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Article

Interaction of Organophosphorus Pesticides with DNA Nucleotides on a Boron-Doped Diamond Electrode

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Eletrodo de diamante foi usado para avaliar a interação dos nucleotídeos guanosina monofosfato (GMP) e adenosina monofosfato (AMP) com os pesticidas clorpirifós, metamidofós e monocrotofós. Observaram-se alterações nas correntes e potenciais de picos dos voltamogramas dos nucleotídeos na presença dos pesticidas dependendo da concentração de pesticida (de $5{,}0 \times 10^{-7}$ a $5{,}0 \times 10^{-5}$ mol L^{-1}) e do tempo de interação (de 1 min a 4 h). Isto é provavelmente devido à ligação dos pesticidas às bases nitrogenadas presentes nos nucleotídeos, o que poderia levar a problemas na replicação do DNA e nas funções biológicas de nucleotídeos. Os pesticidas apresentaram maior interação com AMP que com GMP. Estudos sobre a interação de DNA 50 μg mL^{-1} com os pesticidas (de 30 min até 4 h, e de $1{,}0 \times 10^{-6}$ a $6{,}0 \times 10^{-5}$ mol L^{-1}) não revelaram picos relativos à abertura da dupla hélice ou desenrolamento do DNA.

Diamond electrode was used to evaluate the interaction of the nucleotides guanosine monophosphate (GMP) and adenosine monophosphate (AMP) with the pesticides chlorpyrifos, methamidophos and monocrotophos. Changes were observed in the currents and peak potentials of the nucleotide voltammograms in the presence of the pesticides, with dependence on the pesticide concentration (from 5.0×10^{-7} to 5.0×10^{-5} mol L^{-1}) and the interaction time (from 1 min to 4 h). This is probably due to binding of the pesticides to the nitrogenous bases present in the nucleotides, which could lead to problems in the DNA replication and biological functions of nucleotides. The pesticides showed stronger interaction with AMP than with GMP. Studies of the interaction of 50 μg mL^{-1} DNA with the pesticides (from 30 min to 4 h and from 1.0×10^{-6} to 6.0×10^{-5} mol L^{-1}) did not reveal any peaks relating to double helix opening or DNA unwinding.

Keywords: organophosphorus pesticides, DNA nucleotides, diamond electrode, DNA damage, binding molecules

Introduction

Environmental pollution caused by pesticides and their metabolites is a major ecological and health problem. Residues of these compounds can be present in natural waters, soils, vegetables, and fruits,[1,2] resulting in human exposure.[3] An important group of pesticides currently and widely used for insect control in Brazilian agriculture[4] are the organophosphorus (OP) compounds methamidophos (MET), monocrotophos (MON) and chlorpyrifos (CPF) (Figure 1). In developing countries, the concerns regarding these pesticides arise from a general ignorance of the hazards, and inadequate provision and use of protective clothing, resulting in death and illness among agricultural workers. Contamination of the body with these toxic substances can occur following ingestion, inhalation and skin contact, and the compounds can subsequently accumulate in adipose layers such as breast tissue.[5]

Organophosphorus pesticides interfere in the cellular communication mediated by the acetylcholine molecule. This transmission between cells cannot occur when acetylcholine is not destroyed after performing its function. OP compounds selectively bind to the enzyme (acetylcholinesterase) that acts to destroy acetylcholine. Hence, blocking its action and suppressing the pulse transmission between nerve cells that is essential for coordinating the body vital processes, leading to death.[6] Some OP pesticides can inhibit another esterase, called

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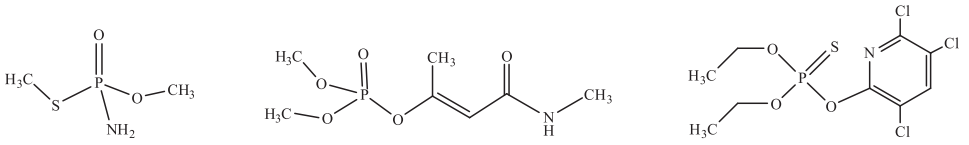

