The SMN structure reveals its crucial role in snRNP assembly

Chenda O. Seng1, Craig Magee1, Philip J. Young2, Christian L. Lorson2 and James P. Allen1,*

1Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ 85287, USA and 2Department of Veterinary Pathology, Bond Life Sciences Center, College of Veterinary Medicine, University of Missouri, Columbia, MO 65211, USA

*To whom correspondence should be addressed. Tel: +1 4809658241; Fax: +1 4809652747; Email: jallen@asu.edu

Abstract

The spliceosome plays a fundamental role in RNA metabolism by facilitating pre-RNA splicing. To understand how this essential complex is formed, we have used protein crystallography to determine the first complete structures of the key assembler protein, SMN, and the truncated isoform, SMNΔ7, which is found in patients with the disease spinal muscular atrophy (SMA). Comparison of the structures of SMN and SMNΔ7 shows many similar features, including the presence of two Tudor domains, but significant differences are observed in the C-terminal domain, including 12 additional amino acid residues encoded by exon 7 in SMN compared with SMNΔ7. Mapping of missense point mutations found in some SMA patients reveals clustering around three spatial locations, with the largest cluster found in the C-terminal domain. We propose a structural model of SMN binding with the Gemin2 protein and a heptameric Sm ring, revealing a critical assembly role of the residues 260–294, with the differences at the C-terminus of SMNΔ7 compared with SMN likely leading to loss of small nuclear ribonucleoprotein (snRNP) assembly. The SMN complex is proposed to form a dimer driven by formation of a glycine zipper involving α helix formed by amino acid residues 263–294. These results explain how structural changes of SMN give rise to loss of SMN-mediated snRNP assembly and support the hypothesis that this loss results in atrophy of neurons in SMA.

Introduction

Ribonucleoprotein complexes mediate key cellular functions with small nuclear ribonucleoproteins, snRNPs, carrying out pre-mRNA splicing in cells (1). Genetic disruption of this process causes a range of diseases with the mechanisms remaining poorly understood at a structural level (2). The assembly of snRNPs into the major splicing unit, the spliceosome, is driven by the SMN complex, which has the SMN protein (the full length form expressed by the SMN1 gene) at its core. In addition, the SMN complex includes the seven proteins termed Gemin2–8 and the UNR-interacting protein, whose molecular arrangement is unknown. The SMN protein assembles the essential SMN/Gemin complex, which in turn mediates the formation of the Sm core domain of the spliceosomal snRNPs (3,4). The major components of the spliceosome are five types of snRNPs, U1, U2, U4, U5 and U6, each containing a small nuclear RNA (snRNA), and seven Sm or Lsm proteins arranged as a heptameric D1-D2-F-E-G-D3-B ring surrounding the RNA (5–7).

The process of assembly is currently known only in broad terms; therefore, to understand the molecular mechanism of snRNP assembly, the three-dimensional structure of the complete SMN protein was determined using protein crystallography. To identify the cellular roles of different domains of this structure, we also determined the three-dimensional structure of a truncated, unstable isoform termed SMNΔ7, the primary product of the SMN2 gene, which is incapable of preventing spinal muscular atrophy (SMA) development (8–11). SMA occurs in...
approximately in 1 in 10 000 live births and is one of the leading genetic causes of infant death (4,8). In most forms of SMA, the SMN1 gene, and correspondingly the SMN protein, is lost, and the health of the patients is dependent upon the SMN2 gene that produces low levels of full-length SMN and an abundance of SMNA7. Importantly, a series of mutant intragenic SMN1 alleles have been identified that primarily encode missense mutations (12–15). Based upon our new structural data, the mutations suggest a functional deficit that is predicated upon subtle structural changes predominately in the C-terminus of the protein. Previous reports have highlighted the propensity of SMN to form stable oligomeric complexes and the correlation of observation of protein self-association with disease severity (4,16,17). Based upon the observation of protein–protein interactions involving a helical region of the protein (18), we propose a dimeric model of the SMN complex. To address the cellular role of SMN in snRNP assembly, the three-dimensional structure of SMN is positioned upon comparison of the protein sequences (21). The Tudor domains are involved in RNA metabolism and structurally are composed of ~60 amino acid residues folded into a barrel-like motif. Examples of such folds are the Sm proteins, which typically are composed of five anti-parallel β strands and function in pre-mRNA maturation (22). The SMN Tudor-1 domain had been previously identified based upon a sequence comparison against Tudor domains from other proteins, although the sequence similarity was low (21). This assignment was confirmed by the structure determination of this domain by NMR and X-ray analysis of a peptide comprised of SMN residues 92–144 (23, 24). The second Tudor domain (Tudor-2) observed in the SMN exons 1–4, SMNA7 and SMN structures was not predicted in the original sequence comparison with other Tudor domains (21).

