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The Dimerization Mechanism of LIS1 and its Implication for Proteins Containing the LisH Motif

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Miller-Dieker lissencephaly, or "smooth-brain" is a debilitating genetic developmental syndrome of the cerebral cortex, and is linked to mutations in the *Lis1* gene. The LIS1 protein contains a so-called LisH motif at the N terminus, followed by a coiled-coil region and a seven WD-40 repeat forming β-propeller structure. *In vivo* and *in vitro*, LIS1 is a dimer, and the dimerization is mediated by the N-terminal fragment and is essential for the protein's biological function. The recently determined crystal structure of the murine LIS1 N-terminal fragment encompassing residues 1–86 (N-LIS1) revealed that the LisH motif forms a tightly associated homodimer with a four-helix antiparallel bundle core, while the parallel coiled-coil situated downstream is stabilized by three canonical heptad repeats. This homodimer is uniquely asymmetric because of a distinct kink in one of the helices. Because the LisH motif is widespread among many proteins, some of which are implicated in human diseases, we investigated in detail the mechanism of N-LIS1 dimerization. We found that dimerization is dependent on both the LisH motif and the residues downstream of it, including the first few turns of the helix. We also have found that the coiled-coil does not contribute to dimerization, but instead is very labile and can adopt both supercoiled and helical conformations. These observations suggest that the presence of the LisH motif alone is not sufficient for high-affinity homodimerization and that other structural elements are likely to play an important role in this large family of proteins. The observed lability of the coiled-coil fragment in LIS1 is most likely of functional importance.

Keywords: homodimerization; protein structure; neuronal migration; lissencephaly; protein stability

Introduction

The Miller-Dieker lissencephaly, or "smooth-brain", is a developmental syndrome caused by mutations in an autosomal gene *Lis1*.[1] The gene codes for a protein which contains an N-terminal motif (residues 8–37) found in numerous eukaryotic proteins and denoted LisH (LIS1-homology motif),[2] followed by a coiled-coil region and a seven-blade β-propeller domain found in a number of signaling proteins of the WD-40 family.[3] LIS1 forms homodimers, and is implicated in interactions with other proteins including the catalytic homo- and heterodimers of the brain PAF-acetylhydrolase (PAF-AH).[4] The homodimerization of LIS1 is essential for its biological function. Heterozygous mice lacking exon 1 (residues 1–63) show a typical lissencephaly phenotype, and the mutant protein no longer interacts with the PAF-AH catalytic subunits.[5]

Recently, two crystallographic studies provided the first insight into the molecular architecture of LIS1. The first described a high-resolution structure of a homodimer of the N-terminal fragment of murine LIS1 (referred to hereinafter as N-LIS1), encompassing residues 1–86 (PDB, 1UUJ),[6] and the second described the complex of LIS1 with the catalytic α_2-homodimer of the PAF-AH (PDB, 1VYH).[7] The homodimeric structure of N-LIS1 is surprisingly asymmetric, because the two helices destined to form a coiled-coil at the C-terminal end,

Abbreviations used: PAF-AH, PAF-acetylhydrolase; a.a., amino acid residue(s); GdmCl, guanidinium chloride; TFE, trifluoroethanol; HSQC, heteronuclear single quantum coherence.

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originate with their axes set at an angle of ~55°, so that one needs to form a distinct kink to allow for a parallel alignment downstream. The coiled-coil fragment, visualized by the crystal structure, had been originally predicted on the basis of the presence of three canonical heptad repeats within the amino acid sequence $(abcdefg)_3$, where *a* and *d* are hydrophobic amino acids, while *e* and *g* carry opposite charges.[8] These heptads indeed form a hydrophobic "zipper", which is vital for the integrity of the coiled-coil. Interestingly, the 3.4 Å resolution structure of the full-length LIS1 in complex with the PAF-AH shows no interpretable electron density for the N-LIS1 fragment,[7] suggesting that this fragment is disordered in the crystals. The N termini of the two β-propeller domains of LIS1 are ~60 Å apart, implying that parallel supercoiling upstream of these domains is virtually impossible. This suggests a possibility that the N-LIS1 domain may alternate between closed (i.e. coiled-coil) and open conformations, and that this flexibility has a functional role.

