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DNA-6MP interactions for electrochemical biosensor development

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Summary: This study presents a novel analytical tool for the detection of 6-mercaptopurine (6MP) through the development of a DNA-based electrochemical biosensor. A specific double-stranded DNA sequence (5′-GGCAGGACGGAG-3′) was identified for its ability to selectively bind 6MP, with an affinity constant of 2.52 × 10^3 M^{-1}, as confirmed by DOSY NMR. Selectivity was further verified by HPLC analysis in the presence of common metabolites and co-administrated drugs. When immobilized on the electrode surface, this DNA-based bioreceptor offers a rapid, cost-effective, and easy-to-use alternative to conventional analytical tools, with promising applications in therapeutic drug monitoring and personalized medicine.

Keywords: 6-mercaptopurine, DNA-drug interaction, selective bioreceptor, electrochemical biosensor, short double-stranded DNA

Introduction

The development of selective bioreceptors remains a key challenge in designing effective DNA-based biosensors for small-molecule drugs. Most current systems rely on isolated DNA fragments, which often lack the specificity required to distinguish the target compound from similar metabolites or co-administered drugs. To address this limitation, this study focused on the use of a specifically selected double-stranded DNA sequence as a bioreceptor for the detection of 6-mercaptopurine (6MP), an active form of an immunosuppressive drug, with the aim of improving both the accuracy and reliability of DNA-type biosensors.

Methods

Custom-synthesized DNA with the sequence 5′-GGCAGGACGGAG-3′ was purchased in double-stranded (*ds*DNA) form. This sequence represents a variant of others previously reported in the literature [1]. To evaluate its potential as a bioreceptor for 6-mercaptopurine, diffusion-ordered spectroscopy (DOSY) NMR was employed. Since the diffusion coefficient (D) value depends on the molecular size—being greater for small molecules such as 6MP and lower for larger molecules such as DNA—binding causes a noticeable shift in D. By comparing the diffusion coefficients of free and bound forms, the binding constant can be determined. For the tested DNA sequence, selectivity studies were conducted via high-performance liquid chromatography. Eleven molecules were chosen as potential interferents: typical metabolites, such as urea, uric acid, glucose, and ascorbic acid; pain killers; nonsteroidal anti-inflammatory drugs, such as acetaminophen, ibuprofen, diclofenac and mesalazine; and two antibiotics, ciprofloxacin, and metronidazole, which are frequently used for the prevention of postoperative recurrence of Crohn's disease. Additionally, metformin, an antidiabetic medication, was included, reflecting its widespread use among the growing population of diabetes patients.

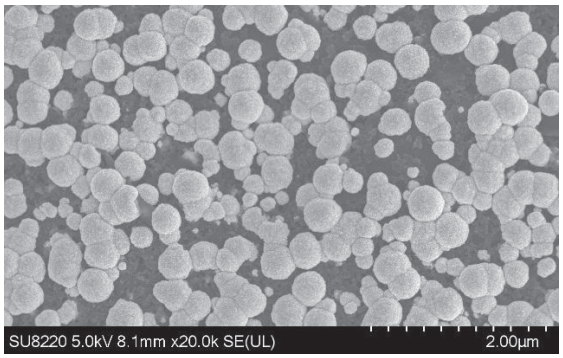

Fig. 1. SEM image of GSPE/Pt.

The developed biosensor employs a graphite screen-printed electrode (GSPE) modified with platinum nanoparticles (PtNPs) to enhance sensitivity via their strong electrocatalytic properties. PtNPs were electrodeposited by Cyclic Voltammetry (CV) technique, from 3 mM H_2PtCl_6 in a

0.5 M H_2SO_4 solution at a scan rate of 50 mV/sec. Figure 1 presents an SEM image of a GSPE with deposited PtNPs. A linear linker, cysteamine (Cys), was used to covalently attach a carboxyl-terminated double-stranded DNA sequence, forming a stable and functional recognition layer.

