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## The solution structure of Sr33

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The recognition of fungal effectors by plant NOD-like receptors (NLRs) is an important step in defense. The coiled-coil (CC) domains of these proteins are known to be necessary and sufficient for response. Two structures have previously been solved, which show highly divergent conformations. The CC domain of the potato NLR, Rx, adopts a compact, monomeric four-helix bundle, while that of a barley protein, Mla10, was observed as an extended homodimer which was thought to be constitutively present, posing problems for mechanisms of self-association induced signaling.

We have solved the solution structure of the CC domain from the related wheat resistance protein Sr33 by NMR spectroscopy. This protein has high sequence similarity to Mla10, but our structure reveals a compact, Rx-like four-helical bundle. We subsequently analysed all three proteins by synchrotron SAXS, supported by MALS and analytical ultracentrifugation. We found that the CC-domains of Sr33, Mla10 and Rx are in fact monomeric in solution, with some evidence of weak self-association. Furthermore, the NMR structure of Sr33 is consistent with the dilute scattering from all three proteins.

Our work thus reconciles the Mla10 structure with existing models of signalling by demonstrating that a stable monomeric fold exists. We suggest that the conformation in the Mla10 crystal is a rare state that may be involved in signaling, and that the combination of this with the NMR structure of Sr335-120 provides a more complete model of the system.

### Keywords

SAXS; NMR; plant innate immunity

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