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Biophysics

Stabilization of Reduced Gold Atoms on the Basis of DNA

Irine Khutsishvili[*], Tamar Giorgadze[*], Shota Gogichaishvili[*], Zaza Melikishvili[**], Vasil Bregadze[*]

[*]*Andronikashvili Institute of Physics, Ivane Javakhishvili Tbilisi State University, Tbilisi, Georgia*
[**]*Chavchanidze Institute of Cybernetics, Georgian Technical University, Tbilisi, Georgia*

(Presented by Academy Member David Mikeladze)

The DNA molecule is amazingly rich in information content and very robust, its unique structural features and powerful recognition capabilities can be of interest for assembling artificial structures for a variety of applications. DNA's unique properties are intensively studied from different points of view: as a molecular wire, as a drug delivery system, as a ladder for ordered arrangements of various nanostructures, as a spacer to control distances between nano objects. The goal of our research is to study the reduction process of gold ions (Au^{3+}) on DNA double helix. The reduction of gold ions to the atomic state in solutions was studied in the absence and presence of DNA, using γ-irradiation (^{137}Cs), and reducing agent – ascorbic acid, the study was carried out by the spectrophotometric method. γ-irradiation of the DNA-Au^{3+} leads to reduction of 26% of the added Au^{3+} ions, whereas ascorbic acid reduces only 22%. We calculated the radiation-chemical yield $G(Au^0)$ for the reduction of Au^{3+} ions in the complex of DNA after 36 hours (237.6 krad) of γ-irradiation, $G(Au^0) = 0.27$, which is the amount of reduced gold atoms per 100 eV of γ-irradiation. Spectroscopic analysis of the influence of ascorbic acid on the reduction of Au^{3+} ions in the absence and presence of DNA showed that in the solution of pure Au^{3+} ions the reduced Au^0 atoms are unstable caused by the oxidation of atoms. Au^0 atoms in the presence of DNA are stable because they bind to DNA bases and are not accessible for oxidation. *© 2023 Bull. Georg. Natl. Acad. Sci.*

DNA, gold ions, reduction, γ-irradiation, nanotechnology

During the past decades, nanotechnology, which involves research and the development of materials and species with lengths 100 nm and less, has been revolutionizing many scientific fields. Nanotechnology is an interdisciplinary field of fundamental and applied sciences, it is based on scientific and experimental foundations, and includes fields of science such as: organic chemistry, molecular biology, semiconductor physics, energy storage, [1,2] engineering, [3] microfabrication, [4] and molecular engineering [5]. The sizes of most of the biological structures are determined by nanoscale, for example the radii of the deoxyribonucleic acid (DNA) double helix is 2 nm. DNA's structural features and powerful recognition capabilities can

be of interest for assembling artificial structures for a variety of applications.

The processes for depositing metal nanostructures on DNA by the reduction of metal ions are known as DNA metallization. One way of DNA metallization is the reduction of metal ions to the DNA surface. Reduction of metal ions can be performed by different methods, by using of reducing agents, for example: ascorbic acid [6-8], sodium borohydride [9,10], by UV-light [11,12], or γ-irradiation [13,14], etc. The poor electrical properties of DNA [15] can be overcome by building metal nanowires in which the double-stranded DNA acts as a template for the seed-mediated growth of silver, gold, or palladium [16-25] wires. DNA based metal nanowires are obtained through different methods. For example, the widespread method is the adsorption of nanoparticles on DNA surface, to obtain copper [26] and palladium [20] nanowires they add metal ions and after reductant to the DNA fixed on the surface.

In recent years, in our laboratory, along with other works, the study of reduction of silver ion by using spectroscopic methods has been done on various surfaces (DNA, G4-PAMAM dendrimer, membrane, hair, cotton) [27-29]. We studied the reduction process of silver ions under the influence of ascorbic acid. The goal of our research is to study the different methods of reduction of gold ions (Au^{3+}) on DNA.

