, selenite or selenate (Table 2). Thus, the effect of salinity changes alone is insufficient to explain the changes in the concentrations of the selenium species during this period of time.

Since the concentration of total selenium varied during the sampling period, aside from the inter-conversion between selenite and selenate, other

Fig. 1. Temporal changes of NR activity (A) and the concentrations of selenite and selenate selenate (B) at the mouth of Chesapeake Bay from 22 January to 8 July 1982. ND: not determined.

Fig. 2. The relationship between NR activity and selenite. The straight line indicates the approximate trend of the data points.
processes such as mixing of waters with different initial concentrations of dissolved selenium and the interactions between the dissolved and particulate phases might also contribute to the changes in the speciation of selenium during the sampling period. However, since the variation in the concentration of total selenium was significantly smaller than the variations of selenite and selenate, these other processes were not likely to be the major controlling processes of the speciation of selenium, although they might mask the effects which resulted from interconversions between selenite and selenate. In the nitrogen system, the excretion of nitrite (an analogue of selenite) from phytoplankton cells during active nitrate (an analogue of selenate) uptake has been observed under various laboratory conditions (Anderson and Roels, 1981; Raimbault, 1986). If the same phenomena occur in the selenium system in the natural environment, i.e. the reduction of selenite to selenite by some enzymatic system and the passive excretion of selenite from living cells, an increase in the concentration of selenite may coincide with an increase in the enzymatic activity. Indeed, although no clear seasonal trend was observed in the concentration of selenite, its concentration maxima on 8 March, 9 April and 18 June coincided with the maxima in NR activity (Fig. 1), and a positive correlation was found between NR activity and selenite (Fig. 2). The correlation coefficient by the linear regression analysis was 0.94 excluding one anomalous point on 18 June.

NR activity did not show a clear temporal trend. This may be the result of temporal and spatial variations in biological productivity at the mouth of Chesapeake Bay. For example, the distribution of phytoplankton is known to be heavily influenced by hydrodynamics which shows short term variations within hours as well as longer term variations in days and months. The species composition might also have varied during the sampling period. NR activity in natural samples is known to be highly species-variable (Kristiansen, 1987). Indeed, the NR activity per unit chlorophyll a or specific NR activity was quite variable, ranging from undetectable to 78.6 × 10^{-9} mol NO_3^- produced/μg chl a/hr with high values occurring on 8 March and 18 June (Table 2). However, these values of specific NR activity were within the range of values reported by other investigators (Holmes et al., 1967; Kristiansen, 1983, 1987).

Although it is not yet clear whether NR itself or other biological processes represented by NR activity are involved, our data indicate that NR activity is closely tied with the formation of selenite in coastal waters. For future research to unequivocally prove the biologically mediated reduction of selenite to selenite, nutrients, particulate selenium and other enzymatic activities also need to be determined.

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References
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チェサビック湾湾口における硝酸塩還元酵素活性とセレンの化学形

高柳和正*1, George T.F. Wong*, Margaret J. Filardo*2

要旨：米国チェサビック湾において、硝酸塩還元酵素活性と亜セレン酸（IV）とセレン酸（VI）の濃度を6か月間モニターした。その結果、硝酸塩還元酵素活性と亜セレン酸の濃度が正の相関を示し、亜セレン酸が沿岸海域海水中で形成され、硝酸塩還元酵素がそれに関与していることを示唆しているようである。