Biosensors for the detection of organophosphate exposure by a new diethyl thiophosphate-specific aptamer

Napachanok Mongkoldhumrongkul Swainson · Pongsakorn Aiemderm · Chonnikarn Saikaew · Kanyanat Theeraraksakul · Pakjira Rimdusit · Charoenkwan Kraiya · Sasimanas Unajak · Kiattawee Choowongkomon

Objective
An aptamer specifically binding to diethyl thiophosphate (DETP) was constructed and incorporated in an optical sensor and electrochemical techniques to enable the specific measurement of DETP as a metabolite and a biomarker of organophosphate exposure.

Results
A DETP-bound aptamer was selected from the library using capillary electrophoresis-systematic evolution of ligands by exponential enrichment (CE-SELEX). A colorimetric method revealed that the aptamer had the highest affinity for DETP, with a mean $K_d$ value ($\pm$ SD) of 0.103 ± 0.014 $\mu$M. The docking results and changes in resistance showed that the selectivity of the aptamer for DETP was higher than that for the similar structures of dithiophosphate (DEDTP) and diethyl phosphate (DEP). The altered amplitude of cyclic voltammetry showed a linear range of DETP detection covering 0.0001–10 $\mu$g/ml with a limit of detection of 0.007 $\mu$g/ml. The recovery value of a real sample of pH 7 was 97.2%.

Supplementary Information
The online version of this article at https://doi.org/10.1007/s10529-021-03158-2.

N. M. Swainson · P. Aiemderm · S. Unajak · K. Choowongkomon (✉)
Department of Biochemistry, Faculty of Science, Kasetsart University, 50 Ngam Wong Wan Road, Bangkok 10900, Chatuchak, Thailand
e-mail: fsclktc@ku.ac.th

C. Saikaew · K. Theeraraksakul
Genetic Engineering Interdisciplinary Program, Graduate School, Kasetsart University, 50 Ngam Wong Wan Rd, Bangkok 10900, Chatuchak, Thailand

C. Saikaew
Division of Occupational and Environmental Diseases, Department of Disease Control, Saraburi 18120, Thailand

P. Rimdusit
Office of Disease Prevention and Control 4, Department of Disease Control, Saraburi 18120, Thailand

C. Kraiya
Electrochemistry and Optical Spectroscopy Center of Excellence, Department of Chemistry, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok 10330, Thailand

K. Choowongkomon
Omics Center for Agriculture, Bioresources, Food and Health, Kasetsart University, Bangkok, Thailand
Conclusions The current method showed great promise in using the DETP-specific aptamer to detect the exposure history to organophosphates by measuring their metabolites, although degradation of organophosphate parent compounds might occur.

Keywords Aptasensor · Capillary electrophoresis · Cyclic voltammetry · Diethyl thiophosphate · Electrochemical impedance spectroscopy · Organophosphate metabolites

Introduction

Organophosphates are typically used as pesticides. Their contamination in the environment may result in their availability in agricultural products, water, soil and air. The metabolism of organophosphates in humans, environmental matrices and microbes generates dialkyl phosphates (Ohshiro et al. 1996; Simaremare et al. 2019).

The metabolites of organophosphates comprise dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl phosphate (DEP), diethyl thiophosphate (DETP) and diethyl dithiophosphate (DEDTP). Each corresponds to the exposure of one or more types of organophosphates. For example, DEP and DETP are metabolites of chlorethoxyphos, chlorpyrifos, coumaphos, diazinon, parathion and sulfoxone. Additionally, DEP, DETP and DEDTP can be generated by the metabolism of disulfoton, ethion, phorate and terbufos (Sudakin and Stone 2011).

A study demonstrated that dialkyl phosphates were commonly found in fruits and vegetables, although the levels of organophosphates were less than legal minimum levels (Zhang et al. 2008). DEP and DETP were found in purchased apples and oranges, including the extracted juices that had been in cool storage for 3 days (Lu et al. 2005). Generally, dialkyl phosphates have greater persistence than their organophosphate parent compounds. Although various aptasensors using an aptamer as the recognition unit have been invented to practically detect organophosphates in real samples (Liu et al. 2020; Selvolini et al. 2018; Zhang et al. 2020), it can be difficult to measure trace amounts of organophosphates following progressive degradation in cold storage or as a result of biodegradation.