Materials and Methods

Materials. In our tests we used the calf thymus DNA (40% GC), obtained from "Sigma-Aldrich". The concentration of nucleic acids was determined by UV absorption using molar extinction coefficients (ε=6600 cm^{-1} M^{-1} at λ=260 nm). The double helix structure of the polymers was proved by their hyperchromicity (~33%) and their typical thermal denaturation transition (0.01 M NaCl).

In tests $HAuCl_4$ salt were used and NaCl served as background electrolytes. For the reduction of gold ions ascorbic acid (AA) and sodium borohydride ($NaBH_4$) was used. $HAuCl_4$, NaCl, AA, and $NaBH_4$ were purchased from "Sigma-Aldrich". All experiments were performed in 0.01 M NaCl.

Instrumentations. Registration of absorption spectra was carried out by compact, precessive, mobile, small power consumption optical fiber spectrometer AvaSpec ULS 2048-USB2 (200-1100 nm).

The study of γ-radiation exposure of DNA samples was carried out on – Radiation tool – "Gamma-kapsula". Source of radioisotope – ^{137}Cs, dose rate of 1.1 Gy/min. Plastic irradiation chamber #3 (10x5sm). Temperature during the irradiation in the chamber – 20±5°C. ^{137}Cs produces gamma rays with an energy of 0.662 MeV and has a half-life of 30.1 years.

Samples in a solution of 0.01 M NaCl in the presence of 0.1 M ethanol in a plastic test tube with a volume of 1.5 ml were irradiated for 4 and 36 hours. The radiation doses were correspondingly 26.4 krad and 237.6 krad.

Results and Discussion

The reduction of gold ions to the atomic state in solutions was studied in the absence and presence of DNA. γ-irradiation, ascorbic acid (AA), and $NaBH_4$ were used as reducing agents; the study was carried out by the spectrophotometric method.

Radioisotope ^{137}Cs was used as a source of γ-irradiation. As known, the hydrated electron and hydrogen radical, formed as a result of the hydrolysis of water caused by radiation, are good reductants. They can reduce the gold ions in solution: $Au^{3+} \xrightarrow{3e^-_{aq}} Au^0$, $Au^{3+} \xrightarrow{3\dot{H}} Au^0$ as well as in $Au^{3+} - DNA$ complex: $Au^{3+}DNA \xrightarrow{3e^-_{aq}} Au^0 - DNA$, $Au^{3+}DNA \xrightarrow{3\dot{H}} Au^0 - DNA$.

Ethanol was added to the samples, during the conversion reaction of ethanol to ethylene glycol, the $\dot{O}H$ radical is joined by ethanol and the $\dot{H}$ radical is released, so called addition reaction.

Table. Radiation-chemical yields G [30] of the primary products of radiolysis of water per 100 eV, Q - the amount of radiolysis products per 1 gold ion after 4 hours (26.4krad) and 36 hours (237.4 krad) of γ- irradiation

	e^-_{aq}	H^+	OH^-	$\dot{O}H$	H_2O_2	H_2	$\dot{H}$
G	2.6	2.8	0.1	2.6	0.7	0.45	0.5
Q (4 hours)	0.22	0.25	0.009	0.22	0.035	0.12	0.14
Q (36 hours)	1.97	2.21	0.08	1.97	0.32	1.10	1.26

Table shows the radiation-chemical yields G of the primary products of water radiolysis at neutral pH after γ-radiation (^{137}Cs) per 100 eV, Q – the amount of radiolysis products per 1 gold ion after 4 hours (26.4krad) and 36 hours (237.4 krad) of γ-irradiation.

Fig. 1 shows the absorption spectra of Au^{3+} ions reduction under different durations of γ-irradiation (^{137}Cs).

