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Aptamer-based Nanosensors: Juglone as an Attached-Redox Molecule for Detection of Small Molecules

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ABSTRACT

Introduction: Among several biosensing approaches, electrochemical-based procedures have been described as one of the most common and useful methods for sensing because of their simplicity, sensitivity, accuracy, and low cost. The electroactive species, which called redox, play a main role in the electrochemical-based approaches. Among several redox molecules used for electrochemical experiments, ferrocene is one of the commonly used redox molecules. However, instability of ferrocenium ion in the chloride containing solutions appeared to be weakness of this redox molecule limiting its utilization. ***Methods:*** In the current study, Juglone was attached (using EDC/NHS coupling method) to the 3'-amino-modified terminus of the immobilized specific aptamer of codeine, which was successfully used in a cyclic electrochemical voltammetry procedure. ***Results:*** The cyclic voltammogram peak of aptamer-attached Juglone was observed in the potential range of +0.4 to +0.9 V and the fabricated aptamer-based sensor was used for detection of different concentrations of codeine in the phosphate buffer 0.1 M solution containing 2 M NaCl. ***Conclusion:*** Based on these findings, it can be suggested that the new aptamer-attached Juglone could be considered as an effective alternative redox molecule in particular with oligonucleotide-based sensing systems.

Introduction

In recent years, significant progress has been made in the development of biosensors for different applications such as medical diagnosis (Song *et al.* 2007; Trkarslan *et al.* 2009), drugs (Alonso Lomillo *et al.* 2003; Joseph *et al.* 2003) and toxins (Liu *et al.* 2004; Pancrazio *et al.* 1998; Stevens *et al.* 2007) tracing, and other purposes (Gao *et al.* 2008; Tombelli *et al.* 2000). Aptamer-based biosensors, called aptasensors, are a new group of biosensors that have been introduced in recent years and attracted a lot of interests in the field of biosensors. Aptamers are single stranded DNA or RNA sequences with high affinity and specificity to a specific target such as antibodies. These properties make aptamers appropriate tool to be used as the recognition part of biosensors in the optical, electrochemical, or other types of affinity biosensors (Lee *et al.* 2008; Song *et al.* 2008; Tombelli *et al.* 2005). Despite of simultaneous progress in the optical and electrochemical sensing, many scientists prefer electrochemical techniques because of their simplicity, sensitivity, accuracy, and low cost (Velasco-Garcia 2009; Wang 2006). In the electrochemical measurements, the biorecognition element, called redox, is one of the main components of assay procedures and acts as a transducer by following methods: these molecules may be used as the electroactive element of the label free electrochemical procedures (being existed in the solution without any attachment to the sensors or surface of the electrode), used as a biomolecule-attached element (being attached to the biomolecular part of the sensor), or used as a surface-attached redox molecules (being attached to the surface of the electrode) (Odenthal and Gooding 2007). Among the mentioned methods, the biomolecule-attached redox molecules are very popular in biosensor designing and ferrocene is one of the commonly used redox molecules in these types of studies (Baker *et al.* 2006; Ferapontova *et al.* 2008; Li *et al.* 2008; Wang *et al.* 2009; Xiao *et al.* 2007). Despite of vast usage of the ferrocene as the redox molecule in the field

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of biosensors, instability of the ferrocenium ion in the chloride containing buffer solutions or in strong nucleophilic reagents is one of the major disadvantages of using this molecule (Hurvois and Moinet 2005; Prins *et al.* 1972; Xiao *et al.* 2007). Moreover, since the redox molecules play an important role as electroactive species, an effective redox molecule could be very important in the improvement and progression of existed techniques in the biosensors fabrication and, therefore, enhance the studies in the field of sensing. The 5-hydroxy- 1,4-naphthoquinone, Juglone, a natural quinone obtained from walnut trees, is an electroactive molecule which has been used as a surface attached redox molecule in recent studies (Baker *et al.* 2006; March *et al.* 2008). Although quinine derivatives has been described as biomolecular-attached redox species before (Chatterjee and Rokita 1994), using of Juglone as an attached-redox molecule in aptasensors is a novel approach in the field of oligonucleotide-based biosensors. Based on our findings, it can be suggested that the new aptamer-attached Juglone could be considered as an effective alternative redox molecule in particular with oligonucleotide-based sensing systems.