Figure 1. Chemical structures of methamidophos (MET), monocrotophos (MON) and chlorpyrifos (CPF).

the neuropathy target esterase, causing a delayed effect known as organophosphorus-induced delayed neuropathy.[7] In a Review, Koureas *et al*.[8] described the negative effects of prenatal exposure to OP pesticides on neurodevelopment and the male reproductive system. Neurologic effects in adults, DNA damage and adverse birth outcomes have also been associated with exposure to OP pesticides.[8-10] In addition, these species may contribute to childhood brain tumors, leukemia and lymphomas, and may also act as liver and respiratory system toxins.[8]

Damage to DNA and/or its structural components (nitrogenous bases, nucleosides and nucleotides) caused by toxic compounds including OP pesticides[5,11] can hinder processes of transcription and replication, resulting in arrest of the cell cycle, cell death and mutations,[12] and defects in DNA can lead to carcinogenesis and premature aging.[13,14] DNA damage caused by CPF,[15-18] MET[19] and MON[20-22] is normally evaluated using the comet assay, which is based on the ability of cleaved and denatured DNA fragments to migrate out of the cell under the influence of an electric field.[23]

The guanosine (GMP) and adenosine (AMP) 5'-monophosphate DNA nucleotides have important biological functions in the body. GMP plays a crucial role in many cellular mechanisms related to metabolism and cardiac activity,[24] while AMP performs important functions in oxidative phosphorylation and trans-membrane signaling processes.[25] Changes in the chemical structures and/or decreased availability of these nucleotides in the body can affect their biological functions, and both effects can result from interaction with OP pesticides.

Electrochemical methods have proved to be highly suitable for use in studies of the interactions of low molecular mass molecules with DNA and its structural components, as well as for detecting DNA damage.[26,27] The electrochemical responses of double stranded (ds) DNA and nucleotides (in terms of current intensity and peak potential) can be modified by the presence of various molecules. It is therefore possible to investigate structural alterations in the double helix of DNA arising from covalent or non-covalent interactions with genotoxic substances, and the possible binding of these compounds with the nucleotides. Nowicka *et al*.[28] used voltammetric and nanogravimetric DNA biosensors to study the toxicity and DNA damage caused by pesticides. The OP pesticide paraoxon-ethyl caused the fastest and most severe damage to nucleic acid. DNA damage caused by carbaril,[29] metals,[30] bisphenol A radicals,[31] diclofenac,[32] triazines,[33] benzopyrene,[34] fluorene nitro derivatives[35] and dyes[36] has been evaluated using modified glassy carbon, carbon paste and graphite electrodes to detect changes in the electrochemical responses of DNA and/or the active agents. A variety of different electrodes have been used to study the interaction of DNA and its components, in solution, with temozolomide,[37] codeine and morphine,[38] Sudan II,[39] calcein,[40] nicotine,[41] actinomycin D[42] and the alkylating agent 4,4-dihydroxy chalcone.[43] This type of investigation can help to identify preferential sites of interaction. For example, variations in the voltammetric signals of guanine and adenine can occur in the presence of active chemical agents when the electroactive sites of the bases are exposed to oxidation due to the breakage or unwinding of double-stranded DNA.[27,44,45] The detection of nucleotides in aqueous solution enables evaluation of the interaction and/or binding of chemical species to the components of the DNA.

In our previous work,[46] detection of the individual or combined DNA nucleotides GMP and AMP using square wave voltammetry (SWV) was performed using boron-doped diamond (BDD) electrodes that had been cathodically pretreated. In the present work, the same voltammetric conditions[46] were used to measure the electrochemical responses of GMP and AMP after interaction with OP pesticides. BDD electrodes are widely used in electroanalysis because they present a very low background current, a wide potential window due to the chemical inertness of the diamond surface, mechanical robustness and compatibility with biological materials.[47-49] Cathodic pretreatment of these electrodes is a simple and effective way of generating highly electrochemically active BDD surfaces, resulting in very low limits of quantification and high data reproducibility.[50,51]

Since OP pesticides can interact with the structural components of DNA, with potentially serious impacts on human health, the objective of this study was to investigate these interactions using a cathodically pretreated BDD electrode. As far as is known, this is the first time that an electrochemical technique employing a diamond electrode is used to explore the binding of OP pesticides with DNA nucleotides.

