Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 3596–3600, June 1985
Biochemistry

Structural effects of substitutions on the p21 proteins

(conformational energy/point mutations/transformation)

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Communicated by H. A. Scheraga, February 4, 1985

ABSTRACT **The conformational effects of different amino acid substitutions (lysine, serine, proline, and D-valine) for glycine at position 12 in the p21 oncogene-encoded proteins have been investigated by using conformational energy calculations. The normal cellular gene codes for a glycine at position 12 in the amino acid sequence, in the middle of a hydrophobic p21-(6–15)-decapepetide from Leu-6 to Gly-15. Mutations that cause amino acid substitutions for Gly-12 result in a protein product that produces malignant transformation of cells. We now find that not only are the preferred structures for the lysine- and serine-containing peptides more restricted and more helical than those for the glycine-containing peptide, but the lowest-energy structure for each substituted peptide is exactly the same as that previously found for the peptide with Val-12, suggesting the existence of a "malignancy-causing" conformation. None of the preferred conformations for the valine-, lysine-, and serine-containing peptides contain chain reversals at positions 11 and 12. However, we find that proline, unlike these residues but like glycine, at position 12 causes helix termination at positions 11 and 12, a result that suggests that the p21 protein product with proline at position 12 may exhibit lowered transforming potential, in agreement with the results of recent genetic recombination experiments.**

Human oncogenes in the *ras* family are homologous to a normal cellular protooncogene that codes for a normal protein product of M_r 21,000 (p21 protein), and several of them such as the EJ bladder carcinoma oncogene (1, 2) differ from it in only the 12th coding triplet, which in the protooncogene is GGC, the codon for glycine (1, 2). Amino acid substitutions at position 12 in the p21 protein encoded by human *ras* oncogenes are known to result in abnormal cellular control and ultimately to cause malignant transformation of cells (1–3).

The sequence of the first 20 amino acids (1, 2) of the p21 protein encoded by the protooncogene is:

$$\overset{1}{\text{Met}}\text{-Thr-Glu-Tyr-Lys-Leu-Val-Val-Val-}\overset{10}{\text{Gly}}\text{-Ala-Gly-Gly-}$$
$$\text{Val-Gly-Lys-Ser-Ala-Leu-}\overset{20}{\text{Thr}}.$$

Malignant transformation of cells occurs when the glycine at position 12 is replaced by a variety of amino acids (1, 2, 4, 5). This Gly-12 occurs in the middle of a long sequence (the transforming region) of 10 hydrophobic or nonpolar amino acid residues, the only such sequence in the p21 proteins.

We have shown previously that glycine at position 12 shows a marked preference for the D* (conformations are explained in the legend to Table 1) conformation that is inaccessible to all naturally occurring L-amino acids (6–8). This result suggested to us that substitution of any other amino acid for glycine at position 12 would result in a mandatory change in chain conformation in the region of residue 12 that may result in malignant transformation (6–8). We also found that, for the "normal" p21 protein, a slightly higher energy conformation existed that was identical to the lowest energy conformer for the transforming p21 protein with valine at position 12 in which the chain reversal (CD*) conformation occurred at residues Val–Gly at positions 12 and 13. These results further suggested that if the "normal" p21 protein were to be present at elevated intracellular levels, where the alternate (higher energy) conformation would exist in significant concentration, malignant transformation likewise would take place. The results of experiments in which malignant transformation of NIH 3T3 cells transfected with the protooncogene spliced to a viral long terminal repeat occurred (3) are fully compatible with these computed results (6–8).

In this paper we examine the structural effects of substituting other amino acids besides valine at position 12, specifically lysine, serine, proline, and the D-form of valine. We compare our results with those obtained for proteins containing glycine and valine at this position (6–8) and with experimental results on the ability of each protein with a different amino acid at position 12 (1, 2, 4, 5) to transform cells.