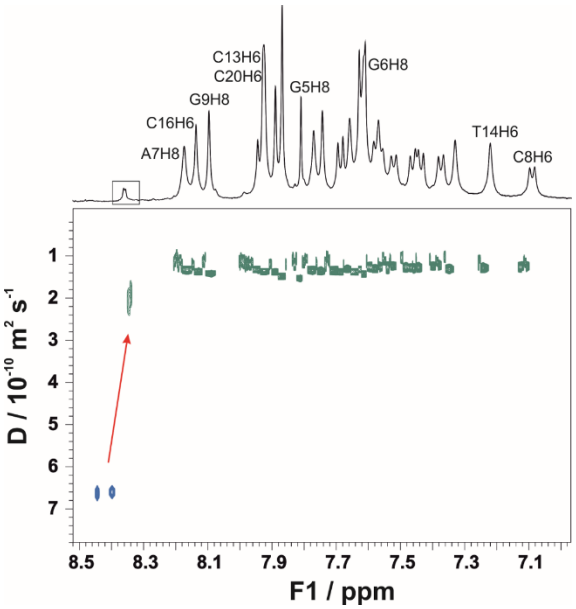

Fig. 2. NMR DOSY spectra of 6MP (blue) and dsDNA (GGCAGGACGGAG)+6MP (green) at a concentration ratio 4 : 1 respectively.

Results

The DOSY NMR spectra of 6MP and its complex with *ds*DNA GGCAGGACGGAG are shown in Figure 2. On the basis of the diffusion coefficient changes upon complex formation, the K_a binding constant was calculated, with a value of $2.52 \cdot 10^3$ M^{-1}. The HPLC results, interpreted as the selectivity of the bioreceptor, summarized in Table 1, reveal different patterns of interference across the various molecules and tested DNA. For comparison, the obtained value for 6MP–27% confirmed strong complex formation with the selected DNA strand, as only 27% of the free DNA remained in the tested solution. Metabolites such as urea, uric acid, etc., and painkillers, with calculated values of approximately 80%, showcase their limited interactions with DNA. Medications such as ciprofloxacin, diclofenac, mesalazine, metformin, and metronidazole also exhibit weak interactions with the studied DNA sequence [2].

*Tab. 1: HPLC selectivity studies presented as the percentage of peak area from the DNA complex (*the error of the analytical procedure is ±2%).*

percentage of peaks area from the DNA complex vs unbound DNA (%)	
6-Mercaptopurine	27
Urea	80
Uric acid	78
Glucose	79
Ascorbic acid	79
Acetaminophen	78
Ibuprofen	80
Ciprofloxacin	79
Diclofenac	101*
Mesalazine	102*
Metformine	80
Metronidazole	100

Voltammetric measurements with the designed biosensor confirmed the presence of DNA on the electrode surfaces, as the oxidation signals of guanine and adenine were recorded. Notably, a significant decrease in the adenine signal was observed following incubation with 6-mercaptopurine, indicating that analyte detection can be achieved indirectly by monitoring this decrease. The developed biosensor exhibited a linear response from 2 to 75 nM (Figure 3), which allows accurate quantification in potential real samples through appropriate dilution, as physiological concentrations typically exceed this range.

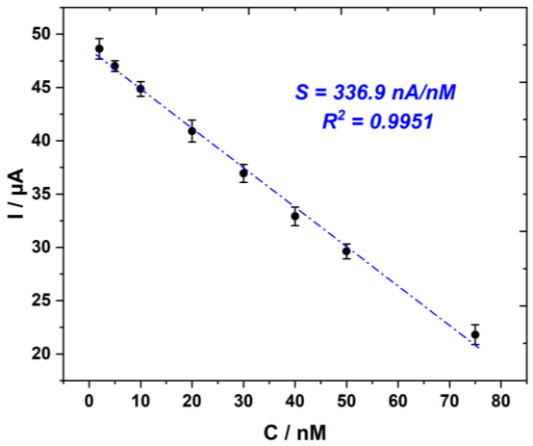

Fig. 3. Calibration curve for 6MP determination on GSPE/Pt -Cys-dsDNA.

References

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