Application of the switchSENSE® technology for real-time study of pesticides interaction with biological molecules

Gulminyam Baratzhanova¹²³*, Jean-Michel Girardet⁴, Agnès Fournier¹, Leyla Djansugurova²³, and Céline Cakir-Kiefer¹

¹Université de Lorraine, INRAE, L2A, F-54000 Nancy, France
²Institute of Genetics and Physiology, Almaty, 050060, Kazakhstan
³al-Farabi Kazakh National University, Almaty, 050040, Kazakhstan
⁴Université de Lorraine, INRAE, IAM, F-54000 Nancy, France

Abstract. Organochlorine pesticides have been extensively utilized in agriculture and pest control, and still contributing to numerous health issues. However, the mechanism underlying the transportation of these compounds through animal and human body is not well understood. The switchSENSE® technology is an original and powerful tool in biosensing, which demonstrates high sensitivity in detecting a variety of biological interaction which involves proteins, nucleic acids and small molecules. There has been a growing interest in using switchSENSE® technology for detecting interactions between proteins and environmental pollutants in recent years. Therefore, the aim of this study is to refine and enhance the methodology of the switchSENSE® technology to facilitate characterization of real time interaction between biological transport molecule, bovine serum albumin (BSA), and organochlorine pesticides. Using this technology, we noticed the conformational change in structure and protein hydrodynamic diameter (DH) of BSA in response to Chlordecone (CLD) and Dichlorodiphenyltrichloroethane (DDT). We also identified the possible obstacles, that should be resolved in future researches.

1 Introduction

Organochlorine compounds (OCP) are pesticides containing chlorinated compounds. These pesticides entered the environment after their utilization in agriculture and in the fight against insects and parasites. Despite their prohibition, residues of OCPs can still be found in the soil, water and food [1, 2]. Furthermore, these pesticides have a chronic toxic effect on both animal and human population [3, 4]. Nevertheless, the understanding of the physiological mechanisms by which OCPs are transported to various peripheral tissues and how these compounds exert harmful effects in the body is still not well understood. There is a hypothesis suggesting that albumin, the predominant protein in blood plasma and serum, exhibits an affinity for binding to pesticides and facilitate their transportation through the body [5].

* Corresponding author: baratzhanova.gulminyam@gmail.com
The switchSENSE® technology (SST) is an original and powerful tool in biosensing, offering high sensitivity for detecting a variety of biological interactions between small molecules, nucleic acids and proteins [6, 7]. This technology can provide information about structures of biomolecules such as size, shape and conformation. Moreover, SST enables to give an understanding of interactions at the molecular level and to provide kinetic research data [8]. This method employs chips featuring a gold surface, overlaid with DNA nanolevers, which are electrically switchable. This feature helps to characterize intermolecular interactions in real-time. For this interaction, the ligand is immobilized on DNA nanolevers, while the analyte flows across the surface. If the analyte binds to the ligand, it may induce a change in dye fluorescence which can be used for determining kinetic and biophysical parameters. To obtain information regarding molecular interaction, the device combines automatic fluid distribution, control over measurements modes (static or dynamic), temperature regulation, chip management, and the implementations of various modifications to the nanolever system. Additionally, the utilization of microfluidics system, minimal sample usage and biochip regeneration make the SST cost-effective and environmentally friendly [8]. SST has demonstrated utility and efficiency in obtaining the association and dissociation kinetic parameters for interactions between small molecules and human serum albumin [9]. It is also possible to detect and characterize the interactions between transport biomolecules and small molecules such as pesticides [10]. In recent years, there has been a growing interest in using SST for the detection the interactions between proteins and pollutants due to its possible applications in environmental monitoring [11], drug discovery and biomedical research [12, 13].

Therefore, the objective of the study was to refine and enhance the methodology of SST to characterize better the real time interaction between albumin and organochlorine compounds such as Chlordane (CLD), Chlordan (CLD-OH), Dichlordiphenyltrichloroethane (4,4’-DDT), dichlorodiphenyldichloroethylene (4,4’-DDE), gamma-hexachlorocyclohexane (γ-HCH), beta-hexachlorocyclohexane (β-HCH). Through a systematic approach involving experimentation and analysis, this research identifies the potential obstacles, including solubility of pesticides molecules, buffer solution composition and the presence of signal noise. Finding and optimization of these possible issues will enhance the efficiency and reliability of the obtained results.