METHODS

The general methods used (9, 10) are based on the program ECEPP (empirical conformational energy for polypeptides and proteins) (11) and have been used successfully to compute the allowed conformations of the naturally occurring amino acids (12), short oligopeptides (13), and long, constrained oligopeptides (14, 15). These methods have been extended to computing the preferred conformations for long, unconstrained polypeptides (6, 16, 17). The methods used to compute the preferred conformations of the hydrophobic p21-(6–15)-decapeptide (from *N*-acetyl-Leu-6 to Gly-15-$NHCH_3$) using ECEPP (11) have been described (6). The allowed conformations for the initial terminally blocked p21-(6–11)-hexapeptide (*N*-acetyl-Leu-Val_3-Gly-Ala-$NHCH_3$) have been determined (6). Each of these allowed conformations was then combined with all of the single-residue minimal-energy conformations (12) for the amino acid residue at position 12, and the energy of each of these combined conformations was minimized as described previously (6). In the case of the single-residue conformational-energy minima for lysine, we selected for each unique backbone conformation the three allowed rotational isomeric states for the first side-chain torsion angle, X_1, as described (16). In all, 25 conformations for lysine were used in the chain build-up procedure by this method. These conformations were sorted by energy, and those whose energies lay within a cutoff value of the energy of the "global minimum" (the

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Table 1. Low-energy conformations for the hydrophobic p21-(6–15)-decapeptides in the transforming proteins *N*-acetyl-Leu-Val_3-Gly-Ala-Lys-Gly-Val-Gly-$NHCH_3$ and *N*-acetyl-Leu-Val_3-Gly-Ala-Val-Gly-Val-Gly-$NHCH_3$ (in parentheses)†

	Conformational state										
Conformer	Leu (Leu	Val Val	Val Val	Val Val	Gly Gly	Ala Ala	Lys Val	Gly Gly	Val Val	Gly Gly)	Energy, kcal/mol
1	A	A	A	A	A	A	C	D*	A	A	0.0
	(A	A	A	A	A	A	C	D*	A	A	0.0)
2	A	A	A	A	A	A	A	A	A	A	0.0
	(A	A	A	A	A	A	C	D*	A	A*	1.0)
3	A	A	A	A	A	A	A	A	A	G*	0.1
	(A	A	A	A	A	A	C	D*	A	D*	1.9)
4	A	A	A	A	A	A	A	A	C	D*	0.7
	(A	A	A	C	D*	A	A	A	A	A	2.0)
5	A	A	A	A	A	A	C	D*	A	A*	0.7
6	A	A	A	C	D*	A	A	A	A	A	0.7
7	A	A	A	A	A	A	A	A	A	C	0.7
8	A	A	A	A	A	A	A	A	A	A*	0.8
9	A	A	A	A	A	A	D	D*	A	A	1.1
10	A	A	A	A	A	A	C	D*	A	G*	1.5
11	A	A	A	A	A	A	A	A	C	C	1.6
12	A	A	A	C	D*	A	C	D*	A	A	1.8
13	A	A	A	C	D*	A	A	A	A	G*	2.0
14	A	A	A	A	A	A	D	D*	A	A*	2.8
15	A	A	A	A	A	A	D	D*	A	G*	2.8
16	A	A	A	C	A	A	A	A	A	A	2.8
17	A	A	A	C	A	A	A	A	C	D*	2.9

Low-energy conformations are defined as those whose energies lie within 3 kcal/mol of that of the global minimum (conformer 1). Conformational states are defined as follows: States in which the dihedral angles are represented by a single-letter code are defined in ref. 11. The familiar states are A (α helix) and E (extended). States E, E*, F, F*, D, and D* are collectively called "β" conformations. The actual dihedral angle ranges for all single-letter states are: A, -110 degrees $\leq \phi < -40$ degrees, -90 degrees $\leq \psi < -10$ degrees; C, -110 degrees $\leq \phi < -40$ degrees, 50 degrees $\leq \psi < 130$ degrees; D, -180 degrees $\leq \phi < -110$ degrees, 20 degrees $\leq \psi < 110$ degrees; E, -110 degrees $\leq \phi < -40$ degrees, -180 degrees $\leq \psi < -140$ degrees or 130 degrees $\leq \psi < 180$ degrees; F, -110 degrees $\leq \phi < -40$ degrees, -180 degrees $\leq \psi < -140$ degrees or 130 degrees $\leq \psi < 180$ degrees; G, -180 degrees $\leq \phi < -110$ degrees, -90 degrees $\leq \psi < -40$ degrees. States indicated by asterisks are obtained by multiplying the corresponding single-letter state by -1 and reversing the inequalities. All energies are relative to the energy of conformer 1, called the global minimum.

