Trapping of Secreted Molecules at Single-Cell Level Using an HPLC Resin Particle Under a Videomicroscope

Tsutomu Masujima,* Kayo Ikeda, Koichiro Ozawa, Atushi Tamura, Yumiko Tada, Hideo Ohya, and Masashi Suzuto

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8551, Japan. Received March 30, 1998; accepted July 1, 1998

Particles of HPLC resins are used for the trapping of secreted molecules from a single cell. The basic molecules, e.g., histamine, are secreted from a single RBL-2H3 cell by granule exocytosis and are trapped by cation-exchange HPLC resins outside the cell. Since quinacrine is concentrated into the exocytotic and acidic microgranules in RBL-2H3 cells, which are used as a model cell line of mast cells, we measured the change in the fluorescence intensity of the quinacrine released from the cells and that of molecules trapped on the resin using a videomicroscope. By measuring the increase in the fluorescence intensity of the resins, we can estimate the real time course of molecular secretion from a single cell.

Key Words: videomicroscopy; HPLC resins; videomicroscopy; quinacrine; RBL-2H3 cells; A23187

Several methods have been used to detect cellular response with a microscope, including the use of Ca²⁺-chelating reagents, membrane potential probes, etc. However, there have been few reports of microanalyses of the molecules released from the single cell. We have been investigating the cell signal pathway of living cells by developing videomicroscopic methods, although it is difficult to detect the released signal molecules at the single-cell level. Here we report on the utilization of the high-performance liquid chromatography (HPLC) resins for the quantitative videomicroscopic analysis of molecules secreted from the cells.

SP-2SW resin (5 μm, TOSOH, Japan), which is a cation-exchange resin for HPLC packing with the sulfonyl propyl group, was used for molecular trapping. The videomicroscope system used in this study consisted of a fluorescence microscope (Zeiss Axiovert 135-TV; Carl Zeiss, Germany), silicon-intensified target (SIT) camera (C2741-08; Hamamatsu Photonics, Japan), PIP-4000 image processor (ADS Co., Japan), GPB-1 image processing board (Ezel-Sharp Co., Japan) and videotape recorder (AG-7355; Panasonic, Japan). The quinacrine and other reagents were all of reagent grade and used without further purification.

The fluorescence of SP-2SW resins (180 μl of 0.05% incubation buffer) in the buffer was monitored by the videomicroscope before and after 20 μl of quinacrine solution was mixed with the solution (Fig. 1). The concentration of basic quinacrine molecules onto the SP-2SW resin was rapid and saturation occurred within 10 sec (Fig. 2a). The distribution ratio was calculated as the ratio of the intensity of the fluorescence of SP-2SW and that of the background (Fig. 2b) and became constant soon after mixing. A decrease in the fluorescence intensity on the SP-2SW resin surface was found after 20 sec due to redistribution of quinacrine to the liquid phase, because no quenching of quinacrine fluorescence intensity was observed in this time frame. The calibration curve between the fluorescence intensity on the resin and

Fig. 1. The Videomicroscope System

* To whom correspondence should be addressed.

Fig. 2. Analysis of the Fluorescence Intensity of SP-2SW Resin

(a) Fluorescence intensity of SP-2SW resin and background. (b) Distribution ratio calculated from the fluorescence intensity of the resin divided by that of the background.

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the total amount of quinacrine in the solution was obtained and used for semiquantitative estimation of the trapped amount.

We then used this resin for the analysis of the cellular release of internal molecular product, i.e., secretion. The secretory granules of RBL-2H3 cells, a rat basophilic leukemia cell line, selectively incorporate quinacrine well, and release of quinacrine from the cells was observed when exocytosis of the granules occurred due to stimulation with antigen or the calcium ionophore A23187. In the present study, RBL-2H3 cells were used as a model cell line to study whether this method can be applied to the analysis of cellular secretion. Prior to the fluorescence videomicroscopic observation, RBL-2H3 cells on a coverslip were incubated in quinacrine solution to incorporate the molecules into acidic secretory microgranules of the cells and then washed twice with incubation buffer. The SP-2SW resin was placed close to a cell and calcium ionophore A23187 was added to the incubation buffer under videomicroscopic observation (Fig. 3a). Figure 3b-d shows sequential images of RBL-2H3 cell exocytosis after the stimulation. A number of microgranules in the cells exhibited quinacrine fluorescence, as seen in Fig. 3b, which began to disappear with the addition of the ionophore A23187, as seen in Fig. 3c, d and then fluorescence intensity of the particles (indicated by arrowheads) increased. It was apparent that quinacrine molecules, which were released by exocytosis, together with the secreted basic molecules inside the granules, e.g., histamine, serotonin etc., were trapped and concentrated on the SP-2SW resin. The fluorescence intensity of the resin close to the cells was also analyzed, as shown in Fig. 4. It was found that the increase in resin fluorescence has two phases with two peaks when the cells are stimulated by calcium ionophore A23187. The fluorescence intensity of the resin without this stimulation did not change from the starting intensity in this time frame. This result corresponds with a previous report in which two different signaling pathways were found to exist in granule exocytosis of RBL-2H3 cells.

The time course of single-cell quinacrine release from RBL-2H3 cells was observed indirectly by this method. The decrease in fluorescence intensity after 4 min seen in Fig. 4b may be due to the redistribution of quinacrine to the liquid phase. Semiquantitative analysis had been done with the calibration curve. The quinacrine released from several cells showed similar intensity to 10 pmol of total quinacrine. Since it is known that quinacrine release coincides with the release of histamine, this method can indirectly measure the amount of released histamine. This method can be applied for the trapping and analysis of various secreted molecules whose amount does not reversely correspond to the molecules remaining in the cells where the molecules are constantly being produced.

Trapping of secreted molecules using HPLC resin and simultaneous videodetection can be applied for the time course analysis of the molecular secretion of cells.

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REFERENCE

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