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Sensing lymphoma cells based on a cell-penetrating/apoptosis-inducing/electron-transfer peptide probe

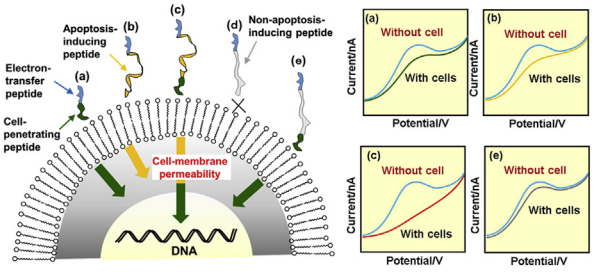
Kazuharu Sugawara [a, *], Hiroki Shinohara [a], Toshihiko Kadoya [a], Hideki Kuramitz [b]

[a] Maebashi Institute of Technology, Gunma 371-0816, Japan
[b] Department of Environmental Biology and Chemistry, Graduate School of Science and Engineering for Research, University of Toyama, Toyama 930-8555, Japan

HIGHLIGHTS

- We constructed a multifunctional peptide probe for the electrochemical sensing of lymphoma cells.
- The peptide probe consists of cell-penetrating/apoptosis-inducing/electron-transfer peptides.
- The electrode response of the peptide probe changes due to selective uptake into the cells.

GRAPHICAL ABSTRACT



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ABSTRACT

To electrochemically sense lymphoma cells (U937), we fabricated a multifunctional peptide probe that consists of cell-penetrating/apoptosis-inducing/electron-transfer peptides. Electron-transfer peptides derive from cysteine residue combined with the C-terminals of four tyrosine residues (Y_4). A peptide whereby Y_4C is bound to the C-terminals of protegrin 1 (RGGRLCYCRRRFCVCVGR-NH_2) is known to be an apoptosis-inducing agent against U937 cells, and is referred to as a peptide-1 probe. An oxidation response of the peptide-1 probe has been observed due to a phenolic hydroxyl group, and this response is decreased by the uptake of the peptide probe into the cells. To improve the cell membrane permeability against U937 cells, the RGGR at the N-terminals of the peptide-1 probe was replaced by RRRR (peptide-2 probe). In contrast, RNRCKGTDVQAWY$_4$C (peptide-3 probe), which recognizes ovalbumin, was constructed as a control. Compared with the other probes, the change in the peak current of the peptide-2 probe was the greatest at low concentrations and occurred in a short amount of time. Therefore, the cell membrane permeability of the peptide-2 probe was increased based on the arginine residues and the apoptosis-inducing peptides. The peak current was linear and ranged from 100 to 1000 cells/ml. The relative standard deviation of 600 cells/ml was 5.0% (n = 5). Furthermore, the membrane permeability of the peptide probes was confirmed using fluorescent dye.

1. Introduction

Microinjection has been a widely used method to transfer DNAs [1], RNA interferences [2], proteins, and peptides [3] into a cell. The microinjection method features high transduction efficiency with

* Corresponding author.
E-mail address: kzsuga@maebashi-it.ac.jp (K. Sugawara).

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control of the dosage and timing [4]. As a new variation, microinjection has also been used to perform therapeutic cloning [5]. Electroporation has been used to introduce non-permeable exogenous substances into cells via a high-voltage electric pulse [6]. The cytotoxicity that is often caused by chemical transfection or viral infection has been lowered via electroporation [7]. On the other hand, peptides that consist of a specific amino acid sequence and structure are able to penetrate a cell membrane [8,9]. Peptides can be introduced to a cell without damaging the cell membrane, and several reports have described basic amino acids such as arginine and lysine residues being carried in cell-penetrating peptides [10–12]. The intracellular uptake of peptides is based on a direct penetration of the cell membrane and on the endocytosis pathway of a cell [13]. Polydisulfides with a peptide can also penetrate a cell membrane [14], which is a phenomenon that is promoted by the polymerization of cell-penetrating moieties. A peptide with a positive charge is incorporated into cells due to its interaction with cell-surface heparan sulfate proteoglycans with a negative charge [15]. Although peptides with basic amino acids and polydisulfides have high levels of membrane permeability, their levels of selectivity are insufficient. Antimicrobial agents composed of amphipathic peptides exhibit a selective membrane permeability that is related to the biological defense mechanisms in living organisms. Wang et al. identified the activity, 3D structure, and mechanism of action for antimicrobial peptides in humans [16]. In addition, computer-assisted design strategies have been used in attempts to solve the difficult problems of relating a primary sequence to a peptide structure [17]. New synthetic antimicrobial peptides have been fabricated with special properties [18], and characterized by their secondary structures: α-helix [19], β-sheet [20], and loop [21]. Antimicrobial peptides penetrate the cell membrane via an interaction with anionic membrane phospholipids [22]. The peptides then disrupt the cell membrane [23,24] and inhibit the protein expression via binding with DNA [25]. The synthesis of macromolecules is suppressed by the binding between RNA and pseudin-2 [26]. This binding causes apoptosis [27], which is influenced by peptide concentration [28] and by the type of the cell species [29].

