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Modified screen-printed electrodes for the investigation of the interaction of non-electroactive quinazoline derivatives with DNA

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Abstract Five morpholino-quinazoline derivatives have been investigated voltammetrically using a competition with the tris(o-phenanthroline) cobalt(III) redox marker for the accumulation at dsDNA modified screen-printed electrodes. An association of quinazolines with DNA was observed at the modified electrodes polarized by the negative potential of –0.4 V vs. Ag/AgCl. This was confirmed by a potentiometric stripping analysis based on the DNA guanine signal. Calibration curves for quinazolines within a concentration range of μmol/L were obtained with DP voltammetry using 5×10^{-7} mol/L $Co(phen)_3^{3+}$ marker. The quinazolines exhibit no effect on the DNA complex with the fluorescent thiazole orange derivative TO-PRO-3. The role of the accumulation potential in the association interaction with DNA is discussed.

Abbreviations DMSO dimethylsulfoxide · DNA deoxyribunucleic acid · DP differential pulse · ds double stranded · DNA/SPE screen-printed electrode modified by DNA · PSA potentiometric stripping analysis · RSD relative standard deviation · SCE saturated calomel electrode · SPE screen-printed electrode

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Introduction

Screen-printing technology represents a route for the simple mass fabrication of solid-state electrochemical transducers. They can be chemically modified by immobilization of the discriminator (biocomponent) within the carbon ink or on the surface of the electrode strip. Screen-printed electrodes have been suggested as inexpensive disposable sensors for decentralized testing or field monitoring of a variety of analytes [1, 2, 3, 4].

The well-known and wide-spread group of quinazoline derivatives [5] is used with respect to their large biological activity in the pharmaceutical industry, medicine and agriculture. Many derivatives act as anticancer active agents [6]. Therefore, a study of the interaction of quinazolines with DNA and simple methods of screening are of interest.

This paper deals with an interaction of some quinazoline derivatives with double stranded (ds) DNA using surface-based and solution methods. For the surface-based study, DNA modified screen-printed electrodes were utilized. Electrochemically inactive quinazolines have been detected voltammetrically with the tris(1,10-phenanthroline)cobalt(III) complex as DNA redox indicator and chronopotentiometrically using the DNA guanine moiety signal. The results are compared with fluorimetric and biological tests. The investigation of the activity of quinazolines towards DNA and the development of analytical procedures for the determination of quinazolines at the DNA biosensors were aims of the study.

Experimental

Apparatus and reagents

A computerized voltammetric analyzer ECA pol, model 110 (Istran, Bratislava) was used for the voltammetric measurement in connection with a screen-printed bielectrode system (FACH, Prešov) including a working electrode and silver/silver chloride reference electrode and a separated platinum auxiliary electrode. Chronopotentiometric measurements were performed with an AUTOLAB

PGSTAT 10 electrochemical analysis system with GPES 4 and GPESSIX software package (Eco Chemie, Utrecht). Here, a screen-printed working electrode, saturated calomel reference electrode and graphite auxiliary electrode were used. The fiber fluorimeter (Royal Veterinary and Agricultural University, Copenhagen) with a 642 nm light emitting laser diode was utilized for optical measurements and the signal output was read using a standard voltmeter.

For DP voltammetry, screen printed electrodes were obtained using a graphite-based ink, Ag/AgCl paste and an insulating paste. A working area of the bare strip was modified by application of the acetate cellulose-based polymer solution containing 0.5 mg/mL dsDNA. For chronopotentiometric measurements, the bare electrodes were prepared using graphite, silver and insulating inks as described previously [7]. They were modified prior to analysis as described in Procedures.

The quinazoline derivatives were prepared as described elsewhere [8, 9] and used as received. The DNA redox marker $[Co(phen)_3](ClO_4)_3$ was synthesized in our laboratory according to literature [10]. Calf thymus DNA was obtained from Merck and used as received. The salmon sperm DNA was from Sigma-Aldrich and purified as described previously [11]. The TO-PRO-3 dye (a monomeric thiazole orange derivative) was obtained from Molecular Probes (Eugene, Oregon).

