Refined crystal structure of the triphosphate conformation of H-ras p21 at 1.35 Å resolution: implications for the mechanism of GTP hydrolysis

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The crystal structure of the H-ras oncogene protein p21 complexed to the slowly hydrolysing GTP analogue GppNp has been determined at 1.35 Å resolution. 211 water molecules have been built into the electron density. The structure has been refined to a final R-factor of 19.8% for all data between 6 Å and 1.35 Å. The binding sites of the nucleotide and the magnesium ion are revealed in high detail. For the stretch of amino acid residues 61–65, the temperature factors of backbone atoms are four times the average value of 16.1 Å² due to the multiple conformations. In one of these conformations, the side chain of Gln61 makes contact with a water molecule, which is perfectly placed to be the nucleophile attacking the γ-phosphate of GTP. Based on this observation, we propose a mechanism for GTP hydrolysis involving mainly Gln61 and Glu63 as activating species for in-line attack of water. Nucleophilic displacement is facilitated by hydrogen bonds from residues Thr35, Gly60 and Lys16. A mechanism for rate enhancement by GAP is also proposed.

Key words: crystal structure/GTP hydrolysis/H-ras/oncogenes/p21

Introduction

Guanine nucleotide binding proteins are believed to be involved in many cellular processes such as signal transduction, protein transport and secretion, and polypeptide chain elongation. They all act as molecular switches. In the ON state or active state they are complexed to guanosine triphosphate (GTP), in the OFF or inactive state to guanosine diphosphate (GDP). In the GTP bound conformation they interact with an effector molecule, and the lifetime of this interaction is regulated by either the intrinsic or GAP mediated GTP hydrolysis.

EF-Tu and p21 has shown that the topological order of secondary structure elements is preserved between these two proteins (Jurnak, 1985; LaCour et al., 1985; deVos et al., 1988; Pai et al., 1989; Tong et al., 1989). In view of the high homology of the G-binding domain between most of the guanine nucleotide binding proteins (Halliday, 1983; McCormick et al., 1985; Dever et al., 1987), it is likely that the tertiary structures of these domains are similar in all such proteins and that the conformational transition between the GTP bound and the GDP bound states during GTP hydrolysis may involve the same structural elements.

As part of the effort directed towards an understanding of this process we have recently determined the three-dimensional structure of a truncated but biochemically fully active form of p21 (residues 1–166) in a complex with the slowly hydrolysing GTP analogue, GppNp (Pai et al., 1989). The structure has now been refined at 1.35 Å resolution. Details of the structure observable at this resolution suggest a mechanism for the hydrolysis of GTP.

Results

Crystallography

The model presented here consists of amino acid residues 1–166 of the p21 protein of the H-ras oncogene. It includes the GTP analogue GppNp and 211 water molecules. The present crystallographic R-factor for 26 806 reflections (78% of the theoretical number of unique reflections between 6 Å and 1.35 Å) is 19.6% (Figure 1). When only reflections over 1σ, 2σ or 3σ are accepted, the corresponding numbers are 19.2%, 18.1% and 17.4%, respectively. The r.m.s. deviations from standard geometry are 0.014 Å for bond lengths and 2.7° for bond angles. The accuracy of atomic positions judged according to Luzzati (1952) is 0.2 Å (Figure 1).

Overall structure

Figure 2 shows a sketch of the main chain conformation as analysed by the program DSSP (Kabsch and Sander, 1983). The structure consists of a central β-sheet and five α-helices. The β-sheet contains six strands, five of which are antiparallel and one of which is parallel. Of the five helices, α1 (residues 16–25) and α4 (residues 127–137) are completely regular α-helices with the corresponding i → i+4 hydrogen bonds. Helix α2 (residues 65–74) and α5 (residues...
152-164) each have a short piece of \(3_{10}\)-helix, \(\alpha_2\) at the N-terminus and \(\alpha_5\) at the C-terminus. In \(\alpha_3\) (residues 87-103) the helix is interrupted at amino acid 92, residue 89 has no hydrogen bond and residue 93 has an \(i-i+3\) and an \(i-i+4\) hydrogen bond.