†The previously calculated (6) low-energy conformers for the Val-12-containing peptide are presented in parentheses below the conformers for the Lys-12-containing peptide for the sake of comparison.

lowest energy conformation; energy of conformer 1 in Tables 1–4) were used in the ensuing chain build-up procedure (6). In this procedure, each remaining amino acid was added one by one to the growing chain in all of its single-residue conformational-energy minima, the energy was minimized, and the above process was repeated. The amino acids considered at position 12 were: lysine, serine, proline in the *cis* and *trans* forms, and D-valine. The cutoffs used for the peptides containing each amino acid were 3 kcal/mol, with the exception of the serine-containing peptide, for which cutoffs of 8 kcal/mol were used.

RESULTS AND DISCUSSION

Substitutions of Lysine and Serine for Glycine at Position 12. The conformational effects from substitution of different amino acids at position 12 are shown in Tables 1–4. In Table 1, in which lysine occurs at position 12, our previous results with valine at this position (6) are included for comparison. In Tables 1 and 2 (lysine and serine at position 12, respectively), it may at once be noted that the global minima (lowest-energy conformations) for the valine-, lsyine-, and serine-containing peptides are identical (compare conformers

Table 2. Low-energy conformations for the hydrophobic p21-(6–15)-decapeptide in the transforming protein *N*-acetyl-Leu-Val_3-Gly-Ala-Ser-Gly-Val-Gly-$NHCH_3$

	Conformational state										
Conformer	Leu	Val	Val	Val	Gly	Ala	Ser	Gly	Val	Gly	Energy, kcal/mol
1	A	A	A	A	A	A	C	D*	A	A	0.0
2	A	A	A	A	A	A	A	A	C	A*	1.5
3	A	A	A	A	A	A	A	A	A	A*	1.9
4	A	A	A	A	A	A	A	A	A	C*	2.0
5	A	A	A	A	A	A	A	A	A	D*	2.1
6	A	A	A	A	A	A	C	D*	A	C	2.3
7	A	A	A	A	A	A	A	A	D	D*	2.4
8	A	A	A	A	A	A	D	D*	A	A*	2.6
9	A	A	A	A	A	A	A	A	D	A*	2.7
10	A	A	A	A	A	A	A	A	A	G*	2.7
11	A	A	A	A	A	A	A	A	A	C	2.9
12	A	A	A	A	A	A	A	A	C	C	2.9

See Table 1 for details.

Table 3. Low-energy conformations for the hydrophobic p21-(6–15)-decapeptide in the nontransforming protein *N*-acetyl-Leu-Val_3-Gly-Ala-Pro-Gly-Val-Gly-$NHCH_3$

Conformer	Conformational state										Energy, kcal/mol
	Leu	Val	Val	Val	Gly	Ala	Pro	Gly	Val	Gly	
1	A	A	A	A	A	D	A	A	A	C	0.0
2	A	A	A	A	A	D	A	A	A	C*	0.2
3	A	A	A	A	A	D	C	D*	A	A	0.3
4	A	A	A	A	A	D	C	D*	A	C*	0.8
5	A	A	A	A	A	D	F	C*	A	A	0.9
6	A	A	A	A	A	D	A	A	A	A	1.0
7	A	A	A	A	A	D	C	D*	A	C	1.2
8	A	A	A	A	A	D	F	C*	A	C	1.3
9	A	A	A	A	A	D	A	A	A	D	1.7
10	A	A	A	A	A	D	C	D*	C	A*	2.0
11	A	A	A	A	A	D	A	A	A	A*	2.2
12	A	A	A	A	A	D	F	C*	C	D*	2.3
13	A	A	A	A	A	D	C	D*	A	D	2.4
14	A	A	A	D	D*	D	F	C*	A	A*	2.5
15	A	A	A	D	D*	D	C	D*	C	A	2.6
16	A	A	A	D	D*	D	F	C*	D	C	2.6
17	A	A	A	D	D*	D	C	D*	C	A*	2.6
18	A	A	A	D	D*	D	C	D*	C	C	2.6
19	A	A	A	A	A	D	C	D*	C	D*	2.7
20	A	A	A	D	D*	D	A	A	C	D*	2.9
21	A	A	A	D	D*	D	F	C*	A	C*	2.9

See Table 1 for details.