Experimental

Reagents and solutions

GMP (> 99%), AMP (≥ 99%), double-stranded calf thymus DNA, and the pesticides CPF (99.9%), MET (98.4%) and MON (99.9%) were purchased from Sigma-Aldrich. Stock solutions of the nucleotides (1.0×10^{-2} mol L^{-1}) and the pesticides MET and MON (1.0×10^{-3} mol L^{-1}) were prepared in pure Milli-Q water ($\rho = 18.2\ M\Omega$ cm). The CPF stock solution (1.0×10^{-3} mol L^{-1}) was prepared in pure methanol (100%). The stock solution of DNA in pure water (5.0 mg mL^{-1}) was stored at 4 °C for 24 h to ensure complete dissolution of the nucleic acid. The nucleotide (monomer units) concentration was calculated using the molar absorption coefficient at 260 nm ($\epsilon_{260} = 6600$ L mol^{-1} cm^{-1}). The ratio of the DNA absorbance intensities at 260 and 280 nm was 1.88, indicating that the DNA was free from protein.[36] For all electrochemical measurements (during the pesticide interaction studies), a pH 7.0 Britton-Robinson (BR) buffer solution (0.1 mol L^{-1}), prepared with analytical grade reagents, was used as the supporting electrolyte.

Apparatus

The electrochemical experiments were performed using the AUTOLAB PGSTAT 30 and GPES 4.9 software packages (Eco Chemie, The Netherlands). The pH of the 0.1 mol L^{-1} BR buffer solution was measured with a pH-meter (Model MPA-210A, Tecnopon), calibrated daily with commercial buffer solutions (pH 4, 7 and 10). The voltammetric studies were carried out using a three-electrode arrangement fitted into a single compartment Pyrex® glass cell (20 mL). The BDD films (Adamant Technologies S.A., La Chaux-de-Fonds, Switzerland) contained 8000 ppm of boron and had a working electrode area of 0.1 cm^2. The reference system was an Ag/AgCl (3.0 mol L^{-1} KCl) electrode, and the counter electrode was a 1 cm^2 Pt foil.

Methodology

GMP and AMP voltammetric signals

The electroanalytical measurements were performed using 0.1 mol L^{-1} BR buffer solution (pH 7.0) containing an equimolar mixture of the GMP and AMP nucleotides (5.0×10^{-4} mol L^{-1}). The SWV conditions were 100 Hz frequency (f), 50 mV amplitude (a) and a step potential (ΔE_s) of 2 mV. The BDD electrode was cathodically pretreated in a 0.5 mol L^{-1} H_2SO_4 solution, applying –3.0 V *vs.* Ag/AgCl for 30 s. The square wave voltammograms were obtained after intermediate cleaning of the electrode surface with pure water, and after surface reactivations by application of an anodic and cathodic pretreatment. The latter consisted of polarizations at +3.0 and –3.0 V *vs.* Ag/AgCl for 5 and 30 s, respectively, in a 0.5 mol L^{-1} H_2SO_4 solution. All the experimental curves were baseline-corrected by application of the moving average with a step window of 5 mV (included in the GPES version 4.9 software).[36,46,52]

Interaction of pesticides with nucleotides in solution

Baseline-corrected square wave voltammograms (f = 100 Hz, a = 50 mV and ΔE_s = 2 mV) were obtained using the cathodically pretreated BDD electrode in 0.1 mol L^{-1} BR buffer solution (pH 7.0). Measurements were made of solutions containing (*i*) individual pesticides at 5.0×10^{-5} mol L^{-1}, (*ii*) an equimolar 5.0×10^{-4} mol L^{-1} mixture of the nucleotides, (*iii*) a mixture of GMP and AMP (both at 5.0×10^{-4} mol L^{-1}) together with each pesticide at 5.0×10^{-5} mol L^{-1}, with different interaction times (1, 10, 30 min, 1, 2, 3 and 4 h), and (*iv*) a mixture of GMP and AMP (both at 5.0×10^{-4} mol L^{-1}) together with different concentrations of each pesticide (from 5.0×10^{-7} to 5.0×10^{-5} mol L^{-1}). The signals were expressed as the relative response, I/I_0, where I_0 is the AMP peak current obtained in an experiment performed prior to the interaction.

