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Particle motion in single acinar cells observed by microscope laser light scattering spectroscopy

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Abstract. We report here the direct observation of the motion of cytoplasmic subcellular organelles and macromolecules within single, metabolically active, pancreatic acinar cells using microscope laser-light scattering spectroscopy (MLLSS). The relative amount and the "effective diffusion coefficient" of the mobile particles shows a marked difference in magnitude between the apical and basal regions of the cell. Secretory stimulation evoked by the cholinergic agonist bethanechol induces changes in the relative motion in the cytoplasm of approximately one fifth of the acinar cells. This study demonstrates the feasibility and utility of a novel technique, MLLSS, for the analysis of intracellular events in regions as small as $(2\,\mu m)^3$ in single, granule secreting cells. It also shows MLLSS to be a powerful tool for the detection and measurement of altered motion in discrete subcellular regions of small mammalian cells after biochemical and pharmacological manipulations.

Key words: laser light scattering, microscopy, acinar cells, granule secretion

Introduction

Cellular secretory mechanisms have attracted wide spread attention. A large number of models have been proposed to describe the process of secretion which in many cells involves a calcium-dependent exocytosis of preformed storage granules. Significant changes in the translocation of ions, the state of the cytoplasm, and intrinsic optical signals are associated with the secretory process (Matthews 1979; Zimmerberg and Whitaker 1985; Salzberg et al. 1985). Though a number of techniques make intracellular observations possible, microscope laser light scattering spectroscopy (MLLSS) is the first fully non-invasive technique of sufficiently high spatial resolution to enable a quantitative analysis of intracellular events within single, small, mammalian cells. Laser Doppler velocimetry and Brownian motion measurements in large non-mammalian cells of the order of 100 μm in diameter (Maeda and Fujime 1972; Piddington and Sattelle 1975; Langley et al. 1976; Mustacich and Ware 1976) have been described, but in smaller cells (red blood cells), only static intensity measurements were reported (Coletta et al. 1982). In contrast, our small volume MLLSS has proven a powerful tool in the investigation of hemoglobin aggregation in normal and sickle cell anemia red blood cells (Nishio et al. 1983; Nishio et al. 1985; Peetermans et al. 1986) and also in the study of eye lens cells (Sun et al. 1984; Peetermans et al. 1987) using dynamic laser light scattering.

We present here the first report of a study with MLLSS on more complex, granule secreting, cells, those of the mammalian pancreatic acinus. These cells release their digestive enzyme content by exocytosis of zymogen granules from the apical aspect of the cell upon stimulation with cholinergic agonists such as bethanechol. The response to the stimulus is generally assessed by measuring the amount of enzyme released into the suspension medium of the acinar cells following stimulation. Such a response is, however, an average over a large number of cells, so that it remained unknown if all cells have the same threshold for stimulation and similar kinetics of secretion, or if only a fraction of the cells secrete, while others remain relatively unresponsive. Using MLLSS, we could establish that, under the conditions of the experiment, the latter was the case. We could also detect differences in the movement of intracellular particles between the zymogen granule containing apical region and the

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basal region of the acinar cells by analyzing the time dependent fluctuations of light scattered from these discrete subcellular regions of single, metabolically active cells. Altering the composition of the suspension medium and the use of a pharmacological stimulant were found to influence subcellular particle motion within single acinar cells.

Materials and methods

Pancreatic acinar cells

Dissociated pancreatic acinar cells from Sprague-Dawley rats of 150–250 g wt were prepared essentially by the technique of Amsterdam and Jamieson (1974a). The dissociated cells were washed three times with Krebs solution of the following ionic composition (mmol l^{-1}; NaCl 118, KCl 4.7, $MgCl_2$ 1.13, NaH_2PO_4 1.15, $NaHCO_3$ 25, glucose 5.6) and suspended in oxygenated medium of similar composition at pH 7.4. The "stimulated" cells were suspended in the Krebs solution supplemented with Ca^{++} (2.56 m*M*) and bethanechol (0.2 m*M*). The isolated cells were kept at 37 °C on a temperature-controlled stage for study by MLLSS. It should be noted here that dispersed pancreatic acinar cells maintain their secretory responsiveness to cholinergic stimulant for at least 4 h after preparation (Amsterdam and Jamieson 1974b); all MLLSS measurements were therefore made within this time period.

