Molecular Modeling of the Amino-Terminal Zinc Ring Domain of BRCA1

Rachelle J. Bienstock,1 Tom Darden, Roger Wiseman, Lee Pedersen, and J. Carl Barrett


ABSTRACT

The equine herpes virus zinc ring domain nuclear magnetic resonance structure was used for homology-based modeling of the amino-terminal zinc ring domain of the BRCA1 breast and ovarian cancer susceptibility gene. The zinc ring domain of BRCA1 is of particular interest because it is the location of significant and frequently occurring missense (Cys48Gly, Cys49Gly, and Cys49Tyr) and frameshift (185delAG) mutations observed in several high-risk kindreds. The BRCA1 zinc ring domain possesses 54% sequence similarity with the equine herpes virus zinc ring domain. The model structure undergoes little conformational variance after 140 ps of solvated molecular dynamics. This model proposes BRCA1 zinc ring domain residues that may play a role in DNA binding and/or protein-protein interactions. These predictions provide a point of departure for the design of mutants to probe BRCA1 zinc ring domain functionality.

INTRODUCTION

BRCA1, the breast and ovarian cancer susceptibility gene on human chromosome 17q21, was recently identified (1, 2). Mutations in this gene account for approximately 95% of families with increased incidence of early onset breast and ovarian cancer and 45% of families with increased incidence of breast cancer alone (3, 4). BRCA1 is believed to encode a tumor suppressor protein, because most mutations appear to result in loss of function. This gene is expressed in several tissues, including breast and ovary, and encodes a 1863-amino acid protein.

The amino-terminal domain of BRCA1 possesses high sequence homology with a zinc finger topology (1). This zinc finger topology, C3HC4, is referred to as the “zinc-ring domain,” “RING finger,” or A-Box (5) and is found in several viral proteins, proto-oncoproteins, and regulatory and transcription factors (6). These include developmental regulatory proteins [XNF-7, SINA, PSC, SU(e), and DG17], cell regulatory proteins (IAP and DROC3H), signal transduction proteins (CRAF1), human oncoproteins (MDM2, MEL18, BIM-1, RET, T81, CBL, and PML), proteins that regulate gene expression (IEII0, VZ61, CG30, PE-38, EPO, EHV, LCMV, and P11), and proteins involved in DNA repair or recombination (RAD5, RAD18, RAD16, RAG-1, and UVS2).

The zinc ring family, first identified in 1991 (7), was found to possess two finger-like domains connected by a linking region and to require zinc for folding. The zinc ring C3HC4 sequence folds independently and is a distinct stable domain (8, 9). This domain differs structurally from other zinc finger domains in that the first and third pairs of cysteines form one zinc-binding site, and the second and fourth pairs of cysteines form the second metal-binding site. This crossed pattern is similar in configuration to a 1—3, 2—4 disulfide bond pattern. A schematic of the BRCA1 amino-terminal zinc ring domain with cysteine and histidine ligated to zinc is indicated in Fig. 1. Although a common functionality for the zinc ring domain has not been identified, it appears to interact with DNA either through direct binding or indirectly by mediating protein-protein interactions. The zinc ring domain of several proteins has been shown to exhibit DNA-binding activity (10) or nuclear localization (11—13).

The BRCA1 zinc ring domain and its structure and function are of particular interest, because it is the location of some of the most frequently occurring mutations linked to breast and ovarian cancer. The 185delAG mutation (deletion of two nucleotide base pairs in exon 2) in the zinc ring domain has been shown to occur in 1 in 100 Ashkenazi Jewish individuals (14, 15). This is in comparison with the estimated 1 in 833 frequency of all BRCA1 mutations in the general population (14, 15). In this article, a three-dimensional model for the zinc ring domain of BRCA1 is presented. This model proposes potential BRCA1 zinc ring domain residues that may play a role in the function of this domain, either binding directly to DNA or mediating DNA binding through protein-protein interactions.

