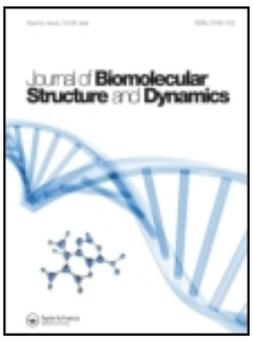

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Oncogenic Mutations and Packing Defects in Protein Structure

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Abstract

Oncogenic mutations in expressed proteins are of primary interest to understand tumor formation but their structural consequences bearing on protein function are not clearly understood. In this contribution I report on two illustrative examples, p21ras and p57, revealing that such mutations have an effect on specific structural deficiencies in the packing of the protein structure, i. e., on backbone hydrogen bonds insufficiently shielded from water attack. These structural deficiencies in the wild type are typically "corrected intermolecularly" by protein complexation or protein-ligand association. However, in the oncogenic mutants, these binding signals are partially or completely suppressed: the mutated residues properly wrap or desolvate the hydrogen bonds intramolecularly. Thus, the interactivity of the proteins becomes impaired: their binding affinity decreases sharply, as there is no thermodynamic benefit from removing water surrounding properly desolvated hydrogen bonds. The results, specialized for p21ras and p53, reveal how oncogenic mutations determine a hindrance to GAP-induced hydrolysis (p21) and decrease binding affinity for DNA (p53). Furthermore, the oncogenic potential of mutations in residues not directly engaged in the interface electrostatics is assessed. The results suggest that a high sensitivity of structural defects to genetic accident might be a necessary condition to establish the existence of a proto-oncogene, an angle that merits a systematic study.

Introduction

Oncogenic mutations are typically highly specific and dramatically affect the interactivity of expressed proteins like p21ras (1-6) or p53 (7-10) which are involved in the signaling pathway for cell proliferation. In p21ras, the mutations induce an inhibition of GAP-mediated deactivation and in p53, they perturb the protein-DNA interaction. Here I report that the oncogenic mutations affect precisely the sites which in the wild type represent inherent structural defects, i. e., backbone hydrogen bonds which are under-protected from water attack (11-14). Such defects actually signal binding sites in the wild type, since there is an energetic advantage associated with the removal of surrounding water upon binding (14): a de-shielding of the effective charges (on the amide and carbonyl groups) enhances the hydrogen-bond electrostatics strengthening them. Since the insufficiently dehydrated hydrogen bonds may be stabilized upon binding, they are in effect signals of binding sites (14).

Backbone hydrogen bonds are determinants of protein structure and they prevail only when they are properly protected or from water attack (12). This is so because water, with its high dipole moment and orientational versatility is a competitor in forming hydrogen bonds with amides and carbonyls. The extent of intramolecular hydrogen-bond protection may be quantified, for instance, by determining the number of hydrophobic groups (CH_n, n=1,2,3) within a desolvation domain typically defined as two intersecting balls of radius 7(±0.4)Å centered at the α-carbons of the hydrogen bonded residues. Such desolvating groups "wrap" the hydrogen

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bond by immobilizing, or removing its surrounding water. Thus, the microenvironment surrounding a hydrogen bond may be assessed with a single hydrophobic wrapping parameter, ρ, indicating the number of desolvating groups.

In a stable protein fold, at least two thirds of the hydrogen bonds are on average wrapped by ρ=18.7±4.9 hydrophobic groups (or ρ=15±3.4 if we count only side-chain groups and exclude those in the hydrogen-bonded residue pair (14)). Under-desolvated hydrogen bonds, here termed *dehydrons*, are then defined as those in the tails of the distribution, i. e. with less than ρ=12 hydrophobic groups in their desolvation domains. Because there is a considerable thermodynamic advantage associated with the removal of water surrounding dehydrons, such bonds have been identified – together with the overexposed hydrophobic groups in the partner molecule- as determinants of protein binding sites (14).

As exemplified here, oncogenic aminoacid mutations in p21ras and p53 contribute to intramolecularly desolvate the hydrogen bonds which in the wild type have a poor hydrophobic wrapping. The net effect of this alteration is thus the impairing of the effective interactivity of the molecules with their hydrolyzing co-catalyst (in p21ras) or with DNA (in p53). Also, this analysis enables one to assess the oncogenic potential of mutations not directly engaged in the p53-DNA interface electrostatics.

