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Probing the light scattering properties of insulin secretory granules in single live cells

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ABSTRACT

Light scattering was recently demonstrated to serve as an intrinsic indicator for pancreatic islet cell mass and secretion. The insulin secretory granule (ISG), in particular, was proposed to be a reasonable candidate as the main intracellular source of scattered light due to the densely-packed insulin semi-crystal in the granule lumen. This scenario, if confirmed, would in principle open new perspectives for label-free single-granule imaging, tracking, and analysis. Contrary to such expectations, here we demonstrate that ISGs are not a primary source of scattering in primary human β-cells, as well as in immortalized β-like cells, quantitatively not superior to other intracellular organelles/structures, such as lysosomes and internal membranes. This result is achieved through multi-channel imaging of scattered light along with fluorescence arising from selectively-labelled ISGs. Co-localization and spatiotemporal cross-correlation analysis is performed on these signals, and compared among different cell lines. Obtained results suggest a careful re-thinking of the possibility to exploit intrinsic optical properties originating from ISGs for single-granule imaging purposes.

1. Introduction

Insulin secretory granules (ISGs) are subcellular organelles of specialized β-cells, with a deceptively simple morphology. In electron micrographs they appear as spheres of around 200–300 nm in diameter comprised of a crystalline, electron dense, core of insulin surrounded by a mantle (or 'halo') of less dense material and enveloped by a simple phospholipid bilayer [1,2]. The granule, however, is far more than just a deposit of the hormone in the cell. It is the site of proteolytic activation of the hormone. Also, it is involved in intercellular communication through the secretion of other biologically active molecules. What is not immediately apparent from electron micrographs is that the insulin granule, perhaps more than any other organelle in the β cell, is a dynamic structure: approximately 10% of the granule population turns over every hour during secretion in live cells [1]. Correct ISGs intracellular trafficking is crucial to assure proper regulation of glucose homeostasis through insulin secretion. In fact, on the other hand, defects in granule trafficking and secretion are recognized hallmarks of pancreatic β-cells dysfunction and, as a consequence, of the onset of Type-2 Diabetes [3]. Current knowledge on granule dynamic behavior mostly owes to both the production of immortalized β-cell lines that retain normal regulation of insulin secretion (for a review see Ref. [4]), and the development of granule-specific markers based on genetically-encoded fluorescent proteins (FPs) (see, among many, Refs. [5–9]). These latter, besides opening new exciting possibilities for the study of ISG dynamics in cells, imply the heterologous over-expression of fluorescently-tagged proteins, which in turn can potentially alter granule functions. Regard to this, Ilegems and co-workers recently demonstrated that light scattering can be used as an intrinsic, label-free indicator for the noninvasive characterization of pancreatic islet morphology and plasticity as well as hormone secretory status [10]. Noteworthy, based on the observed decrease in scattering signal (and concomitant decrease in electron density in TEM micrographs) upon treatment with the Zinc-chelator TPEN, the authors indicate mature ISGs, and in particular the insulin semi-crystal they contain, as the putative primary source of scattered light [10]. This hypothesis, if confirmed, would in turn pave the way to new exciting possibilities for single-granule imaging, tracking, and analysis in live cells and tissues, with no need to over-express fluorescently-tagged granule proteins. This

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intriguing scenario motivated us to investigate the intrinsic optical properties of single ISGs in single live cells by means of a combination of multi-channel imaging of scattering and fluorescence (from selectively labelled ISGs), co-localization assays, spatiotemporal cross-correlation analysis, and comparison between β-like and non β-like cell lines.

To start, primary human cells were disaggregated from Langherans islets, plated on the glass, fixed and immunostained against insulin to distinguish β-cells from non-β-cells (Fig. 1). Then, cells were quantitatively assessed for their light scattering properties, at high resolution, by confocal microscopy. In particular, we recorded the backscattering signal at various laser illumination wavelengths (as schematized in Fig. 1a), that is: a unique detection signal peaking at the incident light wavelength. As can be clearly discerned from Fig. 1b, at any incident wavelength, islet-derived cells show scattering signal as a punctuate pattern, mostly excluded from the cell nuclei, in keeping with previously reported results [10]. The point here is to prove whether such punctuate signals arise specifically from ISGs. In fact, β-cells contain thousands of granules enclosing dense-core crystals, which represent about 10–20% of the cellular volume [11–13]. Under the hypothesis that the insulin-enriched granule lumen is the source of scattered light, β-cells are expected to be much more refractive than non-β-cells, which are devoid of insulin-containing granules. Thus, we selected those cells which are not positive for insulin antibody. Contrary to our expectations, non-β-cells are not distinguishable from β-cells in terms of the overall amount of scattered signal, irrespective of the incident wavelength used (Fig. 1c). Although in line with previous observations [10], this preliminary assay in fixed cells does not support the idea of the ISG as an intrinsic intracellular source of scattering, at least not unique.

