Electrochemical uranyl biosensor with DNA oligonucleotides as receptor layer

Robert Ziółkowski · Łukasz Górski · Sławomir Oszwaldowski · Elżbieta Malinowska

Received: 6 July 2011 / Revised: 6 September 2011 / Accepted: 17 October 2011 / Published online: 8 November 2011 © The Author(s) 2011. This article is published with open access at Springerlink.com

Abstract The feasibility of using gold electrodes modified with short-chain ssDNA oligonucleotides for determination of uranyl cation is examined. Interaction between UO$_2$$^{2+}$ and proposed recognition layer was studied by means of voltammetric and quartz crystal microbalance measurements. It was postulated that ssDNA recognition layer functions via strong binding of UO$_2$$^{2+}$ to phosphate DNA backbone. The methylene blue was used as a redox marker for analytical signal generation. Biosensor response was based on the difference in electrochemical signal before and after subjecting it to sample containing uranyl ion. The lower detection limit of 30 nmol L$^{-1}$ for UO$_2$$^{2+}$ was observed for a sample incubation time of 60 min. Proposed ssDNA-modified electrodes demonstrated good selectivity towards UO$_2$$^{2+}$ against common metal cations, with only Pb$^{2+}$ and Ca$^{2+}$ showing considerable interfering effect.

Keywords Biosensors · Electroanalytical methods · Electrochemical sensors · Mass sensitive sensors · Stripping analysis

Introduction

Uranium and its compounds are used as nuclear power plants fuel, for the production of tanks armor and armor-piercing ammunition, for staining of ceramic products, as well as for electron microscopy investigations of biological samples [1]. All of these applications can possibly be hazardous to human health or environment. That is why rapid and accurate methods allowing for detection of trace levels of uranyl ion in environmental, geochemical or clinical samples are indispensable.

Uranium occurs mainly in two valence states, U$^{4+}$ and U$^{6+}$, with the latter forming water-soluble uranyl ion (UO$_2$$^{2+}$) compounds. The uranium species in aqueous samples were determined using a variety of physical and chemical techniques, with radiospectrometry [2], inductively coupled plasma mass spectrometry [3] and complexometric titration [4] being the most popular. However, all of these techniques require utilization of costly and complicated apparatus. On the other hand, electroanalytical techniques are very useful due to their operation simplicity, low utilization costs, and the possibility of achieving extremely low detection limits.

The most commonly used electrochemical method for determination of uranyl ion at low levels is adsorptive stripping voltammetry [5–7]. The preconcentration and subsequent analysis allows obtaining detection limit of 2·10$^{-9}$ mol L$^{-1}$ [5]. To further improve the selectivity and the lower detection limit, a range of complexing reagents was introduced. These ligands form complexes with uranyl cation with increased adherence to the electrode surface. Some of the compounds used for this purpose are: 2-thenoyltrifluoroacetone-tributylphosphine oxide (detection limit, 10$^{-10}$ mol L$^{-1}$) [4], cupferron (detection limit, 3.7·10$^{-10}$ mol L$^{-1}$) [8], or 2,6-pyridinedicarboxylic acid (detection limit, 0.27·10$^{-9}$ mol L$^{-1}$) [9].

Published in the special issue Analytical and Bioanalytical Science in Poland with guest editor Marek Biziuk.

R. Ziółkowski · L. Górski (✉) · E. Malinowska
Institute of Biotechnology, Department of Microbioanalytics, Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warsaw, Poland
e-mail: lukegor@ch.pw.edu.pl

