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Crystallization and preliminary crystallographic studies of a SAM domain at the C-terminus of human p73α

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p73 is a recently discovered homologue of the tumour suppressor p53 and contains all three functional domains of p53. The α-splice variant of p73 (p73α) contains an additional structural domain near its C-terminus that has sequence homology with the sterile α-motif (SAM) domain. This domain is considered to be responsible for mediating protein–protein interactions. Pyramidal crystals of human p73α SAM domain were obtained by the hanging-drop vapour-diffusion method with ammonium dihydrogen orthophosphate as the precipitant. The crystals diffract to 2.54 Å resolution and belong to the tetragonal space group $P4_12_12$, with unit-cell parameters $a = b = 32.02$, $c = 133.84$ Å. The structure was solved by molecular replacement using the NMR structure of the same protein as the search model.

1. Introduction

p73 is a recently cloned gene that shares high sequence homology with the *p53* tumour suppressor gene (Kaghad *et al.*, 1997). The domain structure of *p73* closely resembles that of *p53* and contains the transcription-activation, DNA-binding and oligomerization domains. Whereas *p53* encodes a unique gene product, alternative splicing of the *p73* gene results in at least six different isoforms, α, β, γ, δ, ε and φ, all having varying transactivation activities (Kaghad *et al.*, 1997; De Laurenzi *et al.*, 1998; Kaelin, 1999; Ueda *et al.*, 1999; Zaika *et al.*, 1999). In addition to the three common p53-like domains, the p73 α-splice variant (p73α) harbours a long C-terminal tail of approximately 200 residues and exhibits substantially reduced p53-like functions (Kaghad *et al.*, 1997). Within this region, a structural module known as the sterile α-motif (SAM) domain has been identified (Ponting, 1995; Thanos & Bowie, 1999). It is considered to be responsible for expanding or regulating protein functions *via* self-association or by association with other SAM domains (for a review, see Schultz *et al.*, 1997). Similarly, the SAM domain of p73α is probably important in down-regulating its p53-like activities (Ozaki *et al.*, 1999; Ueda *et al.*, 1999). The self-association of SAM domains can be visualized in two crystal structures (Stapleton *et al.*, 1999; Thanos *et al.*, 1999). However, homo-oligomerization is not observed in the two reported NMR structures (Chi *et al.*, 1999; Smalla *et al.*, 1999). It will be of interest to see if the crystal structure of p73α SAM domain does contain homotypic oligomerization, thereby revealing its mode of action.

We report the crystallization and structure solution of the SAM domain of p73α, containing residues 487–564. The structure of p73α residues 487–554 has been determined by nuclear magnetic resonance spectroscopy (Chi *et al.*, 1999) and was employed as our search model for molecular replacement.

2. Overproduction and purification

The cDNA sequence encoding p73α residues 487–564 was subcloned into a modified version of the pRSET A vector (Invitrogen) with an engineered thrombin-cleavage site (M. Proctor and M. Bycroft, unpublished work) producing an N-terminal hexahistidine (His_6) tagged protein. The plasmid was transformed into *Escherichia coli* C-41 (Miroux & Walker, 1996) and grown in 2XTY medium at 310 K until the absorbance at 600 nm (A_{600}) reached 0.6–0.9. Protein overproduction was then induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to 0.5 m*M* followed by 16 h growth at 303 K. The cells were lysed by sonication in a sample buffer containing 20 m*M* Tris–HCl pH 7.5, 150 m*M* NaCl and 5 m*M* β-mercaptoethanol. After centrifugation, the His_6-tagged protein was extracted from the supernatant using Ni–NTA resin (Qiagen) according to the standard batch protocol provided by the manufacturer, with a washing buffer containing 20 m*M* imidazole and an elution buffer containing 250 m*M* imidazole. The imidazole was then removed by overnight dialysis against the sample buffer. The His_6 tag was cleaved by 16 h thrombin digestion (10 units per milligram of protein) at room temperature, followed by purification

Table 1
Data-processing statistics for p73α residues 487–564.

The values in parentheses are for the highest resolution shell (2.71–2.54 Å).