Stock solutions of 1×10^{-3} mol/L quinazoline derivatives were prepared in 100% DMSO. The redox indicator stock solution of 1×10^{-3} mol/L was prepared in water. The stock solutions of DNA (5 mg/mL calf thymus DNA and 250 μg/mL salmon sperm DNA) were prepared with a Tris-EDTA solution pH 8.0 (1×10^{-2} mol/L Tris-HCl and 1×10^{-3} mol/L EDTA) and stored at –5 °C. The TO-PRO-3 stock solution was prepared as 1×10^{-4} mol/L in 10% DMSO and stored at –20 °C. All other chemicals were of analytical-reagent grade purity. Deionized, doubly distilled water was used throughout. The experiments were carried out at the laboratory temperature (23 °C).

Procedures

For voltammetric measurement, the electrodes containing DNA immobilized within the cellulose acetate film were utilized. 5×10^{-7} mol/L $Co(phen)_3^{3+}$ was accumulated from its solution or the mixture with quinazoline (in 5 mmol/L phosphate buffer pH 7.0 containing 0.8% DMSO) at the potential +0.600 V for 120 s under stirring. The positive accumulation potential was used similarly to [12, 13]. Then, the DP voltammogram was recorded in the sample solution from –0.400 V to +0.600 V using 100 mV pulse amplitude and 25 mV s^{-1} scan rate. After the subtraction of the curve corresponding to the blank, the DP peak current was evaluated using the standard software.

Another electrode was used for chronopotentiometric measurements. The unmodified SPE was first activated for 360 s at 1.6 V and 60 s at 1.8 V [14, 15], then DNA was fixed onto the electrode surface from its stirred solution (20 μg/mL) in 0.25 mol/L acetate buffer pH 5.0 at 0.5 V vs. SCE for 120 s [7, 15]. Then, the analyte was accumulated from stirred 0.25 mol/L acetate buffer containing 0.8% DMSO at –0.4 V for 120 s. After medium exchange for the acetate buffer blank solution, the potentiometric stripping analysis was performed with a constant current of 6 μA and an initial potential of 0.500 V. The derivative signal was evaluated using the baseline correction.

Fluorimetric assay was performed as described previously [16].

Results and discussion

Two types of the quinazoline compounds have been investigated: one tetrazolo-derivative and four 4-aniline-derivatives (Fig. 1). All of them are non-electroactive within the usual range of potential and the voltammetric investigation of their interaction with dsDNA is based on competitive binding with the DNA electrochemical label $Co(phen)_3^{3+}$. Therefore, the voltammetric behavior of this marker at the screen-printed electrode (SPE) modified with DNA has been investigated. The nature of interaction between DNA used and $Co(phen)_3^{3+}$, the adduct formation constant and binding site size were studied in our previous paper [17]. The modified electrode contains ds-DNA entrapped in a cellulose acetate-based film on the surface of a carbon based SPE. Various carbon materials and polymeric films were tested for this purpose and their composition was optimized.

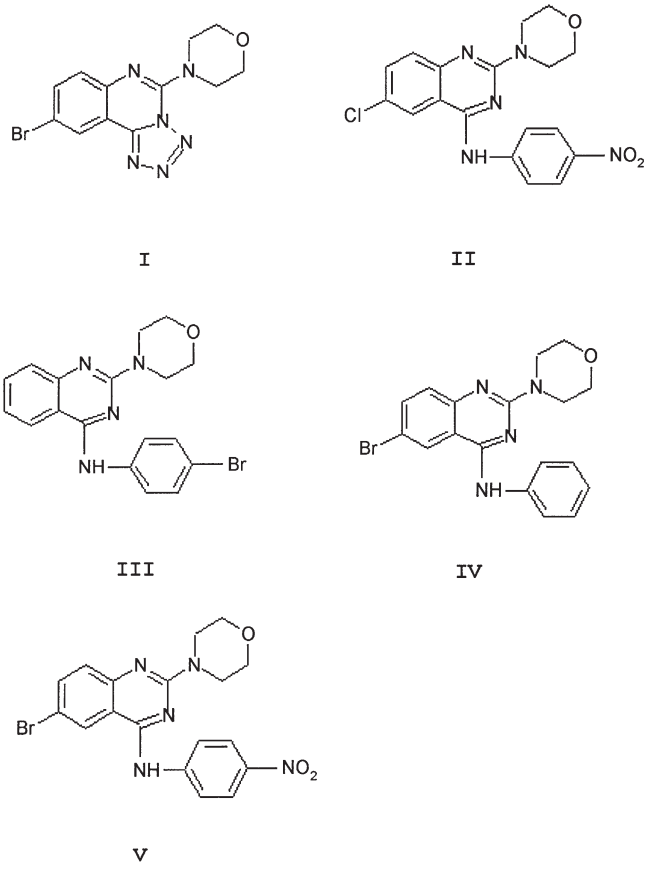

Fig. 1 Structure of quinazoline compounds: *I* 9-bromo-5-morpholino-tetrazolo[1,5-c]quinazoline; *II* 6-chloro-2-morpholino-4-(4'-nitroanilino)quinazoline; *III* 2-morpholino-4-(4'-bromoanilino)quinazoline; *IV* 6-bromo-2-morpholino-4-anilinoquinazoline; *V* 6-bromo-2-morpholino-4-(4'-nitroanilino)quinazoline

