Structural Studies With Importin α And Ku70 NLS Peptide Complex

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The Importin α (ImpA) plays a role in the classic nuclear import pathway recognizing proteins whose has activities in the nucleus. Those proteins contain nuclear localization sequences (NLS) which are characterized by one or two clusters of basic amino acids (monopartite and bipartite NLSs). In this work we present the expression, purification and X-ray crystallography experiments of ImpA from *M. musculus* and the NLS peptide from the DNA repair protein Ku70 to provide more information about the NLS recognition by ImpA. ImpA(70-529) sample was obtained in *E. coli* expression system and purified by affinity chromatography followed by an ion exchange chromatography. A single crystal of ImpA-Ku70NLS complex grew by hanging drop method and collected at a wavelength of 2.6Å (at 100K) using a Synchrotron Radiation Source (Laboratório Nacional de Luz Synchrotron, LNLS, Campinas, Brazil). The crystal was isomorphous to the native and its data was processed using software's from HKL2000 package. Molecular replacement techniques determined the crystal structure. Results of the ImpA- Ku70NLS complex indicated an electron density available only in the major binding site corresponding to the Ku70 NLS peptide. It bounded to the ImpA similarly to the phosphorilated version of the SV40 antigen T NLS showing the possibility of this peptide being a monopartite type in spite of being a bipartite as suggested in previous studies.

Keywords: Importin α, nuclear import, NLS, X-ray crystallography, Ku70.

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