Article

11

Structural basis for signal-sequence recognition by the translocase motor SecA as determined by NMR.

Gelis I, Bonvin AM, ..., Economou A, Kalodimos CG
Cell. 2007 Nov 16; 131(4):756-69

Abstract | Full Text | Related Articles | Citations on Google Scholar | Order Article

Relevant Sections

Export Email Add to MyF1000

Post to

Comments

Only users with full subscription access can leave comments.
No comments yet.

Evaluations

Evaluated by Victoria Ann Higman and Linda Ball | Ian Collinson

This work provides an excellent demonstration of the power of recently developed NMR and isotope labelling methods to provide information on structure, function, dynamics and mechanisms of large, biologically important protein complexes in the solution state.

Evaluated by:
Victoria Ann Higman and Linda Ball
Leibniz-Institut für Molekulare Pharmakologie, Germany
Structural Biology
01 Dec 2010

Rating 6 Recommended

Although solution NMR methods are primarily used to study proteins and complexes with molecular weights below 50kDa, recent advances in isotope labelling schemes and NMR methodology also mean that NMR can now be applied successfully to investigations of much larger complexes. Gelis et al. combine some of these new methods to structurally characterize the interaction of full-length SecA, the 204kDa ATPase motor of the Sec translocase, with functional signal peptides, with the aim of better understanding the extensive promiscuous binding capability of SecA.

Key elements of this study were the selective labelling of Ile, Val, Leu and Met methyl groups within the 204kDa protein and the use of methyl transverse relaxation optimized spectroscopy (TROSY) to record 1H-13C heteronuclear multiple quantum coherence (HMQC) spectra of exceptional quality for all of these residue types. Resonance assignment was achieved by breaking down SecA into smaller constructs, which were easier to assign, and comparing the 1H-13C HMQC spectra of the various constructs with that of the full-length SecA. A high degree of resonance correspondence meant that most assignments could be transferred to the full-length SecA from the smaller fragments. The remaining resonances were assigned with 3D nuclear Overhauser enhancement spectroscopy (NOESY).

The determination of the structure of SecA in complex with a signal peptide was based upon the use of (a) spin labels on the peptide, which affect the SecA methyl-specific spectra and provide important distance constraints, and (b) transferred NOEs to the peptide, which define the peptide conformation when bound to SecA. NOEs and spin labels were also used to great effect in studying inter-domain dynamics in SecA. Finally, chemical shift mapping was used to show that the C-terminal tail of SecA has an auto-inhibitory function. All of these NMR methods are well suited to the study of large protein complexes and this study shows what is possible when they are used in concert. The conclusions drawn from the NMR data were supported by the use of mutagenesis and isothermal titration calorimetry (ITC) to determine the binding affinities of mutated signal peptides, including the spin-labelled mutants. This provided confirmation of the functional conclusions and an understanding of how the spin labels affected binding. The work presented is rather a formidable study, which has significantly advanced general understanding of SecA function. In addition, it illustrates the applicability of NMR to the study of large protein complexes refractory to study by other techniques.

Competing interests: None declared

Cite this evaluation

This paper is important because it describes the first structural arrangement of a signal sequence with a component of a protein translocation apparatus.

Evaluated by:
Ian Collinson
University of Bristol, UK
Structural Biology
20 Dec 2007

Rating 10 Exceptional

Protein targeting and translocation in bacteria can occur by a post-translation route, governed by a motor ATPase component (SecA) and a protein channel (SecYEG).

The study employs NMR to determine a number of constraints to define the nature and consequences of the binding of a signal sequence peptide to SecA. The peptide forms an alpha helix upon binding and, furthermore, associates with only one of two states of SecA identified in solution. These results contribute important information necessary for our understanding of the ATP-driven

mechanics of this reaction.

Competing interests: None declared

Cite this evaluation

Classification Key Changes Clinical Practice | Novel Drug Target | Technique | Clinical Trial | Review

Cite this page