Interaction of pesticides with dsDNA in solution

Baseline-corrected square wave voltammograms (f = 100 Hz, a = 50 mV and ΔE_s = 2 mV) were obtained using the cathodically pretreated BDD electrode in 0.1 mol L^{-1} BR buffer solution (pH 7.0). Measurements were made of solutions containing (*i*) individual pesticides at 5.0×10^{-5} mol L^{-1}, (*ii*) dsDNA at 50 μg mL^{-1}, (*iii*) dsDNA (50 μg mL^{-1}) together with 5.0×10^{-5} mol L^{-1} of each pesticide, with different interaction times of 30 min, 1, 2, 3 and 4 h, and (*iv*) dsDNA (50 μg mL^{-1}) together with different concentrations of each pesticide (from 1.0×10^{-6} to 6.0×10^{-5} mol L^{-1}).

Results and Discussion

GMP and AMP electrochemical signals

Baseline-corrected square wave voltammograms obtained using the BDD electrode in 0.1 mol L^{-1} BR buffer solution (pH 7.0), in the absence and presence of the nucleotides (equimolar mixture at 5.0×10^{-4} mol L^{-1}), are presented in Figure 2. Two irreversible well-defined peaks were observed: at 1.04 V *vs.* Ag/AgCl, reflecting an oxidation

process involving GMP, and at 1.43 V *vs.* Ag/AgCl, which was related to the AMP process. It is known that the processes of electroxidation of GMP and AMP occur in the structures of the nitrogenous bases and that the sugar and phosphate are not electroactive.[53-55] The oxidation processes for the nucleotides show potential values that are higher than those for the bases alone, due to the inductive effect caused by the glycosidic bond in the purine ring, which makes it more difficult to remove the electrons from the bases.[56]

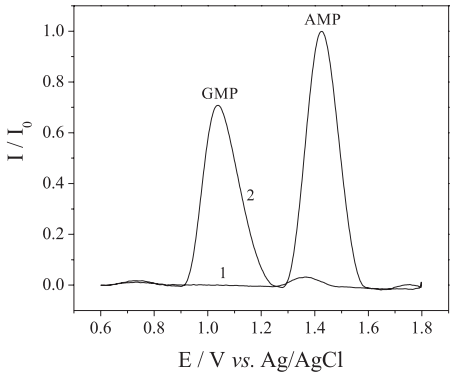

Figure 2. Baseline-corrected square wave voltammograms obtained using the BDD electrode in 0.1 mol L^{-1} BR buffer solution (pH 7.0) in the absence (1) and presence (2) of an equimolar 5.0×10^{-4} mol L^{-1} mixture of the GMP and AMP nucleotides.

An evaluation was made of the effect on the nucleotide voltammetric responses caused by the intermediate cleaning of the electrode surface (by exhaustive rinsing with pure water). There was a reduction in the response, of about 30%, for both the GMP and the AMP signal (scan 4 *vs.* scan 1), due to strong adsorption (or inefficient solubilization) of the reagents or oxidation products on the BDD surface. Electrochemical pre-treatments of the BDD electrode in a 0.5 mol L^{-1} H_2SO_4 solution, at +3.0 and –3.0 V for 5 and 30 s, respectively, were therefore carried out between the electrochemical measurements of the nucleotide mixture. Similar voltammetric responses (n = 4) of the analytes (relative standard deviation (RSD) lower than 5% for GMP and AMP peak currents) were observed after the electrochemical reactivation of the surface. Hence, throughout the pesticide-nucleotide interaction studies, all the voltammetric scans were obtained with a BDD surface that had been satisfactorily reactivated by anodic and cathodic pre-treatment.

