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# Common Conformational Effects of p53 Mutations

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The tumor suppressor gene p53 has been identified as the most frequent target of genetic alterations in human cancers. Most of these mutations occur in highly conserved regions in the DNA-binding core domain of the p53 protein, suggesting that the amino acid residues in these regions are critical for maintaining normal p53 structure and function. We previously used molecular dynamics calculations to demonstrate that several amino acid substitutions in these regions that are induced by environmental carcinogens and found in human tumors produce certain common conformational changes in the mutant proteins that differ substantially from the wild-type structure. In order to determine whether these conformational changes are consistent for other p53 mutants, we have now used molecular dynamics to determine the structure of the DNA-binding core domain of seven other environmentally induced, cancer-related p53 mutants, namely His 175, Asp 245, Asn 245, Trp 248, Met 249, Ser 278, and Lys 286. The results indicate that all of these mutants differ substantially from the wild-type structure in certain discrete regions and that some of these conformational changes are similar for these mutants as well as those determined previously. The changes are also consistent with experimental evidence for alterations in structure in p53 mutants determined by epitope detectability using monoclonal antibodies directed against these regions of predicted conformational change.

**KEY WORDS:** p53; mutation; molecular dynamics; conformation.

## 1. INTRODUCTION

The p53 tumor suppressor gene is the most frequently identified site for mutations in human cancer (Lane, 1994). The encoded wild-type protein is a sequence-specific transcriptional factor that *trans*-activates a number of genes whose products are involved in cell growth regulation (Ko and Prives, 1996). The majority of cancer-related mutations in p53 cluster in several hot-spot regions of the protein that have been highly conserved throughout evolution and occur in the sequence-specific DNA-binding core domain of the protein between amino acid residues 102 and 292 (Fromentel and Soussi, 1992). The mutations in p53 found in malignancies could thus result in substitutions of amino acid residues in these regions that are critical for the determination of the structure, and hence cell-cycle regulatory function, of the protein, contributing to the development of cancer.

Several of the mutations that occur in hot-spot regions of p53 and have been identified in human cancers have been linked to exposures to certain environmentally occurring carcinogens. For example, we previously examined the conformational effects in p53 of amino acid substitutions that have been attributed to vinyl chloride exposure, including His $\rightarrow$ Leu at residue 179, Arg $\rightarrow$ Trp at residue 249, and Ile $\rightarrow$ Phe at residue 255, and demonstrated that each of these substitutions produces common conformational changes in various regions of the DNA-binding domain of the protein (Brandt-Rauf

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[5] Abbreviations: AMBER, Assisted model building with energy refinements; ECEPP, empirical conformational energy program for peptides; rms, root-mean-square.



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*et al.,* 1996; Chen *et al.,* 1999). Other amino acid substitutions in p53 that have been associated with environmental carcinogens include the following: Arg → His at residue 175, Gly → Asp at residue 245, and Pro → Ser at residue 278, which have been identified in asbestos-associated mesotheliomas (Cote *et al.,* 1991); Gly → Asp at residue 245, Arg → Trp at residue 248, and Glu → Lys at residue 286, which have been identified in sunlight-associated squamous cell skin carcinomas (Brash *et al.,* 1991); and Arg → Met at residue 249, which has been identified in radon-associated lung cancers (Taylor *et al.,* 1994). The purpose of the present study was to use molecular dynamics calculations to examine the conformational effects of these additional amino acid substitutions on the structure of the p53 DNA-binding domain and to determine if these diverse substitutions produce any conformational changes that are common among these mutant proteins and similar to those previously identified.

