Particle tracking software allows to discriminate functionally different vesicles involved in gliadin peptide uptake

ID - 202

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Motivation

Gliadin peptides are involved in the pathogenesis of coeliac disease and, although little is known about their processing, there are indications in the literature that they enter the enterocytes. Transcellular transport of the peptide 31-43 has been demonstrated by employing biopsy specimens from untreated coeliac patients. Video time-lapse microscopy, if coupled with particle tracking software, may be employed to monitor vesicle trafficking associated with peptide uptake. A set of software tools originally developed for visualization and statistical evaluation of cellular movements from sequences of subsequent frames, was available in the laboratory and its adaptation to the evaluation of sub-cellular structure dynamics appeared a promising tool towards the understanding of peptide trafficking within the cytoplasm.

Methods

Series of images of cells treated with gliadin peptides were generated by confocal microscopy, used to acquire sets of individual frames spaced by 30 second over a period of 10 min. Cells were seeded on special glass-bottom dishes (3 cm diameter), to allow live observation. The acquired image stack was then elaborated with custom made software, developed by using the PHP language and consisting of a number of objects designed to manipulate the series of images and the tracks followed by particles. It takes as an input a text file containing cartesian coordinates of each followed vesicle, coming out from computer-assisted recording of subsequent positions. The program calculates distance and direction at each time step; then, average speed, direction and tortuosity are evaluated both for each vesicle during the whole observation period, as for the population at each time step. Statistical analysis is used to evaluate the significance of measured speed, direction and tortuosity of moving elements. The list of coordinates is also used to draw the paths covered by the elements of the population studied. Visualization of followed paths may be superimposed onto the original images, and vectors describing the observed movements may be calculated and displayed in the same way.

Results

Live observation was carried out on cells labelled with lissamine-conjugated gliadin peptides (P31-43-liss and P56-68-liss) to study their effects on vesicle dynamics.

After a 30 min at 37C pulse with a mixture of labeled and not labeled peptides, cells were chased with unlabelled peptide and the observation continued in a specially designed incubator (OXO-lab) that can control temperature and CO2. This technique allows to record the live cells for several minutes (10 min), while collecting a series of images every 30 sec. The movement of each vesicle was analysed to study the paths followed by P31-43 and P56-58 labelled vesicles. Analysis of data produced by live observation of cells treated with labeled peptides, allowed to show that vesicle subpopulations may be identified, characterized by different motility, corresponding to P31-43 carrying vesicles, which appear to be slower than those carrying P56-68 both at 30 min. and 3h time point. P31-43-liss labelled vesicles are much slower than P56-68-liss both at 30 min pulse and at 3h chase. P56-68-liss interacting vesicles present longer trajectories (6 microns/10 min. at 30 min pulse and 0.6 microns/10 min. at 3h hour chase) than P31-43-liss interacting vesicles (0.3 microns/10 min at 30 min pulse and 0.3 microns/10 min at 3h chase), suggesting that the latter vesicles do not change their distribution with time because of reduced motility. As a whole, these procedure allowed to suggest that the peptide 31-43 remains in the early endocytic vesicles, causing impaired maturation of these vesicles into late endosomes.

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