Characterizing the pH and Salt Dependence of Neurofilament Grafting Densities

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Introduction:

Neurofilaments
- A class of intermediate filaments
- 10nm-wide protein rods composed of three subunits: low, medium and high (NF-L, NF-M, NF-H)
- Once assembled into filaments, the C-terminus tails of the subunits radiate outward, interacting with the sidearms of adjacent filaments to form extensive neurofilament (NF) networks within the cell
- Stress-buffering members of the axoplasm
- Implanted in maintenance of neuronal cell structure, as support structures (scaffolds) for transport that occurs on microtubules, and in dendritic arborization

Motivation
- Implication of NF’s in such neurodegenerative diseases as Amyotrophic lateral sclerosis, Parkinson disease and Alzheimer disease

Subunit Proteins to Functional Hydrogel

Three Subunit Proteins

Filament

Hydrogel / NF Network

Three Dimers

Tetramer

Grafting Density
- NF-M and NF-H can only dimerize with NF-L
- The ratio of assembled NF-M and/or NF-H to NF-L in the filament

Objective
- To quantify the percentage of NF-M added to the protein solution that has grafted to NF-L at the given assembly buffer salt concentrations and pHs, and to compare this percentage to that obtained for the standard assembly buffer conditions
- Assembly conditions tested include salt concentrations of approximately 40mM, 90mM, 150mM, 240mM and 500mM at pHs of 6 and 8.8
- Initial grafting densities of approximately 12%, 20%, 27%, 34%, 36%, 40%, 46%, 51% and 57% for each buffer condition
- Total of 99 samples tested

Methodology:

Left: the Bradford Assay compares a measured absorbance to a known protein concentration. Measured absorbance of gel phase quantifies subunit presence using the resultant curve.

Above: 15% polyacrylamide gel run on 10% NF-M. Relative intensities for each band in a lane are measured with ImageJ, giving %NF-M for each sample; the stock solution represents initial conditions for all subsequent samples.

Results:

Previous Work
Following the characterization of grafting densities for NF networks at standard conditions, there was a need to determine the pH and salt dependence of grafting density, if any. Quantifying any such effect was the focus of this study.

Current Work
An ideal network grafting density (w% NF-M in gel) is equivalent to the subunit’s initial density prior to assembly (w% NF-M initial), giving a slope of one when plotted against each other. Because subunits may only form dimers containing at least one NF-L (the second subunit may be NF-L, NF-M or NF-H), a saturation point of 50% by unit 62.0%, by weight, gives the maximum possible composition of NF-M. Samples were tested up to 57 weight% NF-M; consequently, only two buffer conditions (150mM at pH 6.04 and 240mM at pH 6.8) displayed clear saturation points within the range tested.

Grafting Densities

Polyacrylamide Gel Analysis

Lane 7 Intensity Histogram (35% 45mM pH 6.82)

Above: Intensity of each band in an eleven acrylamide gels was analyzed using a histogram created in ImageJ (pictured here with the lane it represents). The comparative area of each peak—the first of which represents NF-M, the second NF-L—correlates directly to the w% of the corresponding subunit.

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