Space group	$P4_12_12$
Unit-cell parameters (Å)	$a = b = 32.02$, $c = 133.84$
Resolution (Å)	26.0–2.54
Number of measurements (no cutoff)	17014
Number of unique reflections	2510
Multiplicity	6.8 (6.8)
Completeness (%)	94.9 (97.8)
$R_{\rm merge}$† (%)	7.3 (40.8)
Mean $I/\sigma(I)$	7.6 (2.0)

† $R_{\rm merge} = \sum_{hkl}|I - \langle I\rangle|/\sum_{hkl} I$.

using a second Ni–NTA column. The unbound phase was dialysed against the sample buffer for 8 h at 277 K and further purified by Superdex 75 26/60 size-exclusion chromatography (Pharmacia). The typical yield was 40 mg per litre of culture medium. The pure protein had two additional plasmid-encoded residues (glycine–serine) at its N-terminus.

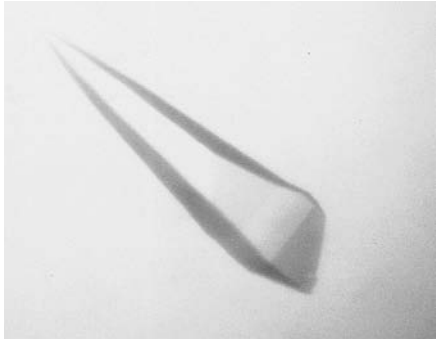

Figure 1
A pyramidal crystal of p73α residues 487–564.

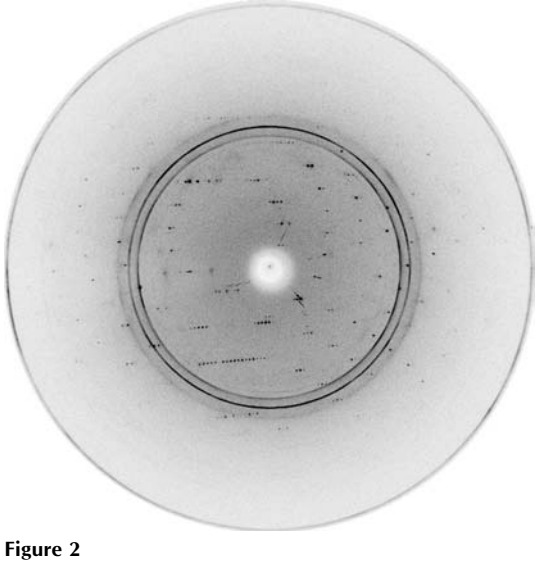

Figure 2
Diffraction image ($\Phi = 44.0$–45.0°) of p73α residues 487–564. Note the presence of ice rings.

Dynamic light-scattering experiments were performed using a DynaPro-801 instrument (Protein Solutions) at 310 K. The concentrated protein sample (29 mg ml^{-1}) was filtered through a 0.1 µm Anodisc filter (Whatman) before the measurements were taken. This experiment gave a polydispersity value of 0.2 nm, which was 10.8% of its average hydrodynamic radius (R_H = 1.85 nm), indicating p73α residues 487–564 to be monodisperse.

3. Crystallization and data collection

Initial screening for crystallization conditions was performed with Hampton Research Crystal Screen and Crystal Screen 2 at 277 and 290 K. Crystals for diffraction data collection were grown using the hanging-drop vapour-diffusion method at 290 K by mixing 1 µl of 16 mg ml^{-1} sample with 2 µl of reservoir solution containing 0.1 *M* Tris–HCl pH 8.5 and 2.0 *M* ammonium dihydrogen orthophosphate. The crystals grew to maximum dimensions of 0.4 × 0.2 × 0.1 mm in 3 d (Fig. 1). The pyramidal crystals were then soaked in various cryoprotectant solutions (Garman, 1999) and tested for stability. The optimal cryoprotection was achieved by diffusing 1 µl of 18% glycerol into the crystallization drop and equilibrating for 2 min at room temperature. The crystals were then cooled in a nitrogen stream at 100 K (Oxford Cryosystems Cryostream).