Pesticide-nucleotide interactions

The OP pesticides studied here did not present voltammetric responses in the potential range from 0.6 to 2.0 V *vs.* Ag/AgCl, under the conditions employed (0.1 mol L^{-1} BR buffer solution at pH 7.0, f = 100 Hz, a = 50 mV and ΔE_s = 2 mV). The interaction of the pesticides with the DNA nucleotides was first investigated using concentrations of the individual pesticides ranging from 5.0×10^{-7} to 5.0×10^{-5} mol L^{-1}, which are representative of levels of OP residues found in the environment and can cause effects in animals and humans. Figure 3 compares the GMP and AMP voltammograms obtained before and after interaction for 1 min with the pesticides at the maximum concentration analyzed.

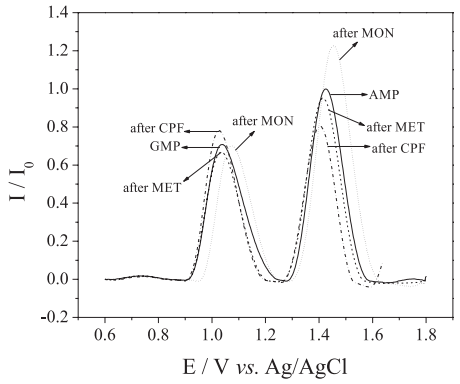

Figure 3. Baseline-corrected square wave voltammograms obtained using the BDD electrode in 0.1 mol L^{-1} BR buffer solution (pH 7.0) containing an equimolar 5.0×10^{-4} mol L^{-1} mixture of the GMP and AMP nucleotides, without interaction and after an interaction time of 1 min in 5.0×10^{-5} mol L^{-1} methamidophos (MET), monocrotophos (MON) and chlorpyrifos (CPF).

The voltammetric response for GMP slightly decreased (by 5.7%) and increased (by 9.8%) after interaction with MET and CPF, respectively (Figure 3). No change occurred in the peak potential after interaction with MET, but a shift of 11 mV towards more negative values was observed after interaction with CPF. The peak current intensity of the GMP signal remained unchanged after interaction with MON, although a peak potential shift of 34 mV towards more positive values was observed. The voltammetric response for AMP decreased after interaction with both MET (by 5%) and CPF (by 20%), and there were shifts of 11 and 22 mV in the peak potential, towards less positive values, after interaction with MET and CPF, respectively. Meanwhile, the AMP signal increased (by 22%) after interaction with MON, and there was a positive displacement of 29 mV in the peak potential.

Figure 4 shows the variations of the nucleotide oxidation current intensities after 1 min of interaction, as a function of OP pesticide concentration. No new voltammetric signals were detected after the pesticide-nucleotide interactions.

Similar profiles of current changes in the presence of individual pesticides were observed for the two nucleotides. For MET concentrations from 5.0×10^{-7} to 1.0×10^{-5} mol L^{-1}, there was a slight increase followed by a decrease of

