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Vision Research 46 (2006) 4568–4574
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Insights into functional aspects of centrins from the structure of N-terminally extended mouse centrin 1

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Received 27 June 2006; received in revised form 28 July 2006

Abstract

Centrins are members of the family of Ca^{2+}-binding EF-hand proteins. In photoreceptor cells, centrin isoform 1 is specifically localized in the non-motile cilium. This connecting cilium links the light-sensitive outer segment with the biosynthetic active inner segment of the photoreceptor cell. All intracellular exchanges between these compartments have to occur through this cilium. Three-dimensional structures of centrins from diverse organisms are known, showing that the EF-hand motifs of the N-terminal domains adopt closed conformations, while the C-terminal EF-hand motifs have open conformations. The crystal structure of an N-terminally extended mouse centrin 1 (MmCen1-L) resembles the overall structure of troponin C in its two Ca^{2+} bound form. Within the N-terminal extension in MmCen1-L, residues W24 and R25 bind to the C-terminal domain of centrin 1 in a target-protein-like geometry. Here, we discuss this binding mode in connection with putative interaction sites of the target-protein transducin and the self-assembly of centrins.

Keywords: Centrin; Calcium-binding protein; EF-hand; Connecting cilium; X-ray structure

1. Introduction

The photoreceptor cells of the vertebrate eye are highly specialized neurons which consist of morphologically and functionally distinct compartments; outer segment, inner segment (nucleus, synaptic terminal), and connecting cilium. The rod photoreceptor outer segment contains hundreds of membrane discs with the visual pigment rhodopsin, and many other proteins of the visual transduction cascade (Dowling, 1970). A slender cellular bridge, the so-called connecting cilium, links the outer segment with the inner segment, which contains the organelles typical for eukaryotic cells (Besharse & Horst, 1990). Centrin isoforms are differentially localized in the ciliary apparatus of the photoreceptor cells (Gießl et al., 2004a; Wolfrum, 1995). The localization of centrin isoforms 1–3 (Cen1, Cen2, and Cen3) is restricted to the inner lumen of the connecting cilium. Here, Cen1 and Cen2 participate in the regulation of the light-dependent bidirectional translocation of the visual G-protein, transducin (G_t), through the connecting cilium via Ca^{2+}-triggered binding to the $G_t\beta\gamma$ subunit complex of the heterotrimeric G-protein (Gießl et al., 2004a; Gießl, Trojan, Pulvermüller, & Wolfrum, 2006 in this issue of vision research; Wolfrum, Giessl, & Pulvermuller, 2002; Wolfrum et al., 2002).

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doi:10.1016/j.visres.2006.07.034

Centrins are relatively small acidic proteins that contain four helix–loop–helix motifs, the so-called EF-hands, which represent potential Ca^{2+}-binding sites (Gießl, Trojan, Pulvermüller, & Wolfrum, 2004b; Salisbury, 1995; Schiebel & Bornens, 1995; Wolfrum et al., 2002). These conserved sites specify them as members of a closely related subfamily within the large superfamily of Ca^{2+}-binding EF-hand proteins including calmodulin (CaM), troponin C (TnC), and parvalbumin (Kretsinger, 1976; Moncrief, Kretsinger, & Goldman, 1990; Nakayama, Moncrief, & Kretsinger, 1992). Four centrin proteins (Cen1, Cen2, Cen3, and Cen4) have been identified so far in mammals (Errabolu, Sanders, & Salisbury, 1994; Gavet, Alvarez, Gaspar, & Bornens, 2003; Lee & Huang, 1993; Levy, Lai, Remillard, Heintzelman, & Fulton, 1996; Madeddu, Klotz, Le Caer, & Beisson, 1996). Gene cloning studies of centrins have been applied for the identification of centrin genes in a variety of species from all kingdoms of eukaryotic organisms such as fungi, plants, and animals (Baum, Furlong, & Byers, 1986; Huang, Mengersen, & Lee, 1988; Zhu, Bloom, Lazarides, & Woods, 1995; Meng et al., 1996). Amino acid sequence comparisons revealed that Cen3 is closely related to the *Saccharomyces cerevisiae* homologue Cdc31, whereas Cen1 and Cen2 are more similar to *Chlamydomonas reinhardtii* centrin (CrCen) (Middendorp, Paoletti, Schiebel, & Bornens, 1997). Moreover isoforms, Cen1 and Cen2, are very similar showing amino acid identities of about 80–90% in vertebrates, whereas sequences of the yeast centrin and the related vertebrate Cen3 isoforms have amino acid identities of about 55% only, compared to the other isoforms. On the amino acid sequence level, Cen4 is closer to the subgroup of Cen1, Cen2, and CrCen than to the subgroup containing Cen3 and yeast centrin (Gavet et al., 2003; Gießl et al., 2004a).