When antimicrobial peptides have a high affinity for a cell, the result is selective sensing. These methods can result in powerful biomedical detection, environmental pollutant monitoring, and drug evaluation. The merits gained by the use of peptides include ease of synthesis for large quantities, lower costs than using proteins, and possibilities for chemical modification [30]. For example, Kafi et al. reported the monitoring of cell-cycle progression using an Au electrode modified with a peptide [31]. To estimate the concentration of pathogenic bacteria, a specific antimicrobial peptide-functionalized quartz crystal micro-balance (QCM) electrode was designed [32]. The cells were immobilized on the electrode based on the interaction between integrin and a peptide, and the electrochemical signals of the cells were measured. In addition, the evaluation of Au electrodes using surface treatment consisting of polyelectrolyte multilayers to facilitate uniform cell attachment was presented based on impedance measurements using a real-time cell growth detector [33]. Moreover, a graphene electrode with an antimicrobial peptide nanotube of folic acid was designed for the detection of cancer cells [34]. Villiers et al. proposed biosensors using a microarray format for high-throughput analyses [35]. The cells, proteins and ions were detected via imaging surface plasmon resonance. Fluorescent bacterial display peptide libraries and fluorescence-activated cell sorting are processes that have been applied to the screening of cells [36]. A nuclear targeting nanoprobe has been fabricated based on peptide-functionalized Au nanoparticles, and its surface enhanced the Raman scattering in living cells [37]. Burlina et al. determined the cellular uptake of cell-penetrating peptides for Chinese hamster ovary (CHO) cells using MALDI-TOF mass spectrometry [38].

We carried out a voltammetric sensing of U937 cells using an arginine-rich peptide probe combined with daunomycin, which is an anticancer antibiotic for U937 cells [39]. Because U937 cells are non-adherent, they were suitable for incubation with the peptide probe in a solution. Daunomycin is electroactive and will penetrate the cell membrane of U937 cells and cause apoptosis. The sensing of the cells was achieved using the changes in the electrode response. The cell sensitivity of the probe was improved when using an arginine-rich peptide rather than daunomycin alone. Thus, the introduction of a cell-penetrating peptide to the electroactive compound contributed to the detection of a cell. In a previous study, the allergen ovalbumin was detected by using the interaction between the protein and a molecular recognition peptide conjugated with an electron-transfer peptide [40]. We carried out a voltammetric sensing of U937 cells using an arginine-rich peptide probe. Because peptide probes are constructed using all of a peptide sequence and are measured in concentrations that are less than 10^{-7} M, the probes exhibit high levels of biocompatibility. Therefore, electron-transfer peptides are suitable for the sensing of both biomolecules and cells.

In the present study, the electrochemical sensing of U937 cells was carried out using an electron-transfer peptide bound to an apoptosis-inducing peptide containing a cell-penetrating peptide. First, arginine residues that would indicate the cell membrane permeability were coupled with an electron-transfer peptide (Fig. 1(a)), which consisted of a tyrosine-rich peptide (Y_4C) due to the phenolic hydroxyl group of tyrosine. Since the cell membrane permeability of a probe is affected by the number of arginine residues, a two-by-two increase in the arginine residues was completed at the N-terminal side. To evaluate the effect of the arginine residues contained in the probe, the decrease in peak current caused by the intracellular uptake of the cell was electrochemically measured. Second, we synthesized a probe with apoptosis-inducing and electron-transfer peptides that could produce apoptosis against U937 cells. We were interested in using protegrin 1 (RGGRLCYCRRRFCVCVGR-NH_2) as an apoptosis peptide because the peptide includes several arginine residues [41,42]. Therefore, a Y_4C peptide was modified with C-terminals of

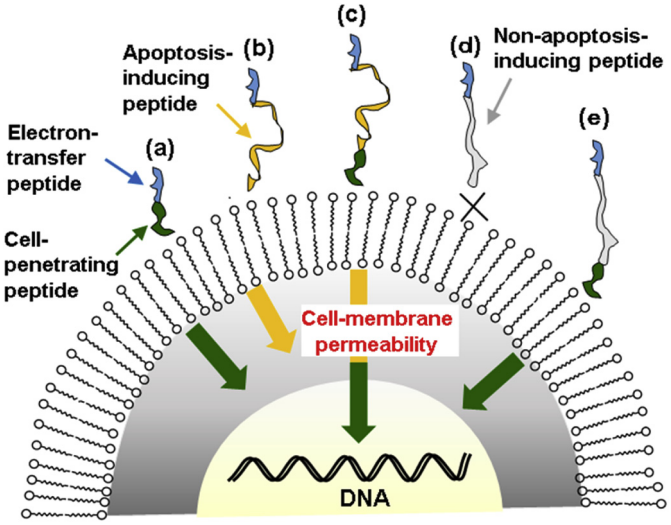

Fig. 1. Membrane permeability of a peptide probe to a U937 cell. (a) Cell-penetrating/electron-transfer peptides, (b) Apoptosis-inducing/electron-transfer peptides, (c) Cell-penetrating/apoptosis-inducing/electron-transfer peptides, (d) Non-apoptosis-inducing/electron-transfer peptides, (e) Cell-penetrating/non-apoptosis-inducing/electron-transfer peptides.