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An Electrochemical DNA Biosensor for the Detection of DNA Damage Caused by Radioactive Iodine and Technetium

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A DNA damage process by radioactive iodine (I-131) and technetium (Tc-99m) is described. The procedure relies on a label-free electrochemical DNA biosensor. Fish sperm double stranded DNA was immobilized onto a carbon paste electrode (CPE) by electro deposition and the changes to the guanine oxidation signal were observed upon exposure to radioactive I-131 and Tc-99m by differential pulse voltammetry (DPV).

The changes to the signal of guanine after exposure to therapeutic doses of Tc-99m and I-131 showed conformational changes in DNA molecules.

Key Words: Electrochemistry, DNA damage, Tc-99m, iodine-131.

Introduction

Electrochemical DNA biosensors provide a rapid and inexpensive method for the determination of DNA damage caused by drug, chemical, or radioactive agents. Electrochemical DNA biosensors are comprised of a nucleic acid recognition layer that is immobilized over an electrochemical transducer. The signal transducer must determine the change that has occurred due to the binding molecules or to the hybridization at the recognition layer, converting this into an electronic signal that then can be relayed to the end user. Observing the pre- and post-electrochemical signals of a DNA-drug interaction provides good evidence of the interaction mechanism to be elucidated. Moreover, this interaction could be used for the quantification of these drugs and for the determination of new DNA-targeting drugs.[1,2]

The role of the nucleic acid recognition layer is to detect the changes that occurred in the DNA structure during interaction with DNA-binding molecules, or to selectively detect a specific sequence of DNA.[3−5] There have been intensive studies of the application of modern voltammetric methods in nucleic acid research and DNA analysis. DNA biosensor technologies are currently under intense investigation owing

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to their great promise for rapid and low-cost detection of specific DNA sequences in humans, viruses, and bacterial acids.[6−8]

DNA electrochemical biosensors enable rapid detection of chemicals that cause irreversible damage to DNA by monitoring the changes in oxidation peaks of the DNA bases guanine and adenine, and have important applications for better understanding and evaluating DNA-drug interaction mechanisms.[9−12]

The guanine oxidation signal was also monitored to detect DNA damage caused by the interaction mechanism between radiation, drugs, and DNA.[3−17] Toxic molecules that interact with DNA could also be detected by monitoring the changes in the guanine oxidation signal in relation to electrochemical DNA biosensors.[18−21]

Element 43, technetium, was discovered in 1937 by Perrier and Segre in a sample of molybdenum that was irradiated by deuterons. This element was named after the Greek word, *technetos,* meaning artificial, as technetium was the first previously unknown element to be artificially made. Since then, 21 isotopes of technetium have been discovered, ranging from Tc-90 through Tc-110. Among these isotopes, Tc-110 has the shortest half-life (0.86 s) and Tc-97 the longest (2.6×10^6 years). All isotopes of technetium are radioactive(19). Tc-99m has a physical half-life of 6.02 h and gamma rays of 140 keV.[1,2] It is the ideal radionuclide for external imaging studies. One of the many desirable attributes of Tc-99m is that it can be produced from molybdenum-technetium (Mo^{99}/Tc^{99m}) parent/daughter generator systems, which are available worldwide. As the molybdenum decays, Tc-99m and Tc-99 build up on the column.[2] The generator yields technetium as the pertechnetate ion TcO_4^-. The advantages of Tc-99m can be summarized as follows: a short but reasonable half-life of 6 h, high photon yield (88%) of 140 keV, no beta radiation (resulting in a low radiation dose), availability in a generator for in-hospital use, chemically reactive, and yields other chemical forms.

The most useful radioisotopes of iodine for nuclear medicine studies are I-123, I-125, and I-131, because of their favorable physical properties. The choice of the isotope to be used is determined by the ultimate application. I-131 has been widely used for clinical diagnosis and therapy for several reasons.[1] I-131 is obtained as a by-product of uranium fusion. It decays by beta emission with a half-life of 8.05 days to Xe-133. The transition energy between I-131 and Xe-133 ground state is 971 keV, and 14 gamma rays are emitted, the most important, with abundance (%), being 364 keV (82%), 637 keV (7%), 284 keV (6%), and 723 keV (2%).

