Data Article

Data on preparation and characterization of an insect odorant receptor based biosensor

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Abstract

Insect Odorant receptors (OrXs) can be used as the recognition element in a biosensor as they demonstrate high levels of sensitivity and selectivity towards volatile organic compounds. Herein, we describe a method to express and purify insect odorant receptors and reconstitute them into artificial lipid bilayers (liposomes). These OrX/liposomes were covalently attached to a gold surface and characterized using quartz crystal microbalance with dissipation monitoring (QCM-D). The interaction of OrX/liposomes immobilized on a gold surface to positive and negative odorants were studied by means of electrochemical impedance spectroscopy (EIS) and QCM-D. The data presented in this article are related to the research article titled "An ultrasensitive electrochemical impedance-based biosensor using insect odorant receptors to detect odorants" [1].

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**Specifications table**

| Subject area                  | Chemistry, Material Science |
|-------------------------------|-----------------------------|
| More specific subject area    | Biosensors                  |
| Type of data                  | Table, graph, and figure    |
| How data was acquired         | QCM-D graphs (Q-sense analyzer (Biolin Scientific)), EIS graphs (PalmSens potentiostat) |
| Data format                   | Analyzed                    |
| Experimental factors          | Gold electrodes functionalization, and electrical properties of OrX/liposome immobilization and ligand binding. |
| Experimental features         | OrX/liposomes were covalently attached to gold surface and QCM-D and EIS studies were utilized to investigate ligand-binding. |
| Data source location          | The University of Auckland, Auckland, New Zealand |
| Data accessibility            | Data are presented in this article |
| Related research article      | Khadka, R., Aydemir, N., Carraher, C., Hamiaux, C., Colbert, D., Cheema, J., Malmström, J., Kralicek, A., Travas-Sejdic, J., 2018 [1]. |

**Value of the data**

- The method used here to purify OrX subunits and integrating into lipid bilayers of liposomes to provide a platform for biosensing studies.
- The QCM-D data after covalent attachment of OrX/liposomes on the gold surface followed by the binding of ligand is very useful to understand the mechanism of signal transduction.
- The EIS capacitance data can be used to compare the sensitivity of this novel insect OrX based biosensor with other relevant OrX based biosensors.

1. **Data**

The western blot analysis of purified OrX subunits are shown in Fig. 1. QCM-D data showing the real time covalent attachment of Or22a/liposomes and Or71a/liposomes on gold sensors followed by the binding of methyl hexanoate and 4-ethylguaiacol respectively, Figs. 2–5. The equivalent electrical circuit model used for fitting the impedance data is shown in Fig. 6 and the calibration plots in terms of change in normalized capacitance values for three EIS sensors (Or10a, Or22a, and Or71a) are presented in Figs. 7–9.

2. **Experimental design, materials, and methods**

2.1. **Preparation of purified OrX subunits**

The purification procedure is a variation on the one detailed in Carraher et al. [2]. To his-tag affinity purify recombinantly expressed OrX subunits from baculovirus-infected SF9 cells, 500 mL of SF9 cells at a concentration of at $2 \times 10^9$ mL$^{-1}$ (measured with a haemocytometer), were infected with baculovirus at a multiplicity of infection (MOI) of 0.1, and incubated at 27 °C for 72 h with shaking at 110 RPM. The cell pellet was collected by centrifugation at 3,800 g for 10 min at room temperature and then resuspended in 40 mL of resuspension buffer A (20 mM Tris/HCl pH 7.5, 100 mM NaCl, 1x protease inhibitor cocktail (Roche Diagnostics GmbH, Germany)), with 25 U/mL Benzonase, then lysed by two passes on an EmulsiFlex C5 emulsifier (Avestin, Germany) at 10,000–15,000 psi. The sample was then centrifuged at 1,000 g for 5 min to remove whole cells and nuclei. The supernatant was removed and spun at 100,000 g for 1 h at 4 °C. The membrane pellet was resuspended in 40 mL of buffer A with 1% w/v detergent (Zwittergent 3–16) and rotated for 1 h at room temperature at 10 rpm. The sample was then centrifuged
at 100,000 g for 1 h at 18°C. The supernatant was removed and loaded onto a 1-mL NiNTA column (GE Healthcare) where the zwittergent 3–16 detergent was exchanged to Fos-Choline 14 (FC-14). The column was washed in ten column volumes of buffer B (20 mM Tris/HCl pH 7.5, 0.36 mM FC-14) with 300 mM NaCl and 20 mM imidazole, and a further ten column volumes of buffer B with 100 mM NaCl and 50 mM imidazole. Protein was eluted with 4 column volumes of buffer B with 100 mM NaCl and 500 mM imidazole. Purity was assessed on Coomassie stained SDS–PAGE gels and by Western blotting.