Typical DP voltammograms of the $Co(phen)_3^{3+}$ complex obtained at the SPEs without and with DNA are shown in Fig. 2. Practically the same picture (curve 1) was obtained at the bare SPE and that covered by cellulose acetate without DNA. Other experimental conditions were the same for all electrodes, i.e. no electrode was activated at the conventional high positive potential [7, 14, 15]. The positive potential was chosen for the accumulation of $Co(phen)_3^{3+}$ according to [12, 13] although the marker in the unchanged redox state can be effectively attached to DNA also at open circuit [18]. The anodic scan was used with respect to a relatively strong competitive effect of

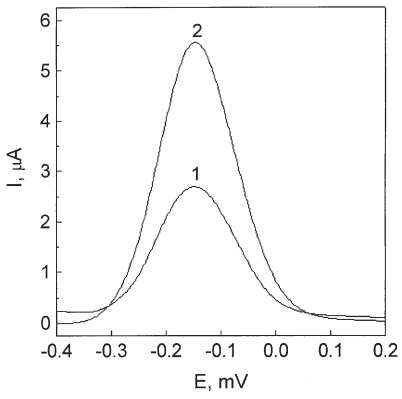

Fig. 2 DP voltammograms of the $Co(phen)_3^{3+}$ complex in 5×10^{-7} mol/L solution obtained at the SPE without (*1*) and with DNA (*2*) after 120 s accumulation at +0.600 V vs. Ag/AgCl, pulse amplitude 100 mV, scan rate 25 mV s^{-1}, 0.005 mol/L phosphate buffer solution of pH 7, 0.8% DMSO

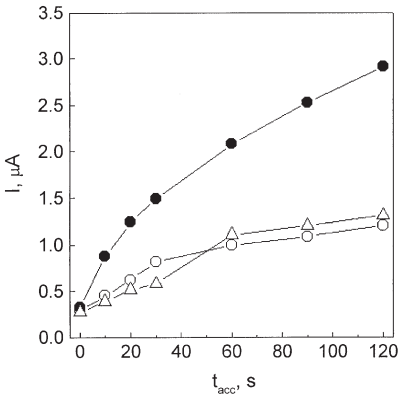

Fig. 3 Accumulation time dependence of 5×10^{-7} mol/L $Co(phen)_3^{3+}$ obtained with three types of working electrodes: a bare strip (△), an SPE covered by the cellulose acetate polymeric film (○) and an SPE covered by the polymer containing DNA (●). Accumulation at +0.600 V vs. Ag/AgCl, scan range from +0.600 V to –0.400 V, other conditions as in Fig. 2

quinazolines under these conditions (see below). A preconcentration effect of the DNA modifier can be seen.

A potential shift of the guest molecule is usually taken as a confirmation of its binding mode. According to published data the interaction between DNA and $Co(phen)_3^{3+}$ at the ionic strength used is either dominantly electrostatic [18] or electrostatic with a significant contribution of the intercalation as it can be deduced from a small change in the marker peak potential obtained with our reagents and conditions [17]. Considering the electrostatic attachment, $Co(phen)_3^{3+}$ has to be bound more strongly than its reduced form $Co(phen)_3^{2+}$ existing at –0.400 V. In fact, the DP peak height for the indicator at the DNA/SPE using the scan from –0.400 V to 0.600 V represents about 77% of that measured at the scan from 0.600 V to –0.400 V.

A positive shift of the peak potential value from –0.150 V at the SPE to –0.148 V at the DNA/SPE together with smaller peak width at the half peak height at the DNA/SPE (0.162 V) compared to SPE (0.172 V) was observed. The small potential shift indicates an equality of the electrostatic and intercalative contributions at the interaction of the complex particle with DNA/SPE. In the case of dominantly eletrostatic binding, a strong negative potential shift could be expected due to the difference in the association constants of $Co(phen)_3^{3+}$ and $Co(phen)_3^{2+}$ with DNA [17, 18]. However, the simple physical adsorption of the marker on the electrode surface may also contribute to its potential characteristics. The strip-to-strip reproducibility for the current signal of 5×10^{-7} mol/L $Co(phen)_3^{3+}$ is characterized by an RSD of 5.6% (n = 10).