Several functions of centrin have been discussed in diverse cells. First, in green algae, centrin fibers contract in response to an increase of the intracellular Ca^{2+}-concentration in the transition zone which is located between the flagellar axoneme and the basal body. The Ca^{2+}-triggered contraction of centrin fibers of the transition zone induces microtubule severing and thereby the excision of the flagellum (Sanders & Salisbury, 1994). Second, in baker's yeast *S. cerevisiae*, Cdc31 functions in the duplication of the spindle pole body. During the first step of the yeast spindle pole body duplication the binding of Cdc31 to Kar1 is required. Furthermore, Cdc31 specifically interacts with other yeast proteins including an essential kinase (Kic1) that probably regulates the spindle pole body duplication (Khalfan, Ivanovska, & Rose, 2000; Sullivan, Biggins, & Rose, 1998). Third, in invertebrates, centrins are ubiquitously expressed and commonly associated with centrosome-related structures such as spindle poles of dividing cells or centrioles in centrosomes and basal bodies (Salisbury, 1995; Schiebel & Bornens, 1995). It has been suggested that Cen3 participates in centrosome reproduction and duplication, while Cen1/Cen2 may play a role in centriole separation preceding centrosome duplication during the cell cycle (Gießl et al., 2004a; Salisbury, 2004).

Fourth, functions of centrins in vertebrate cells are also known. It has been reported that human centrins are a stabilizing component of xeroderma pigmentosum group C protein (XPC) and HRad23B complexes (Araki et al., 2001; Popescu et al., 2003). The XPC containing heterotrimer is involved in recognition of DNA lesions and initiation of global genome nucleotide excision repair. This is an important DNA repair pathway for damage caused by ultraviolet radiation, carcinogens, and chemotherapeutic agents, and impairment of XPC function is associated with the genetic disorder xeroderma pigmentosum. Human centrin 2 (HsCen2) appears to promote DNA binding by XPC both *in vivo* and *in vitro* and increases the specificity of the heterotrimer for damaged DNA (Nishi et al., 2005). The mechanism by which HsCen2 binds to XPC is still not understood. In highly specialized photoreceptor cells, Ca^{2+}-activated Cen1 and Cen2 bind with high affinity to G_t via its $G_t\beta\gamma$ (isoforms $\beta_1\gamma_1$) subunit complex and can thus regulate G_t translocation through the photoreceptor cilium (Gießl et al., 2004a, 2004b; Pulvermüller et al., 2002; Wolfrum et al., 2002).

Here, we discuss the structure of N-terminally extended mouse centrin 1 (MmCen1-L) in comparison with the already known 3D structures of full-length centrin as well as those of N- and C-terminal domains.

2. Structures of centrins

Centrins from diverse species have relatively high sequence homologies (55–85%). Several solution NMR structures of centrin domains as well as a crystal structure of full-length centrin have been reported: the X-ray structure of the human full-length HsCen2 in complex with a peptide derived from the XPC protein (Thompson, Ryan, Salisbury, & Kumar, 2006), NMR structures of the C-terminal domain of HsCen2 in complex with the same XPC-derived peptide (Yang et al., 2006a) and in its unbound state (Matei et al., 2003), the N-terminal domain of HsCen2 (Yang et al., 2006b), the N-terminal domain of CrCen (Sheehan et al., 2006) and the C-terminal domain of CrCen complexed with a peptide derived from the protein Kar1 (Hu & Chazin, 2003). Sequence alignments of centrins with known structures show fairly high homology to mouse centrin 1 (MmCen1; Fig. 1). In all centrin structures determined so far, the N- and C-terminal domains resemble the corresponding domains of troponin C or calmodulin.

