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ARTICLE

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Preliminary crystallographic analysis of the Cks protein p13^{suc1P90AP92A} from *Schizosacharromyces pombe*

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Abstract The p13^{suc1} is the fission yeast member of the Cks (Cdc28-dependant kinase subunit) family of proteins. The Cks proteins bind to and are required for the function of cyclin-dependant kinase (Cdk) proteins during cell cycle progression in eukaryotic cells. Two conformations of Cks have been detected crystallographically; a compact monomer with the C-terminal fourth β-strand inserted into the core of the molecule between strands 2 and 3, and a strand-exchanged dimer where the fourth β-strand is inserted into the core of the dimer partner in an equivalent position. There is a highly conserved "hinge" region consisting of the motif PEP, N-terminal to the fourth β-strand. In the monomer this motif constitutes a β-turn, while in the dimeric structure it is extended, allowing strand exchange. The mutant protein p13^{suc1P90AP92A}, in which alanine residues replace both prolines of the turn, provides an opportunity to examine the role of the prolines in this hinge region and how they may allow for the formation of strand-exchanged dimers by Cks proteins. We have expressed and purified this mutant protein. Two millimolar p13^{suc1P90AP92A} crystallised in 50 mM tris(hydroxymethyl)aminomethane pH 7.5, 30% poly(ethylene glycol) 1500. Diffraction data were collected at room temperature on an MAR345 image plate using Cu Kα radiation from a Rigaku RU200 rotating-anode generator source to 2.70Å. The crystal has unit cell parameters $a = b = 75.1$ Å, $c = 34.9$ Å, $\alpha = \beta = 90°$, $\gamma = 120°$. Diffraction data were indexed to the space group $P6$ and systematic absences $00l$ indicate a screw axis consistent with $P6_3$.

Keywords Cdk proteins · Cks proteins · Cell cycle · p13^{suc1} · p34^{cdc2}

Abbreviations SDS PAGE: Sodium dodecyl sulphate polyacrylamide gel electrophoresis · suc1: Wild-type p13$^{suc\ 1}$ · suc1.PP: P90AP92A mutant of p13^{suc1} · Tris: Tris(hydroxymethyl)aminomethane

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Introduction

The gene SUC1 from the fission yeast *Schizosaccharomyces pombe* encodes a 13 kDa product p13^{suc1} (Hindley et al. 1987), subsequently referred to here as suc1. This protein was first identified by its ability, when overexpressed, to suppress the temperature-sensitive phenotypes of certain fission yeast strains with mutant protein kinase p34^{cdc2} (Hayles et al. 1986a, 1986b). The p34^{cdc2} is an essential regulator of eukaryotic cell division (Nurse and Bissett 1981). It associates with cyclins at various points in the cell cycle (Booher et al. 1989; Moreno et al. 1989) and its kinase activity is required for a number of key transitions during mitosis (Simanis and Nurse 1986; Reed et al. 1985; Nurse and Bissett 1981) involving regulation of substrates including wee1, myt1 and cdc25 (Gautier et al. 1991; Mueller et al. 1995; Booher et al. 1997; Molz et al. 1989). Suc1 itself was subsequently shown to be essential for cell cycle progression (Hayles et al. 1986a; Brizuela et al. 1987; Hindley et al. 1987) and to interact physically with p34^{cdc2} in performing its role (Brizuela et al. 1987).

Conflicting data from functional investigations indicate a diversity of functions for suc1. It is generally agreed that its activity is associated in some way with $p34^{cdc2}$ at several points in the cell cycle but it is unclear whether it acts as an adaptor protein assisting substrate recognition by $p34^{cdc2}$ or if it is an enzyme in its own right (Pines 1996; Vogel and Baratte 1996; Harper 2001).

Three-dimensional structures of suc1 have been obtained using X-ray crystallographic techniques (Bourne et al. 1995; Endicott et al. 1995; Khazanovich et al. 1996). The protein contains four β-strands forming a β-sheet flanked on one side by three α-helices. The first structure of suc1 solved revealed discrete monomers of the protein in a compact fold that associate as a dimer in the asymmetric unit, stabilised by the binding of two zinc ions (Fig. 1a) (Endicott et al. 1995). Following this, two independently solved structures were reported in which suc1 was present as a strand-exchanged dimer with the C-terminal β-strand (i.e. β-strand 4) extended and inserted into the core of the dimer partner (Fig. 1b) (Bourne et al. 1995; Khazanovich et al. 1996). The overall fold of the strand-exchanged dimer is the same as that of the monomeric protein except at the highly conserved residues HVPEPH located between β-strands 3 and 4, which are in an extended conformation in the strand-exchanged dimers and in a β-turn conformation in the monomeric form. This region has been described as a β-hinge because of the alternate conformations it induces in suc1. It has been speculated that the ability of Cks proteins to adopt both conformations indicates a conformational switch that may be functionally significant (Bourne et al. 1995; Pines 1996; Vogel and Baratte 1996; Harper 2001; Rousseau et al. 2004).