MATERIALS AND METHODS

Sequence Alignment. The three-dimensional structures of two zinc ring domains, the EHV and the acute PML proto-oncoprotein, have recently been determined by multidimensional NMR (8, 9, 11). The sequence alignment between the PML and EHV zinc ring domains is shown in Fig. 2 along with the secondary structural elements identified for each protein from the solved NMR structures. Several criteria were used to select the EHV ring domain structure as the basis for the BRCA1 model. The BRCA1 zinc ring domain, for example, possesses greater sequence similarity to EHV than to PML. Using a sequence alignment of Smith and Waterman (16) with standard default parameters, the BRCA1 zinc ring domain possesses 54% sequence similarity and 38% sequence identity with the EHV zinc ring domain, compared with 42.8% sequence similarity and 30.6% sequence identity to the PML domain. Fig. 2 shows the sequence alignment between the BRCA1 and EHV and PML ring domains, respectively.

A BLASTP (Basic Local Alignment Search Tool for Proteins; Ref. 17) search of the Brookhaven Data Bank selected and aligned the EHV with the BRCA1 zinc ring domain, and yielded a P of 9.6×10−4 (chance likelihood of the two sequences relationship). Folding search programs, such as Profiles-3D by Lutyth et al. (18), also indicated that the EHV and BRCA1 zinc ring protein domains have similar folds. Hydrophilicity plots of Kyte and Doolittle (19), surface probability [calculated according to the method of Emini et al. (20)], chain flexibility predictions (Karplus-Schulz), secondary structure predictions of turns and a helices by Chou and Fasman (21), turns prediction by Garnier et al. (22), and the antigenicity index of Jameson and Wolf (23) all support greater similarity of the EHV ring domain structure to BRCA1 than to PML (data not shown). A multiple-sequence alignment of the three sequences (the Pileup algorithm from the package below; Refs. 24 and 25) yields pairwise similarity scores of 0.44 for BRCA1 and EHV, 0.37 for BRCA1 and PML, and 0.42 for EHV and PML. Therefore, EHV was used as the basis for developing a model for the BRCA1 zinc ring domain.

Several multiple sequence alignments have been published previously, aligning the BRCA1 zinc ring domain sequence to the sequences of other known ring domains. The alignment by Borden et al. (11) also shows greater sequence similarity between BRCA1 and EHV than between BRCA1 and
The two zinc ions were entered into the structure by superposition with the herpes virus structure, which gave a general positioning for the zinc ions in the protein. The resulting structure was energy minimized in vacuo using a distance-dependent dielectric constant, as described by Guenot and Kollman (28). The ligand-zinc ion distances were constrained in the minimization to the distances found between the corresponding ligands and zinc in the herpes virus protein. The zinc-cysteine sulfur atoms were pulled together using 500 cycles of conjugate gradients with a force of 100 kcal/mol, followed by minimization with 500 cycles of steepest descents and 1000 cycles of conjugate gradients until the maximum derivative was less than 0.1 kcal/Å. All constraints were then removed, and the structure was reminiimized with 1000 cycles of conjugate gradients until the maximum derivative was less than 0.1 kcal/Å.

Charges were assigned to the zinc ions and cysteine ligands $\text{Sy}$ and histidine N81. Because of the ionic and covalent nature of the zinc ligand interactions, the zinc ion charges are reduced and assigned values of $+1.5$ in the four-cysteine-ligand environment and $+1.9$ in the three-cysteine- and one-histidine-ligand environment. All cysteine Sys have negative partial charges, because a hydrogen is abstracted on binding with zinc. The histidine N81 also has a negative partial charge on binding with zinc. In this model, the cysteine Sys in the four-ligand case were assigned partial charges of $-0.275$, and charges of $-0.450$ were assigned in the three-ligand case. The histidine N bound to the zinc was assigned a partial charge of $-0.670$.

Distances were constrained in a several-step minimization (in vacuo) until the ligand-zinc distance criteria were met. This procedure minimized energy strain and prevented bumping and overlap of atoms. The average zinc ligand distance in the constrained minimized structure is 2.1 Å. The structure was then reminiimized with no constraints. The model was considered acceptable when reasonable zinc ligand distances could be maintained after removal of constraints and reminiimization.

**Molecular Dynamics.** Molecular dynamics simulations were performed to test the stability of the zinc ring BRCA1 model structure. The structure obtained from constrained minimization was solvated in a 5-Å layer of water and subjected to 140 ps of molecular dynamics. No distances were constrained during the molecular dynamics. The molecular dynamics simulation (using the Biosym CVFF) was performed with nonperiodic boundary conditions using the cell multipole method (fourth-order multipole) for treatment of nonbonded Coulombic and van der Waals interactions. A distance-dependent dielectric constant was used (28). The structure was initially reminiimized with solvent, and dynamics were performed beginning with the energy-minimized, solvated structure. The dynamics step size was 1 fs and structures were saved along the trajectory every 2 ps. Calculations were performed on a Silicon Graphics Indigo2 (MIPS R4400; 150-MHz central processing unit) computer.

