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# STRUCTURAL INVESTIGATIONS ON THE G-BINDING DOMAIN OF p21

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## INTRODUCTION

Ever since the ras gene product, p21, was discovered, it became clear, that it is a guanine nucleotide binding protein with significant homology to other G-binding proteins such as protein biosynthesis factors and signal-transducing G-proteins (Barbacid, 1987). Also, a lot of circumstantial evidence suggests that p21 is involved in a growth promoting signal-transduction pathway. Fig.1 shows the scheme for the involvement of p21 in such a process:

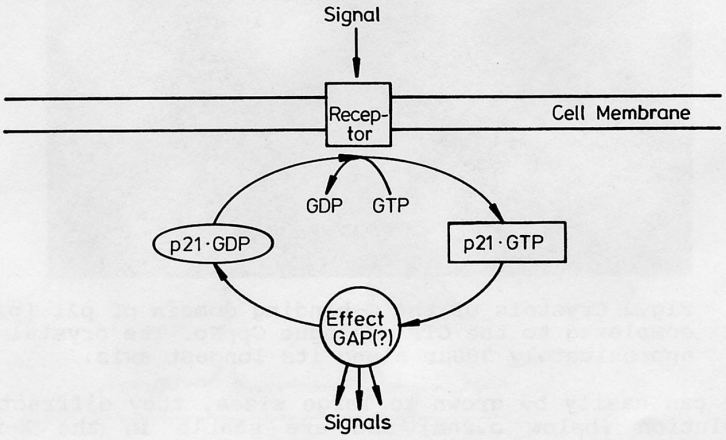


Fig.1 Schematic diagram of the cycling of p21 between an "active" and "inactive" conformation

p21 is cycling between two different conformations, an "inactive" GDP containing and an "active" GTP containing conformation. In its GDP conformation the protein, presumably, receives a signal that catalyzes the exchange of GDP for GTP.

The p21.GTP complex is then able to interact with the effector to transmit a signal. In the process of this interaction, GTP on the protein is hydrolyzed to GDP and the protein returns to the inactive resting state.Although the precise role of p21 is unknown it seems possible that the effector protein has already been discovered. GAP, a protein that has recently been described, is able to interact with p21 only in its GTP conformation and accelerates the GTPase reaction rate of normal cellular p21 (Trahey,M. and McCormick, 1987).

RESULTS

We are interested in describing in structural terms the transition between the two conformational states that are important far all G-binding proteins. For this we use NMR and X-Ray crystallography. Since the structure of the p21xGDP complex has recently been described by Kim and coworkers (deVos et al., 1988) we have concentrated our efforts on the GTP form of p21. We have expressed the G-binding domain of p21 as a C-terminally truncated 1-166 amino acid polypeptide chain called $p21_{C}$'. We have crystallized this protein as a complex with GppNp and GppCp and one of the crystals is shown in Fig.2.

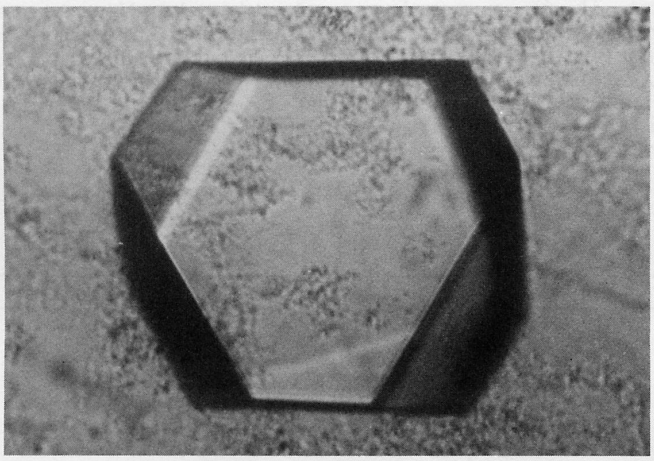

Fig.2 Crystals of the G-binding domain of p21 ($p21_{C}$') complexed to the GTP analogue GppNp. The crystal is approximately 300µm along its longest axis.