Au^{3+} ion has absorption at 310 nm, as a result of irradiation, the intensity of the spectrum decreases after 4 hours of irradiation and after 36 hours of irradiation, the spectrum characteristic of gold ions disappears and the characteristic spectrum of gold atoms appears at~ 530 nm. The decrease in the intensity of the spectrum after 4 hours of irradiation is due to the partial reduction of the Au^{3+} to Au^+, which does not have an absorption spectrum in this area. If we assume that the extinction coefficient of gold atoms is approximately equal to the extinction coefficient of gold particles (per gold atom 12700 $M^{-1}cm^{-1}$), we get that 13% of the gold ions in the solution were reduced to atoms after 36 hours of irradiation. In the presence of DNA in the solution (Fig. 2), due to the high concentration of DNA, the gold ion spectrum is not visible, and after 36 hours of irradiation, the spectrum of the gold atom at ~ 530 nm appeared, and 26% of the gold ions are reduced to the atom. We also studied the effect of γ-irradiation on the DNA double helix [31], showing that γ-irradiation damages DNA, single strand scissions in DNA-NaCl is one distraction per 100 DNA nucleotides. We calculated the radiation-chemical yield [32] $G(Au^0)$ for the reduction of Au^{3+} ions in the complex of DNA after 36 hours (237.6 krad) of γ-irradiation, $G(Au^0)$ = 0.27, which is the amount of reduced gold atoms per 100 eV of γ-irradiation.

Since the goal was to reduce gold ions to the DNA double helix, we decided to use reductants, namely $NaBH_4$ and Ascorbic acid (AA), for the reduction of the ions. It should be noted that

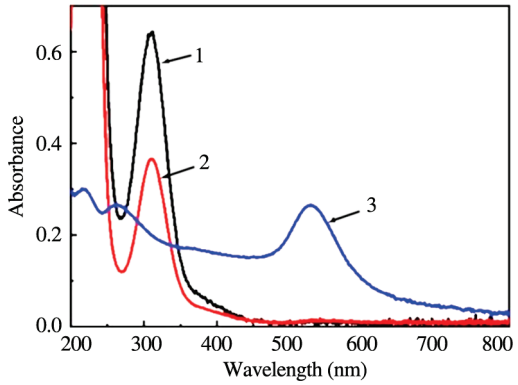

Fig. 1. Absorption spectra of gold ions irradiated with γ-radiation (^{137}Cs). (1) – no irradiation, (2) – 4 hours irradiation (26.4 krad), (3) – 36 hours irradiation (237.6 krad). [Au^{3+}] – $1.6 \cdot 10^{-4}$ M.

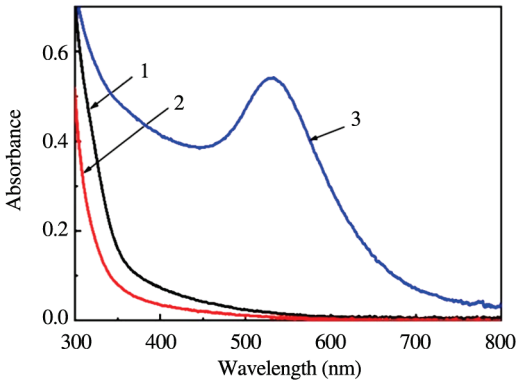

Fig. 2. Absorption spectra of DNA-gold ions complexes irradiated with γ-radiation (^{137}Cs). (1) – no irradiation, (2) – 4 hours irradiation (26.4 krad), (3) – 36 hours irradiation (237.6 krad). [DNA] – $2.8 \cdot 10^{-4}$ M (P), [Au^{3+}] – $1.6 \cdot 10^{-4}$ M.

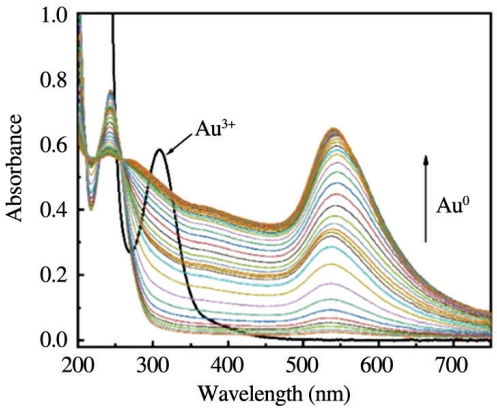

Fig. 3. Absorption spectra showing the reduction process of Au^{3+} ions induced by ascorbic acid. 70 spectra are presented, recorded with an interval of 1 second. $[Au^{3+}] – 1.5 \cdot 10^{-4}$ M, [AA] – $2.5 \cdot 10^{-4}$ M.