1 in Tables 1 and 2). In the case of lysine, another lowest-energy minimum was obtained (conformer 2 in Table 1), which was all α-helix. In each of these cases, it may be noted that no conformation exists in which the helix is broken at residues 11 and 12 as in the glycine-containing peptide, as we previously reported (6–8), although several conformations (such as conformer 4 for valine in Table 1) have their helices terminating before Ala-11. In the case of the lysine- and serine-containing peptides, the α-helical conformation for residues 6–11 is strongly preferred. In fact, a number of conformations exist in which the α-helix is preferred for virtually the whole peptide (such as conformer 2 in Table 1 and conformer 3 in Table 2). In the cases of the lysine- and serine-containing peptides, therefore, the α-helical conformation predominates at least in residues 6–11, as also was found for the valine-containing peptide (6–8) but not for the glycine-containing one (6–8). The global minimal-energy structure for the Lys-12 peptide (conformer 1 of Table 1) is shown in stereo view in Fig. 1. In the case of the glycine-containing peptide, a variety of quite different conformations for residues 6–11 (and in fact for the whole peptide) can exist (6–8). Secondary structure prediction analyses suggest higher α-helix probabilities for valine- and aspartic acid-containing peptides (refs. 2 and 4, respectively) than for the glycine-containing one. Further, the bend-forming tendency was lower at residues 11 and 12 in the valine (2)- and aspartic acid (4)-containing peptides than for the Gly-containing sequence, which in our results shows a high propensity to adopt the CD* conformation at residues 11 and 12.

Thus, three completely different amino acids, valine, lysine, and serine, all promote the same lowest-energy conformation (Tables 1 and 2), in which the chain reversal occurs at residues 12 and 13, while glycine at position 12 causes a chain reversal to occur at residues 11 and 12. These results strongly suggest that, in addition to a "normal" protein conformation, a "malignancy-promoting" conformation (conformer 1 in Tables 1 and 2) may likewise exist. The crucial feature that distinguishes these two basic structures is the existence of a helix-terminating chain reversal at positions 11 and 12, with residue 12 in a left-handed twist (D*) conformation in the normal protein. The question naturally arises as to whether there exists another amino acid at position 12 that might promote a disruption of the α helix at residues 11 and 12.

Substitution of Proline for Glycine at Position 12. We have shown in other studies on the preferred conformations of membrane proteins (16, 17) that proline is very likely to disrupt α helices and other types of regular structures

Table 4. Low-energy conformations for the hydrophobic p21-(6–15)-decapeptide in the protein *N*-acetyl-Leu-Val_3-Gly-Ala-D-Val-Gly-Val-Gly-$NHCH_3$

Conformer	Conformational state										Energy, kcal/mol
	Leu	Val	Val	Val	Gly	Ala	D-Val	Gly	Val	Gly	
1	A	A	A	A	A	A	A	A	C	D*	0.0
2	A	A	A	A	A	A	A	A	A	A	0.9
3	A	A	A	A	A	A	A	A	A	C	1.5
4	A	A	A	D	D*	C	D*	A*	C	D*	1.6
5	A	A	A	D	D*	C	D*	A*	C	A*	1.6
6	A	A	A	C	D*	A	A*	C*	A	G*	2.1
7	A	A	A	A	A	A	A	A	A	D	2.3
8	A	A	A	C	D*	C	D*	A	A	D*	2.9
9	A	A	A	D	D*	A	A*	A	A	C*	3.0

See Table 1 for details.