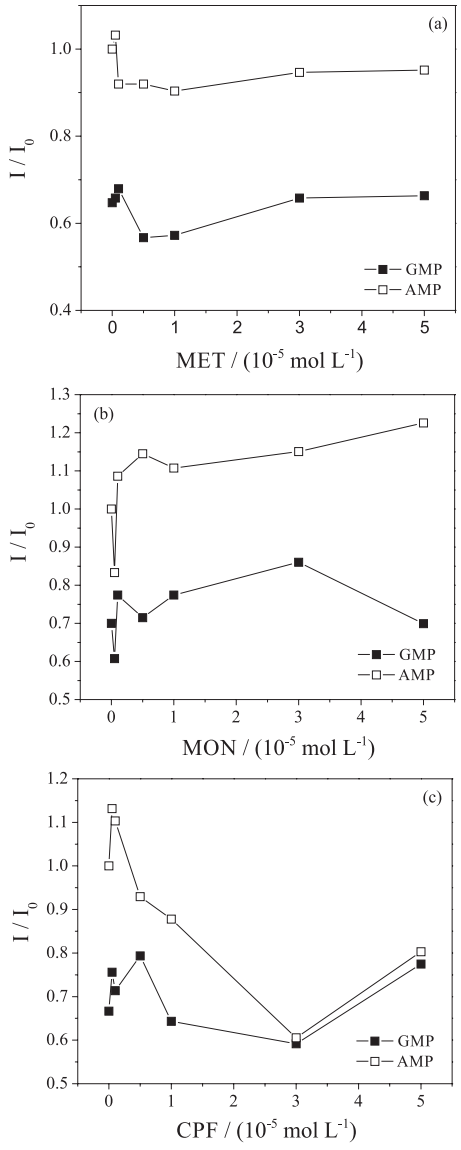

Figure 4. Behavior of the GMP and AMP (equimolar 5.0×10^{-4} mol L^{-1} mixture) current peaks according to the concentration of (a) methamidophos, (b) monocrotophos and (c) chlorpyrifos. Interaction time of 1 min.

the nucleotide currents (Figure 4a). Increases of the nucleotide currents (of 15.9 and 5.42% for the GMP and AMP signals, respectively) were observed for pesticide concentrations from 1.0×10^{-5} to 5.0×10^{-5} mol L^{-1}. In the case of the pesticide MON (Figure 4b), at a concentration of 5.0×10^{-7} mol L^{-1}, the current intensities decreased by 13.3% (GMP) and 16.7% (AMP). There was then an increase in the current values up to 3.0×10^{-5} mol L^{-1} of the pesticide, with the GMP and AMP signals increasing from their original values by 22.8 and 15.0%, respectively. In the presence of 5.0×10^{-5} mol L^{-1} MON, the AMP peak intensity showed a further increase, while the GMP signal presented a significant decrease in intensity. For the pesticide CPF (Figure 4c), increases of around 13% in the responses for both nucleotides were observed for a 5.0×10^{-7} mol L^{-1} pesticide concentration, after which there were decreases of 21.7 and 46.5% for GMP and AMP, respectively (comparing the current intensities at 3.0×10^{-5} and 5.0×10^{-7} mol L^{-1} of pesticide). At the maximum pesticide concentration, the nucleotide peaks presented an increase in intensity of around 30-35%, compared to the current values at 3.0×10^{-5} mol L^{-1}.

The interaction of individual pesticides at the maximum concentration of 5.0×10^{-5} mol L^{-1} with the DNA nucleotides (at concentrations of 5.0×10^{-4} mol L^{-1}) was analyzed for different interaction times between 1 min and 4 h. The behavior of the normalized nucleotide currents according to time of interaction with the pesticides is presented in Figure 5.

