The Structure of the Human Centrin 2-Xeroderma Pigmentosum Group C Protein Complex*]

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Human centrin-2 plays a key role in centrosome function and stimulates nucleotide excision repair by binding to the xeroderma pigmentosum group C protein. To determine the structure of human centrin-2 and to develop an understanding of molecular interactions between centrin and xeroderma pigmentosum group C protein, we characterized the crystal structure of calcium-loaded full-length centrin-2 complexed with a xeroderma pigmentosum group C peptide. Our structure shows that the carboxyl-terminal domain of centrin-2 binds this peptide and two calcium atoms, whereas the amino-terminal lobe is in a closed conformation positioned distantly by an ordered α-helical linker. A stretch of the amino-terminal domain unique to centrins appears disordered. Two xeroderma pigmentosum group C peptides both bound to centrin-2 also interact to form an α-helical coiled-coil. The interface between centrin-2 and each peptide is predominantly nonpolar, and key hydrophobic residues of XPC have been identified that lead us to propose a novel binding motif for centrin.

Human centrin-2 (HsCen-2) is a Ca2+-binding protein of the calmodulin-parvalbumin EF-hand superfamily (2, 3). HsCen-2 and two other human centrins (4) are best known for functions outside the nucleus. Centrins have essential roles in the duplication and segregation of microtubule organizing centers (5, 6). Much research has focused on these functions, because abnormal centrosome duplication may lead to chromosomal instability and then cancer, an idea supported by discovery of supernumerary abnormal centrosomes in different human tumor cells (7–10). In addition to its role in centrosome function, HsCen-2 is found as a stabilizing component of xeroderma pigmentosum complement protein C (XPC) and HRad23B complexes (11, 12). The XPC-containing heterotrimer is involved in recognition of DNA lesions and initiation of global genome nucleotide excision repair. Global genome nucleotide excision repair is an important DNA repair pathway for damage caused by UV radiation, carcinogens, and chemotherapeutic agents, and impairment of XPC function is associated with microtubule organizing centers (5, 6). Most amino acids unique to HsCen-2 from this comparison are highly conserved among centrins across diverse species. An exception is a stretch of 20–25 amino-terminal residues, which are nonconserved among centrins and which are absent in calmodulin and other Ca2+-binding proteins. The carboxyl-terminal sequence 161KKTSLY172 of HsCen-2 is also missing in HsCen-3 and many other centrins of lower eukaryotes lacking centrioles. The aromatic Tyr172 is not found among calmodulins.

Despite high sequence similarity, centrins recognize target proteins distinct from those that partner with calmodulins and other EF-hand family members. Little is known about the structural basis of centrin interactions. Some proteins thought to interact with centrins reveal no clear binding site(s) from amino acid analysis, either by visual inspection or by querying a data base of known sequences and properties of calmodulin targets (23). Residues 847–863 of the human XPC (HsXPC) sequence shown underlined in Fig. 1A, however, were identified as the HsCen-2 binding site after using the calmodulin target data base to suggest sites (11).

There is no information on the structure of full-length centrin either alone or in association with other proteins. Structural details of the C-terminal domain of Chlamydomonas reinhardtii caltractin and a 19-residue peptide from Kar1p protein, a component of spindle pole bodies required for cell integrity in yeast, have been elucidated using NMR methods (24). The formation of this complex is dependent on Ca2⁺ binding (25). Another solution structure of a HsCen-2 fragment (26), including residues Met84–Trp172 shows that a helical portion of the N-terminal domain lies within a hydrophobic binding cavity of the C-terminal domain. A calcium-free solution structure of the HsCen-2 N-terminal domain was just published with a closed conformation (27).
XPC mutant that fails to bind HsCen-2 also reduces binding affinity of XPC for DNA and reduces the specificity for repair of DNA damage (13). We performed structural characterization of the HsCen-2 and XPC complex, because knowledge of the interactions between the proteins would enhance our understanding of the mechanisms of global genome nucleotide excision repair and the mechanisms underlying the target specificity of HsCen-2.