We crystallized MmCen1-L, an N-terminally extended version, as described earlier (Park et al., 2005) and determined the structure at 1.8 Å resolution (to be published elsewhere). MmCen1-L contains 25 additional amino acid residues upstream of the N-terminus of the wild-type MmCen1 sequence. The refined structural model of MmCen1-L contains W24 and R25, which represent the last two amino acids of the N-terminal extension preceding the original start codon of wild-type MmCen1, and residues D53 to K192 out of the 197 residues which constitute MmCen1-L. Superposition of the structures of MmCen1-L

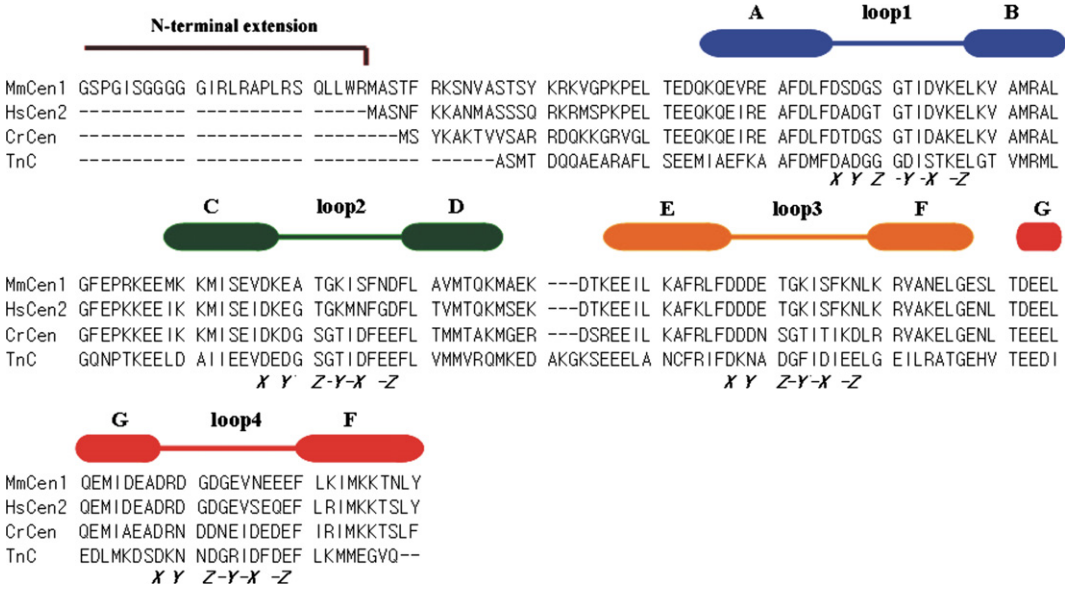

Fig. 1. Sequence comparison of N-terminal extended mouse centrin 1 (MmCen1-L), human centrin 2 (HsCen2), *Chlamydomonas reinhardtii* centrin (CrCen), and chicken skeletal muscle troponin C (TnC). Amino acids potentially involved in metal ion coordination are labeled as X, Y, Z, $-X$, $-Y$, and $-Z$ corresponding roughly to a Cartesian coordinate system centered on Ca^{2+}. The N-terminal extension of mouse centrin 1 is marked by a brown line. The positions of four EF-hand motifs are indicated schematically above the sequence in blue, green, orange, and red colors.

and troponin C (TnC) in its two Ca^{2+} bound form (Satyshur, Pyzalska, Greaser, Rao, & Sundaralingam, 1994; PDB entry 1TOP) reveals close similarities between the conformations of the N- and C-terminal domains of both proteins. The observed difference in relative orientations of N- and C-terminal domains between MmCen1-L and TnC (not shown) is caused by an insertion of three amino acids in TnC (Fig. 1) into the α-helix connecting the N- and C-terminal domains and a different conformation of this α-helix compared to the equivalent structural element in MmCen1-L.