The three-dimensional folds of the Tudor-1 and Tudor-2 domains are very similar despite the differences in their sequences (Fig. 2). The presence of a second Tudor domain is consistent with the observation of multiple Tudor domains in other proteins, with a common arrangement being two successive domains, called a tandem-Tudor domain, often involved in recognition of methylated or acetylated proteins in a cooperative mechanism (25, 26). The assignment of the second region as a Tudor domain predicts a functional role in RNA metabolism, as has been found for the Tudor-1 domain (27). The involvement of both Tudor domains in protein binding is also suggested by the presence of one of the polyproline regions between the Tudor domains as predicted to facilitate binding factors involved in axonal transport such as profilin (28).

The similarity of the three-dimensional structures of SMN and SMNA7 shows that while there are profound functional significances, the minor sequence differences do not lead to a global disruption of SMN structure. The structures reported here demonstrate that SMNA7 is largely intact and therefore likely retains some (albeit, low) functional activity. This is consistent with in vivo activity of the SMNA7 protein in the transgenic mouse models in which over-expression of SMNA7 significantly extends survival and moderates a devastating phenotype (29). However, even massive levels of SMNA7 cannot fully rescue the SMA phenotype, demonstrating a critical role of the C-terminus region in the assembly process that cannot be efficiently accomplished by SMNA7 as its activity is associated with a significant reduction in snRNP assembly (3, 4).

While the majority of SMA cases are due to homozygous deletion of SMN1 alleles, a small but important number of intragenic missense mutations have been identified within SMN1 (12–15). Mutations are found throughout the length of the protein, which had made interpretation of their effects difficult, but the three-dimensional structure of SMN reveals the mutations to be

**Discussion**

The SMN exons 1–4, SMN and SMNA7 proteins have similar three-dimensional structures, including the presence of two Tudor domains. Tudor domains were first identified in the tudor (tud) gene of Drosophila melanogaster, which contains 11 repeats of this domain, and subsequently found in many other proteins based upon comparison of the protein sequences (21). The Tudor domains are involved in RNA metabolism and structurally are composed of ~60 amino acid residues folded into a barrel-like motif. Examples of such folds are the Sm proteins, which typically are composed of five anti-parallel β strands and function in pre-mRNA maturation (22). The SMN Tudor-1 domain had been previously identified based upon a sequence comparison against Tudor domains from other proteins, although the sequence similarity was low (21). This assignment was confirmed by the structure determination of this domain by NMR and X-ray analysis of a peptide comprised of SMN residues 92–144 (23, 24). The second Tudor domain (Tudor-2) observed in the SMN exons 1–4, SMNA7 and SMN structures was not predicted in the original sequence comparison with other Tudor domains (21).