**RESULTS**

Structural differences have been identified between the PML and the EHV NMR-determined zinc ring structures (11). The PML structure is composed of a $\beta$ strand followed by two antiparallel $\beta$ strands, one turn of a $\alpha_10$ helix, and a fourth $\beta$ strand. The EHV structure consists of two antiparallel $\beta$ strands followed by an $\alpha$ helix and a third $\beta$ sheet. Although EHV and PML structural differences have been used to support the idea that the ring motif is structurally diverse, two-layer $\alpha/\beta$ proteins such as EHV and $\beta$ proteins such as PML have been shown to have overall common folds (29). Both PML and EHV structures possess major conserved features: the "cross-brace" $Zn^{2+}$ binding arrangement; the topology of the central antiparallel $\beta$ strands; and packing of the conserved residues in the hydrophobic core (11). In both structures, the $\delta$-nitrogen of the histidine is ligated to the zinc in place of the more common $\epsilon$-nitrogen. The $C_N$ RMS deviation between the two structures for residues comprising the first zinc-binding site (Cys24, Cys27, Cys44, and Cys47) is 1.93 Å, indicating that this binding site is somewhat similar in both structures. The majority of structural differences between PML and EHV occur between the central $\beta$ strands and second zinc-binding sites, where the amino acid sequences of the two ring-domains are dissimilar.

Several subfamilies are beginning to arise within the zinc ring protein family. Although the zinc ring occurs near the amino-terminus

---

**MOLeCULAR MODELING OF BRCA1 AMINO-TERMINAL ZINC RING DOMAIN**

**RESULTS**

Structural differences have been identified between the PML and the EHV NMR-determined zinc ring structures (11). The PML structure is composed of a $\beta$ strand followed by two antiparallel $\beta$ strands, one turn of a $\alpha_10$ helix, and a fourth $\beta$ strand. The EHV structure consists of two antiparallel $\beta$ strands followed by an $\alpha$ helix and a third $\beta$ sheet. Although EHV and PML structural differences have been used to support the idea that the ring motif is structurally diverse, two-layer $\alpha/\beta$ proteins such as EHV and $\beta$ proteins such as PML have been shown to have overall common folds (29). Both PML and EHV structures possess major conserved features: the "cross-brace" $Zn^{2+}$ binding arrangement; the topology of the central antiparallel $\beta$ strands; and packing of the conserved residues in the hydrophobic core (11). In both structures, the $\delta$-nitrogen of the histidine is ligated to the zinc in place of the more common $\epsilon$-nitrogen. The $C_N$ RMS deviation between the two structures for residues comprising the first zinc-binding site (Cys24, Cys27, Cys44, and Cys47) is 1.93 Å, indicating that this binding site is somewhat similar in both structures. The majority of structural differences between PML and EHV occur between the central $\beta$ strands and second zinc-binding sites, where the amino acid sequences of the two ring-domains are dissimilar.

Several subfamilies are beginning to arise within the zinc ring protein family. Although the zinc ring occurs near the amino-terminus
in most protein family members, there are several proteins for which the zinc ring occurs near the middle of the sequence (SINA, PE38, and XNF7) or near the carboxyl terminus (CRAF1, PAS4, CBL, PSC, RAD16, RAG1, and PAF1). Several zinc ring domain proteins have ATPase motifs (HIP116, RAD16, and RAD5). One ring protein, MSL2 (male specific lethal-2) has a metallothionine domain in addition to the zinc ring domain (26). PML belongs to a subfamily of ring proteins that have been shown to possess another cysteine-bistidine motif, identified as a "B-box" following the zinc ring domain (30). PML has two B-box domains followed by an alpha helical, coiled-coil domain downstream from the zinc ring domain (11). The B-box motif and additional alpha helical, coiled-coil domains are absent in BRCA1 and EHV. It has recently been demonstrated that the PML ring finger protein and the HSV type 1 ring domain are not functionally interchangeable. (HSV is required for activation of viral gene expression.) Neither a chimeric HSV protein that contained the PML ring finger nor PML was able to activate gene expression in assays (31). This work suggests that ring finger domains do not possess a common functionality. Different subcategories of zinc ring proteins may reflect structural classes of zinc ring domains with differing functionality.