DNA damage in cells causes serious diseases, including mutations. Detection of DNA damage relies on lengthy and expensive chromatographic and electrophoretic separation assays. In this study DNA damage induced by exposure to radioactive Tc-99m and I-131has been examined.[22,23.]

Experimental Section

Apparatus

The DNA damage caused by radioactive Tc-99m and I-131 was investigated using DPV with an AUTOLAB PGSTAT 30 electrochemical analysis system and GPES v.4.9 software (Eco Chemie, The Netherlands). The 3-electrode system consists of a carbon paste electrode (CPE) as the working electrode, the reference electrode (Ag/AgCl), and a platinum wire as the auxiliary electrode. The body of the CPE was a glass tube (3 mm i.d.) tightly packed with the carbon paste. The electrical contact was provided by a copper wire

inserted into the carbon paste. Carbon paste was prepared in the usual manner by hand-mixing graphite powder (Fisher) and mineral oil (Acheson 38) in a 70:30 mass ratio. The surface was polished on weighing paper to a smoothed finish before use. The convective transport was provided by a magnetic stirring bar.

Chemicals

Double stranded fish sperm DNA (dsDNA, activated and lyophilized) was purchased from SERVA Electrophoresis GMBH (Germany). We prepared 100 mg L^{-1}dsDNA stock solution with TE solution (10 mmol L^{-1} Tris-HCl, 1 mmol L^{-1}EDTA, pH 8) and kept it frozen. More dilute solutions were prepared with 0.50 mol L^{-1}acetate buffer (pH 4, 80). Other chemicals were of analytical reagent grade. All the buffer solutions contained 20 mmol L^{-1} NaCl. Sterilized and deionized water was used in all solutions.

Experimental procedure

DNA damage was performed by applying direct and indirect irradiation methods as described below.

Direct irradiation

We put 10 μL of fish sperm dsDNA sample from a 1000 ppm DNA stock into Eppendorf tubes. Freshly eluted Tc-99m from a Mo-99/Tc-99m generator was added onto DNA samples in fractions of 0.5 mCi (18.5 MBq), 1 mCi (37 MBq), 2 mCi (74 MBq), 5 mCi (185 MBq), 10 mCi (370 MBq), 20 mCi (740 MBq), and 30mCi (1110 MBq) activities, respectively (3 samples were prepared for each radioactivity level). Then, 1 mL of 0.9% NaCl was added to each sample and the tubes were incubated for 6, 12, and 24 h.

Indirect irradiation

Fish sperm dsDNA samples were placed into Eppendorf tubes as described previously. The Eppendorf tubes were put into test tubes in which Tc-99m activities had been previously put. The test tubes were then incubated for 6, 12, and 24 h.

Indirect irradiation with iodine

Fish sperm dsDNA samples were placed into Eppendorf tubes as described above. Then, 5 μCi, 10 μCi, 25 μCi, 50 μCi, and 100 μCi of I-131 activities were put into test tubes, and the Eppendorf tubes were put into the test tubes and incubated for 24 h.

DPV measurements required an immobilization and a detection cycle at a fresh carbon paste surface. A background scan of buffer alone was recorded for each electrode and subtracted from subsequent scans.

After applying direct and indirect irradiation, the damaged DNA was immobilized onto CPE by electro deposition, as described below.

CPE pretreatment: The CPE was activated by applying 1.70 V for 1 min in 0.05 M phosphate buffer solution containing 20 mM NaCl (pH 7.40), without stirring.

DNA immobilization: dsDNA was immobilized on a pretreated CPE surface by applying a potential of 0.50 V to a concentration of 10 ppm dsDNA containing 0.50 M acetate buffer solution (pH 4.80) with 20 mM NaCl at 200 rpm, with stirring. The electrode was then rinsed with 0.50 M acetate buffer solution (pH 4.80) containing 20 mM NaCl for 10 s.

Voltammetric transduction: The electrode was then transferred into the blank 0.50 M acetate buffer solution (pH 4.80) containing 20 mM NaCl for the voltammetric measurement. The differential pulse voltammograms were collected with an amplitude of 10 mV at a 20 mV/s scan rate. The raw data were treated using the moving average baseline correction with a peak width of 0.01 in GPES software. For the measurements performed with the bare CPE, no DNA was adsorbed onto the electrode surface.

Repetitive measurements were carried out by renewing the surface and repeating the above assay format.