The three-dimensional folds of the Tudor-1 and Tudor-2 domains are very similar despite the differences in their sequences (Fig. 2). The presence of a second Tudor domain is consistent with the observation of multiple Tudor domains in other proteins, with a common arrangement being two successive domains, called a tandem-Tudor domain, often involved in recognition of methylated or acetylated proteins in a cooperative mechanism (25, 26). The assignment of the second region as a Tudor domain predicts a functional role in RNA metabolism, as has been found for the Tudor-1 domain (27). The involvement of both Tudor domains in protein binding is also suggested by the presence of one of the polyproline regions between the Tudor domains as the polyproline regions are predicted to facilitate binding factors involved in axonal transport such as profilin (28).

The similarity of the three-dimensional structures of SMN and SMNA7 shows that while there are profound functional significances, the minor sequence differences do not lead to a global disruption of SMN structure. The structures reported here demonstrate that SMNA7 is largely intact and therefore likely retains some (albeit, low) functional activity. This is consistent with in vivo activity of the SMNA7 protein in the transgenic mouse models in which over-expression of SMNA7 significantly extends survival and moderates a devastating phenotype (29). However, even massive levels of SMNA7 cannot fully rescue the SMA phenotype, demonstrating a critical role of the C-terminus region in the assembly process that cannot be efficiently accomplished by SMNA7 as its activity is associated with a significant reduction in snRNP assembly (3, 4).
clustered (Fig. 2). The clusters are spatially removed from each other suggesting that mutations from each cluster affect different cellular properties. SMA patients often have the same missense mutations, notably in exons 6–8, which encode the C-terminal domain, including Pro 245, Pro 247, Gly 261, Ser 262, Tyr 272 and the C-terminus region 279–294 (Fig. 2). The large number of mutations in the C-terminal domain, which also contains the amino acids truncated in SMNΔ7, suggests that this domain plays a critical cellular function that is lost in SMA. Mutations clustered in and around exon 7 have been shown to significantly decrease
self-association and protein stability, suggesting that incorporation into higher order complexes involves this region of the protein and is essential for SMN function (12–15). One section involves amino acid residues exposed on the surface, including residues Gly 2, Gln 15, and Asp 44, from the N-terminal domain. The residues Gly 2 and Gln 15 are positioned to interact with other proteins while Asp 44 appears to have a role in binding with Gemin2 as discussed below. Another set involves the N-terminal domain and Tudor-1 domains, including the amino acid residues Asp 30, Ser 31, Pro 70, Ala 111, Arg 133, Glu 134, Gln 136, Asn 137 and Asn 151. The impact of alterations at residues 30, 31 and 70 on the surface of SMN suggests interactions with other proteins that have not yet been identified. The presence of several missense mutations in the section of the Tudor-1 domain forming a loop and the fourth β strand, residues 130–137 of the domain, suggests a critical role for this section that is associated with binding of the dimethylated arginine residues of Sm proteins (24). Based upon the spatial overlap of the Tudor-1 and Tudor-2 domains (Fig. 2), this region of the Tudor-1 domain corresponds to amino acid residues 184–191 of the Tudor-2 domain, and a role for this corresponding loop is suggested by the identification of the missense mutation of Ala 188 to Ser in the loop.

By combining the three-dimensional structure with biochemical and genetic information (3,4), we propose a structural model that illustrates the importance of the C-terminus region of SMN in snRNP assembly. The resulting molecular assembly model of the SMN complex yields insight into a critical biological function as well as the molecular basis for a devastating neurodegenerative disease (Fig. 3). In the initial step of assembly of the SMN complex, the D1-D2-F-E-G pentamer forms an incomplete ring that is stabilized by Gemin2 through interactions with an extended arm (residues 1–100) (18,19). Docking of SMN to the Gemin2-Sm complex using the program Rosetta (30) showed a preferred position of SMN interacting with Gemin2 and D1, but the orientation was not uniquely determined. As part of the previous crystallization of the pentameric Sm ring with Gemin2, a small portion of SMN, residues 26–62, was included and residues 37–51 were found to form an α-helix interacting with Gemin2, with the residues 26–36 and 52–62 being disordered and not identified in the structure (19). In the full SMN structure, this region is well ordered and part of the N-terminal domain, with residues 40–62 forming a helix-turn-helix motif. The simple α-helix found in the structure of the peptide fragment presumably reflects a lack of protein interactions with the majority of SMN. Assuming that the relative positions of the SMN domains do not change upon assembly, matching the α-helix found in the SMN structure with the α-helix in the published SMN fragment, followed by small adjustments to minimize the energy, we determined a unique placement of SMN into the Gemin2-D1-D2-F-E-G complex, with a large number of interactions between SMN and