## 2. METHODS

Molecular dynamics calculations were performed as described previously (Brandt-Rauf *et al.,* 1996). The starting structure was the wild-type p53 protein from amino acid residues 94–312 generated from the X-ray crystal coordinates (Cho *et al.,* 1994) and energy-minimized as described (Brandt-Rauf *et al.,* 1996). The conformations of the mutant p53 proteins (His 175, Asp 245, Asn 245, Trp 248, Met 249, Ser 278, Lys 286) were constructed from the minimized wild-type structure by substituting each amino acid for the normally occurring amino acid at the respective position in the wild-type structure. In each case, the backbone dihedral angles for the substituted residue were the same as for the corresponding amino acid in the wild-type protein. The starting side-chain conformation for the substituted residue was the lowest energy conformation one for the given backbone conformation as determined by ECEPP (Vasquez *et al.,* 1983). This structure was then subjected to nested molecular dynamics and energy minimizations using ECEPP and AMBER potential functions, as described previously (Brandt-Rauf *et al.,* 1996). Dynamics runs were performed for a total of 700 psec, and the total conformational energies for each mutant structure converged to a minimum value. The last 50 isoenergetic structures on the trajectory were employed in computing the average structure and the coordinate fluctuations. The average structure for each mutant form was superimposed on that for the wild-type protein such that the rms deviation of the coordinates of the backbone atoms of one structure from the other was a minimum. The average rms deviation between the mutant proteins and the wild-type protein was determined for each amino acid residue. Similarly, the average structure for each mutant form was superimposed on every other mutant form and the rms deviations determined.

This approach has been shown to give results that are consistent with experimental data. For example, computed conformational changes in p53 mutants have been confirmed by movement of antibody epitopes (Brandt-Rauf *et al.,* 1996; Chen *et al.,* 1999) and phosphorylation sites (Adler *et al.,* 1997) in the predicted regions of the protein. In addition, when this molecular dynamics approach was applied to the determination of the structure of the Ser 245-p53 as constructed from the wild-type starting structure, it yielded results in excellent agreement with the Ser 245-p53 X-ray crystal structure (overall rms deviation of 0.5 Å and minimal residue-by-residue variation) (Chen *et al.,* 1999).

## 3. RESULTS

Coordinate fluctuations for all residues in each mutant were less than 1.5 Å, confirming that the proteins converge on a specific structure that can be represented by the average structure in each case for sake of comparison to the wild-type structure and to each other.

The overall rms deviations for the average structure of each mutant from the average wild-type structure were considerable and very similar, ranging from 6.0 Å for the His 175 mutant to 6.8 Å for the Asp 245 mutant. On the other hand, the overall rms deviation for the average structure of each mutant from every other mutant was less, ranging from 3.8 to 4.6 Å, and the major differences in these cases were due to large deviations in the N-terminal and C-terminal ends. The individual residue rms deviations for the average structure of each mutant compared to the wild-type protein are shown in Fig. 1, demonstrating large deviations at discrete regions throughout the proteins, most of which are remarkably similar from mutant to mutant. Relatively large (>5 Å) deviations from the wild-type structure can be seen in all the mutant structures in the regions of amino acid residues 94–116, 148–156, 175–185, 218–228, 256–264, and 282–294. Regions of relatively large deviation that occur in some mutants, but not all, include 204–217 and 239–247. In some cases the sites of the mutations are near or within the regions of large deviation, but in many cases these regions are quite distant from the sites of mutation, indicating that common conformational changes can be produced by amino acid substitutions at diverse locations within the same protein structure.