Figure 3 shows the Ramachandran diagram for the refined structure. There are only a few non-glycine residues which have unusual conformations, but these are well defined by electron density, especially Arg149 but also Lys117, which makes hydrophobic interactions with the guanine base. It should also be mentioned that Gly13 is outside the region allowed even for glycines, whereas the phi and psi angles for Gly12 are perfectly normal. Thus it is not surprising that Gly12 can be mutated to Val or Arg without destroying the geometry of the phosphate binding loop (Krengel, U., Pai, E. F. and Wittinghofer, A., submitted). Gly10 and Gly15 are in conformations which are not allowed for other amino acids. This is perhaps the reason why these glycines are completely conserved in the GXXXXGKS/T motif in nucleotide binding proteins and why the structure of the loop is also conserved between these proteins. The value for Ser65 is most probably an artefact. This amino acid lies at the point where the region of multiple conformations changes to a well defined structure, probably taking up errors accumulated in residues 61-64.

**Mobility**

Figure 4 shows the temperature factors for backbone atoms of the refined structure as a function of residue number. For
most residues the B-factors vary between 10 Å² and 30 Å² throughout the protein, the average being 16.1 Å². We do not observe, as described for the structure of the p21–GDP complex (deVos et al., 1988), that the C-terminal half of the molecule generally has lower temperature factors. Residues 7–17, comprising the end of strand β1, the phosphate binding loop, and the beginning of helix α1 have very low B-factors, 9.5 Å² on average, supporting our earlier observation that this region is not flexible, but in fact very rigid (Pai et al., 1989). Residues 116–119, especially Asn116, are also very immobile. Their side chains are involved in binding the guanine base (see below). The residues in the so-called effector region (amino acids 32–40), which has been implicated in binding to GAP (Sigal et al., 1986, 1988; Adari et al., 1988; Cales et al., 1988), show an alternating pattern of low and high temperature factors. Particularly low are those of Phe28, which is involved in an aromatic–aromatic interaction with the

![P21.GMPPNP](image)

Fig. 3. Ramachandran plot of the present p21–GppNp model. +, every residue except glycine, ○ glycine residues.

![p21.GppNp](image)

Fig. 4. Average main chain temperature factors for the 1.35 Å structure of p21–GppNp.

The solvent structure

The complex of truncated p21 and GppNp crystallizes with only 35% of solvent. We found that most of the space between protein molecules is filled with water molecules occupying well defined positions. It is fortunate that in this tightly packed crystal form, the ‘water pockets’ are next to those parts of the protein which change their conformation upon hydrolysis of GTP (Milburn et al., 1990; Schlichting et al., 1990). Therefore, movements of these pieces of the polypeptide chain are not restricted. Differences between wildtype and mutant structures also occur in these regions (Krengel, U., Pai, E.F. and Wittinghofer, A., submitted).

The water molecules with the lowest temperature factors are Wat172 and Wat173, the two axial ligands of the Mg²⁺ ion. Altogether we find nine water molecules within hydrogen bonding distance of partner atoms of the nucleotide GppNp. Two water molecules, Wat175 and Wat189, are close enough to the γ-phosphorus atom to be involved in GTP hydrolysis (see below).

The nucleotide binding site

We have recently shown that guanine nucleotides with two or three phosphates bind to p21 with an affinity that is in the order of 10¹¹/M in the presence of Mg²⁺ at 4°C, whereas the affinity to GMP is six orders of magnitude lower (John et al., 1990). The high affinity of GDP and GTP is reflected in the large number of polar interactions between the protein and the nucleotide GppNp. Figure 5 shows all atoms of either protein or water residues within 3.4 Å of any atom of GppNp along with the corresponding distances.