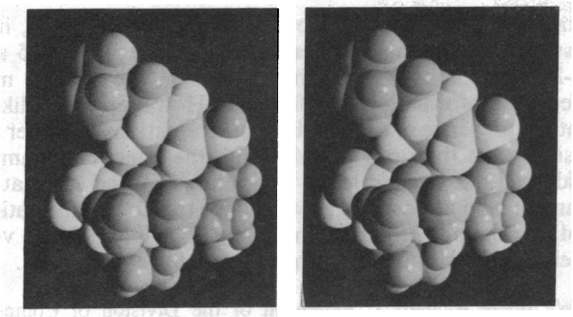

FIG. 1. Space-filling stereo view of the lowest-energy conformation for Lys-12-containing peptide (conformer 1 in Table 1) identical to that for the Val-12-containing peptide (conformer 1 in parentheses of Table 1 and ref. 6). The amino terminus is on the lower left, and the turn occurs in the right middle, where the side chain of residue 12 protrudes.

because the amino acid residue preceding proline makes unfavorable backbone–backbone contacts with proline if it adopts the A (α) state (see the legend to Table 1). Thus, if proline were to occur at position 12, the Ala-11 preceding Pro-12 would be forced to adopt a nonhelical conformation. Therefore, we computed the allowed conformations for the hydrophobic decapeptide with proline at position 12. The results of these calculations are shown in Table 3. It may be noted at once that a number of different conformations occur in which four or more residues are non-α-helical (such as conformer 4) and in some of which, like conformer 14, five consecutive residues are in conformations from the "β" region (see the legend to Table 1). This same result was also found only for the glycine-containing peptide (6). In the case of the lysine-containing peptide (Table 1 and Fig. 1), a number of conformations occur in which certain residues adopt non-α-helical conformations in the middle of the chain (such as conformers 7 and 12). However, no structures with long consecutive segments in non-α-helical conformations (such as conformers 4 and 14 in Table 3) were obtained for this (Lys-12-containing) peptide. Conformers with *cis*-proline were of energies that lay beyond the cutoff value by >10 kcal/mol and, thus, are not listed in the table. As also may be seen in this table, no conformers in which Ala-11 adopts the A conformation are present, as expected. In the two lowest-energy conformers (conformers 1 and 2 in Table 3), the α-helix extends from residue 6 to residue 10 as in the glycine-containing peptide (6–8), and Ala-11 breaks the helix. Fig. 2 is a stereo view of conformer 1 of Table 3. As may be seen from a comparison of Figs. 1 and 2, the presence of proline at position 12 causes a major change in the structure

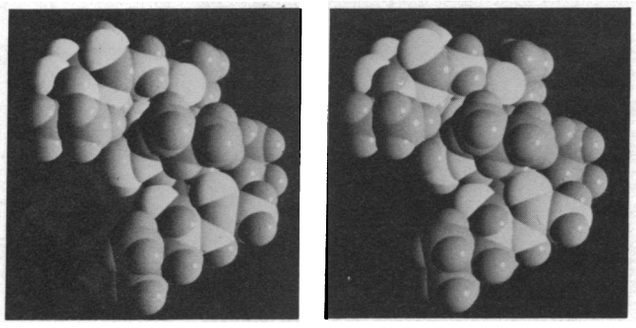

FIG. 2. Space-filling stereo view of the lowest-energy conformation for the Pro-12-containing peptide (conformer 1 in Table 3). The amino terminus is on the lower left. The structure is less compact than that in Fig. 1.

of the peptide, the structure of Fig. 1 being somewhat more compact than the structure of Fig. 2.

While the chain reversal occurs in the same region in the glycine- and proline-containing peptides (i.e., at residues 11 and 12), the conformations still differ somewhat in that the chain "twists" differ in this region because proline cannot adopt the D* conformation. The fact that proline at position 12 disrupts the helix in the same region as glycine and that proline-containing peptides cannot adopt the lowest-energy conformation of the valine-, lysine-, and serine-containing peptides suggests that substitution of proline for glycine at this position may result in a protein with a markedly diminished transforming ability (7, 8). It may still have some residual transforming ability since the conformations of proline- and glycine-containing peptides are not precisely the same.