They can easily be grown to large sizes, they diffract to high resolution (below o.2nm) and are stable in the X-ray beam (Scherer et al., 1989). They have been used, together with heavy atom derivatives to prepare an electron density map of the protein-guanosine triphosphate complex which is currently being fitted to the polypeptide chain.

We have also undertaken biochemical and NMR experiments to investigate the effect of the C-terminal deletion on the properties of p21. We have shown before that the measurement of the GDP off-rate can be a precise monitor of the GDP binding site of p21 (John et al., 1988). In Table1 we show the GDP

Table1. Rate constants of p21 proteins measured in standard buffer (10mM $Mg^{2+}$) at 37°C

| Protein | GDP off-rate $k_{-1}$, $x10^3$,[$min^{-1}$] | GTPase rate $k_2$, $x10^3$,[$min^{-1}$] |
|---|---|---|
| $p21_C$ | 7.9 | 28 |
| $p21_{C'}$ | 7.8 | 37 |
| $p21_T$ | 2.3 | 3.8 |
| $p21_{T'}$ | 3.3 | 3.1 |

dissociation rate constants $k_{-1}$ and the GTPase rate-constants $k_2$ of truncated p21 as compared to normal protein.

One observes that the deletion of 23 amino acids from the C-terminus has no appreciable effect on these reaction rates. Table 1 also shows that the single point mutation Gly12→Val reduces the GDP off-rate and the GTPase rate truncated proteins, although the effect on the dissociation rate is somewhat smaller for p21(1-166). This indicates that the effect of the activating mutation Gly12→Val is preserved in the truncated protein.

We also used NMR spectroscopy to investigate the structural effects of the truncation of the protein. We have shown before that the ß-phoshorous atom of GDP undergoes a large 4ppm downfield shift on binding to p21, which is the same magnitude as with EF-Tu. Fig.3 shows the phosphorous NMR spectra of the $p21_C$xGDPx$Mg^{2+}$ complex compared to the $p21_C$'xGDPx$Mg^{2+}$ complex. One can see that α- and ß-P-resonances have the same chemical shift in the two proteins.

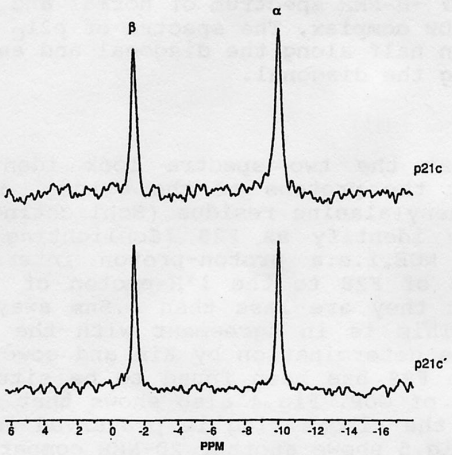


Fig.3 Phosphorous-NMR spectra of the MgxGDP complexes of truncated and normal p21.

Proton-NMR measurements of p21 have been performed in our laboratory to study the solution structure of the protein. Although the protein is too large for a complete 3D structure determination, the aromatic portion of the spectrum is

nevertheless resolved well enough to assign the majority of aromatic protons. In Fig.4 the NOESY-spectrum of the aromatic portion of the proton NMR spectrum between 5.95 and 7.00ppm is shown. With such type of spectrum one can identify through-space interactions between protons that are in close proximity of each other (within 0.5nm).