To better clarify this crucial issue we set out to perform measurements on ISG optical properties in live cells. To this end, we used INS-1E cells, a laboratory standard of β-like insulin-secreting cells. As control, we tested the scattering properties of a non-β-like cell line, i.e. Chinese Hamster Ovary (CHO-K1) cells. Quite surprisingly, but in line the results discussed above, CHO-K1 cells show a similar overall level of scattering as compared to INS-1E, and an analogous punctuate pattern of scattering signal at the intracellular level (Fig. 2a–b). To assess the identity of the scattering sources within tested cells, we performed experiments in presence of fluorescent markers of specific intracellular structures. As shown in Fig. 2c, we first labelled ISGs in INS-1 cells by transient transfection of the Phogrin-mCherry protein. Phogrin is a granule-specific transmembrane protein [14] which, in our aim, does not perturb the insulin intra-granular dense-core crystal, i.e. the putative source of scattered light. In a single-wavelength (i.e. 561 nm), dual channel experiment we combined the simultaneous detection of scattering and fluorescence signals from ISGs. As control, a similar experiment was performed on fluorescently-labelled lysosomes, a well-known intracellular source of scattered light [15]. Worthy of note, already from visual inspection of the two channels (and their superimposition), it is quite clear that punctuate scattering signal from INS-1 cytoplasm does not convincingly correspond to labelled granules. By contrast, in keeping with expectations, lysosomes appear as quite highly-refractive intracellular organelles. A standard co-localization analysis of the scattering and fluorescence signals (e.g. by calculation of Manders' coefficients) would quantitatively support, in principle, visual inspection. Still, in our opinion, it would be prone to artifacts, due to the presence of a background of 'speckle'-like scattering from the cell [16]. To tackle this issue, we set out to perform spatiotemporal cross-correlation analysis on time-lapse imaging of scattering and fluorescence signals. In particular, by using cross-STICS (Spatio-Temporal Image Correlation Spectroscopy) [17,18] analysis, we can highlight the possible dynamic co-localization of signals, i.e. the presence of intracellular dynamic structures which retain both the scattering and fluorescence signals while moving (a feature that does not apply to speckle-like signal). On this basis, we probed fluorescent ISGs and, as a reference, stained lysosomes (Fig. 2c and exemplary S1 and S2 videos). As better described in the Methods section, the amount of cross-correlation measured was used to classify cells as 'positive' or 'negative', in terms of the dynamic co-localization of signals (Fig. 2d). As reported in the histograms in Fig. 2e, only a relatively low amount of measurement on ISGs (about 30%) showed positive cross-correlation with scattering signals. By contrast, almost 70% of the measurements showed positive dynamic co-localization of labelled lysosomes with scattered signals. Extending a similar analysis to additional intracellular organelles is beyond the scope of the present work. Still, one can imagine a similar positive contribution from many other sub-cellular structures, such as mitochondria [19,20]. In general, these results point out that scattering from ISGs, if present, does not afford a dominant contribution to the total cellular refractive properties.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.bbrc.2018.08.029.

The contrast between what shown here and what reported by Ilegems at al [10]. is, in our opinion, only apparent. The authors, in fact, already commented on the evidence that scattering was not coming exclusively from β-cells in their experiments, although quantification of signals from non-β-cells and/or co-localization of the punctuate scattering pattern with fluorescently-labelled ISGs

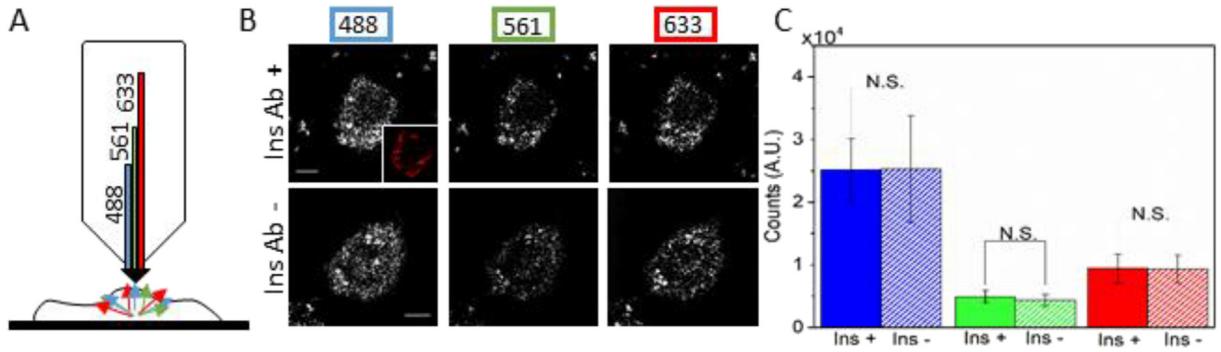

Fig. 1. a) Schematic representation of backscattering signal acquisition. b) Backscattering signal of Human Langerhans islets dispersed cells, either positive for insulin (example of positive immunostaining in the inset of top panel) and negative (lower panel) measured at three different wavelengths: 488 nm, 561 nm and 633 nm. Scale bar: 5 μm c) Comparison of the average backscattering signal at the three selected wavelengths for insulin-positive (n = 10) and insulin-negative cells (n = 10). No statistically significative differences are reported (N.S.) according to Kolmogorov-Smirnov test (significance level: 0.01).

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