Recently, in a series of experiments involving site-specific mutagenesis (5), the codons for each of the 20 naturally occurring amino acids except glycine were substituted for that of glycine in the 12th triplet in the gene coding for the p21 protein. Each of these genes was found to cause transformation except the one coding for Pro-12. The latter was found to be essentially inactive in transformation activity (5). These results coincide closely with our results from conformational energy analysis showing that only glycine and proline at position 12 cause a chain reversal at positions 11 and 12. The experimental results also indicate that the effects of substitutions of amino acids for glycine at position 12 most likely result from conformational effects as opposed to unfavorable steric effects on the binding of the p21 protein to ligands like GTP (18). The latter hypothesis assumes that any amino acid other than glycine will have a side chain that must interact unfavorably with the ligand so that control over enzymatic activity is lost (18). However, proline also has a side chain, the pyrrolidine ring, and likewise should interact unfavorably with the ligand. Yet this substitution significantly reduces oncogenic activity. Moreover, recent results on the binding of GTP to p21 proteins containing glycine and valine at position 12 show that both proteins have the same affinities for this ligand, although the oncogenic proteins shows markedly diminished GTPase activity (19).

Relationship of Conformation to Transforming Ability. In a previous paper, on the basis of our calculations on the hydrophobic decapeptide of the glycine- and valine-containing proteins (6), we proposed a model in which the normal protein bound to a cell element, *E*. Excess amounts of the normal protein would saturate *E* and would be free to cause cell transformation. The abnormal proteins would be unable to bind *E* and be free at low concentrations to cause transformation. Our current results are consistent with this model. Most amino acids, like valine, lysine, and serine, cause the chain to adopt incorrect conformations (no chain reversals at positions 11 and 12) causing a lack of interaction with *E*. The proline-containing peptide, however, can adopt a similar, though not identical, conformation to that of the glycine-containing peptide and, thus, bind *E* though with lower affinity. The fact that the proline-containing peptide can bind *E* lowers its transforming ability.

Substitution of D-Valine for Glycine at Position 12. Because proline at position 12 causes a chain reversal to occur at positions 11 and 12 but cannot adopt the glycine-like conformation (D*) at position 12, we investigated the conformational effect of a D-amino acid that can adopt the D* conformation and that might disrupt the helix at positions 11 and 12. We chose D-valine as the D-amino acid to compare with our earlier results with L-valine (6–8). As may be seen in Table 4, a number of conformers (such as 4 and 5) exist in which the CD* conformation occurs at Ala-D-Val at positions 11 and 12. However, these conformations are not the same as the original glycine minimal-energy conformations (6–8) and

are at least 1.5 kcal/mol higher in energy than the energy for the global minimal-energy conformation (conformer 1 in Table 4), which is almost completely an α helix. These results demonstrate the strong helix-propagating effects of the preceding sequence on the conformation of the D-amino acid at position 12. As may be seen in Table 4, no conformations exist in which an α helix terminates in a CD* conformation at positions 12 and 13, the putative "malignancy-causing" conformation (conformers 1 of Tables 1 and 2). This finding suggests that such a substitution of a D-amino acid at position 12 may lower the ability of the protein to transform cells.

CONCLUSIONS

We have computed the preferred conformations for the hydrophobic p21-(6–15)-decapeptide with six different amino acids at position 12. It is clear that substitution of any L-amino acid for glycine at position 12 causes major structural changes in the peptide. Three of these L-amino acids with noncyclic side chains (valine, lysine, and serine) all seem to promote mainly α-helical structures. The lowest-energy structure for each of these peptides is identical and contains a turn at residues 12 and 13 and may be the one associated with the transforming activity and/or reduced GTPase activity. The substitution of proline for glycine at position 12 also causes structural changes (relative to the lowest-energy structure of the glycine-containing peptide) but preserves the feature that the helix is broken at residues 11 and 12, a structural feature that is unique to the glycine- and proline-containing peptides. These peptides (Gly-12- and Pro-12-containing) also share the further unique property that many of their allowed conformations contain relatively long consecutive non-α-helical segments. This finding does not apply to the allowed conformations of the corresponding peptides with L-amino acids (other than proline) at position 12. Either or both of these conformational features unique to the Gly-12- and Pro-12-containing peptides may explain their low transforming activity. At higher concentrations of the Gly-12 containing protein, the putative "malignancy-causing" conformation may become expressed (6–8). This conformation is not available to the Pro-12-containing peptide unless a substantial energy barrier (8–10 kcal/mol) is overcome. Thus, it is possible that elevated intracellular levels of the Pro-12-containing p21 protein may still not cause cell transformation with high efficiency or may produce transformation only at very elevated protein levels, in excess over that found necessary for the Gly-12-containing protein.