The final minimized BRCA1 zinc ring domain model is shown in Fig. 4 with cysteine and histidine zinc ligands. Superimposing the two minimized BRCA1 structures before and after removing the distance constraints, the RMS deviation is 0.02 Å (all atoms), and the backbone...
RMS deviation is 0.018 Å (N, Cα, C, and O). This demonstrates that the zinc ligand distances are maintained without constraints, and that the structure does not change significantly when the distance restraints are removed.

Superimposing the minimized BRCA1 model structure with the EHV structure yields a total backbone RMS deviation of 3.0 Å. This includes the protein loops, where there is the greatest difference between the two structures. A backbone (N, Cα, C, and O) superposition of the separate secondary structural elements reveals much greater similarity in the structures (as shown in Table 1).

Fig. 5 shows a superposition of the minimized BRCA1 model structure and EHV average NMR structure. The loop regions of the modeled protein are the most difficult to define, because they exhibit the greatest flexibility. The smallest RMS deviations between the EHV structure and BRCA1 model are in regions of the protein that contain a single, well-defined secondary structure (α helix or single β sheet). The greatest difference between the secondary structural elements of the minimized BRCA1 structure and the EHV structure is in the antiparallel β sheet. The β turn between the two antiparallel β sheets differs in both structures. In the EHV structure, this turn (Ala21, Leu22, Pro23, and Cys24) contains a cis-Pro in the i + 2 position and has the characteristic ψ and φ angles of a type V Ib β turn (32). In the BRCA1 model, this turn does not contain a cis-Pro (Thr7, Lys17, Leu22, Pro23, and Cys24) contains a cis-Pro in the i + 2 position and has the characteristic ψ and φ angles of a type V Ib β turn (32). In the BRCA1 model structure, this turn does not contain a cis-Pro (Thr7, Lys17, Leu22, Pro23, and Cys24) contains a cis-Pro in the i + 2 position and has the characteristic ψ and φ angles of a type V Ib β turn (32). In the BRCA1 model structure, this turn does not contain a cis-Pro (Thr7, Lys17, Leu22, Pro23, and Cys24) contains a cis-Pro in the i + 2 position and has the characteristic ψ and φ angles of a type V Ib β turn (32). In the BRCA1 model structure, this turn does not contain a cis-Pro (Thr7, Lys17, Leu22, Pro23, and Cys24) contains a cis-Pro in the i + 2 position and has the characteristic ψ and φ angles of a type V Ib β turn (32).

The accuracy of the model was validated by subjecting the model structure to PROCHECK version 3.0 (33), and WHAT IF version 4.99 (34). PROCHECK assesses the stereochemical quality of the structure, nonbonded contacts within the structure, side-chain conformations, bond lengths and angles, and peptide bond planarity (ω) and reports the deviations from values in well-refined structures. In the BRCA1 model structure, all ω and ψ angles fell within allowed regions of the Ramachandran plot. All the main chain parameters (the peptide bond planarity, number of bad nonbonded interactions, hydrogen bond energies, and α-carbon tetrahedral distortion) were all within acceptable limits from ideal values (in well-refined structures). All of the side chain parameters (χ1 gauche minus and plus and χ1 trans- and χ2 trans-angle values) were comparable to those for high resolution structures. The overall G-factor value given by PROCHECK for this structure was −0.4. Ideally, scores above (less negative than) −0.5 indicate good-quality structures, and values greater than (more negative than) −1.0 are indicative of problems with the structure. A value of −0.4 is approximately equivalent to an X-ray crystal structure with a resolution of 2 Å (33).

WHAT IF checks interatomic distances, atomic packing, torsion and side chain angles, and unsatisfied hydrogen bond donors and acceptors and gives a measure of the overall quality of the structure. The WHAT IF overall quality score for this BRCA1 model structure was −2.8.

The EHV structure on which this BRCA1 model was based gave a WHAT IF quality score of −2.60. As is reasonable, the overall quality and validity of this model appears comparable to that of the NMR structure on which it was based.