Materials and methods

Materials

5-Hydroxy- 1,4-naphthoquinone 97% Acros (Juglone), 3-mercaptopropionic acid ≥98% Merck (3-MPA), N-hydroxysuccinimide ≥99% Merck (NHS), synthesis grade of Merck's N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), Codeine phosphate 99% Iran Temad (CP), and 6-Mercaptohexanol 97% Sigma-Aldrich were purchased and used without further purification. The previously reported specific RNA-aptamer sequence for codeine (Win *et al.* 2006) was synthesized by Microsynth with the 5'-terminus, a C_6 aliphatic thiol, and 3'-tarminus, a C_7 primary aliphatic amin, modifications (5'-SHC_6-GGG ACA GGG CUA GCU UAG UGC UAU GUG AGA AAA GGG UGU GGG GG-C_7NH_2-3').

Instrumentation and procedures

Melting points (obtained by an electrothermal 9100 apparatus), infrared spectra (recorded by a shimadzu (8400) FT-IR spectrometer), and 400 MHz ^{1}H NMRs (recorded on a Bruker Avance instrument, AC 80) had been performed for tracing the synthesized compounds. Column chromatography was performed for further purification by using silica gel 60 (230-400 mesh) and DCM-methaol as stationary and mobile phases respectively.

Electrochemical measurements were performed by a Metrohm *Autolab 302N Potentiostats-Galvanostats* with the three customary electrodes, an Ag/AgCl/KCl 3 M reference electrode; a platinum wire auxiliary electrode; and a gold disk electrode as working electrode (with 2 mm diameter, purchased from Azar Electrode Co.). Total controlling of the electrochemical procedures was carried out by NOVA software (version 1.5, Eco Chemie BV).

All the electrochemical experiments were performed at room temperature and in the 50 mL of different concentrations of CP in phosphate buffer solution (PBS) 0.1 M containing 2 M sodium chloride (NaCl), pH 7.0.

Synthesis of N-Hydroxysuccinimide ester of β((5-hydroxy– 1,4-naphthoquinonyl) thio) propionic acid (Jug-PE)

Jug-PE was prepared by the coupling reaction of β((5-hydroxy– 1,4-naphthoquinonyl) thio) propionic acid (Jug-P) and NHS in presence of EDC (Fig. 1A) (Teh *et al.* 2005; Wu *et al.* 2007). First, the Jug-P was synthesized by the one-step reaction of Juglone and 3-MPA. The synthesis of this intermediate is based on the substitution of thiols on the quinone's rings under a mild condition (Piro *et al.* 2005; Villalba *et al.* 2008). A mixture of Juglone (210 mg, 1.2 mmol) and 3-MPA (123 mg, 100 µL, 1.2 mmol) in 6 mL ethanol were stirred at 40 ^{o}C for 1 hour. The mixture was then cooled to room temperature and remained stirringly for 2-3 hours to complete the reaction. After filtering the precipitate and performing a washing step by cold ethanol, the precipitate was purified by silica gel column (10:1 DCM /methanol) and the product was obtained by evaporating the solvent (Chatterjee and Rokita 1994; Mital *et al.* 2008; Piro *et al.* 2005). The purity of the light orange product, Jug-P, (40% yields, M.p. 216 ^{o}C) was confirmed by TLC. FT-IR νmax (KBr) 3450 (O-H Aromatic), 3100 (O-H Carboxylic acid), 2990, 1650 (C=O), 1600 (C=O) 1453, 1376, 1210, 1100 cm^{-1}.

A 0.25 mmol of synthesized Jug-P (70 mg) was dissolved in 5 mL dry dichloromethane (DCM) and added NHS (0.3 mmol, 35 mg) and EDC (0.3 mmol, 57 mg). The mixture was stirred at room temperature for 17 hours to complete the reaction. Added water (5 mL), and the mixture was extracted by dichloromethane (3 × 10 mL). The organic layers were combined, dried over $MgSO_4$, and evaporation of the solvent afforded green residue. Purification of the residue by silica gel chromatography afforded the desired product, 75% yield, as a mixture of keto and enol forms (Ferapontova *et al.* 2008; Teh *et al.* 2005; Wu *et al.* 2007). FT-IR νmax (KBr) 3470 (O-H Aromatic), 2990, 2850, 1750 (C=O), 1650 (C=O) 1453, 1350, 1210, 1120 cm^{-1}. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 12.1 (s, 1H), 11.9 (s, 1H), 11.6 (s, 1H), 7.70-7.55 (m, 2H), 7.30-7.18 (m, 2H), 6.94 (s, 1H, enol form), 6.62 (s, 1H, enol form), 6.58 (s, 1H, enol form), 3.22-2.80 (m, 8H).