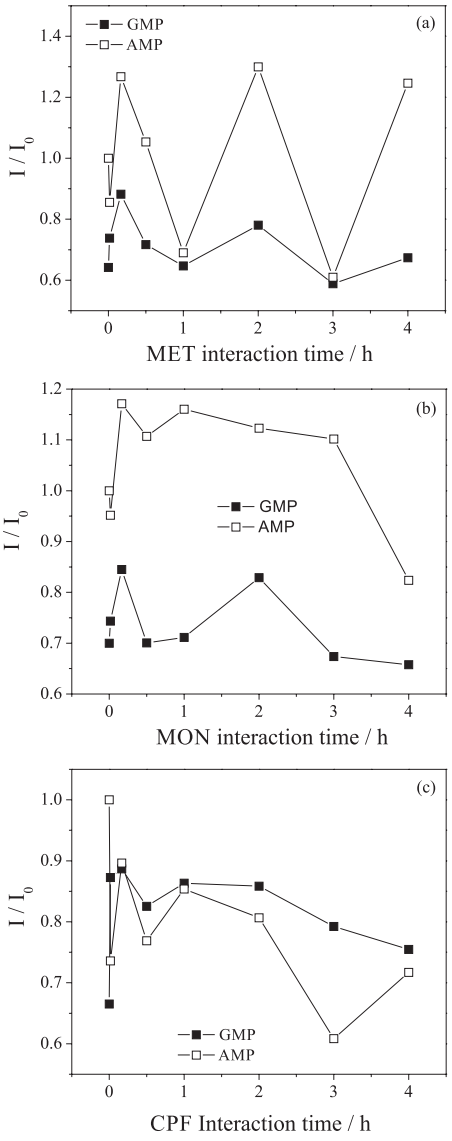

Figure 5. Behavior of the GMP and AMP (equimolar 5.0×10^{-4} mol L^{-1} mixture) current peaks according to the time of interaction with (a) methamidophos, (b) monocrotophos and (c) chlorpyrifos. Each pesticide at 5.0×10^{-5} mol L^{-1}.

The interaction between MET and the nucleotides showed a complex behavior, with alternating increases and decreases of the current intensity throughout the time period employed (Figure 5a). Nevertheless, the temporal trends of I/I_0 were similar for both nucleotides. At 4 h, the GMP and AMP voltammetric signals were 5.0 and 24.6% higher, respectively, compared to the initial values. After interaction with MON for 10 min, the GMP response increased by 20.7%, compared to the initial current value (Figure 5b). During the same time period (0-10 min), there was a slight decrease of the current intensity for the AMP response, followed by a significant increase of 23.0%. Between 10 min and 4 h, there were alternating decreases and increases of the current intensity, especially in the case of the GMP signal. Between 1 and 4 h of interaction with MON, there was a 29.0% diminution of the AMP current intensity. For the pesticide CPF (Figure 5c), the GMP signal increased significantly by 31.1% after 1 min of interaction, and between 1 min and 1 h, there were alternating increases and decreases of the GMP current intensity. Between 1 and 4 h, the GMP peak intensity decreased continuously, with a final difference of 12.6% in the current value. Alternating changes in the AMP signal were also observed between 1 min and 1 h (Figure 5c), similar to the GMP peak. From 1 to 3 h, there was a clear decrease of the AMP peak intensity, with a current value after 3 h of interaction with CPF that was 28.7% lower than the value measured after 1 h of interaction. Between 3 and 4 h, an increase of the AMP peak intensity occurred, in contrast to the continuous decrease in the GMP peak intensity. Three replicate measurements (for each point shown in Figures 4 and 5) were made in the experiments investigating the effects of pesticide concentration and interaction time. RSD was calculated for all points, and was always lower than 5%.

The results of interaction of the three OP pesticides with the DNA nucleotides revealed changes in the intensities of the GMP and AMP voltammetric signals according to both the pesticide concentration and the interaction time. In some cases, displacements of the nucleotide peak potentials were detected after interaction with the compounds, especially MON. The changes in the nucleotide current values may have been due to binding of the OP pesticides to the nitrogenous bases present in the nucleotide structures. A decrease in the voltammetric signal could be explained by possible damage or shielding of the oxidizable groups of guanine and adenine following interaction between the nucleotides and the pesticides.[27,35] On the other hand, the interaction of the pesticides with GMP and AMP could lead to exposure of the electroactive sites of guanine and adenine to the electrode surface, increasing the current intensities. Such interactions could affect the structure of the nucleotides, leading to a decrease of their availability in the body, hence influencing their biological functions. The electrochemical results obtained here suggested a preference of the OP pesticides for adenine sites since there were greater changes in the AMP signal after the interactions compared to the GMP response.

Alkylating agents such as OP compounds are known to be able to cause DNA damage,[57] and alkylation of nitrogenous bases can occur either directly or indirectly via protein alkylation.[19,20,22] Most genotoxins are either intrinsically electrophilic or can be activated to produce electrophilic intermediates that bind to critical macromolecules.[20,22] The heterocyclic nitrogenous bases of DNA can act as nucleophilic agents for such a reaction, and alkylation can take place on nitrogen atoms possessing high electron density.[20,22] According to Wild,[57] the phosphorus moiety in the organophosphorus compound appears to be a good substrate for nucleophilic attack, which can lead to DNA damage by phosphorylation. The results presented here indicate that OP pesticides can undergo nucleophilic attack by the nitrogenous bases present in the nucleotide structures, and that this reaction is involved in DNA damage. It is therefore likely that OP pesticides can modify DNA bases by alkylation.