Molecular Dynamics: Testing the Solvated Structure. Fig. 6 is a plot of the backbone RMS deviation versus time across the 140-ps trajectory. The backbone RMS deviation of the dynamics structure compared with the starting structure equilibrated between 110 and 140 ps to an average value of 1.48 Å. The backbone RMS deviation between the starting and ending structure was 1.65 Å. A backbone superposition of all seventy structures saved along the molecular dynamics trajectory shown in Fig. 7 illustrates that there are no major structural reorganizations along the trajectory. The zinc ligand distances are maintained within tolerance (±0.30 Å) along the 140 ps. Overall, the structure is remarkably stable.

The backbone RMS deviation between the BRCA1 final average molecular dynamics structure and the EHV structure is 3.1 Å, which includes protein loops in the structure. Superposition of the separate secondary structural elements of these structures, as with the minimized BRCA1 structure, also reveals much greater similarity in the structures (as shown in Table 2).

Fig. 5 shows a superposition of the final average molecular dynamics BRCA1 model structure with the minimized BRCA1 model struc-

Table 1  RMS deviation between secondary structural elements of the EHV average NMR structure and minimized BRCA1 model structure

<table>
<thead>
<tr>
<th>Secondary structural element</th>
<th>RMS deviation (Å)</th>
<th>No. of backbone atoms superimposed (N, Cα, C, and O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β sheet 1</td>
<td>1.46</td>
<td>40</td>
</tr>
<tr>
<td>β sheet 2</td>
<td>0.66</td>
<td>32</td>
</tr>
<tr>
<td>Antiparallel β sheets (β sheets 1 and 2)</td>
<td>1.60 72</td>
<td></td>
</tr>
<tr>
<td>α helix</td>
<td>0.69</td>
<td>72</td>
</tr>
<tr>
<td>β sheet 3</td>
<td>0.71</td>
<td>40</td>
</tr>
<tr>
<td>β sheets 1 and 2—α helix</td>
<td>1.81</td>
<td>168</td>
</tr>
<tr>
<td>β sheets 1—3—α helix</td>
<td>1.85</td>
<td>216</td>
</tr>
</tbody>
</table>

MOLECULAR MODELING OF BRCA1 AMINO-TERMINAL ZINC RING DOMAIN

Fig. 5. Superposition of the BRCA1 minimized model (yellow), final average molecular dynamics BRCA1 model structure (blue), and EHV average NMR structure (red).
Fig. 6. Plot of the BRCA1 backbone RMS deviation versus time for the 140-ps molecular dynamics trajectory. The average structure (110–140 ps) has a backbone RMS to the starting structure of 1.48 Å.

DISCUSSION

A specific common functionality for the zinc ring domain has not been identified to date. The domain appears to interact with DNA either through direct binding or indirectly by mediating protein-protein interactions. No member of the zinc ring domain family has been shown to bind DNA in a sequence-dependent manner. However, a synthetic zinc ring peptide was shown to bind to a subset of randomized DNA sequences, and the DNA-protein complex formation was zinc dependent (6). Likewise, the EHV, Mel-18, and XNF7 zinc ring domains have been shown to bind nonspecifically to DNA-cellulose (10, 35). Several zinc ring proteins (i.e., ICPO, RO-52K, and PML) have been shown to exhibit nuclear localization (13). It has been suggested that PML is involved in promoting protein-protein interactions that stabilize PML nuclear body formation (11).

Other zinc finger motifs in addition to the ring domain that possess two independent zinc-binding sites are the LIM domain and the CRD (36). The CRD domain has interleaved zinc ligands, and its recently solved NMR structure revealed the CRD to have a fold similar to that of the ring domain. Both domains consist of a three-stranded β sheet and an α helix. The CRD is in the protein kinase C regulatory domain that is believed to contain the binding sites for diacylglycerol and phorbol esters and to be critical for regulation of kinase activity. The role that the CRD plays, however, is unknown (37).

Many of the proteins containing the LIM domain are transcription factors (the name “LIM” is derived from the three transcription factors in which it was first observed: Lin-11, Isl-1, and Mec-3. However, like the ring domain, the LIM domain has not been shown to have biologically relevant DNA-binding activity. Recent studies (38) have demonstrated a specific protein-protein interaction between two LIM domain proteins, zyxin and CRP. Therefore, it is possible that the ring domain may function similarly to the LIM domain, and that protein-protein interactions in which the ring domain participates may play a role in gene expression regulation. Both the LIM and ring domains are bipartite structures with two zinc-binding domains; therefore, it is possible that they may function similarly to the glucocorticoid receptor, in which one zinc site is required for nucleic acid binding, and the other mediates protein-protein interaction, so that the receptor forms a homodimer. This may be the case, because the HSV Vmw110 zinc ring protein has been suggested to be a multimer in solution (35).