The guanine base of the nucleotide is bound by interaction with the conserved sequence motifs NKXD (residues 116–119) and SAK (residues 145–147), which are identical in the great majority of guanine nucleotide binding proteins (Halliday, 1983; Dever et al., 1987; Gilman, 1987). The carboxylate group of Asp119 makes four hydrogen bonds. One oxygen interacts with the exocyclic amino group and Wat292, the other oxygen binds to the endocyclic nitrogen N1 and to the hydroxyl of Ser145. The keto group at position 6 of the guanine base makes a hydrogen bond to the main chain NH of Ala146.

The side chain amide of Asn116, which has been proposed
Fig. 5. Scheme showing the interactions between GppNp and p21 or water molecules. All dashed lines correspond to hydrogen bonding interactions (distance between donor and acceptor atom <3.4 Å). The corresponding distances in Å are indicated next to the labels, except where one residue makes several bonds in which case the distances are given next to the dashed lines.

Fig. 6. Stereodrawings. (A) Binding site of the base of GppNp. (B) Binding site of the magnesium ion and the phosphates of GppNp.

to be involved in the binding of the O6 oxygen, based on the three-dimensional structure of EF-Tu–GDP (Wooley and Clark, 1989), is seen here to make strong hydrogen bonds to the side chain of Thr144 and to the main chain oxygen atom of Val14. There are weaker interactions with main chain N146 (3.5Å) and N7 of the base, respectively. The main function of Asn116 is thus to tie together the three elements which are involved in nucleotide binding: the phosphate binding loop $^{16}$GXXXGKS, the $^{16}$NKXD and the $^{16}$SAK motifs. It is assisted by Lys117, which links the
Crystal structure of H-ras p21

Fig. 7. Stereopictures of part of the electron density map with the refined model superimposed. (A) Guanine base supported by Lys117 and Phe28. 
Asn116 links Val14 and Thr144. (B) Octahedral coordination of the magnesium ion. The ligands are the hydroxyl groups of Ser17 and Thr35, 
oxygen atoms of β- and γ-phosphate and two waters in the apical positions. (C) View into the 'active site' of p21. Wat175 is bound in a position 
perfect for nucleophilic attack on the γ-phosphates atom. Residues on the right belong to the effector region, those on the left are part of the loop 
L1. Amino acids of loop L4 are seen above the γ-phosphate. The side chain of Gin61 in its active conformation would be above the paper plane 
towards the viewer.

The ribose ring is in the 2'-endo conformation. The angle χ of the N-glycosidic bond is −112° just at the border of 
the anti-range observed for nucleotides with 2'-endo 
puckering of the ribose ring (Saenger, 1984). As reported 
before (Pai et al., 1989), the 2'- and 3'-hydroxyl groups of 
the ribose are rather exposed to the solvent with only weak 
hydrogen bonds to the side chain of Asp30. The 2'-hydroxyl 
is involved in an additional hydrogen bond to the main chain 
carbonyl of Val29. 04' is weakly bound by the ε-amino 
group of Lys117.

The phosphate binding site as shown in Figures 5 and 6B 
is characterized by a large number of interactions. Each of 
the eight phosphate oxygens of GppNp has at least two 
hydrogen bond donors (or the Mg2+ ion) close enough for 
an interaction. The hydrogen bond donors include the main 
chain NH groups of residues 13-18, 35 and 60, the
hydroxyl groups of Ser17 and Thr35 and the phenolic hydroxyl of Tyr32 from a neighbouring molecule of p21 in the crystal lattice which makes contact with the \( \gamma \)-phosphate. The \( \epsilon \)-amino group of Lys16 binds to the \( \beta \)- and \( \gamma \)-phosphate oxygens but is closer to the latter. It should be mentioned that all the main chain nitrogens of residues 13–18 point towards the phosphate groups, thereby creating a positively polarized local electrostatic field. There is a hydrogen bond between the NH of Gly13 and the atom bridging the \( \beta \)- and \( \gamma \)-phosphate, an NH in the case of GppNp. Since this hydrogen bond should be stronger with a bridging oxygen atom, it is likely that this interaction is responsible for the 10-fold lower affinity of GppNp as compared with GTP (see also Schlichting et al., 1990). It could also be responsible for the unusual \( ^{15}\text{N} \) chemical shift of this residue as determined by NMR (Redfield and Papastavros, 1990).