S. Oszwaldowski
Department of Analytical Chemistry, Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warsaw, Poland
It has also been shown that uranyl cation has a strong affinity to phosphate residues [10]. This mechanism was used in various electrochemical sensors for uranyl ion determination with voltammetric or potentiometric transduction. In the case of voltammetric sensors, the recognition layer is deposited on the electrode. Some of the compounds used for such modification are as follows: 2-mercaptoethanol/POCl₃, cysteamine/2-aminoethyldihydrogenphosphate, or (t-butylphenyl)-N,N-di-(isobutyl)carbamoylmethylphosphineoxide [11–13]. The uranyl ion detection limit for the last of abovementioned sensors is at parts per million level. Nevertheless, the analytical procedure in this case was very complicated. The same interaction was also used for the development of potentiometric sensors. PVC membranes containing bis{d[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate} or bis(dicarboxylphosphate) salt as the electroactive components gave near-Nernstian uranyl calibration slopes and selectivity with selectivity coefficients \( K_{UO_2^{2+},Y}^i \) ranging from \( 9 \times 10^{-3} \) to \( 9 \times 10^{-4} \) [14]. It should be also mentioned that \( UO_2^{2+} - \) phosphate interaction was utilized for detection of phosphate ions using electrodes with polymeric membranes containing various uranyl salophene complexes as ionophores [15].

Since the early 1990s, nucleic acids were employed in detection of different chemical compounds [16, 17]. This is possible due to discovery of catalytic and regulatory activities of nucleic acids, which opened completely new fields of their applications. These nucleic acid molecules are called functional nucleic acids—a term that covers DNA and RNA sequences which can act as enzymes, recognition elements, or molecular switches. Ribozymes, microRNAs, or riboswitches are naturally occurring functional RNA oligonucleotides. Nevertheless, there is also a broad range of artificial oligonucleotides representing similar properties. Depending on their acting mechanism, they are called aptamers, deoxyribozymes (DNAzymes), or allosteric nucleic acid enzymes. Such receptors were used for a detection of whole cells (e.g., *Escherichia coli*), small and large peptides (e.g., thrombin and IgE) as well as different organic and inorganic compounds (e.g., adenosine, cocaine, AMP, neomycin, K⁺, UO₂^{2+}, and Hg^{2+}). Electrochemical and optical measurements are the most common detection techniques utilized in combination with abovementioned receptor molecules [18].

In recent years, assays dedicated to uranyl ion detection employing DNAzymes were developed [19, 20]. The reported detection limit (1 nmol L⁻¹) was below the uranium ion concentration level in marine waters. Moreover, the detection range was 1–700 nmol L⁻¹ (in the case of label-free sensor) or 50 nmol L⁻¹–2 μmol L⁻¹ (for labeled sensor). Nevertheless, as the mechanism of the detection is based on the cleavage of oligonucleotide strand and subsequent disassembly of AuNP aggregates at certain temperature, the whole system is sensitive to temperature and ionic strength changes. Additionally, the results also can be influenced by the changes in liquid turbidity [20].

Herein, we report that recognition layer of short DNA oligonucleotides, formed on gold electrode, can be useful for electrochemical uranyl ion detection. Uranyl cation interacts with phosphate DNA backbone, changing the electrostatic balance at the electrode surface [21]. These changes can be directly quantified with the use of redox marker. It is shown that this approach allows for the determination of uranyl ion at the trace level.

**Experimental**

**Apparatus**

Electrochemical measurements were conducted with a CHI 660A electrochemical workstation (CH Instruments, USA). Voltammetric experiments were carried out with a three-electrode system consisting of gold disk working electrode (CH Instruments, USA), a gold wire auxiliary electrode and an Ag/AgCl/1.0 mol L⁻¹ KCl reference electrode (Mineral, Poland). All potentials are reported versus Ag/AgCl reference electrode at room temperature. To restore the gold electrode surface, the electrode polishing kit with alumina powder of different grain sizes was used. The electrochemical solutions were deoxygenated with argon for approximately 15 min prior to data acquisition and were blanketed under an argon atmosphere during the entire experimental period. If not stated otherwise, the cyclic voltammetry (CV) was conducted at a sweep rate of 100 mV s⁻¹ while the square wave voltammetry was conducted at a pulse amplitude of 50 mV, increment of 1 mV, and a frequency of 10 Hz.