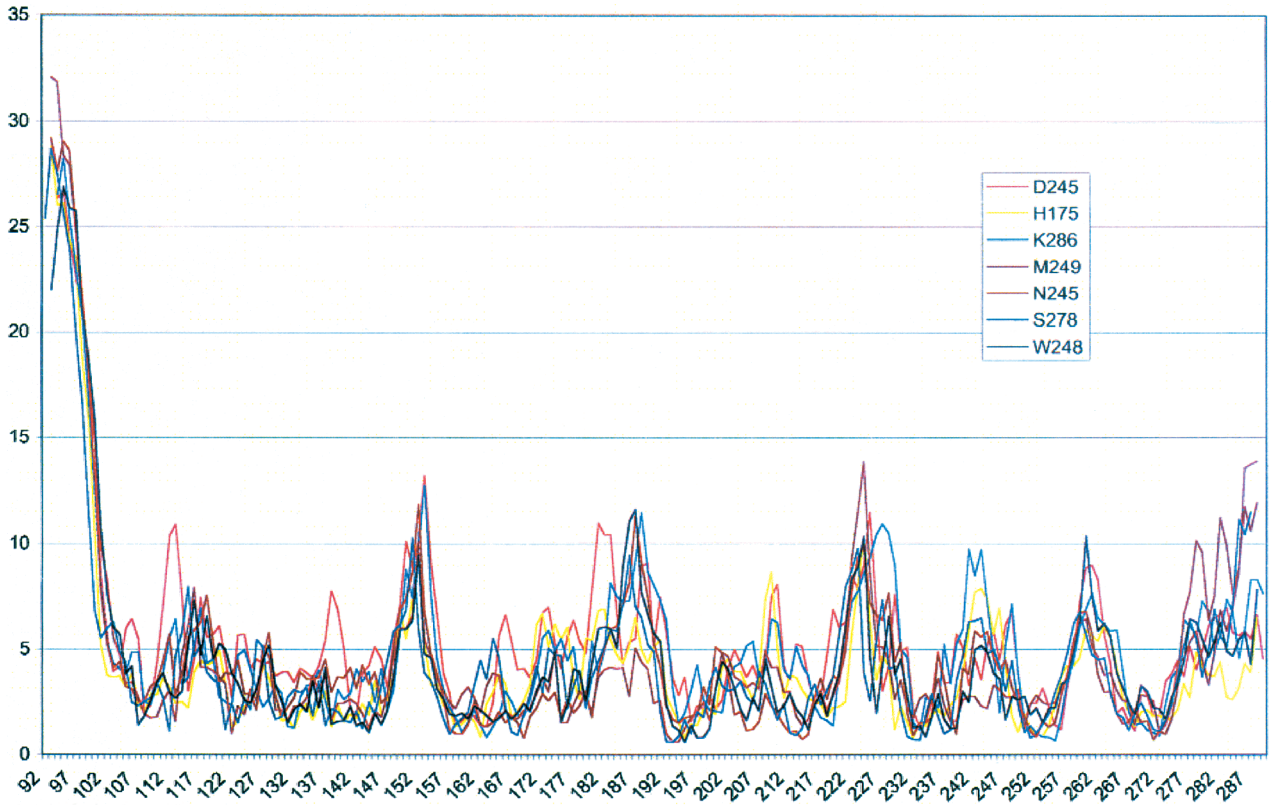


**Fig. 1.** Individual residue backbone deviations for the coordinates of corresponding amino acid residues 94–294 of the indicated mutant p53 proteins from those of the wild-type average structure. Scale is in angstroms.

The specific conformational changes in several of these regions are of interest because of the relationship to noted biological changes in the molecule. For example, the N-terminal region of 94–116 includes part of the epitope for the monoclonal antibody PAb1620 (Ravera *et al.*, 1998). In the wild-type structure, this region can be seen to be exposed on the surface of the protein, whereas in the mutant structures it is folded into the protein structure packing against the hydrophobic core and thus is much less exposed (see Fig. 2, which shows stereo views of the average structure of the His 175 mutant superimposed on the average wild-type structure). The region around amino acid residue 179 forms part of the zinc-binding domain of the protein (Cho *et al.*, 1994). Amino acid substitutions that alter the conformation in this region would have a significant effect on the protein's function since the zinc atom is necessary for its activity. The large conformational changes seen in the mutants in this region are similar to those produced by substitution at amino acid residue 179 (a zinc coordination site) and to those seen by removal of the zinc atom from the structure (Chen *et al.*, 1999). The region of amino acid residues 204–217 is also of interest because it contains the epitope for another monoclonal antibody, PAb240 (Stephen and Lane, 1992). In the wild-type structure this region is folded against the central structure of the protein packing into the hydrophobic core of the β-sandwich structure and is thus not exposed. In several of the mutants, particularly His 175, Asp 245, and Lys 286, this region is bent out and away from the β-sandwich core into an exposed position at the surface of the protein (see Fig. 3, which show stereo views of the average structure of the His 286 mutant superimposed on the average wild-type structure). This is consistent with immunologic findings of epitope binding of PAb240 in some (but not all) mutant p53 proteins but not in the wild-type protein (Gannon *et al.*, 1990).