Feuerstein et al. (1989) have shown that the mechanism of the GTPase reaction follows an associative in-line reaction pathway with inversion of configuration at the \( \gamma \)-phosphate. By using a photolabile GTP precursor nucleotide, caged-GTP, Schlichting et al. (1989) have found that p21 in our crystal form is competent to hydrolyse GTP. The rate of hydrolysis is basically unaltered when compared with hydrolysis in solution (John et al., 1989b; Schlichting et al., 1989, 1990). Thus, it seems reasonable to assume that there is a water molecule in the crystal structure of the p21–GppNp complex which could attack the \( \gamma \)-phosphate. Since direct in-line displacements of phosphorus proceed via a pentacoordinate transition state, where the nucleophile and the leaving group occupy the apical positions of the trigonal bipyramid (see Knowles, 1980), the water molecule must attack from the opposite side of the leaving group, which is the \( \beta \)-phosphate. We could indeed identify two such water molecules, Wat175 and Wat189. They are tightly bound and are close enough to the \( \gamma \)-phosphate. We have reasons to believe (see below) that Wat175 is the attacking nucleophile.

### The \( \text{Mg}^{2+} \) binding site

It is generally believed that phosphotransferases require at least one divalent cation complexed directly to phosphoryl group oxygens for catalytic activity. One can postulate various possible catalytic functions of the \( \text{Mg}^{2+} \) ion, such as shielding of the negative charge on the attacked \( \gamma \)-phosphate, increasing the acid strength of the leaving group \( \beta \)-phosphate) or activation of the nucleophile (Cooperman, 1976; Knowles, 1980). \( \text{Mg}^{2+} \) could also be involved in the stabilization of the transition state of the reaction. The precise role of the metal ion for the guanine nucleotide binding proteins has not been proven and may indeed be different for each enzyme, as can be seen from the fact that there is no preferred ligation pattern of the metal ion to the enzyme or the phosphate atoms for related enzymes (see e.g. Eckstein, 1985). The GTPase activity of guanine nucleotide binding proteins has been shown to be absolutely dependent on the presence of certain divalent ions (Ivell et al., 1981; Gilman, 1987; J.John, unpublished) and the metal ion coordination for GDP or GTP bound to EF-Tu has been investigated by \( ^{55}\text{Mn} - ^{17}\text{O} \) superhyperfine interactions (Eccleston et al., 1981; Kalbitzer et al., 1984). Predictions about the coordination of the phosphate oxygen atoms and the metal ion in p21 have been inferred from EPR studies and those using phosphorothioates (Feuerstein et al., 1987).

As reported before (Pai et al., 1989), \( \text{Mg}^{2+} \) in the three-dimensional structure of the p21–GppNp complex is coordinated to one oxygen each of the \( \beta \)- and \( \gamma \)-phosphates, and to the side chain hydroxyl groups of Ser17 and Thr35, both of which are highly conserved in all nucleotide binding proteins. In contrast to the recent report by Milburn et al. (1990) we found that amino acid Asp57, which is completely conserved as part of the DXXG motif in guanine-nucleotide binding proteins, is completely rigid. In addition, the high resolution structure proves that it is not in the first coordination sphere of \( \text{Mg}^{2+} \) in the triphosphate conformation. Instead, it is hydrogen bonded to a water molecule, Wat173, which is then liganded to the magnesium as shown in Figures 6B, 7B and 8. Asp57 further binds to the side chain of Ser17. The sixth ligand of the metal ion is Wat172, which is held in place by the interaction with the main chain oxygen of Asp33 and the pro-R oxygen of the \( \alpha \)-phosphate.

