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Coupling electrochemistry and TIRF-microscopy with the fluorescent false neurotransmitter FFN102 supports the fluorescence signals during single vesicle exocytosis detection

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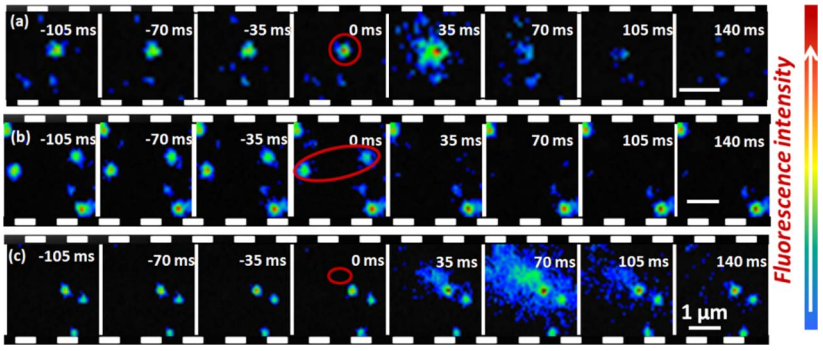
HIGHLIGHTS

- FFN102 is a suitable probe for coupling amperometry and TIRFM during exocytosis.
- The electroactivity of FFN102 arises from the oxidation of its phenolic group.
- The aminoethyl group of FFN102 is the key recognition element by BON N13 cells.
- Coupled analyses in N13 cells allowed us to classify three types of optical signals.
- Preliminary benefits from the coupling involving FFN102 were reported.

GRAPHICAL ABSTRACT

Coupled analyses of FFN102-loaded vesicles allowed us to classify three types of optical signals that probably arise from secretion releases thanks to their concomitant detection with an electrochemical spike.



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ABSTRACT

Applications of the Fluorescent False Neurotransmitter FFN102, an analog of biogenic neurotransmitters and a suitable probe for coupled amperometry and TIRFM (total internal reflexion fluorescence microscopy) investigations of exocytotic secretion, were considered here. The electroactivity of FFN102 was shown to very likely arise from the oxidation of its phenolic group through a CE (Chemical-Electrochemical) mechanism. Evidences that the aminoethyl group of FFN102 is the key recognition element by BON N13 cells were also provided. Amperometric measurements were then performed at the single cell level with carbon fiber electrode (CFE) or Indium Tin Oxide (ITO) surfaces. It proved the disparity of kinetic and quantitative parameters of FFN102-stained cells acquired either at cell top and bottom. Moreover, coupled analyses of FFN102 loaded vesicles allowed us to classify three types of optical signals that probably arise from secretion releases thanks to their concomitant detection with an electrochemical spike. Finally, preliminary benefits from the coupling involving FFN102 were reported in terms of origins of overlapped amperometric spikes or assignment of fluorescence extinctions to real exocytotic events.

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1. Introduction

Vesicular exocytosis, an intracellular membrane trafficking pathway consisting of an outward flux of vesicular contents to the extracellular space, represents a fundamental process of intercellular communications such as synaptic transmission and hormone secretion. As vesicular exocytosis shows great importance in plenty of normal and pathologic events in cells, its investigation has thus attracted extensive research interest for several decades since its first discovery in the late 19th century.[1]The release of biogenic neurotransmitters via exocytosis is one of the most common ways for neuronal communications [2,3]. A unique feature of some well-known biogenic monoamine neurotransmitters is their electrochemical characteristics which promise the possibility of using an electrochemical technique to quantitatively feature the exocytotic process [4–6]. For instance, owing to the electroactivity of catecholamines, the first electrochemical monitoring of exocytotic secretion at chromaffin cells was achieved by amperometric measurement with a carbon fiber ultramicroelectrode (CFE) [7,8]. After almost three decades of development, amperometry has now become the most widely used electrochemical technique for exocytosis investigation because of its remarkable advantages such as facility to implement, remarkable temporal resolution as well as high sensitivity. [5,9,10]As shown in Fig. 1, amperometric measurement of cellular exocytosis could be achieved by placing an electrode against the sample, either on the cell apex (such as CFE, left image) or at the cell bottom (such as ITO microelectrode, right image). Furthermore, in both configurations, an individual exocytotic event is depicted as a single current spike lasting from tens of ms to hundreds of ms as the prompt electrooxidation of biomessengers (secreted by a single vesicle) on microelectrode surface. Therefore, the whole exocytotic process of an individual cell is recorded as a series of oxidation spikes. This quantitative technique gives direct information on the number of molecules emitted per secretory vesicle, the kinetics of individual exocytotic event and the cells secretion frequency.