We describe herein the crystallographic structure of full-length HsCen-2 in complex with a 17-residue synthetic peptide derived from human XPC in the presence of Ca$^{2+}$. Our structure reveals that 1) only the C-terminal domain binds XPC peptide and calcium atoms, 2) the N-terminal lobe exists in a closed conformation with distortions in the loops of its two EF-hands that probably abolish high affinity Ca$^{2+}$ binding, 3) two XPC peptides bound to separate HsCen-2 molecules interact to form an α-helical coiled-coil conformation, 4) the protein-peptide interface is best characterized as nonpolar, since there are very few hydrogen bonds and only one weak ionic interaction, and 5) the majority of the interface with peptide can be described by analysis of the molecular surfaces surrounding the positions of just three hydrophobic HsXPC residues.

**EXPERIMENTAL PROCEDURES**

**General**—Research grade reagents were from Sigma unless otherwise indicated. Protein concentration was determined conventionally by absorbance at 277 nm using an $E_{280}$ of 1.46 mm$^{-1}$ cm$^{-1}$ and after purification by the Bio-Rad (Bradford) method (28).

**Native HsCen-2**—HsCen-2 was expressed and purified as described earlier (29). Crystallization trials were conducted using hanging drop vapor diffusion methods with over 3000 crystallization solutions, which were then optimized for the production of large single crystals. Similar conditions were used for the biosynthesis of selenomethionine (SeMet)-substituted HsCen-2 and are reported below.

**SeMet Human Centrin-2**—SeMet-substituted HsCen-2 was synthesized in Escherichia coli BL21(DE3) (Invitrogen) grown in minimal M9 medium inoculated from starter culture at 37 °C as published (30). After bacterial cultures attained an optical density at 600 of 1.46 mM, methionine biosynthesis was suppressed by the addition of leucine, isoleucine, and valine (50 mg/liter each) and lysine, threonine, and phenylalanine (100 mg/liter each). Selenium methionine was added (25 mg/liter), and protein synthesis was induced 15 min later by the addition of 1 mM isopropyl 1-thio-β-D-galactopyranoside. Cells were grown for 12–16 h at 20 °C, collected by centrifugation, and stored frozen at −80 °C some days before further processing. As described for native HsCen-2 (29), the purification involves protease cleavage of a glutathione S-transferase domain fusion. The protein composition and the incorporation of 10 SeMet residues/molecule were confirmed by SDS-PAGE and native gels and also electrospray mass spectrometry analysis. It is to be noted that expression of the glutathione S-transferase-tagged fusion protein results in the addition of 5 extra amino acids (GPLGS) to the amino terminus of the protein following protease treatment of the fusion protein.

**Peptide Synthesis**—The human xeroderma pigmentosus group C peptide (XPC; NWKLLAKGLLIRERLKR), Asn$^{847}$–Arg$^{863}$ (11) was synthesized using Fmoc (N-(9-fluorenyl)methoxycarbonyl) chemistry and solid phase peptide synthesis in the Mayo Proteomics Research Center Peptide Synthesis Facility. The molecular mass of the XPC fragment was verified by microelectrospray ionization mass spectrometry.

**Preparation of HsCen-2-XPC Complex**—A stock solution of 20 mg/ml SeMet-centrin 2 in 50 mM Tris-HCl, 1 mM diithiothreitol, 1 mM EDTA, pH 7.4, was mixed with enough CaCl$_2$ to allow the binding of 2 mol of Ca$^{2+}$/mol of protein. Then XPC peptide was added to a 1.3 molar ratio of peptide to protein. The XPC peptide is not completely soluble; complex formation was allowed to take place overnight, at which point precipitate was visible. The precipitate consisted of HsCen-2 and XPC peptide. The concentration of the remaining Ca$^{2+}$–HsCen-2 and XPC peptide complex in solution varied from 6.8 to 12 mg/ml.