Our conclusions on the structure–activity correlations for the p21 proteins have been based on our structural calculations for the isolated hydrophobic decapeptide. While it appears that this segment contains strong structural determinants both from our calculations and from extensive homology with other nucleotide-binding proteins (18), it is possible that flanking sequences (such as residues 1–5 and 15–24) and/or long-range interactions in the protein may affect its conformational preferences. However, it is unlikely that the presence of such additional interactions will alter the basic results that: p21 proteins with the noncyclic L-amino acids will not contain turns at positions 11 and 12, that no L-amino acid at position 12 will adopt the D* conformation, and that the p21 protein with proline at position 12 will very likely cause termination of helical structures at Ala-11.

We thank Richard J. Feldmann of the Division of Computer Research and Technology of the National Institutes of Health for the preparation of Figs. 1 and 2.

1. Tabin, C. J., Bradley, S. M., Bargman, C. I., Weinberg, R. A., Papageorge, A. G., Scolnick, E. M., Dhar, R., Lowy, D. R. & Chang, E. H. (1982) *Nature (London)* **300**, 143–149.
2. Reddy, E. P., Reynolds, R. K., Santos, E. & Barbacid, M. (1982) *Nature (London)* **300**, 149–152.
3. Chang, E. H., Furth, M. A., Scolnick, E. M. & Lowy, D. R. (1982) *Nature (London)* **297**, 479–483.
4. Santos, E., Reddy, E. P., Pulciani, S., Feldman, R. J. & Barbacid, M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4679–4683.
5. Seeburg, P. H., Colby, W. W., Capon, D. J., Goeddel, D. V. & Levinson, A. D. (1984) *Nature (London)* **312**, 71–75.
6. Pincus, M. R., van Renswoude, J., Harford, J. B., Chang, E. H., Carty, R. P. & Klausner, R. D. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 5253–5257.
7. Pincus, M. R. & Brandt-Rauf, P. (1984) *Eighteenth Middle Atlantic Regional Meeting of the American Chemical Society* (Am. Chem. Soc., Washington, DC), p. 40.
8. Pincus, M. R. (1985) in *The Molecular Basis of Cancer: An Interdisciplinary Discussion on Basic and Applied Aspects of Cancer*, ed. Rein, R. (Liss, New York), in press.
9. Nemethy, G. & Scheraga, H. A. (1977) *Q. Rev. Biophys.* **10**, 239–352.
10. Scheraga, H. A. (1984) *Carlsberg Res. Commun.* **49**, 1–55.
11. Momany, F. A., McGuire, R. F., Burgess, A. W. & Scheraga, H. A. (1975) *J. Phys. Chem.* **79**, 2361–2381.
12. Zimmerman, S. S., Pottle, M. S., Nemethy, G. & Scheraga, H. A. (1977) *Macromolecules* **10**, 1–9.
13. Simon, I., Nemethy, G. & Scheraga, H. A. (1980) *Macromolecules* **11**, 797–804.
14. Dygert, M., Go, N. & Scheraga, H. A. (1975) *Macromolecules* **8**, 750–761.
15. Miller, M. H. & Scheraga, H. A. (1976) *J. Polym. Sci., Polym. Symp.* **54**, 171–200.
16. Pincus, M. R., Klausner, R. D. & Scheraga, H. A. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5107–5110.
17. Pincus, M. R. & Klausner, R. D. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3413–3417.
18. Wierenga, R. K. & Hol, W. G. J. (1983) *Nature (London)* **302**, 842–844.
19. McGrath, J. P., Capon, D. J., Goeddel, D. V. & Levinson, A. D. (1984) *Nature (London)* **310**, 644–649.