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The effect of Se salts on DNA structure

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ABSTRACT

There is considerable interest in the role of selenium in cancer prevention. Various organic and inorganic Se compounds are considered to be antioxidants. In the present study, the binding modes, the binding constants and the stability of Se–DNA complexes have been determined by Fourier transform infrared (FTIR) and UV–Visible spectroscopic methods. Spectroscopic evidence showed that Na_2SeO_4 and Na_2SeO_3 bind to the minor and major grooves of DNA and the backbone phosphate (PO_2) with overall binding constants of $K(Na_2SeO_4\text{–DNA}) = 5.20 \times 10^4\ M^{-1}$ and $K(Na_2SeO_3\text{–DNA}) = 1.87 \times 10^3\ M^{-1}$. DNA aggregations occurred at high selenium concentrations. No biopolymer conformational changes were observed upon Na_2SeO_3 and Na_2SeO_4 interactions, while DNA remained in the B-family structure.

1. Introduction

Selenium is an important micronutrient and an essential trace element for both humans and animals metabolism. Diet containing selenium includes cereals, grains, nuts, vegetables, meat, and seafood [1–3]. Selenium has been extensively studied for its antioxidant activity and cancer prevention [4–8]. It is found in many dietary supplements and multivitamins in forms of sodium selenite (Na_2SeO_3), sodium selenate (Na_2SeO_4) or selenomethionine (SeMet) [5,9].

The main inorganic selenium compound used in most cancer treatment studies is sodium selenite (Scheme 1). However, a few studies use other forms, such as sodium selenate (Scheme 1) and selenium oxide [1,6,10–14]. Selenium is incorporated as selenocysteine (SeCys) in selenoproteins P, W, and R, as well as in the active sites of the enzymes such as glutathione peroxidases (GPx) and thioredoxin reductases [1,6,15–17]. In cells, these selenoproteins have important antioxidant activities and protect the mitochondria, plasma membrane, and DNA from oxidative damage by reactive oxygen species (ROS) [18–20].

Sodium selenite inhibits the growth of cells and induces apoptosis in human colonic carcinoma [21], brain tumor [22] and breast cancer cells [23]. It inhibits 7,12-dimethylbenz(a)anthracene (DMBA) induced rat mammary tumors [24], human fibrosarcoma tumor cell invasiveness [25] and pulmonary metastasis of melanoma cells in mice [26,27]. The majority of clinical trials provide evidence for selenium as a chemopreventive agent for specific cancers: prostate [28,29], lung [30–32], colorectal [33,34], stomach [19] and multiple cancers [35–38]. However, the results of a recent clinical trial showed that selenium and vitamin E taken alone or together did not prevent prostate, lung or colorectal cancers [39].

Even though much is reported about antitumor activities and protective role of Se compounds in DNA damage [40], little is known about the interaction of sodium selenite and selenate with individual DNA. The interaction of some oxoselenium compounds with DNA have been studied before [41]. Their results showed B-DNA stabilization till 1/1 ratio and a partial B to A-DNA transition at higher ratio of 2/1.

The aim of this study was to characterize the DNA structural changes in the presence of Na_2SeO_3 and Na_2SeO_4. Here, we compared the interactions of Na_2SeO_3 and Na_2SeO_4 with DNA in aqueous solution at pH 7 with Se/DNA (P) molar ratios of 1/120–1/1 by FTIR and UV measurements. It is worth mentioning that the concentration of Se used here is much higher than the physiological concentration of Se found in living cell. Since the FTIR and UV spectroscopic methods are not sensitive to very low Se contents, higher concentrations were used in order to determine the effect of Se on DNA structure. Structural analyses regarding the Se binding site, binding constant, and DNA secondary structures are provided here.

2. Materials and methods

2.1. Materials

Highly polymerized type I calf-thymus DNA sodium salt (7% Na content) was purchased form Sigma Chemical Co. (St. Louis, MO),

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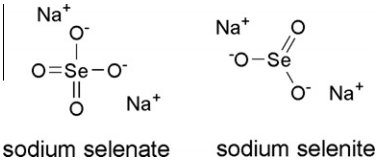

Scheme 1. Chemical structure of Na_2SeO_4 and Na_2SeO_3.

and deproteinated by the addition of $CHCl_3$ and isoamyl alcohol in NaCl solution. Na_2SeO_3 and Na_2SeO_4 were also purchased from Sigma Chemical (St. Louis, MO) and used without further purification. To check the protein content of DNA solutions, the absorbance bands at 260 and 280 nm were used. The A_{260}/A_{280} ratio was 2.10 for DNA, showing that DNA samples were sufficiently free from protein [42]. Other chemicals were of reagent grade and used without further purification.

2.2. Preparation of stock solutions

DNA was dissolved to 0.5% w/v (0.0125 M) polynucleotide (phosphate) (pH 7) in Tris–HCl at 5 °C for 24 h with occasional stirring to ensure the formation of a homogeneous solution. The final concentration of the DNA solution was determined spectrophotometrically at 260 nm using molar extinction coefficient ε_{260} = 66,000 $cm^{-1}\,M^{-1}$ (DNA) (expressed as molarity of phosphate groups) [43]. The appropriate amounts of sodium selenite and sodium selenate (0.1–12.5 mM) were dissolved in water and added dropwise to DNA solution (12.5 mM) to attain the desired Na_2SeO_3 and Na_2SeO_4/DNA(P) molar ratios (r) of 1/120–1/1 with a final DNA(P) concentration of 6.25 mM. The pH values of solutions were adjusted at 7.0 ± 0.2 using NaCl solution. The infrared spectra were recorded 2 h after mixing of the Na_2SeO_3 and Na_2SeO_4 with DNA solution.