If BRCA1 functions as a moderator of protein-protein interactions, it may act similarly to the CRD or LIM domain. In the CRD, residues

![Fig. 7. Backbone superposition of all 70 structures along the molecular dynamics trajectory.](image)

Table 2. RMS deviation between secondary structural elements of the EHV average NMR structure and the BRCA1 final average molecular dynamics structure

<table>
<thead>
<tr>
<th>Secondary structural element</th>
<th>RMS deviation (Å)</th>
<th>No. of backbone atoms superimposed (N, Cα, C, and O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β sheet 1</td>
<td>1.35</td>
<td>40</td>
</tr>
<tr>
<td>β sheet 2</td>
<td>0.59</td>
<td>32</td>
</tr>
<tr>
<td>Antiparallel β sheets (β sheets 1 and 2)</td>
<td>1.38</td>
<td>72</td>
</tr>
<tr>
<td>α helix</td>
<td>0.60</td>
<td>72</td>
</tr>
<tr>
<td>β sheet 3</td>
<td>0.96</td>
<td>40</td>
</tr>
<tr>
<td>β sheets 1 and 2–α helix</td>
<td>1.87</td>
<td>168</td>
</tr>
<tr>
<td>β sheets 1–3–α helix</td>
<td>1.95</td>
<td>216</td>
</tr>
</tbody>
</table>
that are part of the central antiparallel \( \beta \) sheet and \( \alpha \) helix play a significant role in protein binding (37). Although the BRCA1 zinc ring domain possesses only 15% sequence identity and 31% sequence similarity with CRD, in an alignment by Smith and Waterman (16), the central four BRCA1 zinc cysteine ligands, Cys\(^{1}\), Cys\(^{47}\), Cys\(^{61}\), and Cys\(^{64}\), align with the CRD zinc cysteine ligands Cys\(^{115}\), Cys\(^{118}\), Cys\(^{312}\), and Cys\(^{355}\). This corresponds to the central antiparallel \( \beta \) sheet region of the molecule. Cys\(^{332}\) and Cys\(^{355}\) are required for the phorbor ester affinity of CRD. Val\(^{14}\), also required for phorbor ester binding of CRD, is replaced by Leu\(^{3}\) in BRCA1, an aliphatic residue with similar chemical properties. A group of CRD conserved residues (Tyr\(^{109}\), Phe\(^{114}\), Lys\(^{131}\), and Asn\(^{138}\)) have been identified (37) as being positioned for interactions with protein kinase C activators. In BRCA1, Tyr\(^{109}\) is replaced by Lys\(^{38}\), another ionizable residue; Phe\(^{114}\) is conserved; Lys\(^{131}\) is replaced by Gln\(^{60}\); and Asn\(^{138}\) is replaced by Asp\(^{67}\). Asp can potentially deamidate to Asn, and these residues are structurally similar. The Lys\(^{38}\), Gln\(^{60}\), and Asp\(^{67}\) residues are all on the surface of the BRCA1 zinc ring model and have side chains with extended orientations favorable for interaction with other proteins (Fig. 4). The Phe\(^{43}\) residue is buried in the BRCA1 model. These predicted surface residues in BRCA1 (Lys\(^{38}\), Gln\(^{60}\), and Asp\(^{67}\)) may participate in protein-protein interactions if the mechanism of BRCA1 zinc ring domain protein interaction is similar to that of the CRD. Mutations of these residues would probe the BRCA1 zinc ring domain functionality as a mediator of protein-protein interactions.