In vitro, interconversion of suc1 has been shown to occur via an unfolded intermediate (Alonso et al. 2000; Rousseau et al. 2001). It is not known if conversion between the folds could occur under in vivo conditions without substantial unfolding of the monomer or dimer. Whatever the mechanism, understanding the conformation of the HVPEPH sequence in the hinge region between β-strands 3 and 4 is of paramount importance to any equilibrium between monomer and dimer.

The Cks protein family is one of about 40 proteins that are known to be capable of three-dimensional domain swapping (Bennett et al. 1994; Liu and Eisenberg 2002) and analysis of these proteins has revealed that proline repeats commonly occur in the hinge region (Bergdoll et al. 1997). The proline residues of the suc1 hinge, P90 and P92, are highly conserved in the Cks protein family (Fig. 2). We have created a mutant of suc1 in which both these prolines have been substituted with alanine. The three-dimensional structure of this mutant protein, which will subsequently be referred to as suc1.PP, provides an opportunity to examine the structural basis for the high conservation of prolines in this hinge region and the role of these prolines in the formation of strand-exchanged dimers by Cks proteins. We report here the preliminary crystallisation results of this protein.

Materials and methods

Mutagenesis of suc1 expression vector

pRK171suc1P90AP92A was constructed from pRK171 suc1 by site-directed mutagenesis performed using the Quikchange protocol (Stratagene, Germany). The mutations were confirmed by DNA sequence analysis. The following DNA primer was used to substitute the proline residues at positions 90 and 92 with alanine residues:

5′-3′: atg tat gaa gtc cat gtc gca gag gca cac atc ctg cta ttt aag
3′-5′: ctt aaa tag cag gat gtg tgc ctc tgc gac atg gac ttc ata cat

Expression of SUC1P90AP92A

The mutant protein was expressed in *E. coli* BL21(DE3)pLysS transformed with pRK171suc1P90A-P92A and grown in Luria broth with 100 μg ml^{-1} carbenicillin and 34 μg ml^{-1} chloramphenicol at 310 K to

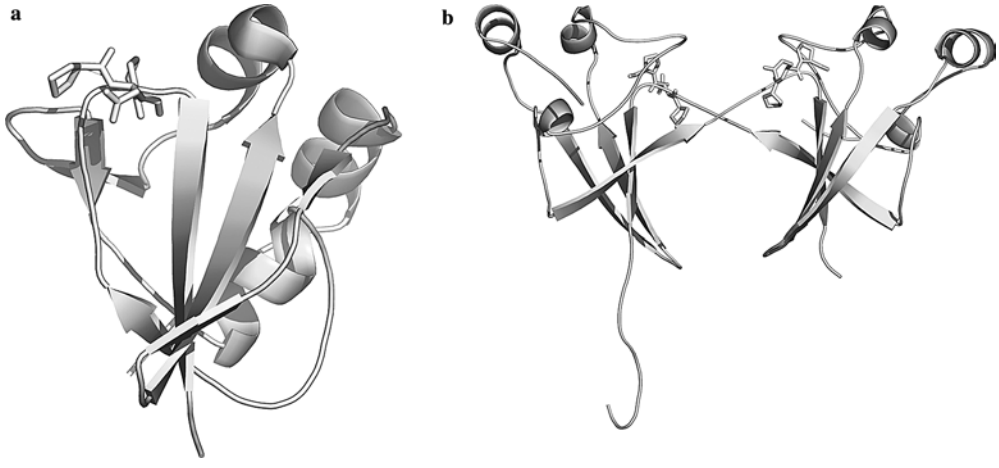

Fig. 1 **a** Structure of $p13^{suc1}$ (suc1) in the compact monomeric fold (coordinates provided by J. Endicott). **b** Structure of suc1 in the strand-exchanged dimeric fold (Protein Data Bank ID SCE1). The structures were prepared in PyMol and are shown as cartoon representations. The hinge region motif PEP is indicated by stick rendering