\( \text{Mg}^{2+} \) ions have been identified in crystals of other substrate complexes of phosphoryl transfer enzymes, such as phosphoglycerate kinase (Watson et al., 1982) and EF-Tu (Woolley and Clark 1989), but a high resolution structure with a well-defined \( \text{Mg}^{2+} \) binding site is only available for phosphofructokinase (Shirakihara and Evans, 1988). In this case the octahedral coordination in the ‘open’ subunit is made by the \( 1 \)-phosphate of fructose-1,6-diphosphate and the \( \beta \)-phosphate of ADP, one aspartic acid and three water molecules. In the ‘closed’ subunit of this allosteric enzyme, \( \text{Mg}^{2+} \) bridges the \( \alpha \)- and \( \beta \)-phosphate oxygens. The rest of the coordination could not be identified with certainty. In the effector site of the same molecule, the \( \text{Mg}^{2+} \) ion makes a bidentate complex with the \( \alpha \)-, \( \beta \)-phosphate oxygens of ADP. Its octahedral coordination is completed by the carbonyl of a glycine, the carboxyl group of aspartic acid and two water molecules. Thus, in both these cases \( \text{Mg}^{2+} \) is complexed to three negative charges and three uncharged oxygen ligands. In EF-Tu, the only other guanine nucleotide binding protein for which a three-dimensional structure has been solved, \( \text{Mg}^{2+} \) appears to be coordinated to the \( \beta \)-phosphate oxygen of GDP, to a threonine and to an aspartic acid residue, corresponding to Ser17 and Asp57 in p21, respectively. One has to keep in mind, however, that these structures have been elucidated only at medium resolution (Jurnak, 1985; LaCour et al., 1985). These data suggest that the details of complexation of \( \text{Mg}^{2+} \) may be similar for guanine nucleotide binding proteins. They are different for other types of proteins, presumably related to the particular enzymatic processes catalysed by these proteins.

### Effector region

Amino acids 32–40 have been shown to be involved in binding of the GTPase activating protein GAP (Sagal et al., 1986; Adari et al., 1988; Cales et al., 1988, Sagal et al., 1988). Out of this stretch of residues, Pro34, Thr35 and Ile36 are particularly interesting. Pro 34 most probably stiffens loop L2. Its neighbour Thr35 is a direct ligand of the magnesium ion when p21 is in the triphosphate conformation but swings further away upon hydrolysis of GTP (Milburn et al., 1990, Schlichting et al., 1990). The hydrophobic side chain of Ile36 is exposed to the solvent in the p21–GppNp complex, but has moved towards the surface of the protein in the GDP form. This arrangement might balance the loss of the interaction energy when Thr35 leaves the \( \text{Mg}^{2+} \) and \( \gamma \)-phosphate coordination, by removing the isoleucine side chain from the solvent.
Mechanism of GTP hydrolysis

We have identified two water molecules (Wat175 and Wat189) which are close enough to the γ-phosphate group to perform an in-line nucleophilic attack. There are two reasons why we prefer Wat175 as the reacting water molecule. It is bound directly opposite to the β,γ-bond, and it would, furthermore, be reasonable to assume that the attacking water molecule would be made more nucleophilic by base catalysis or polarization through an interaction with a group or groups (on the protein or otherwise) which abstract protons or have an affinity for protons. The carbonyl oxygen of Thr35 is close enough to Wat175 to form a hydrogen bond. No such source of potential activation can be found for Wat189.

It has been shown recently that the rate-limiting step of the GTPase reaction of N-ras p21 is a conformational change of the p21-GTP complex preceding GTP hydrolysis (Neal et al., 1990).

\[
p21 + GTP \rightarrow p21\text{-}GTP \rightarrow p21\text{-}GTP^*\]

This change was observed using fluorescent GTP analogues, mant-GTP or mant-GppNp (John et al., 1989a, 1990). Since H-ras p21 shows the same conformational change (J.John, unpublished) and since crystallization of the p21-GppNp complex takes several days, the protein in the crystal should have undergone the conformational change. Since, however, we are dealing with an inhibitor complex which is not able to complete the hydrolysis reaction, we might be looking at an equilibrium mixture of conformations. Further analysis of the size of the fluorescence change associated with the conformational transition should help in answering this question.