The quartz crystal microbalance with energy dissipation (QCM-D) measurements were conducted with Q-Sense E4 instrument and the AT-cut quartz crystal with a fundamental resonant frequency of 4.95 MHz and crystal constant of 17.7 ng/cm² Hz⁻¹ (Q-Sense, Sweden). The effective area of gold sensor exposed to solution is 0.78 cm² with a roughness of less than 3 nm. Such an assay allows to measure the change in frequency and energy dissipation which, with the use of a Voigt-based representation, can be converted into the mass deposited on the gold surface.

For capillary electrophoresis experiments, system with ultraviolet–visible (UV–Vis) detector (Prince Technologies, the Netherlands) and a fused-silica capillary (50 μm (i.d.), 375 μm (o.d.), x cm long, y cm effective length, \( x/y = 82/65 \); Composite Metal, England) was applied. Prior to the daily use, the capillary was pretreated by flushing sequentially for 15 min with 0.1 mol L⁻¹ NaOH, 5 min with water, and 5 min with run buffer. The capillary was also rinsed with...
DNA recognition monolayer was prepared as described in [22]. Briefly, after the electrode cleaning, the 4 μmol L⁻¹ solution of thiolated ssDNA in 1 mol L⁻¹ KH₂PO₄ (pH 4.5) was dropped on the gold working disk electrode. The ssDNA immobilization was carried out for 120 min. Then the solution was removed and the electrode was washed with 1 mol L⁻¹ KH₂PO₄ (pH 4.5), and electrochemical experiments were performed. No diluent thiols (e.g., mercaptohexanol) were used for ssDNA monolayer preparation.

All QCM-D experiments dealing with immobilization and interactions of DNA with uranyl acetate were conducted using the same buffer solutions as in the case of electrochemical assays. Nevertheless, to clean the gold transducers applied in gravimetric experiments, the QCM sensors were subjected to base piranha solution treatment at 70 °C for 15 min. The sensor was washed with abundant amount of water, absolute ethanol, and then dried under argon atmosphere before placing in flow cell of QCM-D instrument, where the ssDNA immobilization process as well as subsequent experiments was carried out. The medium flow was set at 0.2 mL min⁻¹ [23].

Results and discussion

Preliminary experiments

In all experiments conducted in this study, a random 20-base probe sequence was used. No significant effects of DNA sequence are expected, as UO₂²⁺ cation interacts with DNA strand via phosphate groups, identical for all DNA bases.

To evaluate the existence of DNA–uranyl cation interactions, the QCM-D measurements were performed. After the gold transducer cleaning, it was placed in quartz crystal microbalance chamber and the analysis was started. At first (step 1, Fig. 1), the immobilization buffer was passed through the chamber until the baseline frequency was constant. Then (step 2, Fig. 1), the transducer was subjected to solution of 4 μmol L⁻¹ DNA oligonucleotide in immobilization buffer. The frequency dropped dramatically which corresponds to the single-stranded DNA immobilization on the transducer surface. During step 3 of QCM-D experiments, the immobilization buffer was passed through the chamber in order to wash out all oligonucleotides adsorbed on the gold surface. After completion of this step, the 4 μmol L⁻¹ mercaptohexanol solution in immobilization buffer was used to fill transducer surface unoccupied by ssDNA. This step (4, Fig. 1) allows also for elimination of unspecified uranyl–transducer surface interactions. To obtain a baseline for investigation of DNA–uranyl interactions, the immobilization buffer was passed through the
chamber (step 5, Fig. 1). Next, the solution of 1 mmol L\(^{-1}\) uranyl acetate and 1 mmol L\(^{-1}\) ascorbic acid in 50 mmol L\(^{-1}\) Tris–HCl (pH 3.0) was introduced to QCM apparatus (step 6, Fig. 1). The instant frequency increase, observed at the beginning of this step, results from different energy losses at the liquid-crystal interface, corresponding
to different medium composition [24]. Nevertheless, constant frequency decrease indicates the mass deposition at the transducer surface. This originates from the interactions of uranyl ions with phosphate moieties in DNA strand. In the last step of QCM measurements, the immobilization buffer was passed through the apparatus chamber (step 7, Fig. 1). The change of frequency, caused by the uranyl cation deposited at the transducer modified with oligonucleotide monolayer, is indicated by delta sign in Fig. 1.