Traditional methods to detect dialkyl phosphates as a biomarker of exposure include gas or liquid chromatography (LC) coupled with mass spectrometry due to their sensitivity and reliability (Fernandez et al. 2019; Kongtip 2017). However, chromatographic techniques require extensive sample pretreatment, specialist operation and expensive equipment. Thus, these techniques are not suitable for screening dialkyl phosphates on-site.

This research aimed to overcome the false-negative detection of degraded organophosphates and enable the detection of DETP as a biomarker of organophosphate exposure using aptamer-based biosensor techniques. The DETP recognition unit was generated using CE-SELEX, and the highest-affinity aptamer for DETP was selected using a colorimetric method. The aptasensor was further fabricated using electrochemical methods to facilitate the selective measurement of DETP over any other metabolite and to evaluate the sensitivity.

Material and methods

DNA aptamer selection for DETP

The selection of the aptamer from the random single-strand DNA (ssDNA) library was performed using the CE-SELEX method. The ssDNA of the library was composed of 40 variable bases in the middle and primer binding sites flanking the 5' and 3' ends. The capillary column was prerinsed with 50 mM 2-morpholinethanesulfonic acid (MES) buffer at pH 7.4 for 10 min before preheating the ssDNA library, and 1 M DETP or a mixture of the library and DETP was injected into the capillary column for 30 s at 20 kV (Beckman Coulter). The detection was set at a separation voltage of 30 kV and incorporated with the absorbance at 254 nm (Varian). Sample fractions were collected for 2–4 min and used as a template for polymerase chain reaction (PCR) in the next step.

DETP-bound ssDNA enrichment and asymmetric PCR

DETP-bound ssDNA was amplified using a PCR technique. A sample (2 μL) of the template from the
previous step was mixed with buffer, 2 mM dNTPs, 5 μM forward and reverse primers and 1.0 U Taq DNA in a final volume of 50 μL. The reaction started at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 10 s and finally at 72 °C for 10 min. The PCR products were cloned, and the sequences were analyzed as described in the Supplementary Methods.

To amplify forward and reverse strands of OO36, OO40, OO41, OO43, OO47 and OO48, asymmetric PCR was performed as previously mentioned, except that the ratio of the forward to reverse primer was 29:1 when forward strands were generated and 1:29 for reverse strand expansion.

Colorimetric screening for ssDNA candidates

The optimum amount (250 ng in 5 μL) of each PCR and asymmetric PCR product was heated at 95 °C for 10 min and then immediately placed on ice for 5 min. They were then mixed with 37 μL of gold nanoparticles (AuNPs) and 5 μL of targets or water (control) before adding 2.5 μL of 2 N NaCl. Each step was performed at room temperature after 10 min of incubation. The absorbance at 520 nm and 650 nm was measured and compared with the controls. Three aptamer candidates tagged with thiol (-SH) groups were then generated by TianGen Company for further assessment of binding affinity.

Specificity and sensitivity assays by colorimetric method

Three selected aptamers at 0.5 μM were prepared to evaluate their ability to bind DETP and other metabolites as well as pesticides (DEDTP, DEP, DMP, paraquat and carbamate) for cross-selectivity. The sensitivity of the aptamers to DETP was measured using DETP at final concentrations of 0, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 2.5, 5, 10, 25, 50 and 100 μM as the target. The colorimetric method was performed as described previously.

Secondary structure and molecular modeling of DETP-bound aptamers

The secondary structure of selected aptamers was analyzed using RNAfold software (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi).

The DETP-binding sequences and structure were predicted. A 3D loop model of the aptamer, DEDTP, DEP, and DMP was created by Discovery Studio 2019 Client (Dassault Systèmes, San Diego, CA, USA). Molecular dockings were performed by GOLD Ver 2021 Cambridge Crystallographic Data Center, Cambridge, UK) by using the 3D aptamer as a template with GOLDscore. The highest docking pose was chosen and visualized by Discovery Studio.