Preparation of the electrode

For efficient immobilization of aptamer on the surface of the electrode, the impurities have been removed from the electrode's surface by physical and electrochemical procedures which had been described in literature (Baker *et al.* 2006; El-Deab and Ohsaka 2004; Merrill *et al.* 2005; Xiao *et al.* 2007). After complete steps of polishing, the 5'-thiol, 3'-amino-modified RNA-aptamer was immobilized via its 5'-thiolated terminus on the surface of gold electrode by self-assemble monolayer procedure (Wink *et al.* 1997). The self-assemble monolayer has been formed by placing a 50 µL volume of 5 µM modified RNA-aptamer on the polished surface of electrode for 18 hours. The unbound aptamers have been removed from the surface by rinsing with the phosphate buffer (pH 7.0) for several times and the Jug-PE, redox molecule, was attached to the 3'-terminus of aptamer as previously described (Fig. 1B), followed by two-hours treating with 2 mM 1-mercaptohexanol (Ferapontova *et al.* 2008; March *et al.* 2008; Xiao *et al.* 2007). The electrode gets ready in this stage and immediately would being used in experimental sections.

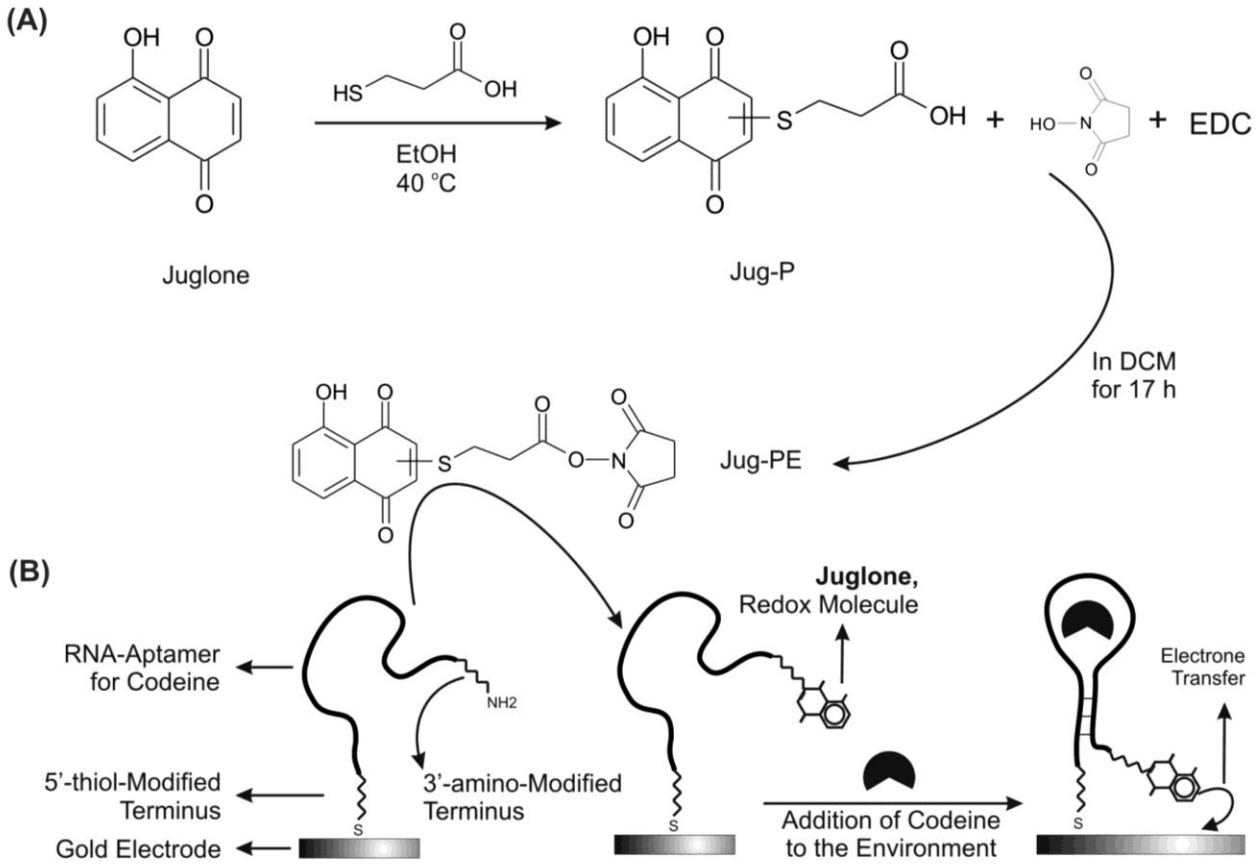

Fig. 1. Juglone (Jug) based nanosensors. A) Schematic representation for synthesis of Jug-PE: The Jug-PE was synthesized from Jug-P (a product of Juglone and 3-MPA reaction) by EDC/NHS coupling reaction. The synthesized Jug-PE would have used as a attached redox molecule for the biosensor designing. B) The codeine's aptasensor: The specific aptamer of codeine was immobilized on a gold surface via its 5'-thiolated terminus. Then, the redox molecule, Jug-PE, was attached to the 3'-amino modified terminus of the aptamer's sequence and use for target sensing.

Results

Electrochemical characterization

For investigating the exact place of aptamer-attached Juglone's oxidation and reduction peaks on the voltammogram, at first, a cyclic voltammetry scan was taken in the potential range of -1 to +1 V and the scan rate of 0.15 V/s in PBS 0.1 M containing 2 M NaCl. Then, the second cyclic voltammetry scan was taken in the same condition as well as a 10 µM concentration of CP. By comparing the obtained scan in the presence of CP with the background scan, it has been investigated that the reduction oxidation and peaks of aptamer-attached Juglone are appeared in the potential range of +0.4 to +0.9 V. The reduction peak appears at 8.5 V and

the oxidation peak appears at 6.5 V on the voltammogram (Fig. 2).

Attached- Juglone as a redox molecule for aptasensor designing

The ability of the attached-Juglone to be used as a redox molecule for aptasensor designing was investigated by codeine addition to the environment. Firstly, a cyclic voltammetry background scan was taken in the potential range of +0.4 to +0.9 V and the scan rate of 0.15 V/s in PBS 0.1 M containing 2 M NaCl. Then, the different amounts of CP were added to the PBS 0.1 M containing 2 M NaCl to prepare different solutions of CP (100 nM, 200 nM, 500 nM, and 1 µM). Comparison of these cyclic voltammograms, taken scans in presence of CP and background scan, shows a significant relation between the CP concentration and the maximum faradic current change in the voltammogram (Fig. 2).