2 Materials and methods

2.1 Sample preparation

OCPs were used as analytes and bovine serum albumin (BSA) as ligand. Chlordane (CLD) (azur isotope A7007), Chlordan (CLD-OH) (azur isotope A7010), 4,4’-DDT (LGC-c12082000), 4,4’-DDE (LGC- FL-35487), β-HCH (LGC- DRE C14072000), γ-HCH (LGC-DRE C14073000) were serial diluted with dilution factor four from 100 μM to 6.25 μM with 10 mM Na2HPO4/NaH2PO4 buffer containing 0.05% Tween 20, 40 mM NaCl, 50 μM EGTA, 50 μM EDTA (PE40 buffer) in the presence of 1% DMSO (Sigma-Aldrich, St. Louis, MO, USA). The concentration of analyte was assessed on ND1000 Nanodrop spectrophotometer (LabTech, Munich, Germany). After the experiment was performed the concentration of analytes was determined by size exclusion chromatography-multiangle light scattering (SEC-MALS) analysis was performed on MiniDAWN TREOS II (Wyatt Technology, Toulouse, France) coupled to Superdex 200 10/300 Increase column mounted on ÄKTA-Purifier™ system (Cytiva,
Uppsala, Sweden) for protein molecular mass and oligomerization determination according to previous work [14]. A covalent conjugate between BSA and a 48 mer ssDNA (complementary nanolever cNLB48) was prepared using the amine coupling kit provided by Dynamic Biosensors (Munich, Germany). Sample containing 200 µg of protein purified applying the anion-exchange chromatographic column (Dynamic Biosensors). The column was initially equilibrated with buffer A (50 mM Na2HPO4/NaH2PO4 pH 7.2 and 150 mM NaCl) and with a linear gradient of 0.15-1 M NaCl. The column then was connected to an ÄKTA-Start™ system (Cytiva).

The extended methodology of conjugate preparation was described by Delannoy et al. [10]. In our study, the bovine serum albumin-DNA conjugate was prepared as a 200 nM solution in PE40 buffer. The experiment was performed using an MPC-48-2-R1-S biochip in a biosensor analyzer switchSENSE® DRX® (Dynamic Biosensors). The concentration of the conjugated sample was assessed on ND1000 Nanodrop spectrophotometer (LabTech, Palaiseau, France) at 260 nm.

Injection of the conjugate into the chip was performed to form fluorescent nanolevers with the complementary ssDNA fixed on the golden surface of the chip [10]. Analysis of the switching dynamics of the sample, and the hydrodynamic diameter (Dh) of BSA was conducted using the Lollipop mathematical model from switchANALYSIS® software. The error was calculated from the sizing results, which corresponds to the global fit error which are at least four measurements.

2.3 Evaluation of the interaction between BSA-DNA conjugates and OCPs

Similar methodology of interaction assessment was performed as described by Delannoy et al. [10]. The ‘conformational change’ analysis as well as the ‘sizing’ analysis were performed between the association and dissociation phases similar to methodology outlined by Hajj et al. [15]. After the flow was stopped, the dynamic response was measured. Relative ΔDR (in %) showed the difference in motion rates between OCP-free nanolevers and OCP-carrying nanolevers. Using switchANALYSIS® software, nonlinear fitting of single-exponential functions was used to analyse all curves. The error corresponds to the global fit error of all fourteen measurements [15].

2.4 Statistic analysis

This study employed an independent two tail t-test analysis to quantify the variance in conformational change between reference (BSA without analytes) and samples (BSA with analytes) group.
3 Results and Discussion

3.1 Characterization of BSA

BSA is a globular protein extracted from cow’s serum and consists of 589 amino acids. Through utilization of SEC-MALS, we have confirmed the presence of two forms of BSA, a monomer (experimental mass of 64.1 kDa), and a dimer (127.3 kDa) with respective proportions of 95.3% and 4.7%. This protein was then covalently conjugated with a complementary nanolever cNLB48 and purified using anion-exchange chromatography FPLC according to the manufacturer’s instructions (Fig. 1).

![Fig. 1. Ion-exchange FPLC to purify BSA conjugated with the complementary ssDNA nanolever of 48 nucleotides (cNLB48). Note: Linear gradient of 0.15-1 M NaCl was applied (in red). A.U. – absorbance unit](Image)

3.2 Interaction between OCPs and albumin

We measured hydrodynamic friction using the frequency of dynamic electrical switching mode, to assess the absolute size and shape of interacting biomolecule and thus allows us to determine the size of albumin. Determining hydrodynamic diameters on a chip allows us to observe conformational changes dynamically. BSA displayed a \( D_H \) of 4.7 ± 0.14 nm confirming the conjugation of the BSA to the cDNA.