Previously, endogenous neurotransmitters including serotonin [11–13], dopamine [14,15], and (nor)epinephrine [16,17], were usually employed as electrochemical reporters for amperometric characterization of exocytosis as they can be easily oxidized at the electrode surface. Recently, we reported that FFN102 (Fig. 2a), a subtly designed analogue of biogenic neurotransmitters, could also act as a novel electrochemical probe. Additionally, its unique optical characteristic makes it an ideal difunctional electroactive/fluorescent probe with our combined fluorescent and electrochemical methods.[18]In particular, aminoethyl group of this molecule is tactfully designed to be the key cellular recognition element, making it possible to be specifically accumulated into the vesicular lumens.[19–24]Its electroactivity is supposed to result from the oxidation of its phenolic group on the electrode surface, similar to those aforementioned neurotransmitters. Furthermore, its pH-dependent fluorescence (pKa = 6.2) exhibits great advantages in the real-time visualization of vesicle motility and in-situ localization of releasing sites because biogenic neurotransmitters are usually non-luminous or very weakly fluorescent.

As we know, TIRFM (total internal reflection fluorescence microscopy) is a special optical technique developed by D. Axelrod in the early 1980s [25] and accounts for a predominant position for exocytosis fluorescent tracking at present owing to its excellent signal-to-noise ratio and distinct spatial resolution. In TIRFM, out-of-focus fluorescence is minimized, hardly any fluorescence background occurs, phototoxic stress for cells is reduced, cell viability improves and longer experiment durations are allowed. In contrast, although amperomery is a quantitative technique, it is completely 'blind' to the 3D movements of the secretory vesicle itself or any labeled regulatory protein before the secretion of vesicular contents since electrical signals can only be detected after the electroactive biomessengers arrive at the electrode surface. In spite of the high temporal resolution and distinct sensitivity, its low spatial resolution becomes the main constraint of electrochemical methodology for investigations of vesicular secretions. However, the combination of amperometry with TIRFM is likely to solve this problem considering the spatial resolution of this optical technique. The benefits of coupling both are to get the excellent temporal resolution of electrochemistry coupled with the good spatial resolution of TIRFM to track a unique vesicle secretion [26,27].

In this manuscript, we carefully investigated the electrochemical properties and cell recognition of this promising probe. Electroactivity of FFN102 was investigated in vitro and was shown to very likely arise from the oxidation of its phenolic group through a CE (Chemical-Electrochemical) mechanism. The importance of the FFN102 aminoethyl group within the coumarin moiety for cell recognition was notably confirmed by comparing cell penetration of FFN102 with its chloro-analogue FFN-Cl (without the aminoethyl moiety) within BON N13 cells. The comparison of vesicular secretions by amperometry at the two poles of BON N13 cells was then carried out by employing synthetic exogenous FFN102 as the electrochemical reporter. Finally, the whole releasing process of individual events could be visualized in real-time by virtue of FFN102's fluorescence property with the coupling method. Coupled electrochemical and TIRFM data were thus obtained with FFN102 and they suggested different types of fluorescent signals arising from secretion in BON N13 cells. This combination also paved the way for confidently assigning fluorescence and amperometric signals that would remain equivocal if they were taken separately.

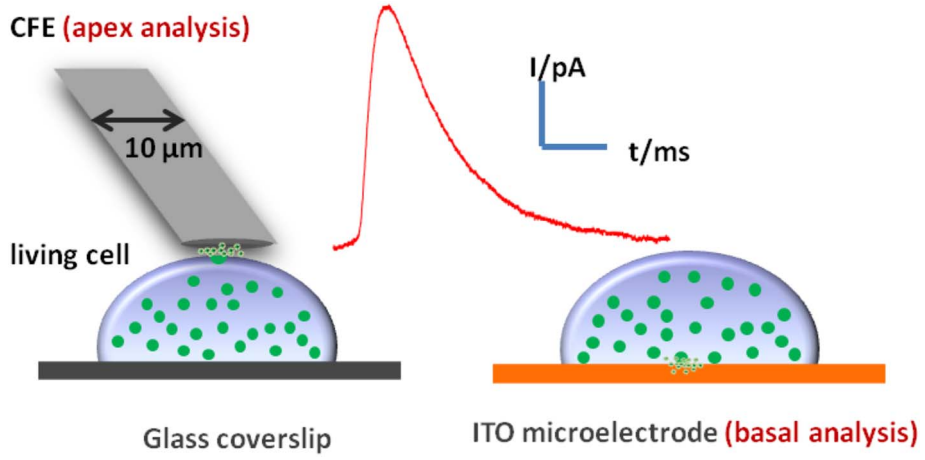

Fig. 1. Schematic illustration of the electrochemical detection of vesicular exocytosis by a single cell as performed at the apical pole (CFE) or at the basal pole (ITO planar electrode). Note that the planar ITO microelectrode is used both as substrate for cell adhesion and to detect oxidation signals.