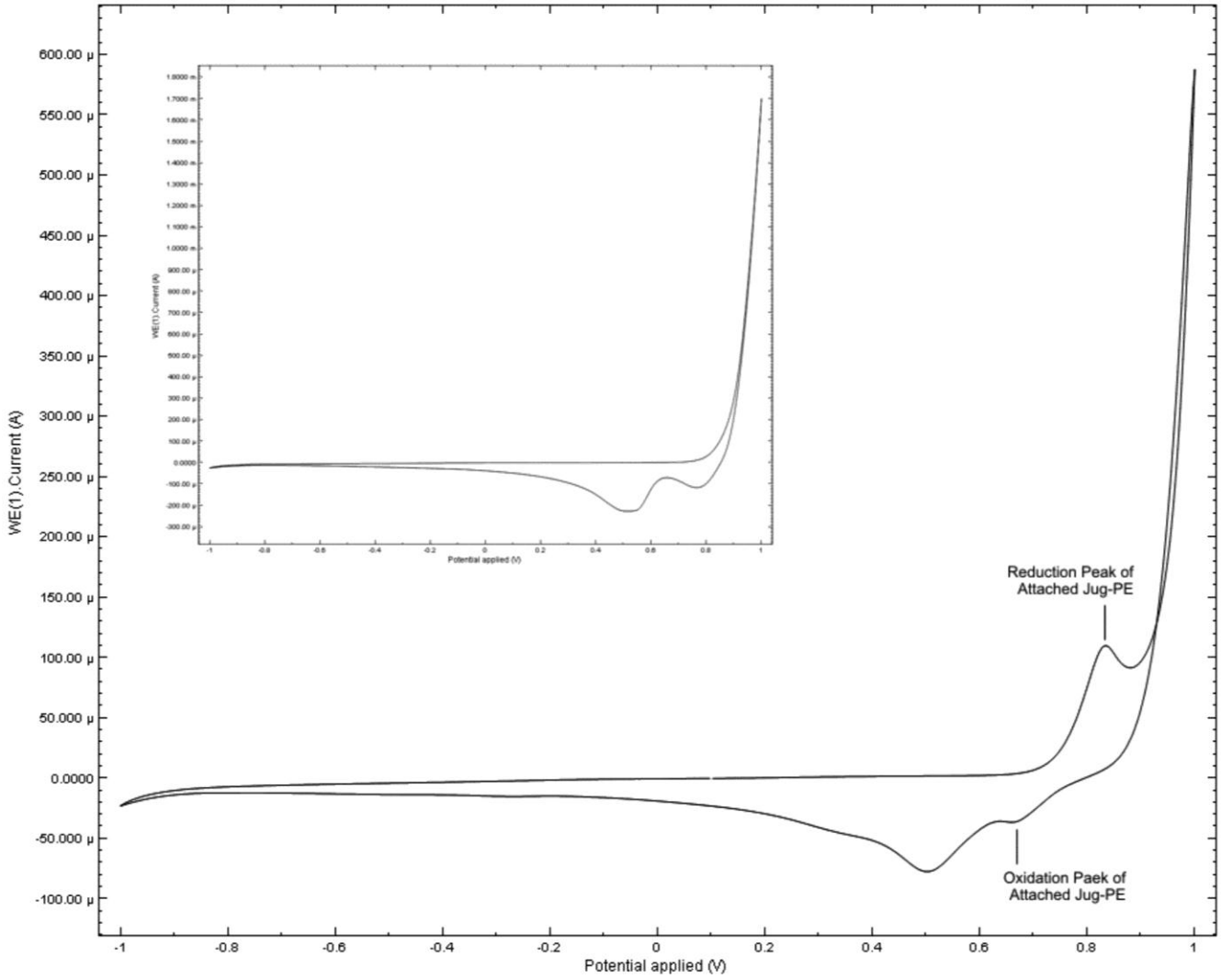

Fig. 2. The cyclic voltammogram of attached Jug-PE: A cyclic voltammetry was taken for a 10 µM concentration of CP in PBS 0.1 M, containing 2 M NaCl (Potential range: –1.0 to +1.0, scan rate: 0.15 V/s). The obtained data show that the oxidation and reduction peaks for this redox molecule appear in the +0.4 to +0.9 V range of potential axis on the voltammogram. The inset shows the background cyclic voltammetry scan which was taken in PBS 0.1 M containing 2 M NaCl in absence of CP.

The obtained data show that the maximum faradic current of working electrode change to 61.6 µA, 73.8 µA, 110 µA, and 211 µA in presence of 100 nM, 200 nM, 500 nM, and 1 µM CP respectively. The obtained data show that the Juglone-tagged aptasensor presents a linear response to the presence of increasing concentrations of CP.

Discussions

According to the obtained data, the place of oxidation and reduction peaks of aptamer-attached Juglone has being observed in the potential range of +0.4 to +0.9 V. This range is different from the previously reported potential range of the oxidation and reduction peaks of surface-attached Juglone (March *et al.* 2008). This significant shift of oxidation and reduction peaks to the right position of the potential axis of voltammogram (upper potential range) is probably because of changes in the electrochemical properties of Juglone because of its attachment to the 3'-terminus of codeine's specific RNA-aptamer.