Results and Discussion

The DPV oxidation signals of guanine obtained with the dsDNA-modified CPE before and after applying direct and indirect irradiation with Tc-99m are shown in Figure 1 (A, B) and Figure 2. The oxidation signal of guanine obtained with the dsDNA-modified CPE before (Figure 1A, B, and Figure 2a) was higher than the those obtained after applying indirect (b) and direct irradiation (c) with Tc-99m and I-131 for 24 h. The decrease in the guanine signals indicated damage in the oxidation site of guanine.[4−7]

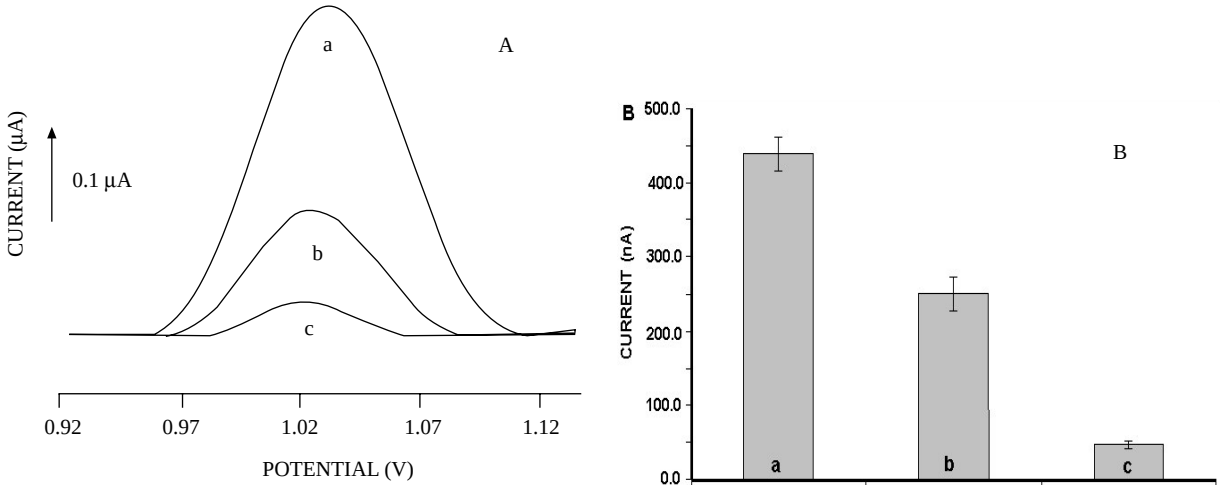

Figure 1. A) Differential pulse voltammogram. **B)** Histogram based on the guanine oxidation signal of 10 ppm dsDNA **(a)** before and **(b)** after exposure, and **(c)** after incubation with Tc-99m for 24 h.

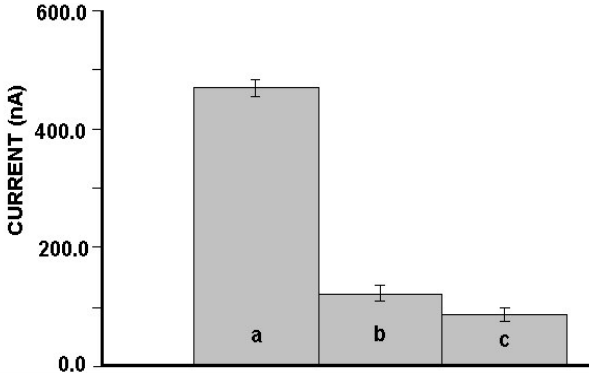

Figure 2. Histograms based on the guanine oxidation signal of 10 ppm dsDNA **(a)** before and **(b)** after exposure, and **(c)** after incubation with I-131 for 24 h.

In Figure 3 the effect of non-radioactive Tc-99m on the DNA is shown. The histograms based on the oxidation signals of guanine obtained with dsDNA-modified CPE before and after direct irradiation with non-radioactive Tc-99m were obtained under the same conditions. There was no remarkable difference between the signals of guanine obtained before and after irradiation using non-radioactive Tc-99m (Figure 2a, b). This shows that the decrease in the guanine signal obtained from dsDNA after direct and indirect irradiation with radioactive Tc-99m resulted from the radioactive effect of Tc-99m.[19]