A variety of zinc finger crystal structures (zif268 (mouse immediate early protein), human globin, Gal4 (yeast transcriptional activator), WT1, and estrogen and glucocorticoid receptors) have shown interactions between the protein zinc finger \( \alpha \) helix and the DNA major groove (39–42). Although there are significant differences between the zinc ring and zinc finger structures, a comparison between the EHV zinc ring and the classic TFIIA-type ring finger (9) showed some structural similarity. Backbone atom superposition of the human enhancer-binding protein TFIIA-type zinc finger and the EHV zinc ring finger yielded an RMS deviation of 2.0 \( \AA \) (9). The first two \( \beta \) strands and the \( \alpha \) helix of EHV overlapped well in length and orientation with those of the human enhancer-binding protein TFIIA-type zinc finger. Hydrophobic core residues and positively charged residues on the outer surface of the \( \alpha \) helix also superimposed in the two structures. It has been shown in zinc finger domain proteins that the \( \alpha \) helix of the zinc finger domain interacts with DNA, usually via hydrogen-bonding between the amino acid side chains of the protein and guanine DNA bases (39–42). The tumor suppressor gene WT1, which encodes a transcription factor, contains a zinc finger domain in which many mutations linked to Wilms’ tumors occur (43). WT1 possesses four zinc fingers and binds to guanine-rich DNA sequences. Cancer-linked mutations in WT1 have been shown to change zinc finger DNA-binding properties (44).

We can extend our model to propose that the \( \alpha \) helix of the BRCA1 zinc finger could bind in the major groove of B-DNA. Mutations of polar residues in the \( \alpha \) helix of the BRCA1 zinc ring domain presumably could interfere with proposed protein-DNA binding. The \( \alpha \) helix of the BRCA1 zinc ring domain consists of Cys\(^{44}\), Lys\(^{45}\), Phe\(^{46}\), Cys\(^{47}\), Met\(^{48}\), Leu\(^{49}\), Lys\(^{50}\), Leu\(^{51}\), Leu\(^{52}\), Asn\(^{53}\), and Gln\(^{54}\). This \( \alpha \) helix contains two amino acids with ionizable side chains, Lys\(^{45}\) and Lys\(^{50}\), and two amino acids with hydrophobic side chains, Asn\(^{53}\) and Gln\(^{54}\). The residues that would be involved in DNA binding, based on the assumption that the BRCA1 zinc ring domain \( \alpha \) helix inserts in the DNA major groove, are Gln\(^{54}\) and Lys\(^{50}\) (hydrophilic residues with side chains that extend toward the surface; Fig. 4). If BRCA1 follows other zinc finger DNA-binding proteins, the side chains of these hydrophilic residues would hydrogen bond with the DNA guanine.

Neither of these positions, however, corresponds to BRCA1 mutations currently linked to breast and/or ovarian cancer. If the BRCA1 zinc ring structure is ultimately shown to bind DNA in a manner similar to other zinc finger transcription factors, mutations at these positions may inhibit binding to DNA. Molecular modeling of interactions between BRCA1 and DNA will be useful in the design of mutants aimed at understanding BRCA1 function.

Several predisposing BRCA1 mutations linked to breast and ovarian cancer have been identified as occurring in the zinc ring domain. One of the original BRCA1 mutations (kindred 1901) contains a frameshift in exon 2 (188del11; Ref. 1), which essentially removes the zinc ring domain from the protein. In addition, the most common BRCA1 mutation seen to date is 185delAG (C24STOP39 in codon 24; Ref. 45), which essentially disrupts the BRCA1 gene product precisely at the first residue of the C\( \alpha \)H\(_{3}\) domain. Three frequent missense mutations, Cys\(^{33}\)Gly, Cys\(^{64}\)Gly, and Cys\(^{64}\)Tyr; Ref. 45) would also disrupt the zinc ring domain structure. The zinc ion in proteins interacts strongly with several ligands, the sulfur in Cys, the nitrogen in His, and the oxygen in Asp, Glu, and water. If the Cys residues were mutated to Gly or Tyr, the zinc ion coordination, no longer tetrahedral, would be unstable. The zinc ion would either be lost from the structure, and the protein would assume a different fold, or water would serve as the fourth ligand for zinc, altering the conformation of the protein. Because the correct protein fold would no longer be maintained, the mutated zinc ring would no longer interact with DNA and/or protein in the same manner. Studies are under way to ascertain whether the zinc ring domain of BRCA1 can bind DNA in a sequence-specific manner, or whether BRCA1 protein-protein interactions are of biological significance.

ACKNOWLEDGMENTS

We are grateful to Howard Smith for his assistance and support with computer graphics.

REFERENCES


9. Barlow, P. N., Lusi, B., Miller, A., Elliot, M., and Everett, R. D. Structure of the
Molecular Modeling of the Amino-Terminal Zinc Ring Domain of BRCA1

Rachelle J. Bienstock, Tom Darden, Roger Wiseman, et al.