**Crystallization/X-ray Data Collection**—The best diffracting crystals resulted from hanging or sitting drop vapor diffusion experiments at 18 or 22 °C. A 2-μl aliquot of SeMet-protein solution was mixed with an equal volume of reservoir containing 2.5–3.2 mM (NH$_4$)$_2$SO$_4$ and 0.1–0.4 M Na$_2$HPO$_4$. Streak seeding was routinely utilized to produce crystals in a reproducible manner with microplates that were allowed to equilibrate 1–2 days beforehand. To transfer crystalline microneuclei, a cat whisker was used for serial dilutions of broken up crystals. A novel method called microseed matrix screening was used later to obtain large crystals (31). Superior cryoprotection resulted by placing crystals in silicon oil saturated with the crystallization solution prior to flash cooling in liquid N$_2$. Table 1 summarizes the statistics for crystallographic diffraction data collection and structural refinement. The data presented were collected from a flash-cooled crystal (100 K) at beamline X12-C (National Synchrotron Light Source, Brookhaven National Laboratory).

**Structure Determination**—The structure was solved by multiwavelength anomalous dispersion using selenomethionine-labeled protein. Diffraction data collected at three different wavelengths were processed with HKL2000 and SCALEPACK (32) (Table 1). 16 of 20 possible selenium atomic positions were identified by SOLVE (33), and RESOLVE (34) built 58% of the HsCen-2 and 95% of the XPC peptide initial atomic coordinates into the experimental electron density map, using phases from multiple wavelength anomalous dispersion, without any user intervention. Programs REFMAC5 (35) and COOT (36) were used for structure refinement and model building. No preexisting atomic coordinates were obligatory for model building. Diffraction data of the low remote wavelength from SeMet-crystals were used during refinement, although native crystals diffracted to 1.3 Å resolution. Native data always contained multiple crystalline lattices, most apparent above 3 Å resolution, despite numerous efforts to overcome the problem. TLS parameters were used to model anisotropic displacements (37) after determining TLS regions, using normal mode analysis with ELNémo.
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(38) on the asymmetric unit contents. The stereochemistry and the agreement between model and x-ray data were verified by CNS (39) simulated annealing omit maps, PROCHECK (40), and SFCHECK (41).

RESULTS

Structural Description—We determined the crystallographic structure (Table 1) of Ca\(^{2+}\)-bound HsCen-2 in complex with the centrin-2 binding peptide derived from human xeroderma pigmentosum group C protein (HsXPC; HsXPC N87NWKLAKGLLIRRKR863) and calcium. The model is refined to 2.35 Å resolution, with R/R\(_f\) free values of 0.192 and 0.245, against diffraction data of the low remote wavelength from SeMet-crystals.

Fig. 1B shows the rainbow-colored backbone trace of molecule A of HsCen-2 with its HsXPC peptide and two Ca\(^{2+}\) atoms (green spheres) bound to the C-terminal domain. The overall dumbbell-like fold consists of two domains, N-terminal (blue) and C-terminal (red), that do not interact. There is a linker region, shown here as an ordered helix. No interactions are observed between the N-terminal domain of HsCen-2 and the XPC peptide; only residues from the C-terminal domain are involved in XPC binding. The complex contains two Ca\(^{2+}\) atoms bound to EF-hand sites III and IV of the C-terminal domain of HsCen-2.

Two complexes are situated within the asymmetric unit as highlighted in Fig. 1C, and no 2-fold noncrystallographic symmetry is found relating them. Atomic coordinates exist for residues 23–172 for the subunit designated as molecule A, whereas molecule B includes residues 25–172.

Atomic coordinates are found for all residues of both XPC peptides and numbered with regard to human XPC protein (Asn\(^{847}\)-Arg\(^{863}\)). Only the C-terminal two XPC residues for the peptide bound to molecule A and the last four for that bound to molecule B are supported by any broken electron density. The entire HsXPC peptide is in \(\alpha\)-helical conformation. The helical dipole is oriented in the same manner as observed in the binding of the Kar1p peptide to the yeast centrin, caltractin (24).