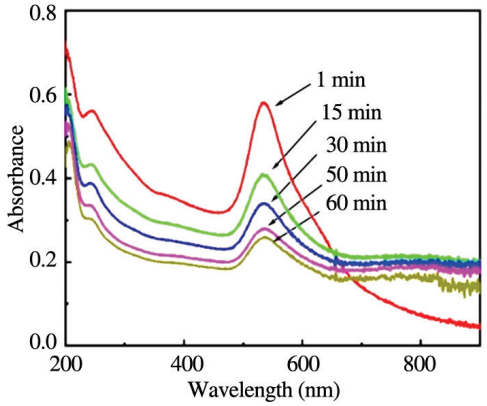

Fig. 4. Decrease of the absorption spectrum of the gold atom from 1 min. to 60 min. $[Au^{3+}] – 1.5 \cdot 10^{-4}$ M, [AA] – $2.5 \cdot 10^{-4}$ M.

reduction of gold ions with and without DNA using the reductant $NaBH_4$ does not occur.

It was established that the complete reduction of the gold ion (Au^{3+}) to the atomic (Au^0) state is possible using ascorbic acid, namely: in Au^{3+}-AA and DNA-Au^{3+}-AA complexes. Fig. 3 presents the time-dependent absorption spectra of Au^{3+} ions reduction induced by AA. The gold ion (Au^{3+}) has an absorption spectrum at 310 nm, which decreases upon addition of AA, and an absorption spectrum characteristic of a gold atom occurs at 530 nm, the absorption increases and reaches a maximum after about a minute, 32% of added gold ions are reduced.

It was found that gold atoms are not stable in the solution, they are oxidized, which is caused by the high concentration of hydroxonium ions in the solution (pH≈6).

Fig. 4 shows the decrease of the absorption spectrum of gold atom after 1 minute, which means the oxidation of the atom, but no Au^{3+} ion spectrum is formed, presumably the oxidation $Au^0 \rightarrow Au^+$ occurs, and the single-charged gold ion does not have an absorption spectrum in the visible region.

Fig. 5 shows the absorption spectra of reduced gold ions (Au^{3+}) in the complex with DNA. The reduction of gold ions takes only couple of minutes.

The trivalent gold ion is a hard metal ion. According to the Pearson's theory [33], hard metal ions (Mn^{2+}, Fe^{3+}, Al^{3+}, Co^{3+}, Au^{3+}) are bound to the phosphate backbone, unlike the soft ions (Cu^+, Ag^+, Pt^{2+}, Hg^{2+}) which interact with bases and form the so-called interstrand cross-link in DNA [27]. In case of gold ions, they interact with phosphates, but after reduction to Au^0 atom, which is a soft metal, probably binds to DNA bases, and is not accessible for oxidation, and the complex DNA- Au^0 is stable.

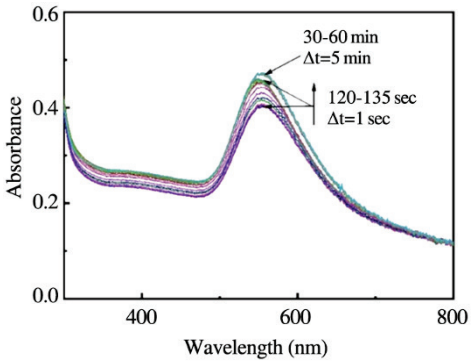

Fig. 5. Spectra for gold ion reduction in complexes DNA-Au^{3+}-AA are presented, recorded with an interval of 1 sec, and after 30 min with an interval 5 min. [DNA] – $0.5 \cdot 10^{-4}$ M (P), $[Au^{3+}] – 1.4 \cdot 10^{-4}$ M, [AA] – $2.5 \cdot 10^{-4}$ M.

