Vibrational Spectroscopy 87 (2016) 27–33

Contents lists available at ScienceDirect

Vibrational Spectroscopy

journal homepage: www.elsevier.com/locate/vibspec

Enhanced Raman spectroscopy coupled to chemometrics for identification and quantification of acetylcholinesterase inhibitors

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ARTICLE INFO

Article history:
Received 15 March 2016
Received in revised form 7 September 2016
Accepted 8 September 2016
Available online xxx

Keywords:
Surface-enhanced Raman spectroscopy
Biosensor
Pesticides
Gold nanoparticles
Acetylcholinesterase

ABSTRACT

In this work, we present a new complete method using Surface Enhanced Raman Spectroscopy (SERS) and chemometrics for the qualitative and quantitative detection of pesticides by measuring the acetylcholinesterase (ACHE) activity. The Raman SERS is not only used for measuring the ACHE activity, but also for the direct detection of pesticides individually and for their identification. Gold nanoparticles (AuNPs) were used as dynamic SERS substrates for sensitive monitoring of ACHE activity in the presence of very low levels of organophosphate and carbamate pesticides, chemical warfare agents that are known to be ACHE inhibitors. The lowest detectable level for paraoxon was determined at 4.0×10^{-14} M and 1.9×10^{-9} M for carbaryl. The use of the enzyme allowed limits of detection for both pesticides that were much lower than the limits obtained by direct SERS analysis of the pesticides. The system shows a linear relationship between the intensity band at 639 cm^{-1} and pesticide concentration. These results suggest that this biosensor could be used in the future for the non-selective detection of all ACHE inhibitors at very low concentrations with possible identification of the inhibitor.

1. Introduction

Organophosphates and carbamates are well-known chemical compounds used in agriculture as insecticides because of their high toxicology properties [1]. Unfortunately this toxicity is not limited to the unique species to be eliminated. They are particularly toxic to humans and animals in connection to their high toxicity to acetylcholinesterase (ACHE) [2]. Indeed, the inhibition of ACHE activity by pesticides can lead to a loss of memory and an impairment of neuromuscular functions [3,4] and finally possible death. ACHE is one important enzyme in the brain. It hydrolyzes the neurotransmitter acetylcholine (ATC) to choline and acetic acid [5,6]. Therefore ACHE activity can be used as a biomarker for this pesticide exposure [7,8].

There are already techniques for the detection of ACHE inhibitors including gas or liquid chromatography and mass spectrometry [9–11]. However the reported techniques involve delicate and complicated sample pretreatment processes. Additionally most of them are specific to each pollutant and they usually require long times of analysis. Various biosensing techniques based on cholinesterase enzyme activity [12] and different transduction technologies (electrochemistry, fluorescence, colorimetric probes etc.) [13,14] were also designed to provide rapid, simple, and selective techniques for toxicity monitoring in environmental, agricultural, food or military applications. However these techniques show several disadvantages, such as poor selectivity, complexity for preparation, time-consuming, and finally the lack of stability of these techniques limits their applications.

In recent years, Surface Enhanced Raman Spectroscopy (SERS) has been developed for many applications such as identification and detection of many types of analytes [15,16]. SERS corresponds to the vibrational Raman signature associated to an important increase of intensity, through the association of the analyte with metallic (such as gold or silver) nano-surface or nanoparticles. This technique, due to its sensitivity, has been implemented in many researches areas such as biology, material science, art, and analytical chemistry [17,18].

The aim of this study is to provide a tool for the fast and sensitive detection of pesticides, by combining the inhibition mechanism of an enzyme for the non selective detection with the Raman SERS for high sensitivity, low cost, simplicity, rapidity, qualitative and quantitative information. Raman SERS was already proposed for detection of cholinesterase inhibitors by ACHE

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http://dx.doi.org/10.1016/j.vibspec.2016.09.005
0924-2031/

activity monitoring using silver nanoparticles to amplify the Raman signal [19]. In this study, a very fast and simple analytical procedure using gold nanoparticles (AuNPs) as active SERS substrates, without any functionalization step, was developed allowing a significant lower limit of detection (LOD) than other SERS studies. Moreover, besides inhibitors quantification, the use of chemometrics allowed the identification of the inhibitor. This detection strategy was tested to quantify and identify paraoxon as organophosphate and carbaryl as carbamate.

2. Materials and methods

2.1. Chemicals

Acetylcholinesterase (ACHE) coming from *Electrophorus electricus* (500U/mg) and acetylcholine chloride (ATCCl) were purchased from Sigma-Aldrich. Phosphate Buffer (PB) was prepared at pH = 8 as a mixture of KH_2PO_4 (1 M) and K_2HPO_4 (1 M). $HAuCl_4$, carbaryl and paraoxon were purchased from Sigma Aldrich. ATCCl was purchased in solid state and was solubilized in PB solution where it gives ATC^+ which is the biologically active form of ATC [20]. ATC (1 mM) is then added to the ACHE solution as substrate for activity measurements. All aqueous solutions have been prepared using deionized water.