Figure 3. Three-dimensional models of the SMN complex (upper) and schematic representation (lower; with the SMN domains and the C-terminal motif delineated). (A) A model of SMN (purple), Gemin2 (G2) (cyan), the D1-D2 Sm dimer (green) and the F-E-G Sm trimer (yellow). The F-E-G Sm trimer is held in place through the amino-terminal region of G2, which includes an α helix/flexible region. (B) In order to form the functional heptameric ring, the opening must expand by movement of the F-E-G Sm trimer and the α helix/flexible region of Gemin2 to allow binding of the B-D3 Sm dimer (red). This binding is proposed to be accomplished by binding of the B-D3 Sm dimer to the C-terminal motif of SMN followed by movement of the proteins into an expanded opening.
Sm proteins undergo a modiﬁcations to lose afﬁnity for SMN (35). The C-terminal region of SmD1 is very consistent with binding interactions found for Tudor domains in other proteins (27). The binding to the methylated C-terminal regions of the Sm proteins is the presence of arginine and glycine amino acids at positions 222–240, with many hydrophobic interactions, with two helices in SmD2, formed by residues Leu 100 to His 123 and Thr 208 to Leu 222. A key salt bridge is evident between Arg 213 of Gemin2 and Asp 44 of SMN, which would be lost in the missense mutation of Asp 44 to Val that shows diminished binding to Gemin2, self-association and snRNP assembly (38).

Identification of this interaction was also found in the structure making use of the small portion of SMN as discussed above (19).

This structural model of the SMN complex matches the emerging picture of the assembly pathway of spliceosomal snRNPs (34, 39). The SMN complex binds Sm proteins and snRNAs to serve as a chaperone to ensure that the Sm cores are assembled on the correct RNA targets (40–42). As has been proposed previously (43), snRNP assembly is predicted to proceed in a classical DNA clamp-loading mechanism, facilitated by the accompanying action of pICln that serves as a chaperone for the assembly.

In vitro, the SMN protein can form a wide range of oligomers with sizes up to 500 kDa while SMNΔ7 does not form such aggregates and is largely monomeric (16, 17). Correspondingly, SMN is much more stable in vivo than SMNΔ7, which is found to rapidly degrade resulting in low cellular levels (4, 17, 45, 46). Previously, the nature of the SMN oligomers has only been roughly established. Self-association of SMN was identiﬁed as involving the regions encoded by exons 2 (residues 28–91) and 6 (residues 242–279) (47). Crosslinking analysis identiﬁed cysteines as being involved in SMN self-association (48). Of the four Cys conserved in vertebrates, Cys 98 and Cys 123 are buried as previously reported for the Tudor-1 domain (22, 23). The remaining residues, Cys 60 and Cys 250, are separated by over 30 Å on different sections of the protein surface. Cys 60 and 250 are not involved in any internal disulﬁde bonds but they could potentially form disulﬁde bonds in crosslinks to other SMN proteins. The three-dimensional structure of a construct of residues 263–281 fused to a maltose binding protein has provided additional information concerning the nature of the protein–protein interactions (18).

This fragment, comprised largely of tyrosine and glycine residues, was found to form an α helix that interacts with itself as a symmetrical dimer similar to glycine zippers found in transmembrane channel proteins.