In the electron density map of the GppNp complex, we observe that amino acids 61–67 in loop L4 have the highest temperature factors (Figure 3). In fact, we can identify alternative positions for at least four of these amino acids, Gln61, Glu62, Glu63 and Tyr64. In one of these conformations for the side chain of Gln61, its carbamoyl group would occupy electron density, with a break in the electron density at the position of the Cα-atom. In this position either the amide nitrogen or the carbonyl atom of the side chain could be brought close enough to Wat175 to make a hydrogen bond. Since a water molecule could be activated for the nucleophilic substitution reaction by polarization via hydrogen bonding from the carbonyl part of the side chain, we have oriented the side chain accordingly. Figure 9 shows a schematic drawing of this situation, which we propose could represent the true triphosphate complex after the conformational change. In such a complex Gln61, assisted by the carbonyl group of Thr35, could activate Wat175. In the same position the amido group is close to the carboxylate side chain of Glu63, which in turn could assist in establishing the proper activating orientation for Gln61 and/or increase its proton withdrawing potential. The finding that a Glu63→Lys mutation partially activates the transforming potential of p21 seems to implicate this residue, at least indirectly, in GTP hydrolysis (Fasano et al., 1984).

In addition to the arguments presented here, there are several other reasons to believe that Gln61 is involved in GTP hydrolysis. It is highly conserved in the small guanine nucleotide binding proteins, except for the proteins encoded by the rap genes, also called Krev (Pizon et al., 1988; Kitayama et al., 1989). The rap p21 proteins have Thr61 instead of Gln61 and have a reduced GTPase activity. Mutation of Thr61 to glutamine increases the GTPase rate to the value found for p21 ras and the mutant protein is also partially responsive to GAP while the wildtype p21 ras protein is not (Frech, 1990, submitted). Substitution of Gln61 in ras p21 by other amino acids in vitro reduces the GTPase rate constant, and the mutant proteins can no longer be activated by GAP (Der et al., 1986a,b; Vogel et al., 1988). Gln61 is conserved in the great majority of the guanine nucleotide binding proteins. In EF-Tu, the corresponding amino acid is His84, and it has been found that the in vitro GTPase rate is smaller than the GTPase of cellular p21, similar to p21(Q61H) (Jacquet and Parmeggiani, 1988). Mutation of His84 to glycine in the nucleotide binding domain of EF-Tu reduces the GTPase activity more than 10-fold (R.H. Cod and A.Parmeggiani, unpublished), which could indicate that histidine in EF-Tu has a similar function to glutamine in most other guanine nucleotide binding proteins.
Figures 5 and 9 show that Mg\(^{2+}\) and the \(\epsilon\)-amino group Lys16 are both coordinated to \(\beta\)- and \(\gamma\)-phosphate. Both of these ligands could therefore assist GTP hydrolysis in two ways: by increasing the electrophilicity of the \(\gamma\)-phosphate group and by increasing the acidity of the leaving group, GDP. A lysine residue is found in the phosphate binding loops of many proteins which bind adenine or guanine nucleotides. It has been shown by several investigators (Sigal et al., 1986; Dombroski et al., 1988; Rao et al., 1988) that this lysine is essential for binding or catalysis. Studies on adenylyl kinase indicate that it may be involved in stabilization of the transition state in the reaction catalysed by this enzyme (Schulz, 1987; Reinstein et al., 1990).

We propose that the conformational state of the molecule shown in Figure 9 is competent for GTP hydrolysis. The fact that loop L4 is mobile with at least two conformations found for each of the residues 61–64 would be consistent with a conformational change of loop L4 being the rate limiting step of GTP hydrolysis. The full conformational change could involve more than the movement of one or two side chains since the half life of the p21–GTP complex in vitro is 20 min at 37°C (John et al., 1988), indicative of a high activation energy for this transition. An obvious candidate for such a slow reaction would be isomerization of a proline residue, and Pro34 is close to the active site.