Electrochemical measurements

Both cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) experiments were carried out on a commercial screen-printed electrode (SPE, Metrohm) with a gold working electrode (4 mm diameter) using ferri/ferrocyanide (Fe(CN)₆³⁻/⁴⁻ in 0.1 M KCl) as the electrolyte. The measurements were conducted using a PalmSens4 potentiostat, and the results were transferred and analyzed using PSTrace software. CV scans were performed with a scanning range from –0.6 V to 0.8 V at a scanning rate of 10 mV/s and used to validate every electrochemical reaction occurring on the aptasensors. EIS was acquired at 5 mV amplitude within the frequency range of 0.1 Hz to 100 kHz.

A bare gold SPE was incubated with 5 μM thiol-modified OO36F aptamer (OO36F-SH) in phosphate-buffered saline (PBS) at room temperature for 3 h, washed with Milli-Q water and subsequently incubated with 1 mM MCH (6-mercapto-1-hexanol) for 10 min to block nonspecific binding on the gold surface. The MCH/aptamer-Au electrode was washed with Milli-Q water, and then the charge transfer resistance (Rct) was measured before being used as an aptasensor to determine targets consisting of 0.025 μg/ml DETP, DEDTP or DEP in PBS. Rct and current (μA) for different concentrations of DETP at 0, 0.0001, 0.001, 0.01, 0.1, 1 and 10 μg/ml in PBS were also measured for the calibration curve. Juices at pH 3 or pH 7 (diluted with PBS at 1:20) were spiked with DETP to study the effect of any interferences in real samples. They were incubated on the SPE for 10 min at room temperature, rinsed with Milli-Q water and subjected to electrochemical measurements. The impedance spectra were presented as Nyquist plots (-Z’/Ω versus Z’/Ω) with a sampling rate of 12 points per decade. The Rct was recorded using the RCRW equivalent circuit.
High-performance liquid chromatography

Different concentrations of DETP in PBS were analyzed by reverse-phase HPLC (Shimadzu and Hitachi, Japan) equipped with a C18 column (TOSOH, Japan) at a flow rate of 1 ml/minute. Mobile phase A (0.1% trifluoroacetic acid (TFA)) was performed for 5 min before the concentration of mobile phase B (100% acetonitrile and 0.1% TFA) was increased to 49.5% for 30 min. The profile was investigated at 220 nm, and data were obtained by Primaide System Manager software.

Method validation

The limit of detection (LOD) was the concentration calculated as the mean of the blank plus three times the standard deviation (SD) of the blank signal. Interference was considered when any deviation from the recoveries was equal to or greater than 5%.

Results and discussion

Aptamer selection

The in vitro selection of DETP-specific aptamers from the library was carried out using CE-SELEX to overcome the drawbacks of conventional SELEX by eliminating stationary support, linker bias and kinetic bias (Mendonsa and Bowser 2004). Initially, ssDNA or pure DETP was analyzed for their mobility rate in CE and eluted at 6–7.5 min (Fig. 1a) or 2.7 min (Fig. 1b), respectively. Afterward, electrophoresis was performed under the same conditions with a mixture of DNA library with DETP. The results are shown in Fig. 1c and revealed two separated fractions eluted at 2.7 and 6.7 min. The eluted fraction at 2.7 min had two peaks with different intensities. This indicated that the formation of the complex with pure DETP shifted the migration rate of the aptamer-target complex closer to the migration rate of the target alone. This could have been due to conformational changes after random ssDNA interacted with DETP, which is a very small molecule. Therefore, DETP-bound ssDNA could have been separated from the ssDNA library by CE-SELEX, and the eluent from 2 to 4 min was collected to enrich the targeted ssDNA aptamer in the next step.