Despite the fact that they belong to the same chemical class, the pesticides showed varying degrees of interaction with the DNA nucleotides, which was probably related to the different chemical structures of the compounds. Methamidophos (*O*,*S*-dimethyl phosphoramidothioate) presented less interaction with the nucleotides, compared to chlorpyrifos and monocrotophos (Figures 3 and 4), with the degree of interaction decreasing in the order MON > CPF > MET. According to Yaduvanshi *et al.*,[22] alkylation is more likely with the methyl ester group than with the ethyl or higher alkyl ester groups, and phosphate esters such as monocrotophos are more reactive than phosphorothionate esters such as chlorpyrifos.

OP pesticides-dsDNA interaction

In order to obtain further information concerning the interaction of the pesticides with the nucleotides inside the DNA double helix, electroanalytical investigation of the OP pesticides-dsDNA interactions was also carried out in the solution phase. Calf thymus DNA, consisting of 41.9 mol% guanine-cytosine and 58.1 mol% adenine-thymine, is a natural DNA that is widely used in studies of the binding of anti-cancer agents or pollutants that affect the structure and function of DNA, and is also used in physicochemical studies of the behavior of these substances in solution.

Firstly, square wave voltammograms (f = 100 Hz, a = 50 mV and ΔE_s = 2 mV) were obtained using the BDD electrode in 0.1 mol L^{-1} BR buffer solution (pH 7.0) containing 50 μg mL^{-1} of calf thymus dsDNA. No oxidation peaks were detected due to the limited accessibility of the nitrogenous base residues contained in nucleotides within the rigid structure of the DNA double helix.[58]

The occurrence of oxidative lesions in dsDNA caused by active compounds lead to the breaking of hydrogen bonds and opening of the double helix (or DNA cleavage), allowing the bases to come into contact with the electrode surface. The electrochemical detection of this oxidative damage can be accomplished by monitoring the oxidation of bases.[58] The interaction of the OP pesticides with calf thymus dsDNA was therefore performed using the cathodically pretreated BDD electrode, with different DNA-pesticide interaction times (from 30 min to 4 h) and different pesticide concentrations (from 1.0×10^{-6} to 6.0×10^{-5} mol L^{-1}). A voltammetric peak related to oxidative damage, opening of the double helix or unwinding of the DNA was detected in no case.

Although no DNA oxidation peaks were detected, this does not confirm that interaction did not occur. In future work, the interaction of the biomolecule with the pesticides could be performed following immobilization of DNA on the BDD electrode, hence facilitating detection of the guanine and adenine moieties and providing further information concerning the binding behavior.

Conclusions

Voltammetric measurements using a BDD electrode showed that the OP pesticides chlorpyrifos, methamidophos and monocrotophos, which are widely used in agriculture, interacted with the DNA nucleotides guanosine monophosphate (GMP) and adenosine monophosphate (AMP). Changes were detected in the oxidation currents and peak potentials of both nucleotides after interaction with the pesticides, especially in the case of AMP. The interactions could be explained by the binding of the pesticides to nitrogenous bases present in the nucleotides. Consequently, there could be changes in the chemical structures of the nucleotides, which could lead to difficulty in DNA replication and a decrease in the concentrations of the nucleotides available in the body, hence affecting their biological functions. On the other hand, no oxidation peaks related to oxidative damage, opening of the double helix or DNA unwinding were detected in studies of the interactions of these pesticides with calf thymus dsDNA, which were also performed using the BDD electrode. Electrochemical measurements employing BDD electrodes could help to identify the preferential sites for binding of toxic compounds to DNA and its component nucleotides.

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