One crystal was used to collect 91 frames and a second crystal was used to collect 12 frames (1° oscillation each) using a MAR 345 scanner at the Medical Research Council Laboratory of Molecular Biology, Cambridge, England. Pseudo-precession images generated with *HKLVIEW* (Collaborative Computational Project, Number 4, 1994) exhibited 4/*mmm* Laue symmetry. Reflection conditions $h = 2n$ (and $k = 2n$) indicated the presence of a screw axis perpendicular to the l axis. Data on the l axis was missing, so the fourfold axis was ambiguous (the four possibilities being $P42_12$, $P4_12_12$, $P4_22_12$ and $P4_32_12$). Assuming there to be one molecule in the asymmetric unit, the Matthews coefficient (V_m) was calculated to be 1.9 Å^3 Da^{-1}. This was within the usual observed range (Matthews, 1968) and corresponded to 36% solvent content.

The two diffraction data sets were processed with *IPMOSFLM* (Leslie, 1992) and were merged, scaled and reduced with programs (*SCALA* and *TRUNCATE*) from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). Owing to the presence of ice rings (Fig. 2), data in the resolution shells 3.64–3.72 Å and 3.84–3.92 Å were omitted. The data was first processed in *P*422 because of the ambiguous fourfold axis. After successful structure solution by molecular replacement (see below), the data was reprocessed in the correct space group, $P4_12_12$ (Table 1).

4. Molecular replacement

We attempted structure solution using the molecular-replacement method (Rossmann, 1972) implemented in the program *AMoRe* (Collaborative Computational Project, Number 4, 1994; Navaza, 1994; Navaza & Saludjian, 1997) by employing the SAM-domain structures deposited in the Protein Data Bank (PDB; Bernstein *et al.*, 1977; Abola *et al.*, 1987) as search models. Early attempts using molecule *B* of the EphB2 receptor crystal structure (PDB entry 1b4f; Thanos *et al.*, 1999) as a search model did not yield any solution over a wide range of search parameters. Next, we used the NMR structure of p73α residues 487–554 (PDB entry 1cok; Chi *et al.*, 1999). We tried searching with the 'most-representative model' (model 7), as well as with a self-prepared minimized averaged model with disordered residues removed, without success. Finally, the structure was solved by using the whole ensemble (18 models) of 1cok as a search model.

Using the script *multi_probe* (see acknowledgements), we prepared three ensemble models from 1cok: an all-atom model, a poly-AG model (all non-glycine side chains truncated to alanine) and a poly-SAG model (all non-glycine/non-alanine side chains truncated to serine). In each case, unstructured residues at both termini (residues 487–490 and 551–554) were removed. All atoms were assigned a uniform temperature factor of 20 Å^2.

The molecular-replacement calculations were performed using data in the resolution range 15–3.5 Å. Rotation-function (RF) searches were carried out with an integration radius of 9 Å. There was no prominent RF peak observed with any of the three search models. All peaks of the RF search were used in the subsequent translation-function (TF) search in the three possible

Table 2
Results of molecular replacement for p73α residues 487–564.

The peaks after a combined translation search/rigid-body refinement were ranked in descending order of correlation coefficient and the top three peaks were listed for each space group.

	Eulerian rotation angles (°)			Translation, fraction of unit-cell edge			Correlation coefficient	*R* factor (%)
	α	β	γ	T_x	T_y	T_z		
$P4_12_12$	85.5	68.3	142.3	0.77	0.65	0.21	52.9	54.6
	22.9	87.0	29.6	1.00	0.31	0.00	28.9	59.9
	58.7	45.8	118.7	1.01	0.22	0.33	28.8	60.2
$P4_22_12$	62.6	77.9	297.1	0.35	0.62	0.31	43.8	57.2
	53.7	30.8	30.4	1.02	0.58	0.23	31.6	60.7
	29.1	82.9	213.9	0.71	0.36	0.24	30.0	60.2
$P4_32_12$	61.9	88.9	204.8	0.01	0.00	0.26	48.9	57.2
	63.0	45.4	115.8	0.96	0.77	0.04	29.3	60.8
	43.9	84.4	110.6	0.72	0.33	0.15	28.2	59.4

space groups $P4_12_12$, $P4_22_12$ and $P4_32_12$, followed by rigid-body (RB) refinement. The space group $P42_12$ was eliminated after several early attempts revealed that it was impossible to have reasonable packing in a unit cell with such a long *c* dimension without a fourfold screw axis. We were only able to determine a structure solution by using the poly-SAG model. The results of the combined TF/RB searches in the three space groups are tabulated in Table 2. The most promising solution was the top peak in $P4_12_12$, with an outstanding correlation coefficient peak and the lowest *R* factor. The solution was examined graphically with the aid of the program *xpack.pl* (Fu & Chen, 1996); this showed that the crystal packing for this solution was good. This solution ranked 27th in the RF search. The molecular-replacement solution was subjected to torsional molecular-dynamics refinement using the program *CNS* (Brunger *et al.*, 1998) and the *R* factor improved to 0.41 with an $R_{\rm free}$ of 0.51. After five cycles of manual rebuilding and refinement, the current *R* factor and $R_{\rm free}$ are 0.33 and 0.38, respectively, and further rebuilding and refinement is under way.