The structural implications of oncogenic mutations are not clearly understood (5, 6, 15, 16). Thus, while we learn that Gly12Val or mutations on Gln61 impose a hindrance on the deactivation of GTP-bound p21ras by GAP-mediated hydrolysis of GTP, the mechanistic details of this impairment remain controversial (4-6, 15, 16).

In this contribution I report that such mutations have a direct effect on specific structural deficiencies found in the packing of the wild-type proteins, i. e., on the under-wrapped or under-desolvated backbone hydrogen bonds. These naturally designed structural deficiencies are typically "corrected" upon complexation or protein-ligand binding (14, 17-19). Thus, such packing deficiencies have been shown both statistically (14) and experimentally (18) to represent sites for protein association or complexation. This is so because the extent of desolvation of pre-formed backbone hydrogen bonds is enhanced intermolecularly as the partner molecule penetrates the desolvation domain of the hydrogen bonds and expels surrounding water. The complete dehydration of such bonds dramatically increases their stability, as the nonbonded state, with the polar amide and carbonyl hindered from proper solvation, becomes highly unstable (14, 18).

As shown here, in oncogenic mutants such packing deficiencies are no longer present: the mutated residues properly wrap or desolvate the hydrogen bonds intramolecularly. Thus, the interactivity of the mutant proteins is severely impaired, as there is no advantage in removing water from the now properly desolvated hydrogen bonds. The results are specialized for p21ras and p53, revealing how oncogenic mutations determine a hindrance to GAP-induced hydrolysis (in p21ras) and decrease binding affinity for DNA (in p53).

In essence, the oncogenic mutations studied in this work are investigated from a structural point of view, and are directly linked to structural defects which were probably unnoticed in previous work (14, 18). The mutations contribute to dehydrate intramolecularly the hydrogen bonds which in the wild type are under-dehydrated, and in so doing, they become deleterious of binding signals. This type of analysis might prove significant as a first step in attempting to identify proto-oncogenes on the basis of the sensitivity of structural deficiencies to genetic accident.

Methods

The extent of desolvation, ρ, of backbone (amide-carbonyl) hydrogen bonds by backbone or side-chain carbonaceous groups (CH_n, n=1,2,3) clustered around them

is easily quantifiable: We may define the desolvation domain of a hydrogen bond as consisting of two spheres of 7Å-radius centered at the α-carbons of the residues paired by the hydrogen bond. These spheres necessarily intersect since the typical minimum distances between non-adjacent α-carbons in secondary structure are in the range 4.7 - 6.1Å (20). The choice of radius is based on the typical cutoff distance used to define pairwise interactions, but the results (i. e., the identification of dehydrons) are robust within the range 7±0.4Å. A hydrogen bond is operationally defined as one satisfying the constraints: N-O (heavy-atom) distance less than 3.5Å, and 45 degrees range in the angle between the NH and CO vectors.

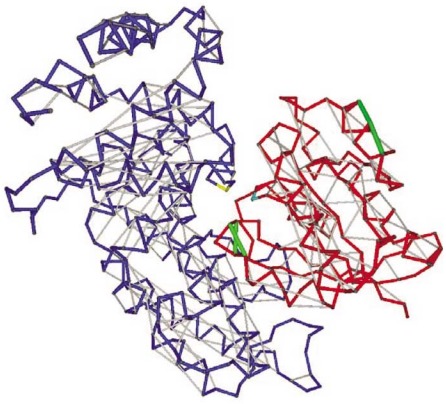
Thus, the extent of wrapping of hydrogen bonds is operationally defined by the number of side-chain carbonaceous groups within their desolvation domains. In the case of a complex, the desolvation shell of an intramolecular hydrogen bond may include carbonaceous groups from residues of the binding partner (if they happen to lie within the desolvation domain upon complexation).

Each residue having carbonaceous groups in the desolvation domain of a hydrogen bond may be regarded as a third body introducing a three-body correlation (hydrophobe-hydrogen-bonded pair). Thus, another cruder measure of the extent of desolvation of a particular hydrogen bond may be obtained by counting the number of three-body correlations in which it is engaged.