A.thaliana		IGVQQSRGWVHYAVHR**PEP**HIMLFRRPLN	
B.belcheri		IGVQQSQGWCHYMKHE**PEP**HILLFRRPKT	
C.elegans 1		LGIQQSPGWMHYMIHG**PER**HVLLFRRPLA	
C.elegans 2		AGVQQSLGWEHYMVHN**PEK**HILLFRRKRH	
D.melanogaster		IGVQQSRGWIHYMIHK**PEP**HILLFRRPLL	
H.sapiens 1		LGVQQSQGWVHYMIHE**PEP**HILLFRRPLP	
H.sapiens 2		LGVQQSLGWVHYMIHE**PEP**HILLFRRPLP	
L.mexicana		LGVQQSQGWVHYMIHK**PEP**HVLLFKRPRT	
M.glacialis		IGVQQSQGWVHYMSHK**PEP**HIA-- RI	
M.musculus		LGVQQSLGWVHYMIHE**PEP**HILLFRRPLP	
O.sativa		IGVQQSRGWVHYAIHR**PEP**HIMLFRRPLN	
P.vulgata		IGVQQSHGWIHYMKHE**PEP**HILLFRRKVT	
P.polyceph.		LGVQQSQGWVHYALHR**PEP**HILLFRREVP	
P.carinii		LGITQSLGWQHYEIHV**PEP**HILLFKRKCD	
S.cerevisiae		LGITQSLGWEHYECHA**PEP**HILLFKRPLN	
S.pombe	**74**	LGITQSLGWEMYEVHV**PEP**HILLFKREKD	**102**
X.laevis		LGVQQSLGWVHYMIHE**PEP**HILLFRRPLP	

Fig. 2 The sequence of the C-terminal residues of the Cks protein suc1 from the fission yeast *Schizosaccharomyces pombe* is aligned with the C-terminal sequences of Cks proteins from a range of species. The alignment was performed using BLASTP. The *S. pombe* sequence starts at residue 74. The highly conserved hinge region consisting of the motif PEP is highlighted in *bold*

an optical density at 600 nm of 0.6. Overexpression was stimulated with 0.4 mM isopropyl β-D-thiogalactopyranoside (Fig. 3) and cells were harvested by centrifugation 3 h later. The cells from 4 l of culture were resuspended in 50 mM tris(hydroxymethyl)aminomethane (Tris) HCl pH 7.5 and 1 mM phenylmethanesulphonyl fluoride and frozen at −86°C.

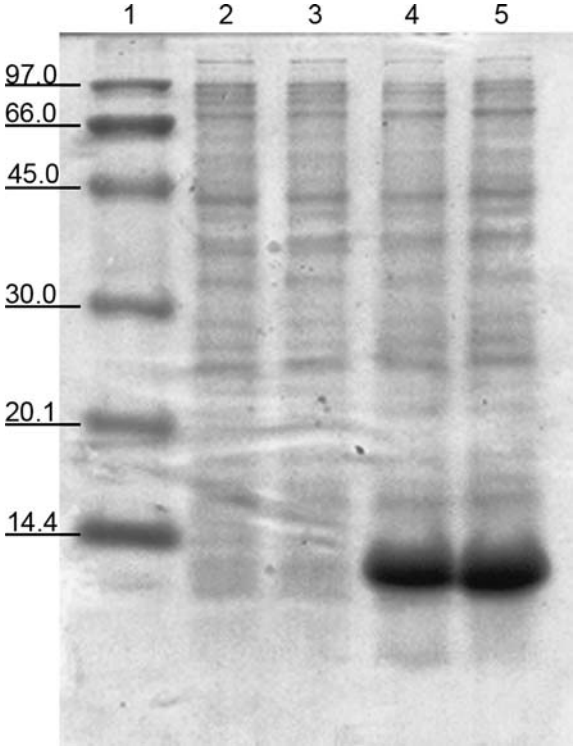

Fig. 3 Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (15%) analysis of BL21pLysS(pRK171suc1P90AP92A) cell lysate prior to (*lanes 2 and 3*) and after induction with isopropyl β-D-thiogalactopyranoside (*lanes 4 and 5*). An Amersham Pharmacia Biotech LMW calibration kit for SDS electrophoresis was used for comparison in *lane 1*