## 4. DISCUSSION

These results suggest that different amino acid substitutions in various regions of the DNA-binding core domain of the p53 protein can produce common conformational changes in several regions of the protein. Several of these regions have been previously identified

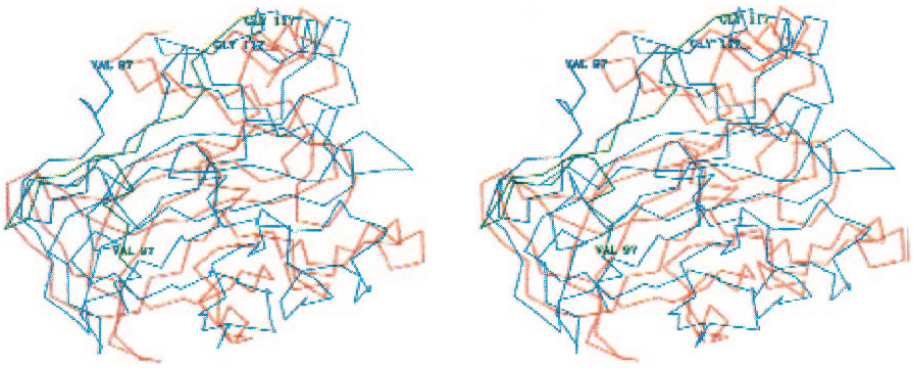

**Fig. 2.** Stereoview of the superimposed Cα traces of the average structures of the wild-type (red) and His 175 mutant (light blue) p53 proteins obtained from molecular dynamics simulations. The residues 97–117 are highlighted in the wild-type (dark blue) and mutant (green) proteins to demonstrate the conformational shift in this region.

in other p53 mutants (Brandt-Rauf *et al.*, 1996; Chen *et al.*, 1999). Comparing these results to those obtained previously demonstrates three different regions that consistently undergo relatively large common conformational changes in all mutant examined to date, including amino acid residues 94–117, 175–185, and 282–294.

The region of amino acid residues 94–117 is of interest because, as noted, it contains part of the epitope for the monoclonal antibody PAb1620 (Ravera *et al.*, 1998). Detection of this epitope has been considered to indicate a "wild-type" conformation for p53 because it is present in the native protein but absent in some (but not all) mutants (including the His 175 mutant studied here) (Vojtesek *et al.*, 1995). The molecular dynamics results here and in prior studies are consistent with this since the epitope region is found to be accessible in the wild-type protein but much less accessible in some of the mutants, such as the His 175 mutant. In other cases (e.g., the Trp 248 mutant), although conformationally shifted in this region, the Pab1620 epitope remains somewhat accessible, consistent with persistent antibody detectability (Legros *et al.*, 1994).

The region of amino acid residues 175–185, as noted, contains one of the coordination sites for the zinc atom in p53 which is important for its function (Cho *et al.*, 1994). Conformational changes seen in this region in the mutant proteins are similar to those seen with mutation at the coordination site or with removal of the zinc