Both independent complexes of HsCen-2, XPC peptide, and calcium are very similar structurally, as shown by superposition in Fig. 1D. The root mean square deviation is 0.39 Å for 144 C\(_\alpha\) atoms. However, there are minor differences found at the two peptide binding sites, which appear due to intermolecular interactions between both HsXPC peptides.

HsXPC Peptides Form Helical Coiled-coil—There are no direct interactions between the C-terminal domains for the two HsCen-2 subunits in Fig. 1C. Rather the crystal forms in part from hydrophobic interactions between the two XPC peptides. The most significant XPC residues involved in the interaction, based on buried surface area, are Leu\(^{850}\), Ile\(^{857}\), and Leu\(^{861}\). The interface also requires a small side chain like the glycine found at position 854 for complete interdigitation of these hydrophobic side chains. Burial of aliphatic carbons from Lys\(^{849}\), Arg\(^{858}\), and Lys\(^{862}\) play a smaller role. The overall conformation of the XPC peptides taken together is that of an \(\alpha\)-helical coiled-coil with a parallel orientation of both helical dipole.

HsCen-2 Interactions with XPC—The buried surfaces at the interface of HsCen-2 and XPC peptide are extensively hydrophobic, and the packing provides for substantial van der Waals interactions. Calculations using the Lee and Richards algorithm (42) reveal roughly 732 Å\(^2\) of the solvent-accessible surface area of HsCen-2 is buried at the interface with XPC peptide; for the peptide, the value is 1020 Å\(^2\). The molecular surface of HsCen-2 at this interface is a highly curved 23 × 22 Å area formed by 30% polar atoms and 70% nonpolar atoms. The complementary surface of the XPC peptide measures 21 × 15 Å, created by 24% polar and 76% nonpolar atoms. Hydrogen bonds play only a small role between 8 and 10 amino acids. The most surprising result was the lack of electrostatic interactions given the basic nature of the HsXPC peptide. Only one ionic interaction, a weak salt bridge, was identified between Arg\(^{856}\) and HsCen-2 Glu\(^{132}\). No bridging solvent molecules mediate peptide binding. Once more, nonpolar interactions predominate.

Shown in Fig. 2A is an \(\alpha\)-helix portrayal of the XPC peptide backbone, labeled by amino acid. The most significant interfacial peptide residues, in order of their nonpolar occluded surface, are Trp\(^{848}\), Lys\(^{849}\) molecule A, Leu\(^{851}\), Leu\(^{852}\), Leu\(^{862}\), and Lys\(^{864}\) molecule B. The high amino acid conservation of these specific XPC residues points to their importance in formation of the binding site. Fig. 2B shows a frequency histogram of a multiple sequence alignment that encompasses the XPC HsCen-2 binding site, an alignment that includes homologous sequences to human XPC from 38 species (the amino acid alignment is provided as supplemental data). Trp\(^{848}\) is completely conserved. The leucines at 851, 855, and 856 are highly conserved; only hydrophobic residues are found at positions 851 and 855. Lys\(^{849}\) is also conserved but can be substituted by the similar arginine or small hydrophobic amino acids. Given our finding that these residues are of prime significance in binding HsCen-2, interactions between proteins homologous to XPC and centrin 2 seem likely throughout eukaryotic phyla.
We also determined the degree to which nonpolar atoms of HsCen-2 amino acids were occluded by the XPC peptide. The results identify in rank order Leu\textsubscript{133}, Leu\textsubscript{112}, Met\textsubscript{145}, Phe\textsubscript{113}, Met\textsubscript{166}, Leu\textsubscript{126}, Ala\textsubscript{109}, Glu\textsubscript{105}, and Val\textsubscript{129}. These nine nonpolar residues cover nearly every residue of the XPC peptide. The arrows in Fig. 2A point to specific HsCen-2 amino acids interacting with each residue of both XPC-derived peptides in the asymmetric unit. To be identified, amino acids must have substantial buried nonpolar surface within 4 Å of an XPC residue. Only minor differences are found among the interactions in both HsCen-2 binding cavities within the asymmetric unit.