Results

The dehydron (ρ≤11) pattern in p21ras within its complex with ras-GAP (pdb.1ctq) is shown on Figure 1a, b: *Strikingly, two of the four dehydrons in the p21ras molecule actually define the p21ras-rasGAP binding site and are located at less than 5A from* the α-carbons *of residues known to undergo oncogenic mutations*. They are: Gln61-Tyr64 and Glu62-Ser65 (there are two other dehydrons, Arg128-Asp132 and Asp132-Ser136, on the opposite site of p21ras whose role in defining protein interactions is unknown to the author at this point).

1a



1b

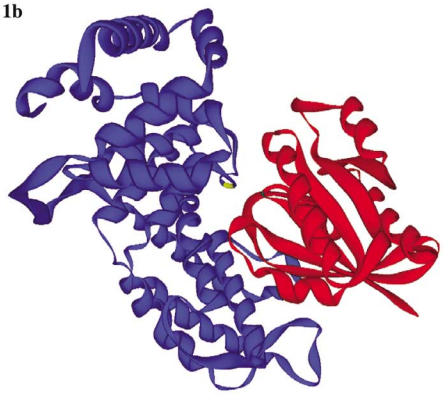
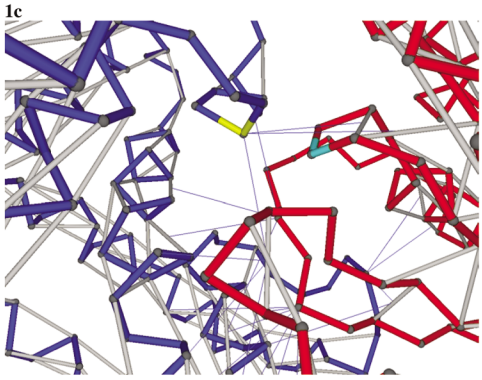


Figure 1a: Dehydron pattern of p21ras (red virtual-bond backbone) complexed with rasGAP (bue backbone). Dehydrons are represented as green segments joining α-carbons and properly desolvated backbone hydrogen bonds are displayed as grey segments. The backbone conformation is represented as a virtual-bond polygonal joining α-carbons. Notice that two of the four dehydrons (in green) on p21ras lie precisely at the binding interface and would be desolvated upon complexation, primarily by Arg789 (in yellow) from rasGAP. Residue Gly12 is indicated in light blue on the p21ras backbone.
Figure 1b: Ribbon representation of the p21ras-rasGAP complex.

1c



1d

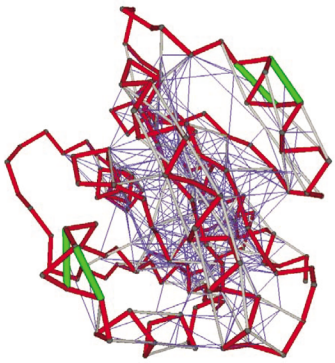
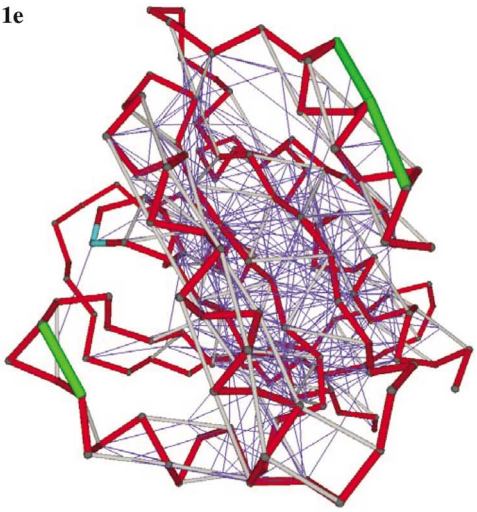


Figure 1c: Detail of the intermolecular desolvation of the two interface dehydrons on p21ras by the aliphatic groups of Arg789 upon complexation. A thin blue line joining an α-carbon of residue *i* with the mid-point of backbone hydrogen bond *(j,k)* represents a 3-body correlation, that is, residue *i* is contributing with its carbonaceous groups to the desolvation of the *(j, k)* hydrogen bond. In this detailed figure, only intermolecular 3-body correlations are represented, that is, *i* belongs to a binding partner and hydrogen bond *(j, k)*, to the other.
Figure 1d: Intramolecular 3-body correlations describing the internal desolvation of hydrogen bonds in the wild-type p21ras molecule.