A contribution of the diffusion of the marker from solution to the total response of the DNA/SPE has also been investigated. In Fig. 3 the accumulation time dependences of the indicator are shown, obtained with three types of working electrodes: a bare strip, a SPE covered by the cellulose acetate polymeric film and a SPE covered by the polymer with entrapped DNA. The differences between the marker signals at the first two electrodes are within experimental errors. The marker accumulation occurring on the electrodes without DNA is evidently due to its adsorption on the carbon strip and not due to an interaction with the polymeric film. Thus the diffusion current of $Co(phen)_3^{3+}$ plays a minor role for the accumulation time of 120 s.

In order to evaluate only the $Co(phen)_3^{3+}$ accumulated at DNA, the total DP peak current of the indicator obtained at the DNA/SPE has been corrected to the signal of the electrode covered with cellulose acetate without DNA by the subtraction of the last one

$$I_0 = I_{0,\,DNA/SPE} - I_{0,SPE}$$

A linear calibration curve (r = 0.9967) within the range 1×10^{-7} to 5×10^{-6} mol/L $Co(phen)_3^{3+}$ was obtained at DNA/SPE and 120 s accumulation.

Using the cellulose acetate covered SPE the marker signal is not affected by the presence of a 10-fold molar excess of quinazolines. However, at the DNA/SPE the indicator peak height decreases in the presence of quinazolines, evidently due to their competitive association with DNA. A content of 0.8% DMSO in the test solution is necessary to ensure the solubility of the quinazolines. The effect of quinazolines depends on the accumulation potential and the potential scan range. Table 1 presents the relative current results obtained with two quinazoline derivatives. The peak current of the marker in the presence of quinazoline obtained at the DNA/SPE ($I_{DNA/SPE}$) was corrected to the signal measured at the electrode without DNA (I_{SPE}) as follows:

$$I = I_{DNA/SPE} - I_{SPE}$$

A strong competing effect of quinazolines on the accumulation within dsDNA was found using the anodic DP scan. The same behavior was observed at a DNA modified carbon paste electrode. According to their structure, the quinazoline derivatives can interact with DNA by the intercalation and displace the marker bound by this mode. With respect to the lower amount of the marker attached

Table 1 Effect of accumulation potential and scan range on the relative response of 5×10^{-7} mol/L $Co(phen)_3^{3+}$ in a mixture with 8×10^{-6} mol/L quinazoline derivative

E_{acc}, mV	ΔE, mV	I/I_0
9-Bromo-5-morpholino-tetrazolo[1,5-c]quinazoline		
+600	+600 → –400	1.006
+600	–400 → +600	0.425
–400	–400 → +600	0.578
6-Bromo-2-morpholino-4-(4'-nitroanilino)quinazoline		
+600	+600 → –400	1.023
+600	–400 → +600	0.867
–400	–400 → +600	0.918

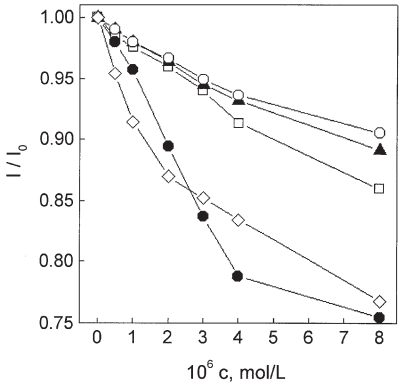

Fig. 4 Calibration curves for quinazoline derivatives in the presence of 5×10^{-7} mol/L $Co(phen)_3^{3+}$ marker measured at the DNA/SPE: 9-bromo-5-morpholino-tetrazolo[1,5-c]quinazoline (□), 6-chloro-2-morpholino-4-(4'-nitroanilino)quinazoline (○), 2-morpholino-4-(4'-bromoanilino)quinazoline (▲), 6-bromo-2-morpholino-4-anilinoquinazoline (●) and 6-bromo-2-morpholino-4-(4'-nitroanilino)quinazoline (◇). Conditions as in Fig. 3

electrostatically at –0.400 V than at 0.500 V (see above), the portion of the total marker displaced by binding of quinazolines at the negative potential is higher (the current of the marker is lower) than that at the positive potential. Calibration curves for quinazoline derivatives in the presence of 5×10^{-7} mol/L $Co(phen)_3^{3+}$ marker are depicted in Fig. 4. The curves can be used for the voltammetric determination of quinazolines in a concentration range from 5×10^{-7} to 4×10^{-6} mol/L. The detection limit (6σ) was estimated to 2×10^{-7} mol/L.