3. Conformation and Ca^{2+}-binding of N-terminal centrin domains

In the crystal structure of the HsCen2-XPC peptide complex (Thompson et al., 2006), two Ca^{2+} ions are bound to EF-hands III and IV of the HsCen2 C-terminal domain, whereas the N-terminal domain is Ca^{2+}-free and adopts a "closed" conformation. This corresponds well to the solution NMR structure of the apo-N-terminal domain of HsCen2 and results from NMR spectroscopy, which was applied to monitor Ca^{2+} titrations of the N-terminal domains of HsCen2 and HsCen1, revealing very weak affinities (10^2–$10^3\,M^{-1}$; Yang et al., 2006b). Upon binding of Ca^{2+} to the HsCen2 N-terminal domain, the "closed" conformation is maintained. The N-terminal domain of MmCen1-L is also found in a "closed" conformation, although the electron density map indicates at least partial metal-binding. Different from these centrins, the N-terminal domain of CrCen adopts an open conformation in the presence of Ca^{2+} ions and is discussed to act as a calcium sensor (Sheehan et al., 2006).

4. Open conformation and high Ca^{2+}-affinity of C-terminal centrin domains

The C-terminal domain of MmCen1-L binds two Ca^{2+}-ions via EF-hands III and IV and adopts an open conformation, which is consistent with the structures of the C-terminal domain of HsCen2 (Matei et al., 2003; Thompson et al., 2006) and the C-terminal domain of CrCen (Hu & Chazin, 2003). The C-terminal domain of HsCen2 has a much higher Ca^{2+}-affinity ($\sim 10^5\,M^{-1}$) compared to the N-terminal domain (10^2–$10^3\,M^{-1}$) (Yang et al., 2006b). Preliminary data using isothermal titration calorimetry (ITC) indicate a strong Ca^{2+} affinity ($\sim 10^9\,M^{-1}$) for the C-terminal domain of MmCen1 (data not shown).

5. Self-assembly mediated by the N-terminal region of centrins

The structural model of MmCen1-L lacks residues 26–52, corresponding to the N-terminus of MmCen1, i.e., residues 1–27 of wild-type MmCen1, because the residual electron density map is largely disconnected and therefore not interpretable. This region is probably highly disordered in the crystal structure. Residues W24 and R25 of MmCen1-L are the only amino acid residues of the N-terminal extension which could be modeled into the electron density map. This was possible in spite of the fact that the preceding 23 residues as well as the following 27 residues were not visible, because W24 is the only tryptophan residue in MmCen1-L. The large indole moiety of its side chain could unambiguously be identified in the F_o–F_c map at an intermediate stage of crystallographic refinement (Fig. 2), and the side chain of R25 is also clearly visible in this map.