The GAP residue Arg789 (indicated in yellow on Figure 1a, b) completes upon complexation the desolvation shell of the wild-type dehydron Gln61-Tyr64, which thus becomes properly desolvated upon complexation. The intermolecular 3-body correlations (***Methods***), whereby the carbonaceous groups of a residue in one binding partner enter the desolvation domain of a hydrogen bond of the other partner are marked as thin lines in Figure 1c, while GTP (not shown) remains bound due to the thermodynamic trade-off associated with removal of surrounding water from the dehydron Glu62-Ser65. *Thus, Arg789 is not only functionally relevant to GTP hydrolysis: Its role would be immaterial if it were not for the energetic and thermodynamic benefit it brings about by squeezing water out of the wild-type p21ras dehydron (61, 64)*. This stabilization is in itself, a major factor driving p21ras-rasGAP association (cf. (14)).

1e



The intramolecular desolvation of hydrogen bonds in wild-type p21ras is described in Figure 1d, represented as a pattern of thin-lined 3-body correlations (see Figure caption). The effect of the oncogenic mutation Gly12Val (light blue site in Figure 1e) is apparent once the *intramolecular* 3-body correlations are examined in the mutant p21ras: The hydrogen bond Gln61-Tyr64 now becomes properly wrapped intramolecularly ($\rho=11\rightarrow\rho=14$), since the methylene and two methyl groups of Val12 lie within the desolvation domain of this hydrogen bond. Thus, exogenous (intermolecular) water removal from this site becomes immaterial since it no longer introduces a significant thermodynamic or energetic advantage*: The oncogenic mutation has suppressed the hot spot for GAP-binding in p21ras and thus p21ras-rasGAP becomes now a low-affinity or loose complex*. Notice that the Gly12Val mutation still preserves the GTP-binding spot, i. e. the dehydron Glu62-Ser65.

On the other hand, the significant oncogenic mutations of Gln61 can have an even more deleterious effect on GAP-mediated hydrolysis, especially if this hydrolysis-functional residue is replaced by a thoroughly hydrophobic residue, or a residue with hydrophobic groups that, upon deletion of the hydrolytic mechanism, can remain attached to the

1f

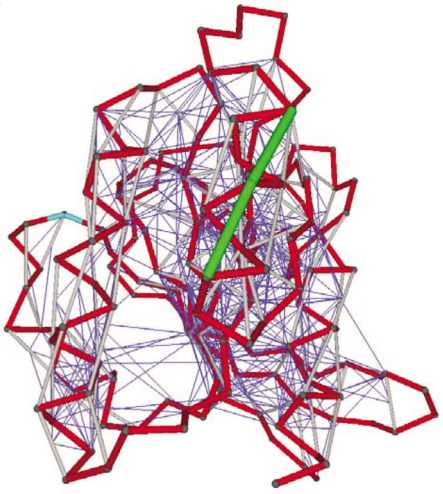
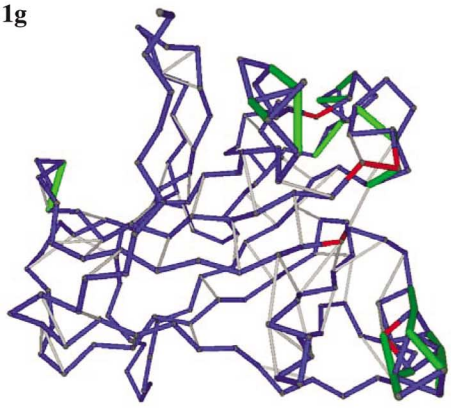


Figure 1e: Intramolecular 3-body correlations in the mutant Gly12Val of p21ras. Notice that Val12 desolvates the hydrogen bond *(61, 64)*, which is thus no longer a determinant for p21ras-rasGAP association.
Figure 1f: The hydrogen-bond internal desolvation pattern of p21ras mutated at Gln61.