Purification was completed with a final size exclusion chromatography (SEC) step. The elution fractions from the NiNTA purification were pooled and centrifuged at 20,000 g for 5 min to remove aggregates and contaminants. Then 5 mL of sample was injected onto a Superdex 200 16/60 column

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**Fig. 1.** Western Blot analysis of OrX/liposomes. Lane 6: Or71a/liposomes (Anti-FLAG). Lane 7: Molecular weight marker. Lane 8: Or10a/liposomes (Anti-FLAG). Lane 9: Or22a/liposomes (Anti-FLAG). Molecular weight of the markers (kDa) are indicated.

**Fig. 2.** Change in frequency and dissipation of a gold surface with self-assembled monolayers (SAMs) of 6-mercaptohexanoic acid (MHA) and N-hydroxysuccinimide (NHS)/1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) modification, followed by Or71a/liposome immobilization on the QCM sensor and then binding of the target ligand 4-ethylguaiaicol.
GE Healthcare) attached to an Akta-Pure chromatography system (GE Healthcare). The sample was run at 1 mL/min in buffer B with 100 mM NaCl, and 2 mL fractions were collected and concentrated using a 100 kDa MWCO Vivaspin filter unit (Sartorius, Goettingen Germany) and stored at −80°C.

2.2. Preparation of liposome integrated OrX subunits

Liposomes were prepared as outlined in Carraher et al. [2].
Fig. 5. Close up view of the change in frequency and dissipation with increasing concentrations of methyl hexanoate (0, 1.6, 8, 20, 40, 100, 200, 500 and 1000 μM) for the Or22a/liposome QCM sensor.

Fig. 6. Equivalent circuit diagram (Randles model) for fitting the non-Faradaic impedance curves where Rs is solution resistance, Q refers to capacitance, Rp is called polarization resistance and W refers to Warburg diffusion.

Fig. 7. Dose response curve in terms of change in normalized capacitance values for the Or71a/liposome EIS based sensor showing detection of the specific ligand 4-ethylguaiacol with high selectivity and sensitivity. Error bars; standard deviation (SD) were generated using four repeats.
2.3. EIS and QCM-D measurements

For QCM-D experiments, 100 nm thick gold sensor crystals were functionalized with self-assembled monolayers (SAMs) of 6-mercapto hexanoic acid (MHA) by incubating them in MHA solution overnight. MHA functionalized crystals were placed in the QCM-D chamber and real time measurements of NHS/EDC activation followed by binding of the OrX/liposomes was monitored. Different concentrations of positive and negative odorants were flowed into the chamber and changes in frequency ($\Delta f$) and dissipation ($\Delta D$) values were recorded.

Fig. 8. Dose response curve in terms of change in normalized capacitance values for Or10a/liposomes on an EIS based sensor showing detection of its specific ligand methyl salicylate with high selectivity and sensitivity. Error bars; standard deviation (SD) were generated using four repeats.

Fig. 9. Dose response curve in terms of change in normalized capacitance values for Or22a/liposomes on an EIS based sensor showing detection of its specific ligand methyl hexanoate with high selectivity and sensitivity. Error bars; standard deviation (SD) were generated using four repeats.
For EIS experiments, a gold disk as a working electrode, platinum coil as counter electrode and Ag/AgCl (3 M NaCl, 0.209 V vs. SHE) as a reference electrode was used. EIS measurements were performed at a fixed voltage of $-0.7$ using a PalmSens potentiostat with degassed PBS solution as an electrolyte.

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**Transparency document. Supporting information**

Transparency data associated with this article can be found in the online version at [https://doi.org/10.1016/j.dib.2018.11.018](https://doi.org/10.1016/j.dib.2018.11.018).

**References**

[1] R. Khadka, N. Aydemir, C. Carraher, C. Hamiaux, D. Colbert, J. Cheema, J. Malmström, A. Kralicek, J. Travas-Sejdic, An ultrasensitive electrochemical impedance-based biosensor using insect odorant receptors to detect odorants, Biosens. Bioelectron. (2018).

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