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References

Abola, E. E., Bernstein, F. C., Bryant, S. H., Koetzle, T. F. & Weng, J. (1987). *Crystallographic Databases – Information Content, Software Systems, Scientific Applications*, edited by F. H. Allen, G. Bergerhoff & R. Sievers, pp. 107–132. Bonn, Cambridge, Chester: Data Commission of the International Union of Crystallography.

Bernstein, F. C., Koetzle, T. F., Williams, G. J. B., Meyer, E. F. Jr, Brice, M. D., Rodgers, J. R., Kennard, O., Shimanouchi, T. & Tasumi, M. (1977). *J. Mol. Biol.* **112**, 535–542.

Brunger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J.-S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T. & Warren, G. L. (1998). *Acta Cryst.* D**54**, 905–921.

Chi, S. W., Ayed, A. & Arrowsmith, C. H. (1999). *EMBO J.* **18**, 4438–4445.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

De Laurenzi, V., Costanzo, A., Barcaroli, D., Terrinoni, A., Falco, M., Annicchiarico-Petruzzelli, M., Levrero, M. & Melino, G. (1998). *J. Exp. Med.* **188**, 1763–1768.

Fu, T. Y. & Chen, Y. W. (1996). *J. Appl. Cryst.* **29**, 594–597.

Garman, E. F. (1999). *Protein Crystallization: Techniques, Strategies and Tips. A Laboratory Manual*, edited by T. Bergfors, pp. 181–195. La Jolla, California: International University Line.

Kaelin, W. G. Jr (1999). *Oncogene*, **18**, 7701–7705.

Kaghad, M., Bonnet, H., Yang, A., Creancier, L., Biscan, J.-C., Valent, A., Minty, A., Chalon, P., Lelias, J.-M., Dumont, X., Ferrara, P., McKeon, F. & Caput, D. (1997). *Cell*, **90**, 809–819.

Leslie, A. G. W. (1992). *Jnt CCP4/ESF–EACMB. Newslett. Protein Crystallogr.* **26**.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Miroux, B. & Walker, J. E. (1996). *J. Mol. Biol.* **260**, 289–298.

Navaza, J. (1994). *Acta Cryst.* A**50**, 157–163.

Navaza, J. & Saludjian, P. (1997). *Methods Enzymol.* **276**, 581–594.

Ozaki, T., Naka, M., Takada, N., Tada, M., Sakiyama, S. & Nakagawara, A. (1999). *Cancer Res.* **59**, 5902–5907.

Ponting, C. P. (1995). *Protein Sci.* **4**, 1928–1930.

Rossmann, M. G. (1972). Editor. *The Molecular Replacement Method.* New York, London, Paris: Gordon & Breach.

Schultz, J., Ponting, C. P., Hofmann, K. & Bork, P. (1997). *Protein Sci.* **6**, 249–253.

Smalla, M., Schmieder, P., Kelly, M., Ter Laak, A., Krause, G., Ball, L., Wahl, M., Bork, P. & Oschkinat, H. (1999). *Protein Sci.* **8**, 1954–1961.

Stapleton, D., Balan, I., Pawson, T. & Sicheri, F. (1999). *Nature Struct. Biol.* **6**, 44–49.

Thanos, C. D. & Bowie, J. U. (1999). *Protein Sci.* **8**, 1708–1710.

Thanos, C. D., Goodwill, K. E. & Bowie, J. U. (1999). *Science*, **283**, 833–836.

Ueda, Y., Hijikata, M., Takagi, S., Chiba, T. & Shimotohno, K. (1999). *Oncogene*, **18**, 4993–4998.

Zaika, A. I., Kovalev, S., Marchenko, N. D. & Moll, U. M. (1999). *Cancer Res.* **59**, 3257–3263.