If we compare the results of gold ions recovered by γ-irradiation and ascorbic acid, we can see that in the presence of DNA, γ-irradiation leads to the

reduction of more gold ions (26%) compared to AA (22%). The opposite occurs in the solution of gold ions, for γ- irradiation leads to the reduction of less gold ions (13%) compared to AA (32%). In our opinion, γ-irradiation is a more effective method for reduction of gold ions, and the fact that we registered fewer gold atoms in a solution without DNA is caused by the time factor, because after irradiation, the spectra were taken after about half an hour, during which time the atoms were oxidized.

Conclusions

Using spectrophotometry, we showed that reduction of the gold ions (Au^{3+}) can be performed with γ-irradiation or adding reductant ascorbic acid, γ-irradiation is more effective but it causes the damage of DNA double helix. We calculated the radiation-chemical yield $G(Au^0)$ for the reduction of Au^{3+} ions in the complex of DNA after 36 hours (237.6 krad) of γ-irradiation, $G(Au^0) = 0.27$, which is the amount of reduced gold atoms per 100 eV of γ-irradiation.

The reduction occurs in the absence and presence of DNA but in the gold ion solution without DNA the gold atom is not stable because of farther oxidation in solution. In case of presence of DNA, gold ions interact with phosphates, but after reduction to Au^0 atom, which is a soft metal, probably binds to DNA bases, and is not accessible for oxidation, and the complex DNA- Au^0 is stable for weeks.

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ბიოფიზიკა

აღდგენილი ოქროს ატომების სტაბილიზაცია დნმ-ის საფუძველზე

ი. ხუციშვილი*, თ. გიორგაძე*, შ. გოგიჩაიშვილი*, ზ. მელიქიშვილი**, ვ. ბრეგაძე*

*ივანე ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი, ანდრონიკაშვილის ფიზიკის ინსტიტუტი, თბილისი საქართველო
**საქართველოს ტექნიკური უნივერსიტეტი, ვლადიმერ ჭავჭანიძის სახ. კიბერნეტიკის ინსტიტუტი, თბილისი საქართველო

(წარმოდგენილია აკადემიის წევრის დ. მიქელაძის მიერ)

სამუშაოს მთავარ მიზანს წარმოადგენდა ხსნარებში ოქროს იონების აღდგენის შესწავლა ატომურ მდგომარეობამდე დნმ-ის თანაობისას და მის გარეშე, ოქროს იონების აღსადგენად გამოყენებულ იქნა γ–დასხივება (^{137}Cs) და რედუქტანტი ასკორბინის მჟავა, მათი შესწავლა განხორციელდა სპექტროფოტომეტრული მეთოდით. ნაჩვენებია, რომ დნმ-Au^{3+} კომპლექსის γ–დასხივება ატომამდე აღადგენს დამატებული ოქროს იონების 26%, ხოლო ასკორბინის მჟავა – 22%. ჩვენ გამოვთვალეთ დნმ-თან კომპლექსში მყოფი Au^{3+} იონების აღდგენის რადიაციულ-ქიმიური გამოსავალი $G(Au^0)$ γ-დასხივების 36 სთ-ის შემდეგ (237.6 კრად) $G(Au^0)$ =0.27, რაც შეესაბამება 100 ევ γ-დასხივების შედეგად აღდგენილი ოქროს ატომების რაოდენობას. დნმ-ის თანაობისას და მის გარეშე Au^{3+} იონების აღდგენაზე ასკორბინის მჟავას გავლენის სპექტროსკოპიულმა შესწავლამ აჩვენა, რომ დნმ-ის გარეშე Au^{3+}იონების ხსნარში აღდგენილი Au^0 ატომები არასტაბილურია, ხსნარში მათი შემდგომი დაჟანგვის გამო. დნმ-ის თანაობისას Au^0 ატომები სტაბილურია, რადგან ისინი უკავშირდებიან ფუძეებს, რაც ხელს უშლის მათ დაჟანგვას.

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