The DETP-bound ssDNA was then amplified using a PCR technique. The PCR products were then cloned. Fifty colonies with expected sizes of PCR products (data not shown) were cultured before their plasmid was extracted and sequenced. The nucleotide sequences of the 50 recombinant plasmids revealed only 39 clones with the insertion of a 40 bp variable region and specific primer sequences. Thus, the PCR products from the 39 clones were further investigated for their affinity to DETP using the gold nanoparticle (AuNP) colorimetric method.

Colorimetric screening for aptamer candidates

In principle, ssDNA or denatured PCR products on the surface of AuNPs prevent aggregation of AuNPs after salt induction. Thus, the ssDNA-coated AuNPs maintain a red-wine color with λmax at 520 nm. The interaction between ssDNA and the target subsequently allows clumping of the AuNPs to occur in the presence of a monovalent positive charge (Na⁺), and the color alters to purple with λmax at 650 nm instead.

The denatured PCR products of the 39 clones were assessed for their ability to detect the presence of DETP and thus allow the aggregation of the AuNPs after NaCl induction. A reduction in the absorbance at 520 nm of the aggregated particles was observed (Fig. 2a) and compared with the reaction where gold aggregation was not interfered with by ssDNA (containing only AuNPs, DETP and NaCl). The PCR products from clones OO36, OO40, OO41, OO43, OO47 and OO48 (gray bars in Fig. 2a) exhibited red-wine gold particle remnants to a similar extent as the control. Clones OO03, OO39 and OO42 also showed promising interactions with DETP; however, their controls (250 ng of ssDNA mixed with AuNPs and NaCl) also changed to purple without DETP added (data not shown). This explained why the increased absorbance at 650 nm was not investigated in this experiment, and only the remaining red-colored particles should be of interest. Thus, the reduction of the remaining ability to absorb light at 520 nm could inversely reflect the reaction rate for AuNP aggregation after conformational change by DETP-ssDNA interaction.

Selected ssDNA, forward (F) and reverse (R), was subsequently amplified using asymmetric PCR with the double strand PCR product from clones OO36, OO40, OO41, OO43, OO47 and OO48 as the
Fig. 1  Electropherogram of CE-SELEX selection Capillary electropherogram of a ssDNA library, b 1 M DETP and c free-DETP, DETP-aptamer complex and separated unbound ssDNA library
Twelve aptamers were successfully generated, and the DETP-ssDNA interaction was investigated. The increase in the A650/520 nm ratio upon target interaction was compared with the positive control comprising AuNPs and NaCl and the negative control without aggregation induction by NaCl. The results (Fig. 2b) revealed 4 ssDNA strands with the highest absorbance ratio: OO36F, OO40F, OO40R and OO47F. In an asymmetric PCR, nontargeted strands or the product of the lower concentration primer are also generated. Therefore, OO40F was selected due to its higher A650/520 nm ratio than OO40R, which was generated from the same template/PCR product. Then, OO36F, OO40F and OO47F were commercially synthesized with thiol group tagging at the 5’ end and further assessed for their specificity and sensitivity using the colorimetric method.

Specificity and sensitivity of thiol-modified aptamers

Three thiol-modified aptamers (OO36F-SH, OO40F-SH and OO47F-SH) were assessed for specificity to distinguish DETP from other metabolites of organophosphates and pesticides using the colorimetric method. In all 3 aptamer reactions, the color of the AuNPs changed after induction by NaCl following DETP detection, but it was not altered when paraquat and carbamate were investigated (Fig. 3, left). This suggested specific recognition by OO36F-SH, OO40F-SH and OO47F-SH of DETP but not the other abovementioned molecules. However, DEDTP, which has a similar structure to DETP, could induce AuNP aggregation before NaCl was applied (data not shown), implying that the small molecule with a thiol group has a similar structure to DETP.
group could autonomously drive the AuNPs to come together and increase the absorption of AuNPs at 650 nm.

After induction by NaCl following the target application, only OO36F-SH had an A650/520 nm ratio of AuNPs in the presence of DETP that was significantly different from DEP, DMP, paraquat, carbamate and the control and was not lower than that for DEDTP (Fig. 3, right). This experiment led to the conclusion that the OO36F-SH coating on AuNPs bound DETP more selectively than OO40F-SH and OO47F-SH.