To validate the assay, chronopotentiometric measurements have been performed utilizing the DNA/SPEs prepared by the DNA fixation immediately before the analysis. The conventional procedure based on the adsorption of DNA from its solution was used for the electrode modification. Using the simple procedure described in Experimental, electrodes with an irreversible adsorbed portion of ssDNA as well as dsDNA were obtained [7, 12, 13, 14, 15, 19, 20]. The amount of DNA and the stability of its adsorbed layer can be enhanced by more sophisticated procedures [21, 22]. With our electrode a typical guanine-residue PSA peak [15, 19] was observed at about 1.04 V vs. SCE. The treatment of the DNA/SPE at 0.500 V or –0.400 V in acetate buffer blank solution (a simulation of

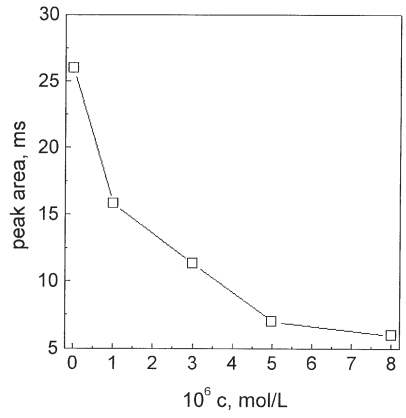

Fig. 5 Calibration plot of the PSA signal for 9-bromo-5-morpholino-tetrazolo[1,5-c]quinazoline. Conditions: 120 s accumulation at –0.400 V vs. SCE in 0.25 mol/L acetate buffer pH 5 and 0.8% DMSO, measurement in pure acetate buffer using 6 μA constant current and initial potential of 0.500 V

the accumulation step) leads to similar PSA peaks, however, with an increased background current in the later case. The accumulation of 9-bromo-5-morpholino-tetrazolo[1,5-c]quinazoline on the DNA/SPE at 0.500 V for 120 s followed by the medium exchange does not change the guanine-residue signal. On the other hand, the negative accumulation potential of –0.400 V causes a decrease of the PSA signal together with a small shift in the background current to less positive potential values. These changes depend on the concentration of the quinazoline derivative. In Fig. 5 the plot of PSA signal vs. quinazoline concentration is shown. It can be used for the determination of the analyte within the μmol/L concentration range.

A fluorimetric investigation has also been carried out using the long wave thiazole orange dye TO-PRO-3 [23] and the purified salmon sperm DNA. The measurement in optical fiber is based on a release of the dye from the TO-PRO-3:DNA complex in the presence of analyte. No effect of the quinazolines has been observed within the concentration range of 10^{-7} to 10^{-5} mol/L. Similar results were obtained in the cell cycle analysis by flow cytometry. The 4-aniline-quinazoline derivatives caused no change in cell cycles of L1210 line cells [24]. However, the 9-bromo-5-morpholino-tetrazolo[1,5-c]quinazoline induces DNA single stranded breaks in V79 cells [25].

From our investigations it can be deduced that the quinazoline derivatives under study bind to dsDNA when applying the negative accumulation potential. Generally, the potential may affect not only a guest particle (e.g. its redox state and charge) but also the structure of the host DNA. To test this point, experiments have been performed with the DNA/SPEs pretreated at various potentials in blank supporting electrolyte. Then the $Co(phen)_3^{3+}$ marker was accumulated for 120 s at an open circuit and the DP voltammogram was recorded with the scan from negative to positive potential values. The signal increases significantly with the negative potential used for the electrode pretreatment. Hence, an influence of the potential on the binding ability of DNA itself can be expected.

Conclusion

The DNA modified electrodes represent suitable tools for the detection of an interaction of analytes with DNA. The analyte-DNA association can be significantly forced by the potential value. For the determination of traces of non-electroactive molecules, the signals of both the DNA redox marker and the DNA-guanine residue can be used. Electrochemical studies can provide valuable additional information for the investigation by conventional biological and optical methods.

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