Using the albumin-conjugated chips and SST, real time molecular interactions between BSA as the ligand and CLD, CLD-OH, DDE, DDT, \( \beta \)-HCH, \( \gamma \)-HCH as analytes were investigated. The effects were presented as relative size changes with respect to the BSA \( D_H \) in the absence of pesticides. Before injection of new analytes, the flow channel was washed with running buffer (PE40 containing 1% DMSO) to remove any presence of the previous effector. In each measurement, pesticides were injected after the determination of the initial \( D_H \). We observed changes in the \( D_H \) of BSA after interaction with CLD and DDT (Table 1). The calculated volume of BSA (considered as a sphere) increased by 1.4 and 1.9 times after interaction with CLD and DDT, respectively (Table 1).

![Table 1. Interactions between OCPs and BSA](Image)
Fig. 2 illustrates the result from the effect of these molecules on the conformational change of BSA. Significant differences were observed between the reference spots (for which no CLD or DDT are bound onto the nanolevers) and the sample spots, meaning that these pesticides were bound onto the immobilized BSA.

However, the further results did not show conformational change of BSA after CLD-OH, DDE, γ-HCH, β-HCH injection (Table 1). Unfortunately, we could not also detect binding affinity of any of these interactions.

Therefore, it can be said that the switchSENSE® technology compared to other existing methodologies provides depth understanding of interaction between biological molecules determined in real time, with structural information on shape, size and conformation. SST was used previously by our lab for characterizing the interaction between CLD, CLD-OH and lipoproteins. Delannoy et al. [10] obtained interesting results, showing that CLD binds to low density lipoproteins while its metabolite CLD-OH shows preference to high density lipoproteins.

By our research we wanted to optimize experiment for revealing interaction of OCPs to serum albumin. The conformational change analysis as well as the sizing analysis revealed interaction between BSA and CLD, DDT. BSA responds to these compounds by alterations in structure (unveiled by sizing measurements) or shape (measured by conformational change), however we still do not know how these changes in DH influence on binding affinity between BSA and OCPs. The conditions of using protocol did not provide essential data about KD of the reactions between BSA and studied pesticides. It might be explained by the following possible obstacles: (i) A low affinity between BSA and OCPs would be undetectable with SST, as exchanges could be rapid and in favour of dissociation during

### Table 1. Interaction between OCPs and bovine serum albumin

<table>
<thead>
<tr>
<th>Samples</th>
<th>Sizing $D_H$ (nm)</th>
</tr>
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<tbody>
<tr>
<td>BSA</td>
<td>4.7 ± 0.14</td>
</tr>
<tr>
<td>BSA-CLD</td>
<td>5.25 ± 0.07</td>
</tr>
<tr>
<td>BSA-DDT</td>
<td>5.85 ± 0.07</td>
</tr>
<tr>
<td>BSA-γHCH</td>
<td>4.75 ± 0.07</td>
</tr>
<tr>
<td>BSA-CLD-OH</td>
<td>4.65 ± 0.21</td>
</tr>
<tr>
<td>BSA-DDE</td>
<td>4.55 ± 0.07</td>
</tr>
<tr>
<td>BSA-βHCH</td>
<td>4.55 ± 0.07</td>
</tr>
</tbody>
</table>

Note: The mean of fourteen measurements of each condition (red and blue) are shown. Note: DR – dynamic response, Ref. – reference. Data were analysed by t-student test (*p < 0.05 vs. reference)
analyte flow or (ii) sensitiveness of SST maybe insufficient due to small size of OCPs, as only fluorescence proximity sensing is measured using the static mode for kinetics determination.

4 Conclusion

This paper presents an additional potential application and optimization of switchSENSE® technology for the identification of interactions between transport biomolecules and pollutants such as organochlorine pesticides. The advantages of this approach over the other conventional methods for studying these interactions is explained by its capacity to enable real-time measurements, thereby offering unprecedented insight into molecular processes. In summary, the utilization of SST has unveiled notable conformational changes and an increase in size of BSA in the presence of CLD and DDT. However, to fully grasp the specific impact of these changes on the binding affinity, further investigations are needed for a deeper understanding of the molecular mechanisms underlying the transport of pesticides inside of the body. Additionally, our study has identified the possible obstacles that should be addressed in future researches. These obstacles may include methodological challenges such as the sensitivity of SST which could be inadequate because of small size of pesticides, due to fluorescence proximity sensing was measured in the static mode for kinetics determination, and low binding affinity of OCPs to BSA could be undetectable with SST. Addressing these challenges will be important for understanding mechanism of pesticide action in body and adaptation of this technology for usage in different fields. In conclusion, the utilization of this technology revealed an interaction of pesticides and transport biomolecule. It should be noted that further researches are essential for understanding the specific impact of these changes on the binding affinity between albumin and OCPs.

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