Microscope laser light scattering spectroscopy (MLLSS)

This experimental technique differs from the conventional dynamic laser light scattering technique (Chu 1974) in that the scattering volume in MLLSS is some 10^5 times smaller making it as low as $(2\ \mu m)^3$. It therefore becomes possible to analyze the motion of particles inside small biological cells and to determine the relative contribution of mobile and immobile scattering material. Here we give only a brief description of the technique; further details can be found elsewhere (Nishio et al. 1983, 1985; Peetermans et al. 1986).

The scattered light intensity from a cell is composed of two parts. One part originates from non-moving scatterers such as the cell membrane and protein aggregates or organelles of sizes comparable to the cell diameter. Its corresponding scattered light intensity, $I_{imm}(t) = \langle I_{imm} \rangle$, is static. The other part originates from mobile scatterers such as granules and macromolecules within the scattering volume and it gives rise to a scattered intensity that fluctuates around its average value: $I_m(t) = \langle I_m \rangle + \Delta I_m(t)$. The total scattered light intensity $I(t) = I_m(t) + I_{imm}$ contains information on all scatterers and is analyzed in the form of an intensity autocorrelation function $c(\tau)$: $c(\tau) = \langle I(t)\, I(t+\tau) \rangle_t$.

The basic concept of intensity autocorrelation spectroscopy is that the fluctuations of the scattered light intensity at two different times are highly correlated if the time separation τ between the measurements is smaller than the time required for the scatterers to move over the characteristic length scale L at which the light scattering experiment probes. This length scale L is the inverse of the scattering vector, which is determined by the wavelength λ of the laser light in the cytoplasm and by the angle θ between the incident and the detected scattered beam: $L = \lambda/(4\pi \sin\theta/2)$.

If the time separation τ between successive intensity measurements is larger than the time needed for a scattering particle to move over this length L, the scattered light intensity fluctuations are uncorrelated. As a result, the intensity autocorrelation function $c(\tau)$ is a decaying function of time. The functional dependence on time is determined by the type of motion undergone by the scatterers. For particles in Brownian motion, $c(\tau)$ is an exponential function with the characteristic decay inversely proportional to the diffusion coefficient $\bar{D}$ of the particles that scatter light: $T = L^2/\bar{D}$. In the experiments presented in this report, L was approximately 200 nm.

In the case of highly concentrated, polydisperse protein solutions, like the cytoplasm, the motion of the proteins may not be a free diffusive motion and the diffusion coefficient $\bar{D}$ observed by laser light scattering may not represent the motion of individual protein particles. Rather, the observed diffusion coefficient is inversely proportional to the correlation length ξ, which represents the average distance beyond which particles move independently of one another. When two particles are within a distance smaller than ζ, their motion is affected by each other's presence and their motion is correlated. The correlation length ξ is the hydrodynamic radius of the individual particle in the absence of interactions between the particles. The viscoelastic properties of the cytoplasm can be easily altered by changing the interaction between the constituents (Ishimoto and Tanaka 1977). In this work, the correlation functions $c(\tau)$ are essentially exponential and we have extracted an "effective diffusion coefficient" $\bar{D}$ by fitting a single exponential curve to $c(\tau)$.

Besides $\bar{D}$, another quantity is of importance in this report: the ratio R_I of the average light intensity scattered by mobile particles relative to the total

average scattered light intensity (Peetermans et al. 1986): $R_I = \langle I_m \rangle / (\langle I_m \rangle + \langle I_{imm} \rangle)$. The quantity R_I characterizes the relative amount of mobile particles. It should be pointed out that intensity fluctuations due to changes in the number of large scatterers in the scattering volume may also contribute to the marked differences which we measured in different parts of the acinar cells under the conditions of our experiment (see below).