To evaluate the dissociation constant (K_d), DETP was added to the aptamer-coated AuNPs in a dose-dependent manner, accompanied by NaCl aggregation induction. Color alterations of the AuNPs were observed by the naked eye (Fig. 4, top). Subsequently, the values of the absorption ratio (A650/A520 nm) were measured and plotted against various DETP concentrations. The K_d values were analyzed using nonlinear regression (curve fit), as demonstrated in Fig. 4. It was clear when observed with the naked eye that the AuNPs altered the color after 0.5 μM DETP was added and detected by the AuNPs coated with OO36F-SH (Fig. 4a, top). However, there were inconclusive color changes in the reactions involving OO40F-SH and OO47F-SH (Fig. 4b and 4c, top). The dose–response curve revealed that signal saturation occurred when 5.0 μM DETP was added to interact with the OO36F-AuNPs (Fig. 4a, bottom curve). With higher amounts of DETP, 10.0 μM was required to interact with OO40F-SH and OO47F-SH to reach saturation (Fig. 4b and 4c, bottom curve). The K_d analysis demonstrated that OO36F-SH had the highest affinity for DETP (0.103 ± 0.014 μM), while OO40F-SH and OO47F-SH had less affinity (K_d...
0.388 ± 0.093 μM and 0.593 ± 0.046 μM, respectively. The \( R^2 \) value was greater than 0.9000 (suggesting a reliable curve fit) only for OO36F-SH and OO47F-SH (\( R^2 \) values of 0.9248 and 0.9651, respectively). This experiment indicated that OO36F-SH had the highest affinity to DETP compared with the other aptamers and that it was suitable for use as the recognition unit in a colorimetric sensor to detect DETP at amounts greater than 0.5 μM by the naked eye.

Secondary structure and 3D molecular docking results

The sequences of 3 selected aptamers were used to predict the secondary structures based on RNAfold. The results revealed bulges and hairpin loops in all 3 aptamers (Supplementary Results). Such secondary structures in the length region of 10–15 nucleotides could hypothetically bind to a target (Zhou et al. 2010). Only the secondary structure of the hairpin loops of OO36F with the highest free energy of -8.07 kcal/mol from the prediction is shown in Fig. 5a. The docking results with a hairpin loop structure of 3’-TGACACAGTCA-5’ showed the best potential binding site for all organophosphate derivatives. Furthermore, it confirmed our finding that DETP showed the highest docking score with the OO36F aptamer (26.06 kcal/mol) than DEP (23.76 kcal/mol) and DEDTP (22.96 kcal/mol). The complex of DETP with the OO36F aptamer was stabilized by hydrogen bonds between DETP and dG17 and dT18 and hydrophobic interactions with dA15 (Fig. 5b-c).

This research unprecedentedly selected aptamers that bind DETP, a metabolite of organophosphates, using CE-SELEX and a colorimetric aptasensor. Previous work used modified SELEX to screen ssDNA interacting with four organophosphates (Wang et al. 2012). Although it could select aptamers that bind isocarbophos, omethoateas, phorate and profenofos simultaneously, it required 12 rounds of SELEX, immobilization of the ssDNA library and elution steps. Such challenging steps for conventional SELEX could prevent full interaction between the immobilized ssDNA and the target and could retain the highest-affinity ssDNA in the column during the elution step (Mendonsa and Bowser 2004). These limitations
could be overcome by performing CE-SELEX, which did not require immobilization and elution.

Recent studies have used synthesized ssDNA as binding units of pesticides or organophosphates, with AuNPs as the optical sensor. Acetamiprid at amounts higher than 2.5 mM could be visibly detected (Tian et al. 2016). Omethoate in the range of 0.1–0.5 µg/ml could also be investigated based on the same principle of the biosensor (Liu et al. 2020). The colorimetric aptasensors demonstrated the limit of detection for phorate at 10 µM (Bala et al. 2016) and for malathion at 1 pM (Bai et al. 2015).