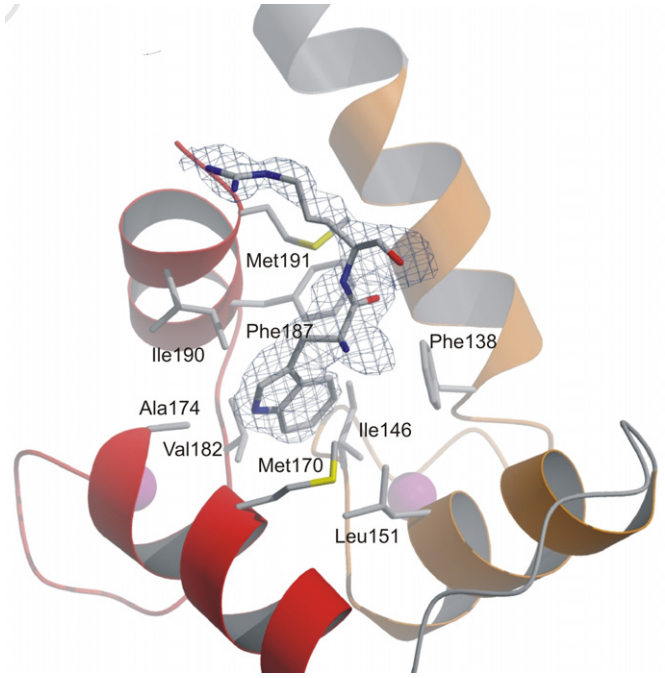

Fig. 2. Close-up view of the C-terminal domain of MmCen1-L, showing residues W24 and R25 of the N-terminal extension of mouse centrin 1 in contact (distances less than 4 Å) with hydrophobic amino acid side chains. Two calcium ions bound to this domain are represented as spheres. The same color scheme as in Fig. 1 was used. A section of a F_o–F_c map is shown which was calculated at an intermediate stage of structure refinement when the model did not contain the residues of the N-terminal extension, and contoured at 2σ. This figure and Fig. 3 were drawn using MOLSCRIPT (Kraulis, 1991), CONSCRIPT (Lawrence & Bourke, 2000), and RASTER3D (Merritt & Bacon, 1997).

W24 and R25 interact with a hydrophobic surface exposed by the C-terminal domain of MmCen1-L in its "open" conformation.

Our observation that the tendency of wild-type MmCen1 to aggregate is reduced in MmCen1-L might be correlated with these interactions. It has been shown for HsCen2 that the 25 N-terminal amino acid residues are highly disordered in the crystal structure (Thompson et al., 2006) or are of irregular and dynamic structure in solution (Yang et al., 2006b) and might have an active role in self-association of HsCen2 by forming intermolecular interactions with the C-terminal domain of a second centrin molecule (Yang et al., 2006b). Assuming that the observed aggregation of MmCen1 is caused by self-association, the reduced tendency of MmCen1-L to aggregate could be caused by competitive binding of the N-terminally extended sequence. The N-terminal extension in MmCen1-L would bind to the same hydrophobic surface which interacts in the proposed self-assembly mechanism with the N-terminus of wild-type MmCen1. The distance between the C_α-atoms of R25 and D53 in MmCen1-L is 30.7 Å, short enough to be covered by the intermediate 27 residues missing in the crystal structure, thus facilitating an intramolecular interaction between the N-terminal extension and the C-terminal domain. As the polypeptide backbone linking R25 and D53 cannot be traced in the electron density map of the MmCen1-L crystal, the alternative possibility cannot be excluded. In this alternative scenario the residues W24 and R25 which bind to the C-terminal domain originate from a neighboring molecule in the crystal and the mode of interaction is intermolecular. However, the model of an intramolecular mode of interaction in MmCen1-L would be more suitable to explain the reduced tendency to aggregate.

In contrast, the N-terminal stretch of wild-type MmCen1 is most likely too short to reach the exposed hydrophobic surface at the C-terminal domain and therefore can only be involved in intermolecular interactions. This would explain why the wild-type interaction leads to oligomerization of centrin molecules, whereas in MmCen1-L the N-terminal extension reduces oligomerization due to an entropically more favorable intramolecular interaction.

6. Target-protein recognition

In the superimposed crystal structures of MmCen1-L and the complex between HsCen2 and a XPC-derived peptide, residues W848 and K849 of the XPC-derived peptide fit strikingly well to W24 and R25 of MmCen1-L (Fig. 3). In both the crystal structure of the HsCen2/XPC-derived peptide complex and the solution structure of the HsCen2 C-terminal domain with the XPC-derived peptide (Yang et al., 2006b), W848 of the peptide was identified to be the