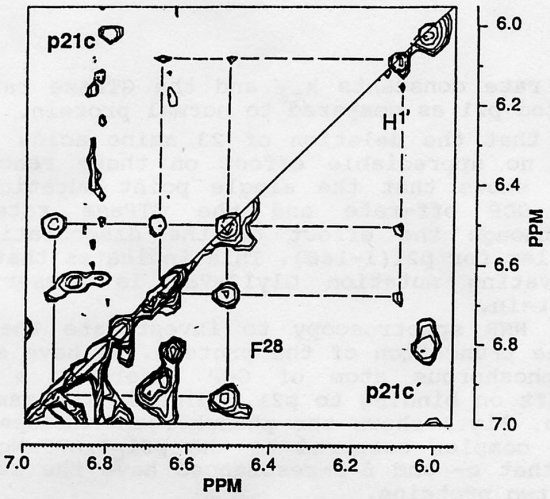


Fig.4 NOESY $^{1}$H-NMR spectrum of normal and truncated p21 in theMgxGDP complex. The spectra of $p21_C$ and $p21_C$' were both cut in half along the diagonal and each half recombined along the diagonal.

One can see that the two spectra look identical. We have assigned earlier the protons of the ortho-, meta- and para-position of a phenylalanine residue (Schlichting et al., 1988) that we can now identify as F28 (Schlichting, unpublished). Fig.4 shows an NOE,i.e.a proton-proton interaction, of the aromatic protons of F28 to the 1'H-proton of the GDP-ribose, which means that they are less than 0.5nm away from the 1'H-proton of GDP. This is in agreement with the finding of the crystal structure determination by Kim and coworkers (deVos et al., 1988) where F28 has been found to be situated on top of the guanine base of GDP. Fig.4 also shows that the position of F28 relative to the ribose ring is preserved in the truncated protein $p21_C$'. Fig.5 shows another 2D-NMR comparison of the two proteins in form of the COSY-spectra, and here again the two half-spectra are aligned along the diagonal middle line. With a COSY spectrum one is able to identify interactions between protons that are near neighbours through covalent bonds.
The two spectra show one characteristic difference, namely the appearance of an additional peak which can be identified as a tyrosine. The reason for the appearance of this new tyrosine residue is not known but it indicates that this tyrosine changes its environment when the last 23 amino acids are deleted, possibly by becoming more mobile.

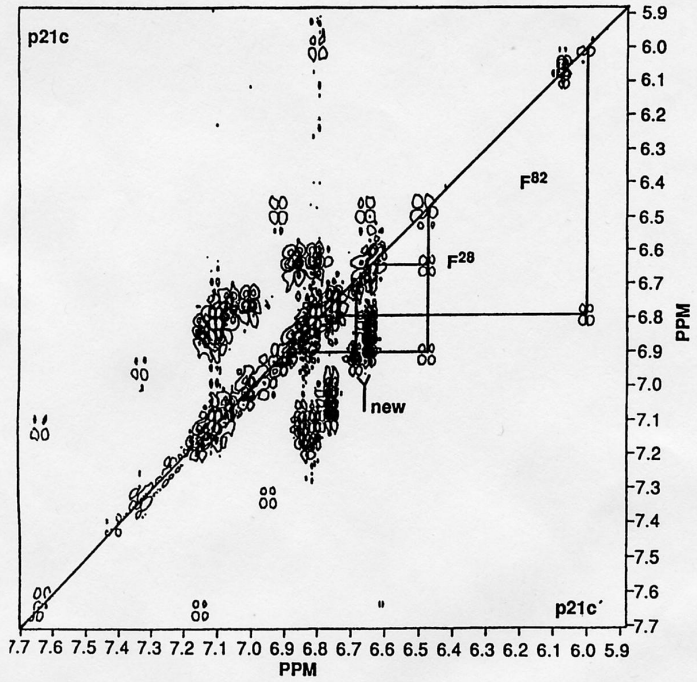


Fig.5 COSY $^{1}$H-NMR spectra of truncated and normal p21 both complexed to MgxGDP The two spectra are aligned along the diagonal as in Fig.4.

We have also tested the biological function of the truncated protein by microinjecting the $p21_{T}$' into PC12 cells (data not shown). Not surprisingly we find that the C-terminal truncation abolishes the biological effect of the protein. In conclusion we can say that although the p21 1-166 polypeptide is biologically inactive its biochemical and structural properties resemble very much those of the intact protein.

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