In all the structures of p21 that we have analysed, however, Pro34 is always in a trans conformation. Another possibility would be that the structural conversion involves the breaking and reformation of several hydrogen bonds in a more or less concerted way. Crystal structure analyses of oncogenic p21 mutants hindered in the flexibility in loop L4 lend some support to this interpretation (Krengel, U., Pai, E.F. and Wittinghofer, A., submitted).

Millburn et al. (1990) report that in the complex p21–GppCp, Tyr32 binds to the \(\gamma\)-phosphate of the nucleotide analogue. In the structure described here, it is Tyr32 of a neighbouring molecule in the crystal lattice which takes this place. Obviously, the p21 protein can accept side chains from other proteins at this position. In vivo, this part may be played by a residue of GAP. Possible roles for such a side chain could be to desolvate part of the active site, or it could increase the positive potential created by the main chain nitrogens of loop L1 and the side chain of Lys16. Other effects exerted on p21 by GAP might involve protonation of the leaving group and/or stabilization of the ‘active’ conformation of loop L4 and possibly also of helix \(\alpha 2\).

To shed further light on the exact nature of the GTP hydrolysis reaction it will be necessary to determine the crystal structure of the p21–GTP (or p21–GTP-analogue) complex directly after binding, before the conformational change has taken place. Our work on initiating the GTPase reaction in crystals by photolysis of caged GTP at the active site suggests that this may be possible after several technical improvements have been realized (Schlichting et al., 1989, 1990). This could lead to a description of the structural changes occurring here and later in the GTPase cycle at atomic resolution.

Materials and methods

Cloning, over-expression and purification of the truncated form of p21 (amino acids 1–166) were performed as described (Tucker et al., 1986; John et al., 1988). The complex of p21 (1–166) and the slowly hydrolysing GTP analogue GppNp was prepared according to John et al. (1990). Crystals were grown following the procedure of Scherer et al. (1989). The crystals belong to the trigonal space group P \(\overline{3}\) 2 1 with cell constants of \(a = b = 40.3\, \text{Å},\) and \(c = 162.2\, \text{Å},\) and contain one molecule per asymmetric unit (Scherer et al., 1989).

X-ray intensity data were recorded at 4°C on an electronic area detector (Siemens/Nicolet, Madison, WI, USA) as described (Durbin et al., 1986; Pai et al., 1989). For data reduction we used the program package XDS (Kabsch, 1988). From each of two crystals two data sets at 1.5 Å resolution were collected and scaled \((R_{merge} = 0.075)\) to the data set of 5378 independent reflections used to solve the structure at 2.6 Å resolution (Pai et al., 1989). For refinement we used the molecular dynamics program package X-PLOR (Brünger, 1987). The standard protocol was followed, except for the dielectric constant which was set to 4 to prevent electrostatic interactions from dominating the refinement. Refined individual temperature factors were included in the refinement. With the 1.5 Å data 5 ps of molecular dynamics (4 ps at a temperature of 2000 K) and 3150 rounds of energy minimization were run using the 2.6 Å model as the starting structure. This took the R-factor from 38.9% to 25.6%.

When large crystals became available two more native data sets were measured and merged with the 1.5 Å data to bring the limit of resolution to 1.35 Å \((R_{merge} = 0.111)\). Two more rounds of refinement separated by one minor rebuilding session resulted in a final R-factor of 19.6%. All electron density maps were calculated using the method developed by Read (1986) to reduce model bias. Water molecules were included in the model when corresponding density was in hydrogen bonding distance from a possible donor or acceptor atom and when its temperature factor after refinement was <80 Å². The r.m.s. deviations from target values are 0.014 Å for bond lengths and 2.7° for bond angles.

The colour pictures were prepared using the program FRODO (Jones, 1978) adapted to an IRIS 4GT (Silicon Graphics, Mountain View, CA, USA) by C.M. Cambillau.

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Crystal structure of H-ras p21


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