Results and discussion

Figure 1 is a diagram of an acinar cell. Two distinct regions are seen: the apical region within which most granules are stored, and the more transparent looking basal region. The nuclear region was avoided in gathering the data. An optical fiber, embedded in one of the eyepieces, picks up the scattered light and guides it to the detection system for photon correlation spectroscopy. It is shown in Fig. 1.

Figure 2 shows intensity autocorrelation functions taken on cells from the bethanechol-stimulated sample. Approximately one fifth of the cells in this sample showed increased motion in the basal region of the cells after bethanechol stimulation. This is clearly demonstrated in Fig. 2a which gives the correlation functions taken on the basal region of three such cells and the same functions taken on the basal region of three cells in the unstimulated sample. No difference was found between the signal recorded from acinar cells in the unstimulated sample and the apparently non-stimulated cells in the bethanechol containing sample. In the apical region, all cells, including the ones showing increased motion in the basal region, showed correlation functions that hardly decay at all, as is illustrated in Fig. 2b. Most striking is the marked difference between the correlation functions obtained from the apical and basal regions of the stimulated cells. The functions collected from basal regions decay substantially, whereas the functions taken from apical regions show little if any decay. This may be interpreted as follows. These cells, even though primed for secretion, contain a pool of granules which are closely packed in one aspect of the cell with restricted freedom of motion. In the basal regions, intracellular proteins and small organelles together with some granules, are more free to move. Accordingly, the intensity autocorrelation functions collected from these basal regions show appreciable decay.

So far, we have studied the cells in the two reported suspension media: a solution with no added Ca^{++} or secretory stimulant (unstimulated cells) and a solution containing bethanechol and Ca^{++} (stimulated cells). Out of approximately fifty cells inspected in the stimulated sample, about one fifth showed the substantially increased motion of intracellular macromolecules in the basal region which was presented above. This finding is of considerable importance in view of the fact that the receptors for stimulant recognition are restricted to the basal aspect of acinar cells but secretion occurs from the apical pole. Transmission of a secretory signal through the basal cytoplasm is therefore an

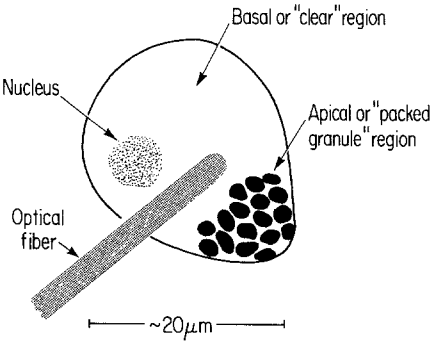

Fig. 1. Diagram of a pancreatic acinar cell showing the "packed granule", apical and the "clear", basal regions of the cell, and the cell nucleus. The optical fiber pick-up for the scattered light is also shown