The aptamer OO36F presented the lowest K_d at 0.1 µM and could apparently detect a minimum amount of DETP at 0.5 µM with the naked eye. However, none of our aptamers could work efficiently in the presence of DEDTP because it is a small molecule with a thiol group and hence could induce the aggregation of AuNPs before NaCl induction. Thus, the colorimetric assay to detect DETP is not specific when investigating exposure to disulfoton, ethion, phorate or terbufos, which are the parent compounds of both DETP and DEDTP. Hence, an application is still required that does not rely on the aggregation of AuNPs.

Electrochemical impedance spectroscopy

A thiol-modified aptamer was integrated with SPE to selectively detect DETP, DEDTP or DEP. The changes in Rct are presented in a Nyquist plot (Fig. 6a). The Rct on the surface of the aptamer coated with SPE increased when 0.025 µg/ml DETP, DEDTP or DEP was detected compared with MCH blocking. The percentage change in Rct was calculated using \( \frac{\text{Rct of target} - \text{Rct of MCH}}{\text{Rct of MCH}} \times 100 \), and the mean values are presented in Fig. 6b. The binding of DETP to the aptamer increased the Rct (± SD) to 47.5 ± 8.8%, which was significantly higher than the altered Rct of DEDTP at 14.3 ± 1.5% and of DEP at 7.0 ± 1.4%. The EIS results were consistent with docking scores and demonstrated that blocking the gold surface on the electrode by MCH could overcome the simultaneous aggregation of AuNPs caused by DEDTP during the colorimetric assay. Thus, the OO36F aptamer could be used to detect the metabolites of organophosphates with higher selective binding to DETP than DEDTP and DEP.

To achieve a calibration curve, different concentrations of DETP in PBS were measured using CV and EIS and then compared to HPLC. A linear decrease in the amplitude of voltammetric waves was obtained over the concentration range of 0.0001–10 µg/ml DETP (Fig. 7a). However, a linear increase in Rct was detected at the higher concentration of 0.01 µg/ml (Fig. 7b). Similarly, it has been reported that the CV technique was more suitable to detect capsaicin at low concentrations but EIS was more appropriate at higher concentrations (Randviir et al. 2013). The range of concentrations detected by the aptasensor was more sensitive than HPLC, which had an area of peak detectable at 0.1 µg/ml or higher. This range of DETP concentrations detected by HPLC covered the same linearity range of dialkyl phosphate concentrations previously investigated by LC–MS/MS over the concentration of 0.1–1 µg/ml (Fernandez et al. 2019). The LOD calculated from the CV measurement was 0.007 µg/ml.

The practicability of the aptasensor was assessed to detect DETP in a real sample that was simply prepared. DETP was spiked in a commercial guava juice or in the juice after dilution (1:20) in PBS at pH 3 or pH 7, respectively. The amount of DETP in guava juices and the recovery value were calculated using the calibration curve obtained from CV (Table 1). The measurement of the juice at pH 7 showed no interfering effect, with a recovery value of 97.2%. However, the changes in the amplitude of the sample at pH 3 surpassed the calibration curve. Thus, the recovery of pH 3 could not be calculated under the same conditions as the calibration curve because different pH values could affect the conformation of any aptamers. Due to the aim of using the current aptasensor on-site and preventing retention of the analytes during processing, neither centrifugation nor filtration were required during sample preparation. Thus, the recovery of HPLC techniques was not pertinent. The current measurement indicated the sensitivity of OO36F coupling with the EIS or CV method that can efficiently be used to detect DETP in real samples in the field.

Biosensors using EIS and CV have been used to detect organophosphates due to their sensitivity,
specificity and reproducibility. The aptasensors reported a linear range of chlorpyrifos at $0.1 \text{–} 105 \text{ ng/ml}$ and carbendazim at $0.01 \text{–} 10 \text{ ng/ml}$ (Eissa and Zourob 2017; Jiao et al. 2017). However, the current work unprecedentedly emphasized using aptasensors to detect the metabolite DETP, as this could provide the history of exposure of organophosphates that could be detoxified and transformed into dialkyl phosphates (Sudakin and Stone 2011). Furthermore, any other interferences in real samples, such as pigment/color and turbidity, should be investigated.