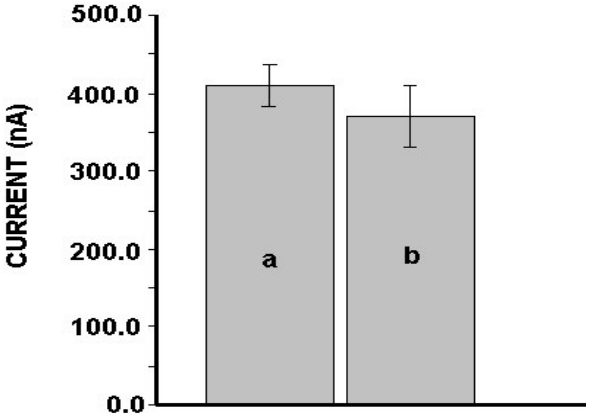

Figure 3. Histograms based on the guanine signal of 10 ppm dsDNA **(a)** before and **b)** after dsDNA incubation with non-radioactive technetium.

The effects of various radioactivity levels of Tc-99m on the guanine signal of dsDNA studied using a dsDNA-modified CPE surface are shown in Figure 4. It was observed that after indirect irradiation of dsDNA with varying Tc-99m radioactivity levels, the guanine signal decreased gradually with a radioactivity level of up to 5 mCi of Tc-99m. After application of 5 mCi, the signals increased gradually with increasing radioactivity level up to 5 mCi, above which it started to level off until the level of 50 mCi. The increase in the signals indicates that Tc-99m breaks the hydrogen bonds between DNA bases.[22,23]

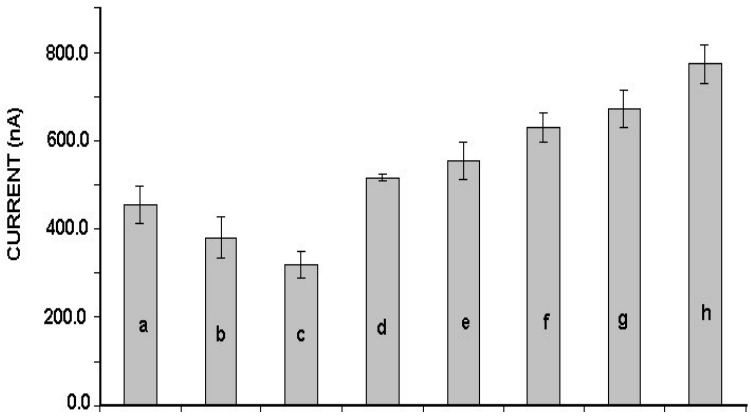

Figure 4. Histograms based on the guanine oxidation signal obtained from dsDNA modified CPE before irradiation **(a)** and after irradiation with **(b)** 1 mCi, **(c)** 2 mCi, **(d)** 5 mCi, **(e)** 10 mCi, **(f)** 20 mCi, **(g)** 30 mCi, and **(h)** 50 mCi Tc-99m for 24 h.

In Figure 5 the results of the same experiment conducted using I-131 under the same conditions are shown. After indirect irradiation of dsDNA with varying I-131 radioactivity levels, the signal of guanine

decreased gradually with radioactivity levels of up to 5 μCi (Figure 5b), above which it started to level off until the value of 100 μCi (Figure 5e). The decrease in the guanine peak obtained from dsDNA damaged by I-131 indicates damage in the oxidizable group of guanine.[19]

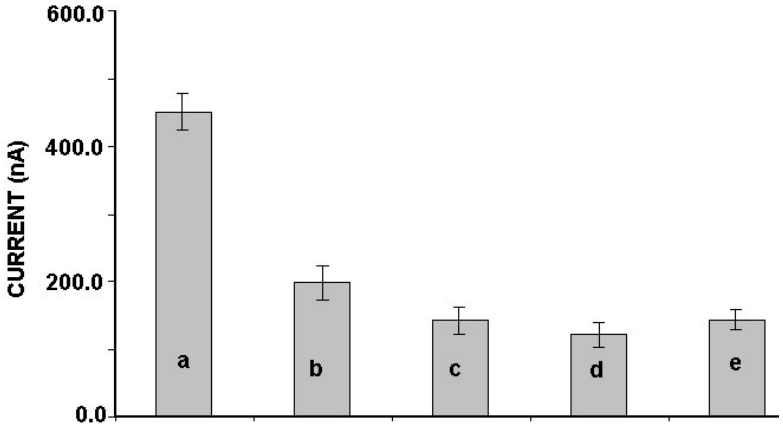

Figure 5. Histograms based on the guanine oxidation signal obtained from dsDNA modified CPE before irradiation **(a)** and after indirect irradiation with **(b)** 5μi, **(c)** 10 μCi, **(d)** 50 μCi, and **(e)** 100 μCi I-131 for 24 h.