The LisH (LIS1-homology) sequence motif (residues 8–37) is found in numerous proteins in all eukaryotic genomes, including human. At least three proteins containing the LisH motif are implicated in genetic syndromes: the transducin β-like 1X (TBL1), which causes ocular albinism with late-onset sensorineural deafness;[9] the OFD1, involved in oral-facial-digital syndrome type 1;[10] and TCOF1, implicated in the Treacher-Collins-Franeschetti syndrome.[11] TBL1 contains a LisH domain (a.a. 54–87), an F-box, and seven WD-40 repeats in its C-terminal region. Mutations in the fly ortholog, Ebi, affect multiple processes including neuronal differentiation through the epidermal growth factor receptor pathway.[12,13] The OFD1 syndrome is an X-linked dominant disease that is lethal in males and in females it is characterized by malformations of the face, oral cavity, and digits, and by a highly variable presentation. The phenotype may also include mental retardation or polycystic kidney disease.[10] The gene product mutated in this disease, OFD1, contains both a LisH motif (a.a. 69–102) and several coiled-coil domains.

Considering the ubiquitous nature of the LisH motif and its potential biological significance, we investigated in detail the LisH-dependent mechanism of homodimerization of N-LIS1. Using NMR, spectroscopic techniques and chemical denaturation, we show that the LisH motif accounts for only a part of the free energy of the homodimerization of N-LIS1, while the other part originates primarily from a single residue downstream of the LisH motif, Trp55. By contrast, the coiled-coil fragment is very labile, and has the ability to alternate between "closed" and "open" conformations depending on the ionic strength of the solution and possible other factors *in vivo*. These results illustrate the complexity of the intermolecular interactions involving the LisH-containing proteins.

Results

N-LIS1 dimer

The crystal structure of the N-LIS1 fragment[6] is shown in Figure 1(a). Briefly, the LisH motif, built of two short helices, forms a dimer of tightly packed four-helix bundles with a clearly defined hydrophobic core. A comparison of various LIS1 sequences shows that the amino acid conservation pattern corresponds to the location of buried, hydrophobic residues (Figure 1(a)), suggesting that the overall tertiary structure of the homodimer is preserved in other proteins similar to LIS1 (Figure 1(b)). Downstream of residue Leu37 the structure forms a solvent-exposed loop with a labile conformation, and with some helical content between residues Asn40 and Leu50. The sequence, which initiates the coiled-coil, i.e. LEKKWT, is the most conserved hexapeptide in all known LIS1 homologues from fungi to man, with only one conservative change (MEKKWT) in *Ciona intestinalis*. This oligopeptide adopts an α-helical conformation upstream of the three heptad repeats and the axes of the two helices form an angle of ~55° (open scissors). The helices run across the homodimer interface so that the side-chains of Trp55 actually cross over to adjacent monomers. Downstream of Trp55 one of the helices forms a dramatic kink, so that Thr56 and Ser57 adopt non-helical

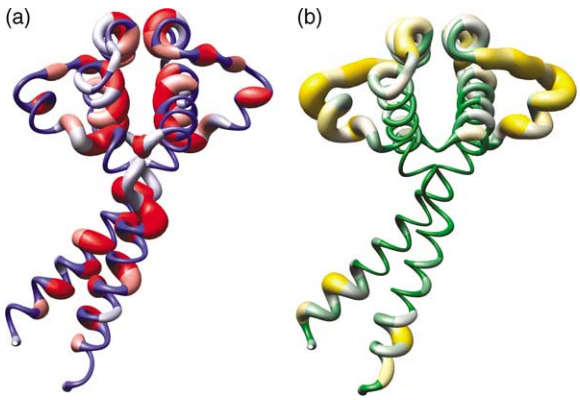

Figure 1. (a) Overall structure of the N-LIS1 fragment represented as a "worm" ribbon colored by hydrophobic scale (red to blue) and depicted by varying worm thickness (thick hydrophobic to thin hydrophilic). (b) Alignment-based phylogenetic conservation of residues in the LIS1 family mapped onto the LIS1 structure (green conserved, yellow variable). Conservation is illustrated by variability in worm thickness, where thin means conserved and thick denotes variable. The hydrophobic residues coincide with conserved regions in LIS1. High residue conservation could be observed at the beginning of the coiled-coil structure covering the Trp55 residue. Structure used to generate (b) was prepared on the ConSurf server based on the PSI-BLAST similarity search with the *E*-value inclusion threshold of 2×10^{-3} (http://consurf.tau.ac.il).[32] Both Figures were generated with Chimera software using the Render by Attribute extension (http://www.cgl.ucsf.edu/chimera).[35]