In the full SMN structure, residues 263–279 form an α helix that is part of the C-terminus α helix-extension motif and has a substantial surface exposure. Pairing two SMN complexes as a dimer with an approximate 2-fold symmetry axis centered on the α helices establishes the glycine-zipper helix–helix interactions as well as a number of stabilizing interactions involving the Tudor-2 and N-terminal domains, in particular between Gemin2 and several regions encoded by exon 2, including an α helix (residues 52–62) and a loop formed by residues 85–91 (Fig. 4). Associated with the loss of the C-terminus is a reduction in the oligomerization of SMN, which has been proposed to be

Gemin2 stabilizing the complex. This placement of SMN is in agreement with a number of other experiments including studies elucidating SMN–Gemin2 interactions and electron microscopic studies of the SMN complex (20, 31).

In our proposed model of the SMN/Gemin/Sm complex, SMN is located adjacent to the opening of the Sm ring and would have a key role in the assembly of the full ring (Fig. 3). The opening between SMN and the Sm partial ring is too small for the B–D3 dimer to be inserted and complete the ring. However, the many possible interactions between SMN and the B–D3 dimer suggest that the C-terminus region of SMN can extend and wrap around the B–D3 dimer resulting in movement of the B–D3 dimer into the proper position while shifting the F–E–G proteins to enlarge the opening. With this placement, the SMN has multiple interactions with the B–D3 dimer, primarily with the Tudor-1 and the polyproline regions. The model predicts that SMN and Gemin2 are required to assemble the heptameric D1–D2–F–E–G–D3–B ring of Sm proteins with a critical role for the C-terminal region of SMN.

This proposed model is supported by several experimental observations. This model places SMN adjacent to the B, D1 and D3 Sm proteins, which have much stronger associations with SMN than the other Sm proteins (17, 32–34), with the speciﬁcity for SMN binding being spatially speciﬁed by the heteromorphic nature of the Sm ring (35). The close interactions between the C-terminal region of SMN and SmB in the model (Fig. 4) agree with the observation that this region of SMN plays a critical role in the binding of SmB (17). A common feature of these three Sm proteins is the presence of arginine and glycine amino acids at the C-terminal regions, which are required for binding to SMN (33–35). Prior to binding to the SMN complex, the B, D1 and D3 Sm proteins undergo a modiﬁcation, the dimethylation of the C-terminal arginine residues. The modiﬁed C-terminal Sm regions are found to lose afﬁnity to SMN when either the Tudor-1 domain is not present or when key residues of the Tudor-1 domain are modiﬁed (36). The interactions of the modiﬁed C-terminal Sm regions with the Tudor-1 domain and the C-terminal region of SMN support their roles in binding to the Sm proteins. The binding to the methylated C-terminal regions of the Sm proteins is very consistent with binding interactions found for Tudor domains in other proteins (27). The C-terminal region of SmD1 also interacts with SMN, although primarily with the loop formed by residues 222–240, with many hydrophobic interactions, with two helices in SmD2, formed by residues Leu 100 to His 123 and Thr 208 to Leu 222. A key salt bridge is evident between Arg 213 of Gemin2 and Asp 44 of SMN, which would be lost in the missense mutation of Asp 44 to Val that shows diminished binding to Gemin2, self-association and snRNP assembly (38).

Identification of this interaction was also found in the structure making use of the small portion of SMN as discussed above (19).

This structural model of the SMN complex matches the emerging picture of the assembly pathway of spliceosomal snRNPs (34, 39). The SMN complex binds Sm proteins and snRNAs to serve as a chaperone to ensure that the Sm cores are assembled on the correct RNA targets (40–42). As has been proposed previously (43), snRNP assembly is predicted to proceed in a classical DNA clamp-loading mechanism, facilitated by the accompanying action of pICln that serves as a chaperone for the assembly.