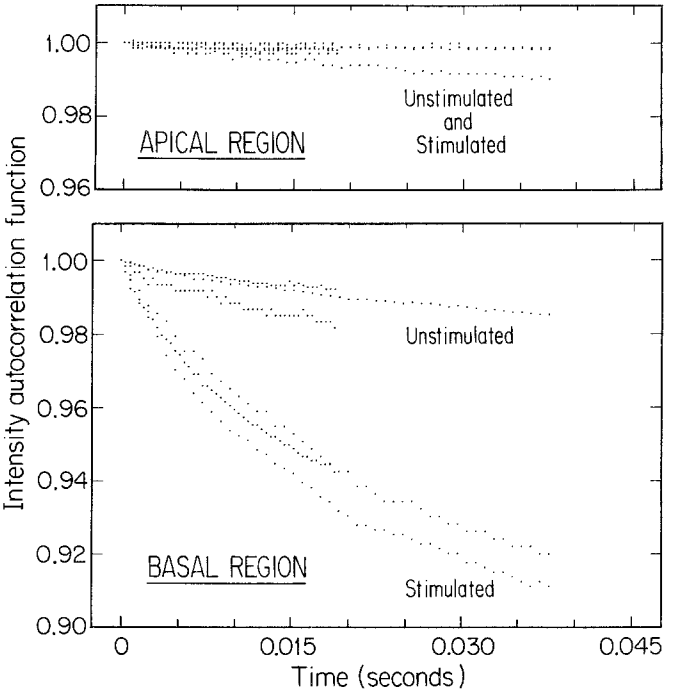

Fig. 2. Intensity autocorrelation functions for the apical (top) and basal (bottom) region of 3 unstimulated and 3 stimulated acinar cells at the same scattering angle (21 °)

Table 1. Measurements of the effective diffusion coefficient, $\bar{D}$, of the basal region, and the relative intensity of mobile scatterers, R_I, for the basal and apical regions, of stimulated or unstimulated pancreatic acinar cells

Cell type	$\bar{D}$ [cm²/s] basal	R_I basal	R_I apical
Unstimulated	3.54×10^{-9}	0.065	0.034
	1.92×10^{-9}	0.040	0.002
	0.58×10^{-9}	0.042	0.010
Stimulated	12.80×10^{-9}	0.153	0.031
	11.29×10^{-9}	0.142	0.021
	5.32×10^{-9}	0.164	0.134

essential and integral part of the secretory process. The fact that only a fraction of the cells in the bethanechol containing sample actually were stimulated is also very important for as Amsterdam and Jamieson (1974 b) pointed out in their first experiments measuring secretion from dispersed acinar cells, receptors for stimulant molecules might be located only on *some* individual cells of an acinus, others coupled via low-resistant pathways being excited indirectly. Only a single cell technique such as MLLSS could reveal such information.

The effective diffusion coefficient $\bar{D}$ of the basal region and the relative intensity of mobile scatterers R_I for the basal and the apical regions derived from the correlation functions of Fig. 2 are given in Table 1. The value of R_I is systematically higher in the basal region than in the apical region, reflecting the presence of the densely packed granules in the apical regions and relatively more mobile scatterers in the basal region of the acinar cells. The coefficient $\bar{D}$ of the basal region is increased about 3-fold in stimulated cells compared to unstimulated cells. The table gives $\bar{D}$ and R_I values for the 3 unstimulated and 3 stimulated cells on which the correlation functions of Fig. 2 were taken. These results were taken from correlation functions collected during 30 s on average. The reproducibility for repeated measurements at the same location of the cell is excellent.

In future studies it should be possible to determine, by use of a flow system for medium transfer, and by autocorrelation at different scattering angles, the magnitude of granule translocation within the apical region itself before and after stimulation of the same cell. Finally, MLLSS opens up the very important possibility of using selective pharmacological agents to probe the mechanisms underlying transfer of the secretory signal from the basal to the apical aspect of the cell and the dynamic control of the secretory process within single cells.

Conclusion

We have demonstrated that measurement can be made by photon correlation spectroscopy of intracellular movement in single pancreatic acinar cells. The difference in signal between the "clear" or basal and "packed granule" or apical region of one individual cell is striking. Furthermore, changes can be detected in the mobility of particles in the basal region of about one fifth of the cells exposed to a secretory stimulant in the conditions of the reported experiments.

These results clearly demonstrate the usefulness of MLLSS for non-invasive intracellular analysis and pave the way for a high spatial resolution study of events in discrete regions of single, metabolically active, secretory cells. Our preliminary data on pancreatic acinar cells form an example to illustrate the power of this technique in the detection of modified movement in subcellular sections of small mammalian cells resulting from exposure to a pharmacological agent. These data obtained here on pancreatic acinar cells also illustrate the way in which pharmacological agents may be used together with MLLSS to analyze the secretory process at the subcellular level.

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