doi:10.1017/S1431927611001747
Microsc. Microanal. 17 (Suppl 2), 2011

Substitutionally Enhanced STEM (SE-STEM) Imaging of Protein Aggregates Inside Cells

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The aberrant folding and subsequent aggregation of proteins into insoluble plaques known as amyloid fibrils is the underlying mechanism behind a number of diseases, including Alzheimer's and Parkinson's diseases [1]. The exact role that these aggregates play in the disease process is not yet well understood [1]. Part of the reason for this lack of understanding is that it is extremely challenging to visualize the interactions between the protein aggregates and human cells or tissue due to the difficulties that arise when attempting to identify the carbon-rich aggregates within the carbonaceous cellular environment [2]. Traditional strategies to overcome this lack of contrast have involved the use of stains or tags that can potentially be either unreliable or intrusive.

In this work we have taken a fragment of the Alzheimer's-related Aβ peptide and replaced the naturally occurring sulfur that is present in the methionine amino acid with a selenium atom. Human phagocytic cells were exposed to different aggregate species formed from the selenium-enhanced Aβ fragment and its selenium-free analogue to examine how these aggregates are taken up by the cells, where they localise to within the cells and how their structure changes after uptake. Cells exposed to the selenium-enhanced aggregates were imaged using high angle annular dark field scanning transmission electron microscopy (HAADF-STEM), an electron microscopy technique in which only electrons that are scattered to high angles on interaction with the sample are used to generate an image. Electrons that are scattered to relatively high angles have undergone Rutherford scattering, the cross-section of which is dependent on Z^n (n~2) [3], HAADF-STEM is therefore extremely sensitive to local variations in atomic number. The replacement of the naturally occurring sulfur with a selenium atom increases the electron scattering cross-section of the atom in question by a factor of approximately 4.5. This allows the selenium-enhanced aggregates to be identified reliably within the cellular environment without the use of any other stains. Energy dispersive X-ray spectroscopy (EDX) was used to confirm the presence of selenium.

The high spatial resolution of substitutionally enhanced STEM (SE-STEM) imaging allows the localisation of specific aggregates to be determined in different subcellular locations, from the nucleus to the plasma membrane, without the use of any stains. The imaging method also allows the examination of the structure of individual aggregates and their stability within the cellular environment.

This is a high-resolution imaging technique that could be extended to the visualisation, within a cellular or tissue environment, of any specific protein or biological molecule that naturally contains a sulfur atom. It therefore holds great potential for the examination of both disease-related and physiologically healthy biological systems. [4]

References

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[4] This work was supported by the UK Engineering and Physical Sciences Research Council.

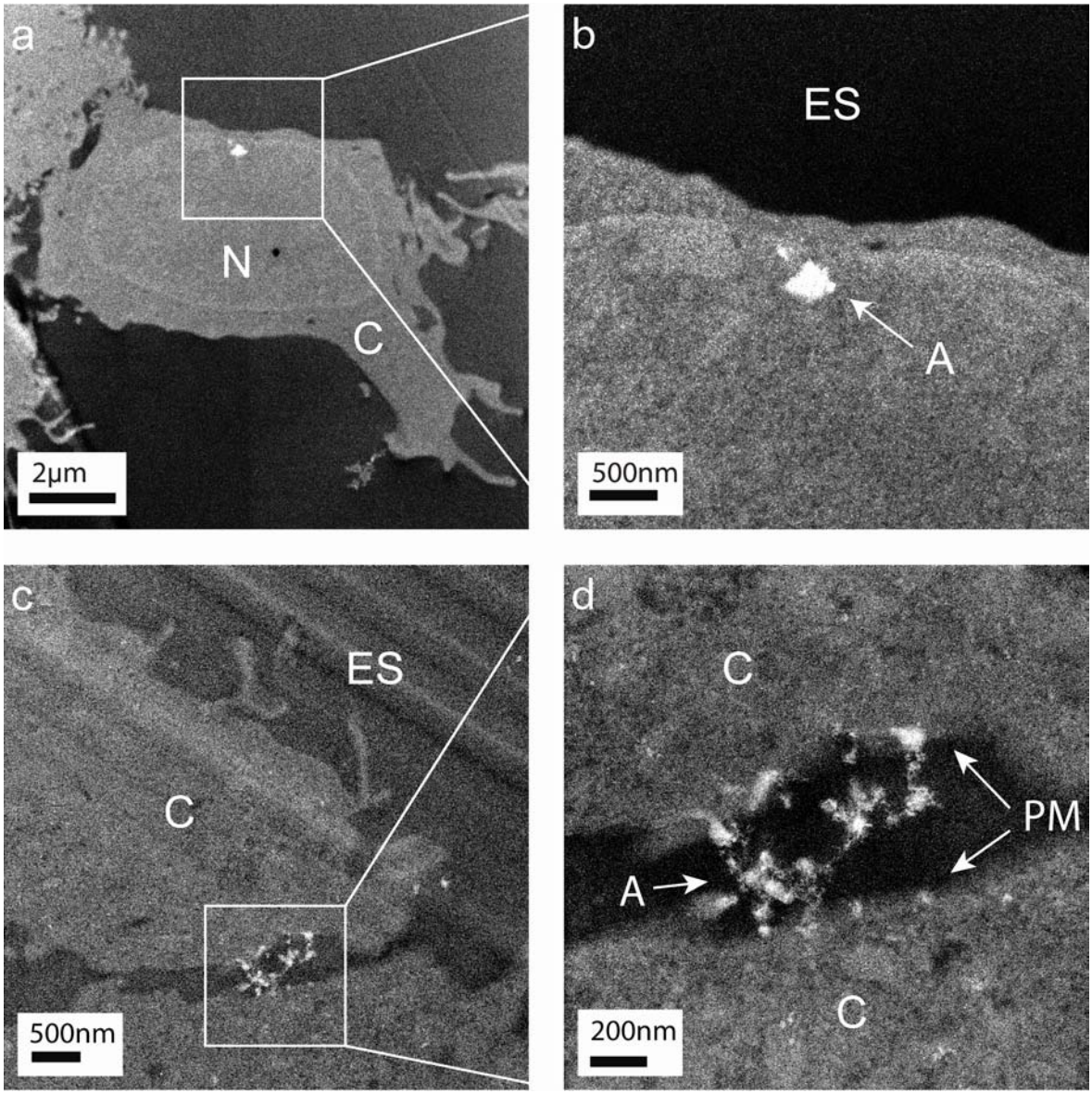

FIG. 1. HAADF-STEM images of cells exposed to selenium-enhanced protein aggregates. Aggregates can be clearly seen in the cell nucleus (a & b), and at the plasma membrane (c & d) and their structure can be assessed. N = nucleus; C = cytoplasm; ES = extracellular space; A = aggregate; PM = plasma membrane.

https://doi.org/10.1017/S1431927611001747 Published online by Cambridge University Press