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Fluorescent Imaging of Live Hippocampal Neurons with Platinum Nanoclusters

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1. Introduction

Fluorescent imaging is a powerful technique to reveal how target cells or biomolecules work in biological tissues and cells. So far, organic dyes, fluorescent proteins, and quantum dots have been developed and widely used as a fluorescent probe. However, these fluorescent probes do not work well in certain conditions: they have cytotoxicity and steric hindrance due to inclusion of harmful atoms and/or their sizes. Hence, metal nanoclusters, that are composed of several metal atoms and emit fluorescence owing to quantum size effect, have been anticipated as a newly categorized fluorescent probe. Since the fluorescent wavelength depends on their sizes, it is possible to control the fluorescent wavelength by adjusting the number of composed atoms [1].

We have synthesized platinum nanoclusters (Pt NCs). We succeeded in synthesizing blue and green fluorescent Pt NCs by using PAMAM as a template molecule [2]. Furthermore, by using polyethyleneimine (PEI) as a template molecule, we synthesized yellow fluorescent Pt NCs [3]. These Pt NCs were less photo-bleaching than organic dyes, and the size is smaller than that of semiconductor quantum dots. Thus, Pt NCs can be used for longer observation time compared with organic dyes and for a less cytotoxic fluorescent probe because they can prevent the steric hindrance compared with quantum dots. In this presentation, we will show that AMPA receptors on neurons were labeled with Pt NCs and the axons and neurites in hippocampal neurons were clearly visualized by fluorescent imaging.

2. Experiment

We used PEI as a template molecule to synthesize Pt NCs. A PEI solution (400 μL) was mixed into a pure water (2.52 mL), and a H_2PtCl_6 solution (80 μL) was added into the solution drop by drop. Then, the solution was stirred for 2 h until the complex was formed. Next, L-AA (80 μL) as a reducing agent was added into the mixture solution and the solution was stirred for 1 week at 95°C to complete the reduction. Synthesized Pt NCs were found to emit yellow fluorescence, and the fluorescent wavelength can be controlled by pH adjustment using the PEI solution.

To label AMPA receptors with the Pt NCs, we used anti-glutamate receptor 2 (GluR2) as an antibody which specifically binds to AMPA receptors. Since both of PEI and GluR2 have the amino group, we used glutaraldehyde for conjugating Pt NCs to GluR2. A glutaraldehyde (2.5 μL, 8% w/v) solution was mixed with a PBS solution (2.5 μL), and then the Pt NCs solution (2.6 μL) was added. The solution was stirred for 2 h at 37°C, and a GluR2 (41.7 μL, 1 mg/mL) solution was added into the mixture. After that, the mixture was incubated over night at 4°C.

We cultured hippocampal neurons with Pt NCs conjugated to antibodies, and also unconjugated Pt NCs for a control experiment.

3. Results

We succeeded in observing the axons and neurites clearly by a laser scanning confocal microscope (E_x: 488 nm, E_m: 540-560 nm, NA: 1.20) as shown in Fig. 1. When the unconjugated Pt NCs were used, not axons or neurites but nucleus were observed. It is considered that the unconjugated Pt NCs were accumulated at the neucleus. From these results, we confirmed that the AMPA receptors on the neurons were surely labeled with Pt NCs.

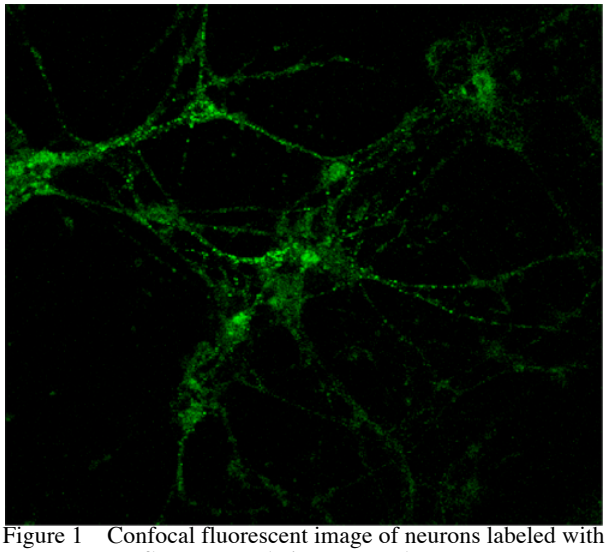

Figure 1 Confocal fluorescent image of neurons labeled with fluorescent platinum nanoclusters

4. Conclusions

We succeeded in labeling AMPA receptors with Pt NCs and observing the axons and neurites in hippocampal neurons by the laser scanning confocal fluorescence microscope.

References

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