**Fig. 6** EIS responses of DETP, DEDTP and DEP. a Nyquist plots of responses of $0.025 \mu g/ml$ DETP (red diamond), DEDTP (green square) and DEP (blue triangle) and aptamer-coated electrodes that were blocked with MCH (gray circle). A modified Randles equivalent circuit was used to fit the impedance spectra and is presented in the inset. b Comparative aptasensor response presented as percentages of altered Rct of DETP, DEDTP and DEP. The average of 3 individual experiments is presented in the bar graph, and error bars indicate SD. The percentage of altered Rct was analyzed using one-way ANOVA with a significant difference from DETP at $p < 0.001$ (***).
Conclusion

Aptamers that specifically bind to DETP, a metabolite of organophosphates, were successfully generated using CE-SELEX. The selection of the highest-affinity aptamer for DETP was performed by observing the color change of AuNPs. OO36F had the lowest $K_d$ of $0.103 \pm 0.014 \mu M$, with a clear color change of the AuNPs observed with the naked eye at $0.5 \mu M$. The aptamer also selectively bound to DETP rather than other organophosphate metabolites, such as DEP and DMP. However, the colorimetric sensor using AuNPs coated with OO36F was not suitable for the detection of DEDTP, which is another metabolite of the same parent compound as DETP.

The thiol-modified OO36F aptamer was then used to fabricate an aptamer-based electrochemical sensor. The aptamer-immobilized gold electrode detected the change of $Rct$ or amplitudes when the aptamer was bound to targets on the gold surface that blocked nonspecific binding by any thiol processing molecules, especially DEDTP. DETP was detected at $0.025 \mu g/ml$ or $0.148 \mu M$ and had a significantly higher $Rct$ value than either DEDTP or DEP at the same concentration. The current aptasensor demonstrated high sensitivity to DETP by covering the linear range of concentrations at $0.0001–10 \mu g/ml$ with an LOD of $0.007 \mu g/ml$. However, HPLC had detectable concentrations equal to or higher than $0.1 \mu g/ml$ of DETP. When guava juices at pH 7 were prepared, this sensor had a recovery of $97.2\%$. These values are presented as

Table 1

| pH of guava juice | Response ($\Delta \mu A \pm SEM$) | Concentration of DETP ($\mu g/ml$) | Recovery value (%) |
|-------------------|----------------------------------|----------------------------------|-------------------|
| 3.3               | $-83.333 \pm 0.333$              | 1                                | Spiked            |
| 7.0               | $-39.545 \pm 0.026$              | 1 $0.972 \pm 0.002$              | Calculated        |

Fig. 7 Calibration curves and HPLC Chromatograms of DETP in PBS. Calibration curves of the DETP concentration vs. a the change of amplitude ($\mu A$), b the change of $Rct$ or c the peak area of the chromatogram. d HPLC chromatograms depicting the detectable concentration of DETP from 0.1 to $10 \mu g/ml$ with a retention time of 6.3 min, as shown in the dashed frame. A goodness of linear regression fit was measured and presented as R-squared.
results indicated the great potential to use the aptasensor to indicate the history of exposure of organophosphates by detecting trace amounts of the metabolite DETP, although biodegradation of organophosphates in the samples might occur.

Acknowledgements The authors would like to thank the Kasetsart University Research & Development Institute (KURDI/FF(KU)25.64) and International SciKU Branding (ISB), Faculty of Science, Kasetsart University for language editing services.

Funding This study is supported by the National Research Council of Thailand, Kasetsart University Research and Development Institute (grant no. KURDI (FF(KU) 25.64)), and the Omics Center for Agriculture, Bioresources, Food and Health, Kasetsart University (OmiKU).

