Probing Peptide-metal Ion Interactions on a Phosphorylated α-Synuclein Fragment

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ABSTRACT: To understand the causal relationship between phosphorylation and aggregation of α-synuclein, a protein involved in Parkinson's Disease, it is important to know how the phosphorylation of the protein alters its metal binding properties and tendency towards aggregation. Synthetic peptides corresponding to a C-terminal segment of α-synuclein containing a phosphorylated tyrosine residue (pY125) have been studied extensively by using terbium (Tb$^{3+}$) luminescence spectroscopy, ESI-mass spectrometry, circular dichroism and $^{31}$P NMR. The 1:1 dissociation constant for Tb$^{3+}$: pY125 peptide was determined by fluorescence and ESI-MS titrations to be $K_D = 0.3(5)$ μM at pH 7.0. Two additional metal binding events were observed at higher Tb$^{3+}$ concentration. Interaction of this peptide with other di- and trivalent metal ions was monitored by competitive displacement of Tb$^{3+}$. The pY125 peptide has a high binding affinity for Fe$^{3+}$, which was confirmed by ESI-MS studies. Fe$^{3+}$ and Al$^{3+}$ are metal ions that uniquely form cross-linked species of the pY125 peptide.