In vitro, the SMN protein can form a wide range of oligomers with sizes up to 500 kDa while SMNΔ7 does not form such aggregates and is largely monomeric (16, 17). Correspondingly, SMN is much more stable in vivo than SMNΔ7, which is found to rapidly degrade resulting in low cellular levels (4, 17, 45, 46). Previously, the nature of the SMN oligomers has only been roughly established. Self-association of SMN was identified as involving the regions encoded by exons 2 (residues 28–91) and 6 (residues 242–279) (47). Crosslinking analysis identified cysteines as being involved in SMN self-association (48). Of the four Cys conserved in vertebrates, Cys 98 and Cys 123 are buried as previously reported for the Tudor-1 domain (22, 23). The remaining residues, Cys 60 and Cys 250, are separated by over 30 Å on different sections of the protein surface. Cys 60 and 250 are not involved in any internal disulfide bonds but they could potentially form disulfide bonds in crosslinks to other SMN proteins. The three-dimensional structure of a construct of residues 263–281 fused to a maltose binding protein has provided additional information concerning the nature of the protein–protein interactions (18).

This fragment, comprised largely of tyrosine and glycine residues, was found to form an α helix that interacts with itself as a symmetrical dimer similar to glycine zippers found in transmembrane channel proteins.

In the full SMN structure, residues 263–279 form an α helix that is part of the C-terminus α helix-extension motif and has a substantial surface exposure. Pairing two SMN complexes as a dimer with an approximate 2-fold symmetry axis centered on the α helices establishes the glycine-zipper helix–helix interactions as well as a number of stabilizing interactions involving the Tudor-2 and N-terminal domains, in particular between Gemin2 and several regions encoded by exon 2, including an α helix (residues 52–62) and a loop formed by residues 85–91 (Fig. 4). Associated with the loss of the C-terminus is a reduction in the oligomerization of SMN, which has been proposed to be
associated with reduction of snRNP assembly (4). This α helix of the C-terminal region has many Tyr and Gly residues (Fig. 1) that are part of the YG box associated with self-oligomerization and the region of SMN most frequently found altered in the form of missense mutations (Fig. 2) (12,47,49). Although additional interactions involving other surface features could result in higher ordered complexes of isolated SMN, these interactions would most likely arise due to the lack of Gemin2, SmB and other proteins that are part of the SMN complex, such as residues 52–91 encoded by exon 2b (Fig. 3). This model demonstrates the feasibility of a dimeric SMN complex during snRNP assembly consisting of SMN, the heptameric Sm proteins and the Gemin proteins, although this model only includes Gemin2. The enhanced stability of oligomers of SMN and SMNΔ7 compared with SMNΔ7 alone can be explained if there is an equilibrium between monomeric and oligomeric states, with the monomeric form of SMNΔ7 being unstable. Thus, the SMN complex most likely forms a glycine-zipper-mediated dimer in the cell but not any higher ordered structures.

The three-dimensional structure of the SMN protein reveals its key role in the formation of a robust multimeric complex that assembles a diverse cohort of RNP complexes, including snRNPs. Interactions involving the C-terminus of SMN and the B-D3 dimer play a critical role in the completion of the Sm ring. Comparison of the SMN and SMNΔ7 structures supports the loss of snRNP assembly as being the key contributor to the atrophy of neurons. While other functions that have not yet been identified may contribute, the structural differences show that SMNΔ7 has limited stability, loss of ability to assemble the Sm ring and cannot mediate adequate levels of snRNP assembly. This outcome has implications for amyotrophic lateral sclerosis, ALS, which like SMA is characterized by the progressive degeneration of motor neurons (50). Genetic screening of ALS patients has revealed altered genes encoding proteins associated with the SMN complex and RNA metabolism, and spliceosome integrity is lost in both diseases (51–53). These common features between SMA and ALS, in the context of the SMN model, suggest that some forms of ALS may arise due to genetic loss of RNP function.

Materials and Methods

The purification and crystallization of three proteins, SMN, SMNΔ7 and SMN exons 1–4, are presented below. SMN is the full-length protein (294 amino acid residues) identical to SMN for the first 278 amino acid residues but exon 7 is skipped by alternative splicing, leading to the incorporation of four amino acids from what would normally be the 3’ UTR for full-length SMN. SMN exons 1–4 is a non-biological form of the protein consisting of the protein encoded by exons 1–4 (amino acid residues 1–202).

