Volammetric DNA biosensor for the study of mechanism of action of anticancer drug-adriamycin

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The interaction of anticancer drug adriamycin with DNA has been studied using voltammetric DNA biosensor. The binding mechanism of adriamycin was elucidated by using differential pulse voltammetry (DPV) at DNA modified glassy carbon fiber electrodes (GCFE). The decrease in guanine oxidation peak current at +0.9 V was used as an indicator for interaction mechanism in acetate buffer (pH 4.5). The studied drug-DNA interaction mechanism at charged electrode surface is similar to the in-vivo DNA-drug complex formation, where DNA is in close contact with charged phospholipid membranes and proteins. Thus, the fabricated biosensor helps in understanding the in-vivo mechanism of action of this anticancer drug.

Keywords: Modified electrode, Biosensor, Adriamycin, Anthracycline compound

The antitumor activity of compounds is based on their ability to interact with DNA, causing changes in its structure and base sequence, which inhibits the DNA cross linking reaction. Some carcinogens\textsuperscript{1} and oxidising substances\textsuperscript{2} cause oxidative damage to DNA structure and thus the study on the oxidative damage can prove highly useful in preventing perspective. In recent years, the use of modified electrodes has proved to be highly useful for the study of interaction mechanism between substances of medicinal importance\textsuperscript{3}. Such electrodes work as electrochemical biosensors\textsuperscript{4} providing a simple and inexpensive technology for the diagnosis of genetic diseases and the detection of pathogenic biological species\textsuperscript{5,6}. It has been demonstrated that the sequence specific hybridization of nucleic acids can be detected directly or by DNA intercalators\textsuperscript{7-11} which generally work as anticancer drugs.

Adriamycin, an antibiotic drug of anthracyclines family, possesses a wide spectrum chemotherapeutic applications and antineoplastic action. Antitumor properties of adriamycin are known for more than three decades but, the pharmacokinetic and biochemical studies to establish its in-vivo mechanism of action and to improve its administration and anticancer activity are still important goals to achieve. A survey of literature records that adriamycin and other analogous anthracyclines behave as DNA intercalator and their activity accumulates in nuclear genome\textsuperscript{12-15}.

The work reported in the present paper is aimed at the fabrication of voltammetric DNA-biosensor on a GCFE and study the interaction of adriamycin \textit{in situ} with ds-DNA at a charged surface i.e. DNA modified GCFE and to propose a suitable mechanism for the DNA-Adriamycin interaction.

**Experimental Procedure**

The chemicals used were of Anal-R/BDH grade. Calf thymus DNA (Himedia Ltd., Mumbai) and Adriamycin hydrochloride received as gift sample from Ms. Dabur Pharma. Ltd. Baddi (distt. Solan H.P.) were used in the present study. Solutions of the chemicals were prepared in conductivity water.

An Elico (Hyderabad, India) \textit{µ}-polarographic analyser model CL-362 was used for voltammetric studies. Glassy carbon fibers (NF-12, sixty Elititigoitit, U.K.) were used for the fabrication of GCFE, a saturated calomel electrode and coiled platinum electrodes were used as reference and counter electrode respectively. The pH-measurements were made on a systronic (India) \textit{µ}-pH system-361.

**Fabrication of modified electrodes**

\textit{Adriamycin adsorbed GCFE}

For the fabrication of adriamycin adsorbed modified GCFE, the electrode was dipped in a
solution of adriamycin 5 µg/mL for 300 s at the deposition potential of +0.4 V. The electrode was thoroughly rinsed with water and then used for voltammetric measurements. To study the adriamycin-DNA interaction the modified electrode was dipped in a voltammetric cell containing 80 µg/mL DNA and 0.1 M acetate buffer of pH 4.5±0.1, and the DP voltammogram was recorded.

**Thick layer ds-DNA modified GCFE**

Thick layer ds-DNA modified GCFE was fabricated by dipping GCFE in 25 mg/mL solution of ds-DNA for 10 min. It was then taken out and allowed to dry. The electrode was then dipped in a solution of adriamycin (5 µg/mL) for varying time intervals. The electrode was then taken out of adriamycin solution, washed with distilled water and allowed to dry. The dried electrode was dipped in a solution of acetate buffer (0.1 M) of pH 4.5±0.1 and the voltammograms were recorded.

**Thin layer ds-DNA modified GCFE**

A thin layer of ds-DNA on the GCFE surface was mounted by immersing the GCFE in 80 µg/mL ds-DNA solution at +0.40 V applied potential for 10 min. This ds-DNA modified GCFE was dipped in adriamycin solution for 3 min, taken out of the solution and rinsed with water and transferred to a polarographic cell containing acetate buffer solution of pH 4.5±0.1 and the differential pulse voltammogram was recorded.