Another important issue that had to be addressed before the construction of DNA-based uranyl sensor was the stability of receptor layer in the presence of UO$_2^{2+}$. It was reported that in presence of reductive compounds (e.g., ascorbic acid), uranyl ion can lead to DNA degradation [25]. Although the abovementioned phenomena was reported for double-stranded long deoxyribonucleic acids, it is crucial to investigate the possibility of similar phenomenon in the case of single-stranded 20-mer oligonucleotide which will be used as receptor layer in this work. Based on the results shown in Fig. 1, cleaving effect is not evident, although these observations are not conclusive. To further explore this problem, capillary electrophoresis investigations were carried out [26]. As it can be seen in Fig. 2a, electropherogram of 20-mer oligonucleotide shows only one well-developed peak. To evaluate the influence of uranyl acetate solution (in combination with ascorbic acid) on tested oligonucleotide, samples containing 1 mmol L$^{-1}$ uranyl acetate, 1 mmol L$^{-1}$ ascorbic acid (pH 3.0), and 4 μmol L$^{-1}$ of 20-mer ssDNA were prepared. After 1 h of incubation, no distinct changes in retention time, peak height and area were observed (Fig. 2b). These results definitely excluded the possibility of receptor layer degradation during UO$_2^{2+}$ determination.

Fig. 3 Square wave voltammograms for gold disk electrode modified with ssDNA (a); unmodified electrode (b). (I) Analysis in 50 mmol L$^{-1}$ Tris buffer solution, pH 3.0; (II) analysis in 50 mmol L$^{-1}$ Tris buffer solution, 3.0, containing 50 μmol L$^{-1}$ methylene blue; (III) analysis in 50 mmol L$^{-1}$ Tris buffer solution, pH 3.0, containing 50 μmol L$^{-1}$ methylene blue and 1 mmol L$^{-1}$ uranyl acetate.

Fig. 4 Calibration curve towards uranyl acetate for ssDNA-modified electrode: a 60 and b 5 min incubation time. Each experiment was repeated three times with the use of the same electrode.
Electrochemical investigations

First, electrochemical measurements were conducted without any external redox marker, relying on the redox properties of UO$_2$$^{2+}$ ion. However, these experiments were not successful, as current signal, registered for ssDNA-modified electrode in uranyl acetate solution, was about five times lower, as compared with unmodified Au electrode (data not shown). It seems that the electron and mass transfer is limited by ssDNA immobilized at the electrode surface.

For further investigations, a well described DNA redox label, methylene blue, was employed (it was not introduced in preliminary QCM experiments due to its low mass and resulting small frequency changes). The postulated mechanism of analytical signal generation is as follows: after subjection of DNA-modified gold electrode to uranyl acetate solution, the total negative charge of receptor layer decreases due to complexation of UO$_2$$^{2+}$ with DNA backbone phosphate groups. This weakens the interaction of cationic methylene blue with DNA receptor layer. As a consequence, methylene blue molecules are replaced by UO$_2$$^{2+}$ ion, allowing electrochemical determination of uranyl cation. Indeed, comparison of curves (II) and (III) in Fig. 3a reveals a dramatic drop in the measured current. Analogous voltammograms for bare gold electrode show almost identical current values registered for methylene blue solution with or without the presence of uranyl cation (Fig. 3b). Based on these results, it is evident that ssDNA immobilized on the electrode interacts with UO$_2$$^{2+}$. Significant change of analytical signal after the contact of receptor layer with analyte cation, encouraged us to utilize this mechanism for quantitative uranyl cation determination.

Further experiments were carried out in order to investigate the analytical parameters of proposed biosensors. As the proposed mechanism is based at the uranyl preconcentration at the electrode surface, the incubation time of 60 min was chosen. This was intended to saturate the oligonucleotide strands with analyte cation, namely UO$_2$$^{2+}$. To prepare the calibration curve, the difference in current peak surface for methylene blue before and after the contact of the proposed sensor with sample solutions was plotted against uranyl ion concentration.