1g



1h

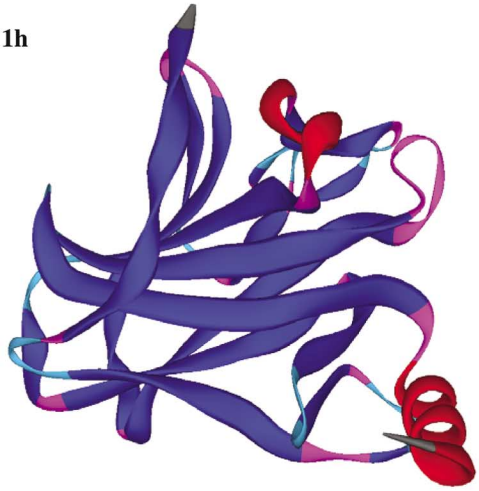


Figure 1g: Dehydron pattern in p53, with blue virtual-bond backbone. Notice that the positively charged Arginines (red) engaged in protein-DNA association, all contribute to the partial desolvation of dehydrons which in turn favor water removal at the protein-DNA interface.
Figure 1h: Ribbon representation of p53.

backbone, thus desolvating it. Thus, as shown in Figure 1f, mutations on this residue lead to a proper intramolecular desolvation ($\rho=11\rightarrow\rho=14$ or $\rho=11\rightarrow\rho=15$) of the adjacent backbone hydrogen bonds *(61, 64)* and *(62, 65)*, with concurrent suppression of the GAP-mediated hydrolysis of GTP, now loosely bound to p21ras.

The other two mutations with deleterious effect on the hydrolysis-related dehydrons involve residues Gly60, Ala66 and Arg68. As such residues are replaced by others, like Gln or Lys, with longer aliphatic chains, the relevant wild-type dehydrons would now get properly desolvated ($\rho=11\rightarrow\rho=13$, 14 or 15, depending on the substitution), thus their oncogenic potential resides in their ability to impair p21ras deactivation by hindering GAP-mediated hydrolysis of GTP.

The dehydron pattern in p53 (pdb.1kzy chain A) is shown in Figure 1g, while the ribbon representation of the molecule is shown in Figure 1h. Significantly, the positively charged residues Arg175, Arg248, Arg249, Arg273, Arg282 involved in protein-DNA interaction belong to the desolvation domains of spatially adjacent dehydrons or clusters of dehydrons. This warrants the effectiveness of protein-DNA recognition, since not only the Arginine charges are matched with the phosphate charges, but the removal of water surrounding the electrostatic interactions -and thereby enhancing them- is thermodynamically and energetically prompted by the presence of vicinal dehydrons in p53. This synergy between a favored water removal and the electrostatics is revealed by the coincident distribution on the protein surface of dehydrons and positively charged groups.

Water removal is favored precisely due to the proximity of dehydrons to the DNA-binding Arginines (Fig. 1g). Thus, Arg175 is engaged in the desolvation of dehydrons His168-Glu171 and Cys176-Glu180; Arg248 and Arg273 are engaged in desolvation of Ser240-Arg248; Arg248 is also in the desolvation domain of Cys242-Asn247; Arg273 contributes to the desolvation of Cys277-Asp281; and Arg282 desolvates the dehydrons Gly279-Arg283, Asp281-Glu285 and Arg283-Glu286.

Besides the perturbation of protein-DNA interactions brought about by the obvious mutations of the four Arginines, there are other predicted mutations with oncogenic potential which should suppress the interface p53 dehydrons, thus leading to severe screening of the protein-DNA electrostatics. For instance, the mutation of Gly245 to a residue with hydrophobic groups (Gln) has a desolvating effect on the former dehydron Cys242-Gly245, as well as on Ser240-Arg248, both of whom get above the $\rho=11$ threshold upon mutation. Thus, such a mutation should be oncogenic since it disfavors water removal at the protein-DNA interface. Other residues whose mutation is expected to be oncogenic for the same reasons are: His179, Ser240, Gly279, Arg283. To a lesser extent water removal at the interface would be disfavored by mutating Thr284 or Glu285 because of the presence of a cluster of eight adjacent dehydrons in that region (Fig. 1g).

Conclusions

The two cases analyzed, p21ras and p53, reveal that oncogenic mutations occur precisely at sites in close proximity to packing defects in wild-type protein structure. Such defects, here termed dehydrons, are naturally designed to foster the interactivity of the wild-type protein. Thus, the oncogenic nature of mutations arises due to their deleterious effect on such dehydrons: As dehydrons are removed, the protein loses its binding affinity.

As suggested by the results presented in this work, a high sensitivity of structure wrapping to genetic accident might be a necessary condition to determine the existence of a proto-oncogene. In this work only point mutations have been considered, but more complex cases such as deletions or insertion of mobile genotypic regions which preserve structural motifs should and will be studied. Of course, a

wrapping hypersensitivity cannot be a sufficient diagnosing factor, functional considerations are needed to rule out adventitious effects of mutations.

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