Several nonpolar interactions are of seemingly great consequence. 1) Both Met\textsuperscript{145} and Phe\textsuperscript{113} form the greater part of a deep pocket shown in Fig. 2C into which the invariant Trp\textsuperscript{968} of HsXPC is inserted. The Trp\textsuperscript{968} side chain also forms a weak hydrogen bond to the Met\textsuperscript{145} main chain. 2) Leu\textsuperscript{145} of HsXPC fits a shallow pocket formed largely by Leu\textsubscript{10} and again Phe\textsubscript{113}. 3) Leu\textsuperscript{455} of HsXPC associates most with Leu\textsubscript{133}, Leu\textsubscript{112}, and Phe\textsubscript{113}. 4) The packing of the aliphatic side chain of Lys\textsuperscript{849} of the peptide with Met\textsubscript{166} buries substantial nonpolar surface.

**Closed Conformation for N-terminal Domain**—The interhelical angles within α-helices within EF-hand protein structures have been used to classify and measure conformational state. The angular values for HsCen-2 indicate the N-terminal domain without calcium bound resides in a “closed” conformation (Table 2), although differences are found compared with those reported for a solution structure of the HsCen-2 N-terminal domain alone (27). Values for the C-terminal domain unsurprisingly indicate an open state. Data from equivalent computations on other homologous proteins are maintained on an internet Web site called the CaBP Data Library (available on the World Wide Web at structbio.vanderbilt.edu/cabp_database/).

Fig. 3 depicts an overlay of backbone traces from the N-terminal HsCen-2 domain (blue) on that of the C-terminal domain (red) in a cross-eye stereoimage. The α-helices are numbered I–IV to correspond with past literature on homologous EF-hand folds and with the interhelical angle data in Table 2. The bound peptide (green) and both Ca\textsuperscript{2+} atoms are presented as well. Looking at the blue ribbon, it is obvious that no helical peptide could bind within the N-terminal domain within its existing structure.

Fig. 3 also allows comparison of the more distorted loops of EF-hand sites I and II in the N-terminal and without calcium present to sites III and IV. Site I differs from the more canonical HsCen-2 site IV (or EF-hand I in calmodulin) mainly by the replacement of an aspartate important for Ca\textsuperscript{2+} binding by Thr\textsuperscript{45}. An analogous comparison suggests two substitutions reduce Ca\textsuperscript{2+} affinity at site II, Glu\textsuperscript{79} rather than an aspartate and Asp\textsuperscript{88} rather than a glutamate.

**DISCUSSION**

We report, for the first time, the structure of full-length HsCen-2 complexed to calcium and XPC peptide. As such, the structural studies herein may offer insights into the manner in which centrin interacts with proteins such as XPC.

**Comparison with Homologous Structures**—The overall positioning and orientation of both domains of HsCen-2 together reveals a unique conformation in comparison with homologous protein structures. However, the C-terminal residues 101–172 of HsCen-2 overlay coordinates of calmodulin bound to a peptide from rat calmodulin-dependent protein kinase I (Protein Data Bank code 1MXE) with a root mean square deviation of only 0.7 Å.

Fig. 4 shows the superposition of the C-terminal domain of HsCen-2 with model A from the solution structure of yeast (centrin) caltractin bound to a peptide derived from Kar1p protein (24). C-terminal backbone C\textsubscript{n} atoms can be overlaid with a root mean square deviation of 0.8 Å. The interface made by the interaction of caltractin with the peptide is

### TABLE 2

| Interhelical angles within HsCen-2 |     |     |     |     |     |     |
|-----------------------------------|-----|-----|-----|-----|-----|-----|
|                                   | α I | α II | α III | α IV | α II | α III |
| N-terminal                        | 138 | −87  | 127  | 116  | −52  | 143  |
| C-terminal                        | 93  | −139 | 124  | 126  | −45  | 96   |

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**FIGURE 3.** The two domains of HsCen-2 are compared. A cross-eye stereo image is shown of a superposition of the N-terminal HsCen-2 domain (blue) on the C-terminal domain (red). The N-terminal domain exists in a closed conformation. Relative positions of the two bound calcium atoms (dark green) bound to EF-hands III and IV and the XPC peptide (green) with Trp848, Leu851, and Leu855 are drawn. The helices are numbered with regard to past convention and Table 2 herein.