Protein purification, crystallization and diffraction measurements

The SMN, SMNΔ7 and SMN exons 1–4 constructs, which have been previously described (16,45), were inserted into pRSETc with a 6-Histidine tag at the N-terminus in Escherichia coli strain BL21 (DE3). Cell cultures were grown in sterile super optimal broth medium in the presence of 10 mg/l ampicillin, and incubated at 37°C and 250 rpm for 10–12 h. Protein expression was induced with 1 mm isopropyl β-thiogalactoside, and cells were grown at 37°C for 4–6 h. The protein was expressed in inclusion bodies that were harvested using centrifugation at 5,000 × g for 10 min. The protein was bound to a Ni-affinity column, refolded by slow removal of urea while bound to the column, and eluted using imidazole. The eluted protein was purified using size exclusion chromatography, and the purified protein was concentrated to 10–15 mg/ml for crystallization using the hanging drop method.

For SMN exons 1–4, the protein was mixed equally with the reservoir containing 50% polyethylene glycol 4000, 0.1 M Tris- HCl 8.0 and 0.3 mM sodium chloride. Protein crystals appeared within 3–4 weeks at room temperature, and X-ray diffraction was measured using an in-house Rigaku X-ray generator (RUX200) and RAXIS IV imaging plate. Diffraction data were measured to a resolution limit of 2.7 Å from the crystals that were found to belong to the space group P21. The collected data had 53,732 total reflections and 4201 unique reflections, which were 99.9% complete with a Rmerge of 15.8%, an average I/σ(I) of 6.4, and a multiplicity of 4.3. For SMNΔ7, the protein was mixed equally with the reservoir containing 40% polyethylene glycol 4000, 0.1 M Tris- HCl, pH 9.0 and 0.3 mM sodium chloride. Diffraction data were collected at the Brookhaven National Laboratory to a 3.0 Å resolution limit. The crystals were found to belong to the space group C2. The collected data had 22,532 total reflections, 7286 unique reflections, which were 88.9% complete with a Rmerge of 13.2%, an average I/σ(I) of 4.1 and a multiplicity of 3.1. For SMN, the protein was mixed equally with the reservoir containing 1.8 M ammonium sulfate, 0.1 M MES, pH 6.5 and 0.01 M cobalt chloride. Diffraction data were collected at the Advanced Light Source (ALS) beamline 5.0.1 at the Lawrence Berkeley National Laboratory. Diffraction data were measured to a 5.5Å resolution limit. The space group was determined to be C2. The data had a total of 24,090 reflections, 7946 unique reflections, which were 61.3% complete with a Rmerge of 14.5%, an average I/σ(I) of 4.6, and a multiplicity of 3.0. These data are summarized in Table 1.