Purification of suc1.PP

Cells were lysed by a freeze/thaw cycle and sonication. Suc1.PP was purified from the lysate by adsorbtion onto Q-Sepharose (50 ml), followed by elution with an NaCl gradient of 0–0.8 M in 20 mM Tris pH 7.5 at a flow rate of 1 ml min^{-1}. Fractions containing suc1.PP were identified using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) on 15% polyacrylamide followed by Coomassie Blue staining. Pooled fractions were made 60% saturated with ammonium sulphate and the precipitate, after centrifugal harvest, was redissolved in a minimum volume of 50 mM Tris pH 7.5 20% saturated with ammonium sulphate. This protein solution was dialysed overnight at 277 K in 5 l of 50 mM Tris pH 7.5 using Spectapor dialysis membrane 3 kDa molecular weight cut-off. Pure suc1.PP was obtained by subjecting the dialysate to size-exclusion chromatography on Sephacryl S200 HR (260 ml), eluting with 50 mM Tris pH 7.5 at a flow rate of 0.5 ml min^{-1}. The purity of the fractions was assessed by silver staining (Wray et al. 1981) SDS PAGE gels. Pure fractions were combined and concentrated to 4 mM in 50 mM Tris HCl pH 7.5. Crystallisation conditions were screened using Hampton Screen 1 and 2 in hanging drop format.

Results

Crystallisation and data collection

Needlelike crystals formed at room temperature in 50 mM Tris HCl pH 7.5, 30% poly(ethylene glycol) 1500 (Fig. 4). Diffraction data were collected at room temperature (attempts at cryo-freezing were unsuccessful) to 2.7 Å on an MAR345 image plate using Cu Kα radiation from a Rigaku RU200 rotating-anode

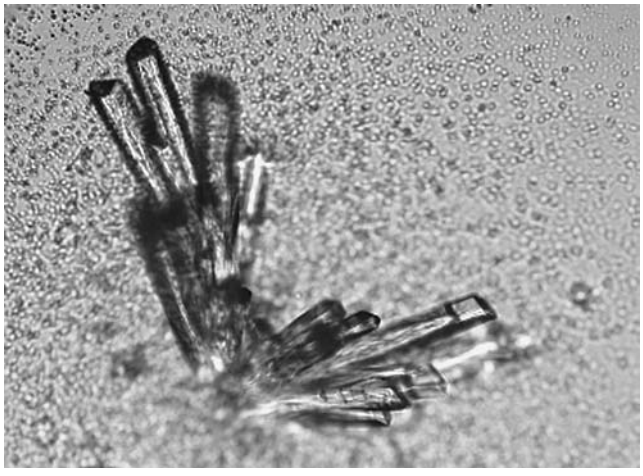

Fig. 4 Crystals of purified P90AP92A mutant of suc1 grown in 50 mM tris(hydroxymethyl)aminomethane HCl pH 7.5 and 30% poly(ethylene glycol) 1500, at room temperature. The average crystal dimension is 0.8×0.01 mm

Table 1 Diffraction data and processing statistics

Resolution range (Å)	24.6–2.7
Detector distance (mm)	140
Oscillation range (degrees)	1.25
Exposure time (min)	40
Observed reflections	8,553 (771)
Unique reflections	3,150 (299)
Completeness (%)	98.3 (98.7)
Redundancy	2.72 (2.58)
R_{merge} (%)	10.9 (71.8)
I/σ (I)	9.67 (1.31)
Number of images	91
Number of molecules per asymmetric unit	1
Matthews coefficient (Å^3 Da^{-1})	2.2
Solvent content (%)	43.3

Table 2 Completeness and R_{merge} statistics

Resolution		Completeness (%)	R_{merge} (%)
25.00	5.80	96.8	4.2
5.80	4.61	99.4	5.2
4.61	4.03	99.4	6.1
4.03	3.66	99.1	9.2
3.66	3.40	98.1	12.9
3.40	3.20	100.0	20.2
3.20	3.04	97.7	30.6
3.04	2.91	99.7	45.0
2.91	2.80	95.3	58.3
2.80	2.70	98.7	71.8

generator source. The relevant data collection statistics are shown in Table 1, and were obtained using the HKL suite of programs (Otwinowski and Minor 1997). Reflections were indexed to a unit cell with primitive hexagonal symmetry and dimensions $a=b=75.1$ Å, $c=34.9$ Å, $\alpha=\beta=90°$, $\gamma=120°$. Further processing with HKL revealed the presence of a screw axis indicated by systematic absences found along 00*l*. The determination of phases using molecular replacement methodologies is to proceed. Diffraction data and processing statistics are shown in Table 1, and Table 2 contains completeness and R_{merge} statistics.

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