CRYSTAL STRUCTURE OF IMPORTIN-ALPHA FROM NEUROSPORA CRASSA COMPLEXED WITH A CLASSICAL NUCLEAR LOCALIZATION SEQUENCE
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Neurospora crassa is a filamentous fungus that is widely used as a model organism to study different aspects of eukaryotic cells, including the classical nuclear import pathway. The protein Importin-α (Impα) is a key piece in this process, recognizing nuclear localization sequences (NLSs) in the macromolecules that will be transported. To understand and compare the nuclear import pathway in fungus, this work presents the first structure of Impα from a filamentous fungus (NcImpα) complexed with a classical NLS (SV40NLS). The aim is characterize and compare the Impα structure from fungus complexed with a classical NLS with others Impα complex. The recombinant protein Impα was expressed and purified by affinity chromatography for crystallization and other biophysical experiments. X-ray diffraction data were collected from a single crystal of NcImpα/SV40NLS using a synchrotron radiation source. Calorimetric analysis were also performed to determine the affinity and thermodynamic parameters of the interaction between Impα and SV40NLS peptide. The final NcImpα/SV40NLS structure was obtained with a high resolution (1.75 Å) allowing unambiguously observe the peptide binding sites. The structure showed a high similarity with the Impα from yeast and rice that belongs to the same subfamily α1. SV40 NLS peptide binds at major and minor binding sites of NcImpα, however the binding at the minor site is more similar with rice Impα, with a lysine and arginine at P1’ and P2’ positions, respectively. Therefore, comparing the binding of different peptides with Impα from others organisms, this arrangement seems to be the most favorable. Calorimetric analysis permitted a direct comparison with mammalian Impα/SV40 NLS complex and were compatible with crystallographic results, showing the binding differences of peptide at the NLS minor site between both structures. The results highlight the differences between Impα from subfamilies α1 and α2, besides enhance the importance for minor site in the specificity of Impα to recognize NLSs.

Keywords: Importin-α; Neurospora crassa; NLS.
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