**FIGURE 4.** The C-terminal domain of the HsCen-2 XPC complex is positioned on yeast centrin with Kar1p peptide (Protein Data Bank code 1oqp). HsCen-2 and XPC peptide are shown in rainbow colors with N terminus (blue) and C terminus (red), Trp848 (marked by W), Leu851, and Leu855 of HsXPC are drawn. The Kar1p peptide trace is white, and the yeast centrin trace is purple, with both N termini marked blue and C termini in red.

similar to but unlike that of HsCen-2 in detail. The position of the Kar1p peptide is displaced from the XPC α-helix, but the orientation of the peptide and positioning of its tryptophan are roughly the same. The Kar1p peptide residues Trp248, Leu251, and Leu252 fit hydrophobic pockets, and Glu245, Lys247, and Arg250 mediate electrostatic interactions. Again, electrostatic interactions play a smaller role in the interface formed by HsCen-2 and XPC peptide. In addition, XPC lacks a large hydrophobic residue to match the Leu855 of Kar1p, position 5 in Fig. 1A. Like Kar1p, many peptides that are targets of calmodulin also have a large hydrophobic residue at this location. Finally, the importance of Leu855 as an anchoring interfacial residue in XPC, which Kar1p substitutes with an aspartic acid, is reflected by the comparison denoted by the red asterisk between the extended conformation of the Kar1p peptide away from the binding cavity and the alternate helical conformation of XPC within the cavity.

Disordered HsCen-2 N-terminal Residues—One aspect missing from the HsCen-2 structure is the sequence of amino acids numbered 1–22 from the N-terminal domain, which were determined to be disordered. The region is of interest, because it is not present in any other members of the calmodulin-parvalbumin superfamily. Titrating HsCen-2 (26, 43) and other centrins (44) to Ca\(^{2+}\) saturation is complicated by aggregation of high order oligomers, an aggregation of reversible nature. Deletion of amino acids 1–24 has little effect on protein stability, global secondary structure, or metal binding (29) but does prevent aggregation; thus, these residues must be involved in the self-assembly (45). The formation of fibers consisting of centrin may be of physiological importance in the duplication and segregation of microtubule organizing centers (21). However, as yet no evidence exists that multiple centrin molecules are involved in interactions with XPC-containing complexes or that HsCen-2 self-assembly is of importance in DNA repair.

Coiled-coil Conformation of HsXPC Peptide—More effort is required to prove that the α-helical coiled-coil structure of the HsXPC peptides is of physiological interest, since the interactions observed may be of importance only in the peptide and not the XPC protein. A comparison of 38 homologous amino acid sequences with the HsXPC peptide suggests putative α-helical coiled-coil character in many but not all species. Coiled-coil α-helices are a highly common structural motif for strong affinity protein-protein interactions and often found in cytoskeletal or fiber-like molecules. Few proteins have been reported as centrin binding targets; the list includes XPC, Kar1p, Shi1p, and three proteins not yet introduced, ninein, the nuclear envelope protein MPS3p, and the N-acetylglucosamine phosphate mutase. Of these, only the mutase has no region predicted as helical coiled-coil.

HsCen-2 C-terminal Residues—The carboxyl-terminal sequence 167KKTSLY172, of HsCen-2 is observed in both molecule A and B, but this region is disordered and probably would not have been observed if Tyr172 did not interact with the XPC peptide (see Fig. 2). As mentioned in the Introduction, this region is missing in HsCen-3 and other centrins of lower eukaryotes lacking centrioles, and Tyr172 is not present among calmodulins. The region is of interest, because phosphorylation of Ser170 has been proposed to occur at the G2/M phase of the cell cycle to effect regulation of centrin levels in the cytoplasm, a process that seems linked to centriole separation (46). In our structure, Ser170 is a surface residue that seems accessible to kinase.