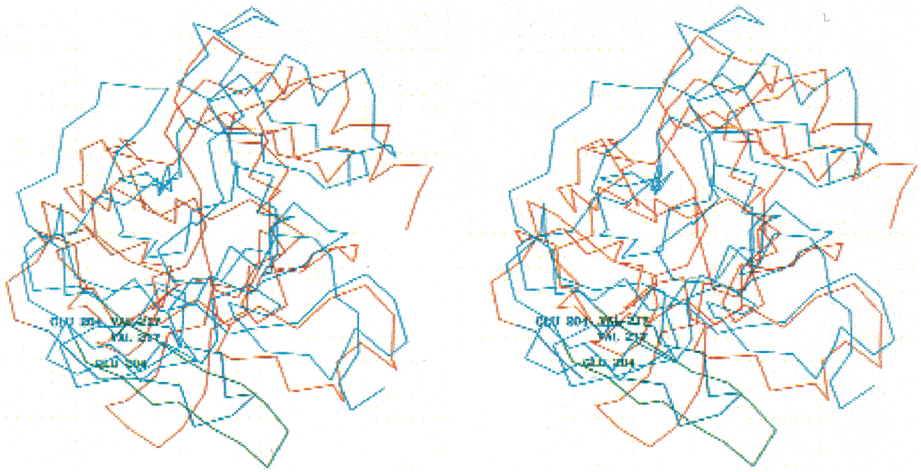

**Fig. 3.** Stereoview of the superimposed Cα traces of the average structures of the wild-type (red) and His 175 mutant (light blue) p53 proteins obtained from molecular dynamics simulations. The residues 204–217 are highlighted in the wild-type (dark blue) and mutant (green) proteins to demonstrate the conformational shift in this region.

atom itself (Chen *et al.,* 1999), which may largely explain the loss of function seen in the p53 mutants. This region also contains the epitope for another monoclonal antibody, DO-11, which has been detected in many p53 mutants (including the His 175 mutant studied here) but not in the wild type (Vojtestek *et al.,* 1995). Once again this is consistent with the molecular dynamics results since this region moves considerably in the His 175 mutant in such a fashion that instead of being packed against the hydrophobic core (as it is in the wild type), it is bent out and away from the rest of the structure and is exposed on the surface.

The region of amino acid residues 204–217 is also significantly shifted in several p53 mutants. Once again, this is consistent with the molecular dynamics results. As noted, in the His 175 mutant this region has a major deviation (>8 Å) in such a way that leaves the PAb240 epitope exposed at the surface of the protein rather than concealed as occurs in the wild-type structure. This is consistent with the results of antibody detectability of the epitope (Legros *et al.,* 1994). On the other hand, several mutants studied here show this region to be much less deviated leaving the Pab240 still partially concealed in the core structure of the protein. For example, in the Trp 248 mutant this region moves less than 5 Å and the epitope is not exposed, consistent with the lack of antibody detectability of the epitope (Legros *et al.,* 1994). We previously demonstrated that detection of this epitope in biological samples was a reliable indicator of the presence of several different mutations in p53 produced by vinyl chloride (Brand-Rauf *et al.,* 1996; Chen *et al.,* 1999). However, since this epitope is not moved and revealed in all p53 mutants, these results cannot be expected to be generalizable to the detection of any and all p53 mutations that could occur by other mechanisms.

Another region where there is frequent significant deviation is amino acid residues 256–264. This region was shifted in all seven mutants studied here but not in several of the previously studied mutants. Once again, this region is known to contain an antibody epitope for DO-12 that is not detectable in the wild type protein but is detectable in many mutants, including the His 175 mutant (Vojtestek *et al.,* 1995). The molecular dynamic results here are consistent with that. This region is packed against the hydrophobic core in the wild-type and is concealed but it is significantly shifted to the surface of the protein in the mutants, including the His 175 mutant.

Overall, the results of this study indicate that various amino acid substitutions at diverse sites in the p53 core DNA-binding domain produce conformational changes in the protein that are common for many of the mutants. The changes found by molecular dynamics are consistent with experimental results based on the detection of antibody epitopes in the regions of conformational change. As has been found in other studies, these regions of conformational change may represent important "effector" domains of the protein that are involved in critical intermolecular interactions with other cellular macromolecules and thus may become attractive targets for further study of the mechanisms of action of p53's diverse functions.

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