**Results and Discussion**

**Adriamycin analysis at bare GCFE**

When a solution of adriamycin (5 µg/mL) in a 0.1 M acetate buffer of pH 4.5±0.1, was electrolysed using bare GCFE as working electrode and scanning the potential in cathodic direction, it produced two reduction peaks (Fig. 1) at -0.45 V and -0.60 V due to the reduction of 5,12-diquinone groups to produce a highly reactive semiquinone radical\(^{16}\). However, if the solution is electrolysed performing positive potential scanning of the working electrode, the resulting differential pulse voltammogram produced a well defined peak (Fig. 2) at +0.56 V, due to the oxidation of 6,11-dihydroquinone of adriamycin\(^{17}\).

**Adriamycin-DNA interaction at bare GCFE**

Looking at the possibility of oxidation/reduction of adriamycin at the GCFE, attempts have been made to study the possible interaction of the anticancer drug with ds-DNA. For the said purpose experimental sets of solutions were prepared by taking a fixed concentration of adriamycin (20 µg/mL) in 0.1 M acetate buffer of pH 4.5±0.1, and varying the concentration of DNA from 20 to 200 µg/mL. The oxidation of adriamycin was investigated for each set. The first set i.e. without DNA, produced a DPV adriamycin oxidation peak at +0.56 V, which shifted to more electro-positive potential with increasing DNA concentration and the peak current shortened. The shift in \(E_p\) value and shortening of peak current may be explained on the basis of change of species that is oxidized at the GCFE surface, i.e. due to the formation of drug-DNA complex\(^{18}\).
Although, the above findings approve the formation of Adriamycin-DNA complex, but to have a clear-cut understanding on the mechanism of the drug-DNA interaction at charged surfaces, the GCFE has been modified in three different ways:

**Adriamycin adsorbed GCFE**

The voltammogram at adriamycin adsorbed GCFE clearly shows a big peak at +0.50 V due to oxidation of adsorbed adriamycin and the other peaks may be due to oxidation of purine bases of DNA. It is based on the presumption that DNA diffuses from bulk of the solution on to electrode surface and the chemisorbed adriamycin intercalated into the double helix of DNA. As such, the distortion of double strand takes place, which allows the oxidation of purine bases.

Besides, if after the first scan, a potential of -0.60 V was applied for 60 s, and then the voltammogram was recorded (Fig. 3), it produced a peak at +0.40 V. The appearance of this peak is due to the interaction of adriamycin in ds-DNA at guanine rich region.

**Thick layer ds-DNA modified GCFE**

Adriamycin thick layer ds-DNA modified GCFE produced a well-defined voltammetric oxidation peak with $E_p$ value +0.50 V. The height of the adriamycin oxidation peak with respect to the time of immersion of the thick layer ds DNA modified GCFE in adriamycin solution was investigated (Fig. 4). The results showed a linear relationship between the peak height and time of immersion of the electrode in adriamycin solution i.e. 0.00 to 60 min, and then it attained a constant value. Thus, indicating the preconcentration of adriamycin at the thick layer ds-DNA modified electrode surface, which was not possible with bare electrode.

Significantly reproducible peak currents were observed for the similar time of immersion of the thick layer ds-DNA modified GCFE in adriamycin solution for the first scan only. If the differential pulse voltammogram is recorded using the same modified electrode, an abrupt decrease in the peak current is observed. This suggests a fast consumption of the neoplastic drug at the modified electrode surface.

However, if the voltammograms are recorded separately using bare GCFE and thick layer ds-DNA modified GCFE and scanning the potential from -0.70 to -0.00 V, the bare GCFE produced only one peak at -0.56 V. Whereas, at thick layer ds-DNA modified GCFE two peaks (Fig. 5) were observed at -0.60 and -0.70 V.

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Fig. 3—Differential pulse voltammogram for 80 µg/mL ds-DNA solution in 0.1 M acetate buffer at pH 4.5 ± 0.1, after applying a potential of -0.60 V during 60 s at adriamycin modified GCFE, pulse amplitude -50 mV, scan rate 12 mVs$^{-1}$, current range 10 µA.

Fig. 4—Differential pulse voltammogram in 0.1 M acetate buffer at pH 4.5 ± 0.1, obtained with time of immersion of the thick layer ds-DNA modified GCFE in 20 µg/mL adriamycin solution during 15-60 min, pulse amplitude -50 mV, scan rate 12 mVs$^{-1}$, current range 10 µA.

Fig. 5—Differential pulse voltammogram in 0.1 M acetate buffer at pH 4.5 ± 0.1, obtained with a thick layer ds-DNA modified GCFE after being immersed in 20 µg/mL Adriamycin solution during 180 s with applying negative potential from -0.70 to -0.00 V during 60 s, pulse amplitude -50 mV, scan rate 12 mVs$^{-1}$, current range 10 µA.
-0.45 V respectively. The observed new peak at -0.45 V indicates a different interaction mechanism of adriamycin-DNA, at the modified GCFE surface.