2.3. FTIR spectroscopy measurements

Infrared spectra were recorded on a Bruker ATR spectrometer (Ettlingen, Germany) equipped with an external water cooled high power Hg-arc source and was accessible with a room temperature operated deuterated triglycine sulfate (DTGS) detector and a ZnSe beam splitter. The spectra of Na_2SeO_3 and Na_2SeO_4/DNA solutions were recorded using a cell assembled with ZnSe windows. Spectra were recorded using the Opus software supplied by the manufacturer of the spectrophotometer. The spectra of the solutions were recorded after 2 h incubation of the Na_2SeO_3 and Na_2SeO_4 with DNA solution, using ZnSe windows. The ratios used for infrared were Se/DNA(P) molar ratios (r) of 1/120, 1/80, 1/40, 1/20, 1/10, 1/5, 1/2 and 1/1 with a final DNA(P) concentration of 6.25 mM. For each spectrum, 100 scans were recorded with resolution of 4 cm^{-1}. The difference spectra [(polynucleotide solution + Na_2SeO_3 and Na_2SeO_4 solution) − (polynucleotide solution)] were obtained using a sharp DNA band at 968 cm^{-1} as internal Refs. [44,45]. This band, which is due to sugar C–C and C–O stretching vibrations, exhibits no spectral change (shifting or intensity variation) upon Na_2SeO_3 and Na_2SeO_4–DNA complexation, and cancelled out upon spectral subtraction. The intensity ratios of the bands due to several DNA in plane vibrations related to A–T, G–C base pairs and the PO_2 stretching vibrations were measured with respect to the reference bands at 968 cm^{-1} (DNA) as a function of Se concentrations with an error of ±3%. Similar intensity variations have been used to determine the ligand binding to DNA bases and backbone phosphate groups [46]. The plots of the relative intensity (R) of several peaks of DNA in-plane vibrations related to A–T, G–C base pairs and the PO_2 stretching vibrations such as 1710 (guanine), 1662 (thymine), 1610 (adenine), 1492 (cytosine), and 1226 cm^{-1} (PO_2 groups) versus Se concentrations were obtained after peak normalization using $R_i = I_i/I_{968}$, where I_i is the intensity of the absorption peak for DNA in the complex with i as the ligand concentration, and I_{968} is the intensity of the 968 cm^{-1} peak (DNA internal reference). The plot of intensity was drawn for r = 1/120–1/1.

2.4. Absorption spectroscopy

The absorption spectra were recorded on a LKB model 4054 UV–Visible spectrometer, the quartz cuvettes of 1 cm were used. The absorption spectra recorded with Na_2SeO_3 and Na_2SeO_4 concentrations of 0.005–0.5 mM and constant polynucleotide concentration of 0.5 mM. The binding constants of the Na_2SeO_3 and Na_2SeO_4–DNA complexes were calculated as reported [47]. It is assumed that the interaction between the ligand [L] and the substrate [S] is 1:1; for this reason a single complex SL (1:1) is formed.

The relationship between the observed absorbance change per centimeter and the system variables and parameters is as follow;

$$\frac{\Delta A}{b} = \frac{S_t K_{11} \Delta\varepsilon_{11}[L]}{1 + K_{11}[L]} \tag{1}$$

where $\Delta A = A - A_0$ from the mass balance expression $S_t = (S) + (SL)$, we get $(S) = S_t/(1 + K_{11}(L))$.

Eq. (1) is the binding isotherm, which shows the hyperbolic dependence on free ligand concentration. The double-reciprocal form of plotting the rectangular hyperbola $\frac{1}{y} = \frac{f}{d}\cdot\frac{1}{x} + \frac{e}{d}$, is based on the linearization of Eq. (1) according to the following equation,

$$\frac{b}{\Delta A} = \frac{1}{S_t K_{11} \Delta\varepsilon_{11}[L]} + \frac{1}{S_t \Delta\varepsilon_{11}} \tag{2}$$

Thus the double reciprocal plot of $1/\Delta A$ versus $1/(L)$ is linear and the binding constant can be estimated from the following equation

$$K_{11} = \frac{intercept}{slope} \tag{3}$$

3. Results and discussion

3.1. FTIR spectra of Na_2SeO_4–DNA adducts

Evidence related to Na_2SeO_4–DNA complexation comes from the infrared spectroscopic results shown in Figs. 1A and 2A. The spectral changes (intensity and shifting) of several prominent DNA in-plane vibrations at 1710 (G and T, mainly G), 1662 (T, G, A, and C, mainly T), 1610 (A and C, mainly A), 1492 (C and G, mainly C), 1226 (PO_2 asymmetric stretch), and 1088 cm^{-1} (PO_2 symmetric stretch) [48–52] were monitored at different Na_2SeO_4–DNA molar ratios, and the results are shown in Figs. 1A and 2A.

At r = 1/120, 1/80, major reduction in intensity was observed for the bases and phosphate PO_2 vibrations (Figs. 1A and 2A). The intensity of the guanine band at 1710 cm^{-1} decreased by 18%, the thymine band at 1662 cm^{-1} decreased by 15%, the adenine band at 1610 cm^{-1} decreased by 22% and the asymmetric phosphate vibration decreased by 6% (Figs. 1A and 2A). The observed intensity decreases can be related to DNA helix stabilization upon Na_2SeO_4 complexation. At higher concentrations (r = 1/40, 1/20), intensity of the G, T, A and asymmetric PO_2 bands increased. The observed increase in intensity can be related to DNA helix destabilization as a result of extended binding of Na_2SeO_4 to bases and phosphate group (Fig. 2A). At r = 1/10, 1/5, reduction in the intensity of the bases and phosphate bands can be attributed to DNA aggregation upon Na_2SeO_4 interaction (Fig. 2A). At r = 1/2, increase