Table 1. Summary of X-ray diffraction data and refinements

<table>
<thead>
<tr>
<th>SMN</th>
<th>SMNΔ7</th>
<th>SMN exons 1–4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Space group</td>
<td>C2</td>
<td>P2₁</td>
</tr>
<tr>
<td>Resolution limits (Å)</td>
<td>3.0</td>
<td>2.7</td>
</tr>
<tr>
<td>Unit cell lengths</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a (Å)</td>
<td>107.1</td>
<td>55.1</td>
</tr>
<tr>
<td>b (Å)</td>
<td>62.2</td>
<td>49.4</td>
</tr>
<tr>
<td>c (Å)</td>
<td>57.1</td>
<td>68.2</td>
</tr>
<tr>
<td>Angles (°)</td>
<td>90, 95.1, 90</td>
<td>90, 120, 90</td>
</tr>
<tr>
<td>Reflections Total</td>
<td>22,532</td>
<td>53,732</td>
</tr>
<tr>
<td>Unique</td>
<td>7,286</td>
<td>4,201</td>
</tr>
<tr>
<td>Multiplicity</td>
<td>3.1 (3.0)</td>
<td>4.3 (4.0)</td>
</tr>
<tr>
<td>I/σ (I)</td>
<td>4.1 (2.5)</td>
<td>6.4 (3.5)</td>
</tr>
<tr>
<td>Rmerge (%)</td>
<td>13.2 (25.0)</td>
<td>15.8 (35.4)</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>88.9 (88.3)</td>
<td>99.9 (99.9)</td>
</tr>
<tr>
<td>Rwork (%)/Rmerge (%)</td>
<td>32.7/34.0</td>
<td>22.8/28.8</td>
</tr>
<tr>
<td>No. in asymmetric unit</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>RMSD bond length (Å)</td>
<td>0.009</td>
<td>0.010</td>
</tr>
<tr>
<td>RMSD bond angle (°)</td>
<td>1.743</td>
<td>2.402</td>
</tr>
<tr>
<td>Ramachandran plots</td>
<td>Allowed (%)</td>
<td>100</td>
</tr>
<tr>
<td>Outlier (%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

RMSD, root mean square deviation.

*NA: Due to the limited resolution, only the backbone trace was determined.
Structure determination

Initially, the structure of SMN exons 1–4 was solved using the Tudor-1 domain (22,23) (PDB ID: 1MHN) as the search model. A unique position and orientation for the search model was obtained, and the chain trace for the SMN exons 1–4 model was completed. The resulting SMN exons 1–4 model agreed well with the data with a $R_{work}$ of 22.8% and 28.8%, respectively. The completed SMN exons 1–4 model was subsequently used as a search model for the SMNΔ7 data. A unique position and orientation was obtained, and the chain trace for the SMNΔ7 model was completed. The resulting SMNΔ7 model has all residues built except for the first seven that are disordered in the crystal. The model has excellent geometry, with good agreement with the data as measured by the $R_{work}$ and $R_{free}$ of 32.7% and 34.0%, respectively. The availability of the SMNΔ7 model allowed us to accurately determine the structure of SMN despite the resolution limit of 5.5 Å. The SMNΔ7 model was used as a search model and a unique set of positions and orientations for the three proteins in the asymmetric unit was obtained. The model was refined using rigid body refinement, with the largest peaks in the difference electron density maps being in the C-terminal domain. At this stage, the model had a $R_{work}$ and $R_{free}$ of 36.2% and 39.4%, respectively, and the resulting electron density map showed the position of the C-terminus region, notably residues Gly 279–294. After building the C-terminus region, the resulting SMN structure showed no significant differences in the electron density maps. While the model could be refined with 30.6 and 32.9% values of $R_{work}$ and $R_{free}$, respectively, due to the limited resolution of the diffraction data, we report only on the differences at the C-terminus region that were evident and built using the initial electron density map. Structure determination was performed using the CCP4 and PHENIX suites (54,55) with model building being performed using COOT (56). Validation of the diffraction data and structural model was performed using Procheck, Sfcheck and Validation (55–58). The diffraction data and refinements of these three structures are summarized in Table 1. The coordinates are freely available from the Protein Data Bank under the accession codes 4NL6 and 4NL7 for SMN and SMNΔ7, respectively.

Acknowledgements

We thank Chadwick Larson for his assistance in the development of the purification protocols of SMN and SMNΔ7 and Chad Simmons for assistance in collection of the X-ray diffraction data. Atomic coordinates and structure factors have been deposited with the Protein Data Bank under the accession codes 4NL6 and 4NL7 for SMN and SMNΔ7, respectively.

Conflict of Interest statement. The authors declare no conflicts of interest.

Funding

The work was partially supported by a grant from the CureSMA (JFA).

References


31. Cauchi, R.J. (2010) SMN and Gemins: we are family. or are we? Bioessays, 32, 1077–1089.