Since, adriamycin is irreversibly adsorbed at the bare GCFE surface, it becomes necessary to clean the electrodes each time before use whereas, the thick layer ds-DNA modified GCFE did not require cleaning of the GCFE. This clearly reveals that all the adriamycin is intercalated inside the ds-DNA film and could not reach the electrode surface. As such, it could be concluded that the voltammetric peaks, are observed due to adriamycin which was intercalated into thick layer of ds-DNA. Since, the voltammograms were recorded in the acetate buffer supporting electrolyte solution only, the possibility of any contribution to the voltammetric peaks from adriamycin present in solution is ruled out. As such, a critical comparison of Fig. 2 and Fig. 5, the observed new peak at -0.45 V in Fig. 5 may be attributed to the adriamycin-guanine site (in DNA) interaction leading to a charge transfer reaction to from adriamycin semiquinone and guanine radical cation. However, as regards to the peak at -0.60 V (Fig. 5) it may be attributed to the reduction of the adriamycin. Reduction of adriamycin at bare GCFE produces a peak at -0.56 V the shift in the peak potential for the adriamycin reduction at the two different electrode surfaces may be explained on the basis of the change in the electrode surfaces.

However, when the ds-DNA modified GCFE after being dipped in adriamycin for 10 min, rinsed and immersed in a buffer of pH 4.5±0.1, was subjected to a potential of -0.60 V for about 60 s and then the voltammogram was recorded by positive scanning of the modified electrode potential, the voltammogram produced two new peaks (Fig. 6), one at +0.80 V and other at +1.1 V. The former peak may be due to guanine oxidation and the later due to adenine oxidation.

**Thin layer ds-DNA modified**

Differential pulse voltammogram at thin layer ds-DNA modified GCFE (Fig. 7) shows a well defined peak with peak potential of +0.50 V. The peak may be attributed to the oxidation of 6,11-dihydroquinone group of the adriamycin molecule.

However, after recording the oxidation peak, a negative potential of -0.60 V was applied on the modified electrode for 60 s followed by positive potential scanning. The voltammogram recorded now showed two new peaks in addition to the adriamycin oxidation peak (Fig. 8). The peak at +0.90 V may be attributed to 8-oxo-G oxidation. A clear separation of the peak due to 8-oxo-G and adriamycin can be explained on the basis of non-uniform coverage of the GCFE surface by DNA and adsorption of adriamycin at these uncovered surfaces. The results are in good agreement with those observed using thick layer DNA modified GCFE. This shift of 8-oxo-G peak to less positive potential informs about the DNA-adriamycin interaction (damage to DNA).

**Mechanism**

Adriamycin generates a free radical by way of transfer of an electron to its quinone portion. The highly reactive adriamycin radical formed at -0.60 V may

![Fig. 6—Differential pulse voltammogram in 0.1 M acetate buffer at pH 4.5±0.1, obtained with a thick later ds-DNA modified GCFE after being immersed in a 20 µg/mL adriamycin solution for 60 s at potential -0.60 V, pulse amplitude -50 mV, scan rate 12 mVs⁻¹, current range 10 µA.](image6)

![Fig. 7—Differential pulse voltammogram in 0.1 M acetate buffer at pH 4.5±0.1, obtained with a thin layer ds-DNA modified GCFE after being immersed in 20 µg/mL adriamycin solution during 180 s, pulse amplitude -50 mV, scan rate 12 mVs⁻¹, current range 10 µA.](image7)
oxidise the guanine site of ds-DNA in which it is intercalated within double helix. This argument is based on the results obtained by other methods\textsuperscript{1,20}. In addition to this, peak at +0.56 V as observed in case of pure Adriamycin oxidation, at bare GCFE shifts to less positive side i.e. +0.45 V, which may be explained on the basis of interaction between Adriamycin and 8-oxo-G which is formed as a result of interaction of Adriamycin in guanine rich region of ds-DNA. As such, one electron transfer from guanine moiety to quinine leading to guanine cation formation appears to be the probable reaction. However, due to the tendency of guanine cation to undergo hydrolysis, and finally the semiquinone is further reduced to form adriamycin, and 8-oxo-G is formed.

**Conclusion**

Voltammetric in-situ sensing of DNA oxidative damage caused by reduced Adriamycin intercalated into DNA is possible using ds-DNA modified GCFE. The results show that Adriamycin intercalated in double helix DNA can undergo oxidation or reduction and react specifically with the guanine moiety and thus form mutagenic 8-oxo-G residue. A mechanism model for the reaction may be proposed.

The developed biosensor is of utmost relevance because the mechanism of interaction of DNA-Adriamycin at charged interfaces is parallel to in-vivo DNA-drug (Adriamycin) complex reaction, where DNA is in close contact with charged phospholipid membranes and proteins rather than when intercalation is in solution. It also promises the use of voltammetric techniques for in-situ generation of reaction intermediates. As such, it is a complementary tool for the study of biomolecular interaction mechanism.

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