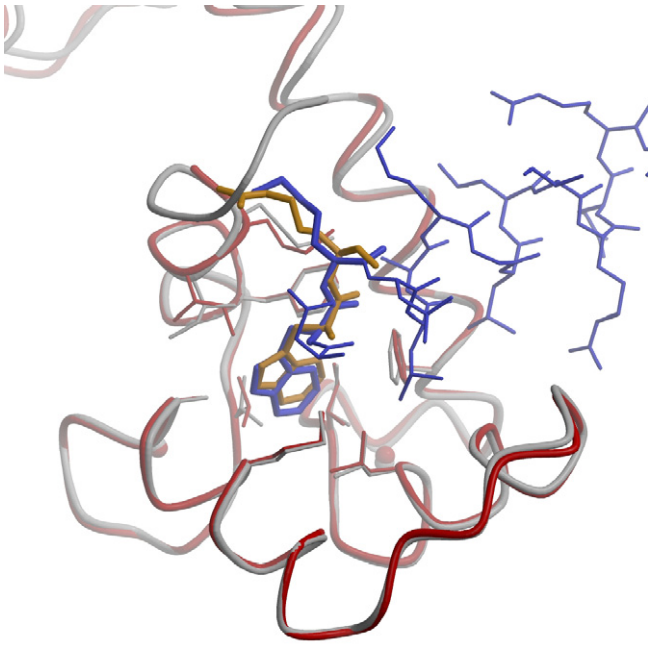

Fig. 3. Superposition of the C-terminal domains of MmCen1-L (red) and HsCen2 (grey; PDB code 2GGM); the latter in complex with a peptide derived from xeroderma pigmentosum group C protein (XPC), which is shown as blue sticks. The rms deviation is 0.4 Å for main chain atoms. View similar as in Fig. 2. W24 and R25 of the N-terminal extension of MmCen1-L are shown as thick orange sticks. The hydrophobic residues shown to interact with W24 and R25 of MmCen1-L are strictly conserved in HsCen2, where they interact with W848 and K849 (thick blue sticks) of the XPC-derived peptide.

Table 1
Sequence alignment and motifs of centrin-binding proteins

XPC (841–856)	K	R	A	L	G	N	W	K	L	L	A	K	G	L	L	I
Kar1 (242–257)	E	L	I	E	S	K	W	H	R	L	L	F	H	D	K	K
hSfi1 (131–120)					E	K	W	E	E	F	V	K	R	L	L	R
$G_t\beta$ (57–72)	K	I	Y	A	M	H	W	G	T	D	S	R	L	L	L	S
$G_t\beta$ (76–91)	D	G	K	L	I	I	W	D	S	Y	T	T	N	K	V	H
$G_t\beta$ (93–108)	I	P	L	R	S	S	W	V	M	T	C	A	Y	A	P	S
$G_t\beta$ (163–178)	D	T	T	C	A	L	W	D	I	E	T	G	Q	Q	T	T
$G_t\beta$ (205–220)	D	A	S	A	K	I	W	D	V	R	E	G	M	C	R	Q
$G_t\beta$ (291–306)	D	F	N	C	N	V	W	D	A	L	K	A	D	R	A	G
$G_t\beta$ (326–340)	A	V	A	T	G	S	W	D	S	F	L	K	I	W	N	

Aligned are sequences of the centrin targets xeroderma pigmentosum group C protein (XPC, Swiss-prot entry: Q01831), cell division control protein Kar1(Swiss-prot entry: P11927), KIAA0542 protein from human (hSfi1, Swiss-prot entry: O60289) (Nagase et al., 1998; Sheehan et al., 2006; Thompson et al., 2006; Yang et al., 2006a) and $G_t\beta$ (PDB entry: 1GOT, bovine $G\beta_1$). Note that the sequence of the repeat in hSfi1 (residues 120–131) is reversed.

first of three bulky hydrophobic residues. These residues are at the relative positions 1, 4, and 8, are located within an α-helix and interact with a hydrophobic molecular surface at the C-terminal domain of HsCen2. The tryptophan residue is completely conserved according to sequence alignments of XPC homologous proteins (Thompson et al., 2006) and of other centrin targets (Yang et al., 2006b). This suggests that the interactions of residues W24 and R25 of the N-terminal extension with the C-terminal domain of MmCen1-L can be considered to be target-peptide-like.

