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Development and optimization of chromatographic methods for the separation of recombinant tau proteins

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In neurons, tau proteins play key role in the stability of microtubules and in the efficiency of axonal transport through regulated interactions with tubulins, which are affected by isoform variations and phosphorylation. However, pathological modifications, specifically hyperphosphorylation, lead to microtubule disruption and to the formation of tau aggregates, including oligomers and neurofibrillary tangles. These aggregates negatively impact neuronal function and contribute to neurodegenerative diseases. The intercellular transfer of these aberrant tau proteins induces neuronal cell death and the advancement of neurodegenerative diseases, particularly in Alzheimer's disease.

The objective of this study was to design an effective chromatographic process for the separation of tau protein isoforms. The inherent similarity in primary structure among these isoforms complicates separation due to their comparable physicochemical characteristics. The potential of size-exclusion chromatography, which enables separation within a narrow size range, was explored. Additionally, hydrophobic interaction chromatography was investigated to leverage differences in hydrophobicity among isoforms.

To enhance separation efficiency, size-exclusion chromatography columns with varying pore sizes, connected in series with increasing pore diameter, were tested. Buffers of higher ionic strength were employed to eliminate non-specific interactions. Some isoforms, despite their minor molecular weight differences, were successfully separated using a 0.01M Tris buffer (pH 7) supplemented with 0.5M NaCl and GuHCl as mobile phase.

Initial experiments using hydrophobic interaction chromatography were conducted with a reversed-phase column and a 5-80% acetonitrile gradient in 0.1% formic acid as mobile phase. Preliminary chromatograms showed visible peaks, suggesting potential separation of tau protein isoforms.

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