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

References

Bai W, Zhu C, Liu J, Yan M, Yang S, Chen A (2015) Gold nanoparticle-based colorimetric aptasensor for rapid detection of six organophosphorous pesticides. Environ Toxicol Chem 34:2244–2249

Bala R, Sharma RK, Wangoo N (2016) Development of gold nanoparticles-based aptasensor for the colorimetric detection of organophosphorous pesticide phorate. Anal Bioanal Chem 408:333–338

Eissa S, Zourobor M (2017) Selection and characterization of DNA aptamers for electrochemical biosensing of carbendazim. Anal Chem 89:3138–3145

Fernandez SF, Pastor A, Yusa V, Montesinos L, Pardo O (2019) Development of a novel methodology for determination of dialkyl phosphates in human urine using liquid chromatography-tandem mass spectrometry. J Chromatogr B Analys Technol Biomed Life Sci 1130–1131:121810

Jiao Y, Hou W, Fu J, Guo Y, Sun X, Wang X, Zhao J (2017) A nanostructured electrochemical aptasensor for highly sensitive detection of chlorpyrifos. Sens Actuators, B Chem 243:1164–1170

Kongtig P et al (2017) The impact of prenatal organophosphate pesticide exposures on Thai infant neurodevelopment. Int J Environ Res Public Health. https://doi.org/10.3390/ijerph14060570

Liu DL, Li Y, Sun R, Xu JY, Chen Y, Sun CY (2020) Colorimetric detection of organophosphorus pesticides based on the broad-spectrum aptamer. J Nanosci Nanotechnol 20:2114–2121

Lu C, Bravo R, Calabianio LM, Irish RM, Weerasekera G, Barr DB (2005) The presence of dialkylphosphates in fresh fruit juices: implication for organophosphorus pesticide exposure and risk assessments. J Toxicol Environ Health A 68:209–227

Mendonsa SD, Bowser MT (2004) In vitro evolution of functional DNA using capillary electrophoresis. J Am Chem Soc 126:20–21

Ohshiro K, Kakuta T, Sakai T, Hirota H, Hoshino T, Uchiyama T (1996) Biodegradation of organophosphorus insecticides by bacteria isolated from turf green soil. J Ferment Bioeng 82:299–305

Randviir EP, Metters JP, Stainton J, Banks CE (2013) Electrochemical impedance spectroscopy versus cyclic voltammetry for the electroanalytical sensing of capsaicin utilising screen printed carbon nanotube electrodes. Analyst 138(10):2970–2981. https://doi.org/10.1039/c3an00368j

Selvolini G, Bajan I, Hosu O, Cristea C, Sandulescu R, Marrazza G (2018) DNA-based sensor for the detection of an organophosphorus pesticide. https://doi.org/10.3390/s18072035

Simaremare SRS, Hung CC, Hsieh CJ, Yiin LM (2019) Relationship between organophosphate and pyrethroid insecticides in blood and their metabolites in urine: a pilot study. Int J Environ Res Public Health. https://doi.org/10.3390/ijerph17010034

Sudakin DL, Stone DL (2011) Dialkyl phosphates as biomarkers of organophosphates: the current divide between epidemiology and clinical toxicology. Clin Toxicol (Phila) 49:771–781

Tian Y, Wang Y, Sheng Z, Li T, Li X (2016) A colorimetric detection method of pesticide acetamiprid by fine-tuning aptamer length. Anal Biochem 513:87–92

Wang L, Liu X, Zhang Q, Zhang C, Liu Y, Tu K, Tu J (2012) Selection of DNA aptamers that bind to four organophosphorus pesticides. Biotechnol Lett 34:869–874

Zhang J et al (2020) An interdigitated microelectrode based aptasensor for real-time and ultratrace detection of four organophosphorus pesticides. Biosens Bioelectron 150:111879

Zhang X, Driver JH, Li Y, Ross JH, Krieger RI (2008) Dialkylphosphates (DAPs) in fruits and vegetables may confound biomonitoring in organophosphorus insecticide exposure and risk assessment. J Agric Food Chem 56:10638–10645

Zhou J, Battig MR, Wang Y (2010) Aptamer-based molecular recognition for biosensor development. Anal Bioanal Chem 398:2471–2480

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.