As shown in Fig. 4a, the obtained lower detection limit (LDL) for UO$_2$$^{2+}$ was 30 nmol L$^{-1}$. Moreover, calibration curve has fairly high slope (5.216) and good linear correlation ($R^2$=0.9998). The upper detection limit was slightly below 0.1 μmol L$^{-1}$, resulting in relatively narrow

![Graph showing current changes for various ions](image)

**Table 1** Comparison of chosen uranyl ion determination methods

| Technique                  | Analysis time (min) | Selectivity | LDL (mol L$^{-1}$) | Detection range (mol L$^{-1}$) | Reference |
|----------------------------|---------------------|-------------|--------------------|-------------------------------|-----------|
| Adsorptive stripping voltammetry | 3                   | ND          | 2·10$^{-8}$        | ND                            | [5]       |
| Electrochemical sensor      | 21                  | ND          | 1·10$^{-6}$        | 1–10·10$^{-6}$                | [11]      |
| Potentiometry               | 0.35                | 9·10$^{-4}$ | 9·10$^{-2}$        | 9·10$^{-2}$–10$^{-5}$         | [14]      |
| Optical biosensor           | 30                  | Excellent   | 50·10$^{-8}$       | 2·10$^{-6}$–5·10$^{-7}$       | [20]      |
| Electrochemical biosensor   | 6                   | Good        | 5·10$^{-8}$        | 1·10$^{-6}$–5·10$^{-8}$       | This work |
The selectivity of proposed biosensor was examined for incubation time of 5 min, at the concentration of 0.4 μmol L\(^{-1}\) for all cations tested. The effect of chosen interfering ions on electrochemical methylene blue signal is shown in Fig. 5. Only for Pb\(^{2+}\) and Ca\(^{2+}\) cations more significant response was observed, although still over twice lower than for UO\(_2\)^{2+}. The statistical analysis of these results was conducted. Test of statistical significance of the differences of mean responses (\(t\) test) was performed for each cation in comparison to uranyl ion. Analysis of each sample was repeated four times (\(n=4\)). In each instance, the mean and the standard deviation were calculated and the subsequent computation was performed to achieve experimental values of \(t\) statistics. It can be concluded that the selectivity for all cations was significantly different from uranyl ion results (\(\alpha=0.05\), critical value of \(t=2.45\); experimental values of \(t\) for interfering cations were as follows: Pb\(^{2+}\), 8.90; Cu\(^{2+}\), 8.40; Ca\(^{2+}\), 15.58; Mg\(^{2+}\), 15.46; Fe\(^{3+}\), 26.21; Cd\(^{2+}\), 15.26; and Sr\(^{2+}\), 22.00). There is a possibility that selectivity could be further increased after optimization of immobilized oligonucleotide length, as well as the density of probes on the transducer. Nevertheless, the presented sensor allows for uranyl ion determination in samples without abovementioned interferences.

Conclusions

It has been shown herein that gold electrodes modified with short-chain ssDNA oligonucleotides can be used for trace-level voltammetric determination of uranyl cation. According to our knowledge, this is the first report on the use of DNA-modified biosensor for UO\(_2\)^{2+} analysis. The proposed ssDNA recognition layer functions via the strong interaction between phosphate DNA backbone and UO\(_2\)^{2+} ion. It was found that the usage of redox properties of uranyl cation for generation of analytical signal is impractical; accordingly, external redox marker, namely methylene blue, was employed for electrochemical measurements. Due to the competition between UO\(_2\)^{2+} and methylene blue, current signal decreases proportionally to the analyte concentration.

Proposed ssDNA-modified electrodes showed good selectivity towards UO\(_2\)^{2+}, against some common metal cations. Only Pb\(^{2+}\) and Ca\(^{2+}\) cations showed considerable interfering effect, although the response towards UO\(_2\)^{2+} was still over twice higher.