Conclusion

The detection of DNA damage by direct and indirect irradiation with radioactive Tc-99m and I-131 was electrochemically studied. The decrease in the guanine oxidation signal after irradiation with Tc-99m and I-131 can be attributed to conformational changes in the DNA double helix. The increase in the guanine oxidation signal after irradiation with varying Tc-99m radioactivity levels can be attributed to the hydrogen bonding break down, which results from increasing radioactivity levels of technetium.

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References

1. S. Rauf, J.J. Gooding, K. Akhtar, M.A. Ghauri, M. Rahman, M.A. Anwar and A.M. Khalid, **J. Pharm. Biomed. Analysis 37,** 205-217 (2005).
2. J.J. Gooding, **Electroanalysis 14,** 1149-1156 (2002).
3. P.B. Dervan, "**Nucleic Acids and Molecular Biology",** 2nd ed., ed. Eckstein, F. and Lilley, D.M.J., Springer-Verlag, Berlin, pp. 49-64, 1998
4. S.R. Mikkelsen, **Electroanalysis 8**, 15-19 (1996).
5. K.M. Millan and Mikkelsen, S.R., **Anal. Chem. 65** 2317-2323 (1993).
6. E. Palecek, **Talanta 56,** 809-819 (2002).

7. A.M. Oliviera Brett, M. Vivan, I.R. Fernandes, and J.A.P. Piedade, **Talanta 56,** 959-970 (2002).
8. F. Jelen, M. Tomschik and E. Palecek, **J. Electroanal. Chem. 423,** 141-148, (1997).
9. M. Burcu, K. Kerman, D. Ozkan, P. Kara and M. Ozsoz, **Electroanalysis 14,** 1245-1250 (2002).
10. M. Tomschik, F. Jelen, L. Havran, L. Trnková, P.E. Nielsen and E. Palecek, **J. Electroanal. Chem. 476,** 71-80 (1999).
11. A.M. Oliveira-Brett, V. Diculescu, and J.A.P. Piedade, **Bioelectrochemistry 55**, 61-62 (2002).
12. P. Kara, D. Ozkan, A. Erdem, K. Kerman, S. Pehlivan, F. Ozkinay, D. Unuvar, G. Itirli and M. Ozsoz, **Clin. Chim. Acta 336,** 57-64 (2003).
13. K. Kerman, Y. Morita, Y. Takamura and E. Tamiya, **Electrochem. Commun. 5,** 887-891 (2003).
14. J. Wang, A.-N. Kawde, A. Erdem and M. Salazar, **Analyst 126,** 2020-2024 (2001).
15. J. Wang and A.-N. Kawde, **Analyst 127,** 383-386 (2002).
16. A. Erdem and M. Ozsoz, **Electroanalysis 14,** 965-974 (2002).
17. J.A.P. Piedade, I.R. Fernandes and A.M. Oliveira-Brett, **Bioelectrochemistry 56**, 81-83 (2002).
18. B. Meric, K. Kerman, D. Ozkan, P. Kara, A. Erdem, O. Kucukoglu, E. Erciyas and M. Ozsoz, **J. Pharm. Biomed. Anal. 30,** 1339-1346 (2002).
19. J. Wang, G. Rivas, M. Ozsoz, D.H. Grant, X. Cai and C. Parrado, **Anal. Chem. 69,** 1457-1460 (1997).
20. K. Kerman, B. Meric, D. Ozkan, P. Kara, A. Erdem and M. Ozsoz, **Anal. Chim. Acta 450,** 45-52 (2001).
21. M. Ozsoz, A. Erdem, P. Kara, K. Kerman and D. Ozkan, **Electroanalysis 15,** 613-619 (2003).
22. R.J. Kowalsky and J.R. Perry, "**Radiopharmaceuticals in Nuclear Medicine Practice**". Appleton and Lange**,** Norwalk**,** 81-83, 1987.
23. R.E. Henkin, "**Nuclear Medicine".** Mosby-Year Book, Inc., St. Louis, 1996.