Assuming that a similar mode of interaction involving a tryptophan residue might occur upon binding of the target-protein G_t to Cen1 via its $G_t\beta\gamma$ subunit complex (Pulvermüller et al., 2002), we searched for potential centrin binding sequences in the $G_t\beta$ and $G_t\gamma$ subunits. A typical 1–4–8 pattern of bulky hydrophobic residues as found in HsCen2 and CrCen target proteins is also present in the β-subunit of G_t, consisting of W332, F335, and W339, and three additional tryptophan residues of this subunit are part of polypeptide segments which contain two residues at positions compatible with the 1–4–8 pattern (Table 1). However, within $G_t\alpha\beta\gamma$ the tryptophan residues of the β-subunit are not exposed to the surface according to the crystal structure of transducin (Lambright et al., 1996; PDB code 1GOT), as expressed by a relative solvent accessibility, calculated using NACCESS (Hubbard & Thornton, 1996), which does not exceed 5%. Of these tryptophan residues, W99 and W332 are shielded from the solvent by the $G_t\alpha$ subunit and therefore not available for Cen1 binding. In order to prove whether simple changes in side chain conformations of the tryptophan residues in $G_t\beta$ would facilitate Cen1 binding, we generated all possible side chain rotamers of these residues in the transducin crystal structure and superpositioned the tryptophan side chain atoms in the resulting structural models with the corresponding atoms of W24 in the MmCen1-L structure. All resulting models proved to be stereochemically meaningless, as indicated by numerous clashes between atoms of MmCen1 and transducin. That none of the bulky hydrophobic residues of $G_t\beta$ listed in Table 1 is involved in an interaction mode similar to the one reported for HsCen2 and CrCen target proteins (Fig. 3; Hu & Chazin, 2003; Thompson et al., 2006; Yang et al., 2006b) is also likely due to the fact that none of these residues is found within α-helical segments, rather they are parts of β-strands. As a consequence, such a binding mode would require gross conformational changes of the corresponding sequence motifs.

While by NMR techniques, titration of the N-terminal domain of CrCen with a fragment of the known centrin target Sfi1 revealed the binding of this fragment to a discrete site on the CrCen N-terminal domain (Sheehan et al., 2006), no comparable interactions were observed between the HsCen2 N-terminal domain and the target XPC-derived peptide (Thompson et al., 2006). Preliminary binding experiments performed in our laboratory with full length and N-terminally truncated mutants of MmCen1 indicate that the N-terminal domain is involved in complex formation with G_t. This would support the observation of Sheehan et al. (2006) that also centrin's N-terminal domain can represent a binding site for target proteins.

7. Conclusion

In MmCen1-L, centrin's C-terminal domain interacts with W24 and R25 of the N-terminal extension preceding the original MmCen1 amino acid sequence. The binding mode of these two residues closely resembles the target-protein interactions known for the C-terminal domains of HsCen2 and CrCen. However, analyses of the amino acid sequences and comparison of the three-dimensional structure of G_t and target proteins of other centrins exclude this binding mode for the G_t/Cen1 interaction. It seems to be possible that both the C- and the N-terminal domains of MmCen1 are involved in the interaction with G_t. The tendency of centrin to aggregate is reduced in MmCen1-L which might be due to competitive binding of W24 and R25 and the original N-terminal segment of MmCen1 to the same site at the C-terminal domain. It therefore seems to be possible that the exposed surface of the "open" C-terminal domains of MmCen1 and other centrins have a dual role in the interaction with target proteins and in self-assembly.

Acknowledgments

Hui-Woog Choe gratefully acknowledges the support by the CNBU funds for overseas research, 2006. We thank Victor Lamzin and Paul Tucker and the scientific and technical staffs at EMBL outstation (DESY) for continuous support of this project. The project was supported by the DFG (WO 548/6 to U.W., PU 186/2 to A.P., ER 294/1-1 to O.P.E.) and Sfb 449 (to K.P.H. and O.P.E.) and Sfb 498 (to N.K.).

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