The remarkably low detection limit of 30 nmol L\(^{-1}\) for UO\(_2\)^{2+} ions could be achieved by extending the sample incubation time to 60 min. For short sample incubation periods (5 min), upper detection limit of 1 μmol L\(^{-1}\) and lower detection limit of 50 nmol L\(^{-1}\) were registered. The possibility of adjusting the performance of proposed modified electrodes to predicted concentration of UO\(_2\)^{2+} in sample solution is of special importance.

Obtained results, while still preliminary, are very promising in the comparison with other UO\(_2\)^{2+} sensors, as shown in Table 1. To further improve the working parameters of proposed biosensors, including the elimination of interfering effect from certain cations, efforts are currently in progress to optimize the length and packing density of oligonucleotides used for electrode modification. Moreover, the use of impedance spectroscopy and redox markers other than methylene blue for generation of the analytical signal is also planned.

Acknowledgments This work was co-financed by the Polish Ministry of Science and Higher Education (research project N N204 125237) and Warsaw University of Technology.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

1. Hammond CR (2000) In: Handbook of chemistry and physics, 81st edn. CRC, Boca Raton
2. Holzbeche J, Ryan DE (1980) Anal Chim Acta 119:405–408
3. Caddia M, Iversen BS (1998) J Anal At Spectrosc 13:309–313
4. Mlakar M, Branica M (1994) Marine Chem 46:61–66
5. Djogić R, Branica M (1995) Anal Chim Acta 305:159–164
6. Wang J (1985) Stripping analysis: principles, instrumentation and applications. VCH, New York
7. Mlakar M (1993) Anal Chim Acta 276:367–372
8. Kefala G, Economou A, Voulgaropoulos A (2006) Electroanalysis 18:223–230
9. Gholivand MB, Nassab HR, Fazeli H (2005) Talanta 65:62–66
10. Zobel CR, Beer M (1961) J Biophys Biochem Cytol 10:335–346
11. Becker A, Tobias H, Mandler D (2009) Anal Chem 81:8627–8631
12. Banerjee R, Katsenovich Y, Lagos L, Semen M, Najad M, Balsamo V, Pannell KH, Li CH (2010) Electrochim Acta 55:7897–7902
13. Shervedani RK, Mozaffari SA (2005) Surf Coat Tech 198:123–128
14. Moody GJ, Slater JM, Thomast JDR (1988) Analyst 113:699–703
15. Wojciechowski K, Wróblewski W, Przygórzewska J, Rokicki G, Brzózka Z (2002) Chem Anal 47:335–346
16. Ellington AD, Szostak JW (1990) Nature 346:818–822
17. Hianik T, Wang J (2009) Electroanalysis 21:1223–1235
18. Dolatabadi JEN, Mashinchian O, Ayoubi B, Jamali AA, Mobed A, Losic D, Omidy, de la Guardia M (2011) TRAC—Trend Anal Chem 30(3):459–472
19. Brown AK, Liu J, He Y, Lu Y (2009) Chembiochem 10:486–492
20. Lee JH, Wang Z, Liu J, Lu YJ (2008) J Am Chem Soc 130:14217–14226
21. Privett BJ, Shin JH, Schoenfisch MH (2010) Anal Chem 12:4723–4741
22. Ziolkowski R, Górski L, Zaborowski M, Malinowska E (2010) Bioelectrochemistry 80:31–37
23. Camillone N (2004) Langmuir 20:1199–1206
24. Talib ZA, Baba Z, Kurosawa S, Sidek HAA, Kassimb A, Yunus WMM (2006) Am J Appl Sci 3:1853–1858
25. Yazzie M, Gamble SL, Civitello ER, Stearns DM (2003) Chem Res Toxicol 16:524–530
26. Cohen AS, Terabe S, Smith JA, Karger BL (1987) Anal Chem 59:1021–1027
27. United States Environmental Protection Agency. Available at http://www.epa.gov. Accessed 5 July 2011
28. Haba FR, Wilson CL (1963) Microchim Acta 51:305–312