HsCen-2 Metal Binding and EF-hands—The affinities of Ca\(^{2+}\) binding sites within HsCen-2 and other centrins are lower than for calmodulin (43, 47–49). Calmodulin Ca\(^{2+}\) binding affinities for its four sites are ~10\(^5\) to 10\(^6\) M\(^{-1}\), which clearly permits response to intracellular Ca\(^{2+}\) levels of 1–10 μM (50). Resting nuclear Ca\(^{2+}\) ranges from 0.1–0.3 μM but 0.35–1.2 μM levels occur when cells are stimulated (51). In comparison, studies involving integral HsCen-2 or just its C-terminal domain report macroscopic binding constants of 1.5 × 10\(^5\) and 7.5–8.1 × 10\(^3\) M\(^{-1}\) and a [Ca\(^{2+}\)]\(_{50}\) of 29–30 μM (26, 43). We speculate that Ca\(^{2+}\) binding affinity of HsCen-2 is increased with XPC peptide bound, since there exists a linked three-party reaction, and Ca\(^{2+}\)-centrin-2 has
27-fold higher affinity for the XPC peptide used herein compared with Ca\(^{2+}\)-free centrin-2 (11). The affinity of apocalmodulin for Ca\(^{2+}\) increases over 1000-fold when bound to target proteins (52). Ca\(^{2+}\) affinities for an N-terminal domain fragment of HsCen-2 are reported as \(\sim 10^2\) to \(10^3\) M\(^{-1}\) in the absence of bound polypeptide (27).

Unique HsCen-2 Target Recognition—The XPC peptide shown in Fig. 1A was predicted to adopt amphiphilic-helical structure with charged and hydrophobic surfaces and initially chosen from prior knowledge of calmodulin binding sites (11). Based on the positions of hydrophobic residues conserved among sequences from diverse XPC protein species, however, the HsCen-2 binding region does not correspond precisely to the 1-5-8-14 motif seen in Figs. 1A and 3), which categorizes binding sites of many Ca\(^{2+}\)/calmodulin-dependent proteins (53). Two structures of peptides of smooth muscle myosin light chain kinase and skeletal muscle myosin light chain kinase bound to calmodulin belong to this 1-5-8-14 classification (54, 55), and their sequences are provided as examples in Fig. 1A. Our structural results support a centrin-2-based binding motif of 1-4-8, corresponding to XPC residues Trp\(^{648}\), Leu\(^{651}\), and Leu\(^{855}\) shaded pink in Fig. 1A. The HsXPC residue at position 14 is a hydrophobic leucine, in perfect agreement with the premise of a novel motif defined by residues at positions 1-4-8, with an invariant tryptophan at position 1 and generally hydrophobic leucine at position 14 forms part of the \(\alpha\)-helical coiled-coil interface between XPC peptides and does not interact with HsCen-2.

Conclusions—The interface formed by full-length HsCen-2 and human XPC can be characterized by extensive nonpolar interactions involving just three hydrophobic residues of the XPC peptide. The result is a binding motif that probably binds with higher affinity to centrin-2 in comparison with calmodulin. This novel motif is defined by positions 1-4-8, with an invariant tryptophan at position 1 and generally hydrophobic leucine at position 14, for two positions in homologous XPCs from diverse species. In calmodulin, there is a similar motif defined as 1-5-8-14. The overall conformation of HsCen-2 was found to be unique, although the N-terminal and C-terminal domains alone closely match existing structures of EF-hand superfamily members. Only the C-terminal domain of centrin-2 was found to bind XPC peptide and calcium atoms, and the N-terminal domain was found in a closed conformation. N-terminal residues prior to Pro\(^{612}\) predicted to be disordered were not observed in electron density. Tyr\(^{72}\) of centrin-2 interacts with XPC peptide and anchors at least five somewhat disordered C-terminal residues thought to be of importance in the regulation of centrin-2. Interestingly, bound XPC peptides interact to form an \(\alpha\)-helical coiled-coil conformation. However, more studies will be required to determine whether any coiled-coil structure is typical of the centrin-2 binding site in XPC protein.

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