2.2. Synthesis of gold nanoparticles

Citrate reduced gold colloids were prepared according to a well-known process (see for instance reference [21]). Briefly, AuNPs were obtained by mixing 500 ml of 0.01% HAuCl4, boiled under stirring in a round bottom flask equipped with a condenser, and 7.5 ml of 1% sodium citrate. After 20 min, the mixture was extracted and cooled to room temperature.

2.3. Inhibition of ACHE by paraoxon and carbaryl

ACHE (5 μl of 1 $U.ml^{-1}$) was incubated with 5 μl of varying concentrations of paraoxon (from 4×10^{-5} to 4×10^{-15} M) and carbaryl (from 4×10^{-5} to 4×10^{-10} M) in 0.01 M PB (pH 8) at room temperature. Next, 5 μl of 1 mM of ATC in PB was added to this mixture, and the SERS spectra of the resulting mixture were recorded after addition of ATC.

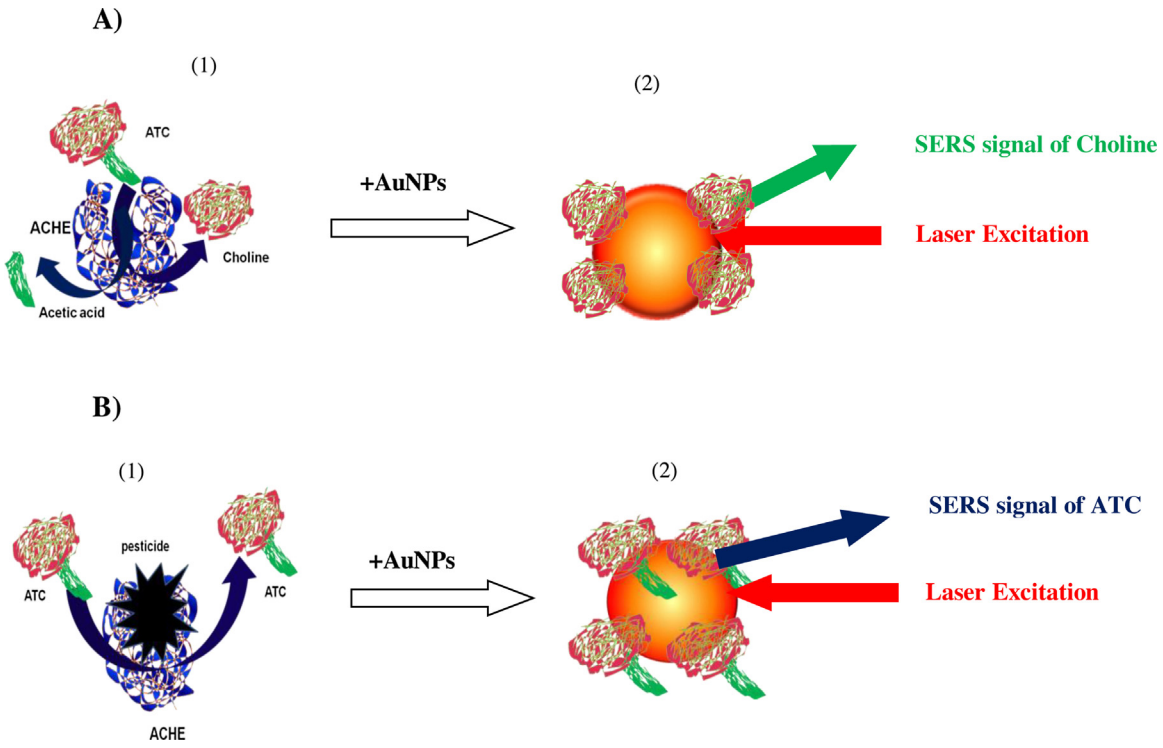
2.4. ACHE activity measurement

The activity of ACHE was first monitored in the absence of pesticide. In this case, according to Scheme 1A, ATC added to the enzyme solution was totally hydrolyzed in choline and acetic acid. When AuNPs are added, they bind to choline.

In the presence of pesticide, ACHE is inhibited and ATC cannot be totally transformed in choline and is still present in solution. Then with pesticides in the analyte solution, when AuNPs are added, they bind to ATC molecules (Scheme 1B) that were not hydrolyzed by ACHE. In this case, the Raman signal which is observed is very close to the Raman spectra of pure ATC showing the typical bands of this molecule. This reaction (Scheme 1B) was used for monitoring the inhibition of the ACHE activity in the presence of pesticides by Raman spectrometry.

2.5. Raman SERS measurements

To collect the SERS spectra, 2 μl of the analyte solution and 2 μl of AuNPs solution were mixed before deposition on a glass slide. For all analysis, SERS spectra were acquired at 5 different positions on the slide and an average spectrum was generated. SERS spectra were recorded with a Raman X-Plora spectrometer from HORIBA. The 638 nm excitation wavelength was provided by a laser diode



Scheme 1. Schematic representation of the protocol for measuring the acetylcholinesterase (ACHE) activity using Raman SERS. (A): (1) The ACHE catalyses the hydrolyze reaction of acetylcholine (ATC) to choline and acetic acid, (2) then the addition of gold nanoparticles (AuNPs) gives rise to the Raman signal of choline. (B): (1) The pesticides block the ACHE activity and ATC cannot be hydrolyzed, (2) The addition of AuNPs gives rise to the Raman signal of ATC.