Faradic current change of working electrode in presence of CP could be described by the previously reported mechanism (Fig. 1B) (Ferapontova *et al.* 2008). In the absence of CP, because, the structure of aptamer is open, the Juglone stay away from the surface of electrode. In this situation, the background scan has been taken. In presence of CP, the structure of aptamer changed to closed format and this phenomenon causes the vicinity of the Juglone to the electrode's surface. The significant change in the spatial structure of aptamer in presence of CP causes to change in faradic current. Since, the more concentrations of CP affect on the more numbers of aptamers, by increasing the concentration of CP the faradic current of working electrode would being increased. So, the faradic current of working electrode could be described as an index for the involved aptamers and it could be introduced as an index for the codeine's concentration in the environment indirectly.

Conclusion

The new redox molecule was used in the PBS buffer containing 2 M NaCl successfully. According to the literature, the faradic current of working electrode is related to the ionic strength of the medium (Xiao *et al.* 2007), so the ability of this redox molecule to be used in the environments with relatively high concentration of chloride salt could be considered as an important advantage of this molecule. The voltammetric results show that the new aptamer-attached Juglone has a significant oxidation and reduction peak in the µA limit on the voltammogram. Thus, based on these findings, it can be suggested that the new aptamer-attached Juglone could have been considered as an effective alternative redox molecule in particular with oligonucleotide-based sensing, for example oligonucleotide-based biosensors.

Ethical issues

None to be declared.

Conflict of Interests

Authors declare no conflict of interests.

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