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Crystallization and preliminary X-ray diffraction data of the complex between human centrin 2 and a peptide from the protein XPC

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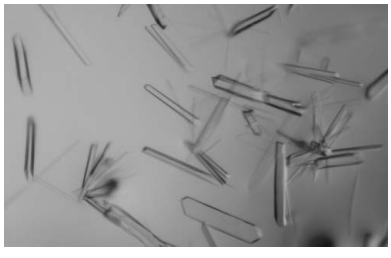

Centrins are highly conserved calcium-binding proteins involved in the nucleotide-excision repair pathway as a subunit of the heterotrimer including the XPC and hHR23B proteins. A complex formed by a Ca^{2+}-bound human centrin 2 construct (the wild type lacking the first 25 amino acids) with a 17-mer peptide derived from the XPC sequence (residues Asn847–Arg863) was crystallized. Data were collected to 1.65 Å resolution from crystals grown in 30% monomethyl polyethylene glycol (MPEG) 500, 100 m*M* NaCl and 100 m*M* Bicine pH 9.0. Crystals are monoclinic and belong to space group *C*2, with two molecules in the asymmetric unit. The unit-cell parameters are $a = 60.28$, $b = 59.42$, $c = 105.14$ Å, $\alpha = \gamma = 90$, $\beta = 94.67^\circ$. A heavy-atom derivative was obtained by co-crystallization with Sr^{2+}. The substitution was rationalized by calorimetry experiments, which indicate a binding constant for Sr^{2+} of $4.0 \times 10^4\ M^{-1}$.

1. Introduction

Centrins are Ca^{2+}-binding proteins belonging to the EF-hand superfamily that are highly conserved in eukaryotic cells. There are three human centrin isoforms (HsCen1–HsCen3), each having four potential Ca^{2+}-binding sites, with variable physicochemical and binding properties (Durussel *et al.*, 2000; Cox *et al.*, 2005). The nuclear fraction of HsCen2 was found to be associated with the nucleotide-excision repair (NER) pathway through a physical interaction with XPC, forming the heterotrimer HsCen2–XPC–hHR23B (Araki *et al.*, 2001). XPC plays a key role in the initial phase of the global genome NER, consisting of the recognition of the damaged DNA and the recruitment of other components of the repair pathway (Volker *et al.*, 2001). The XPC–HsCen2 interaction is thought to stimulate NER activity by enhancing the specificity of damage recognition (Nishi *et al.*, 2005), but understanding of the detailed regulatory mechanism requires structural data at the atomic level.

We recently identified a high-affinity centrin-binding site in the C-terminal part of XPC and characterized the interaction between a 17-residue peptide (P1-XPC; Asn847–Arg863) from this region of XPC and HsCen2 (Popescu *et al.*, 2003). The high affinity ($K_a \simeq 10^8\ M^{-1}$) and the moderate Ca^{2+} sensitivity of the HsCen2–XPC interaction suggest that the complex pre-exists in the nucleus prior to any DNA-damaging events. Here, we report the crystallization and preliminary X-ray diffraction data of the complex formed by a HsCen2 construct and P1-XPC.

2. Results and discussion

2.1. Preparation of the complex

The recombinant protein (HsCen2) was overexpressed in *Escherichia coli* as described previously (Durussel *et al.*, 2000). The cDNA of the deletion construct ΔN25-HsCen2, which lacks the first 25 residues, was obtained by polymerase chain reaction from the cDNA of the wild type and was subcloned into the expression vector pETBlue-1 (Novagen). The protein was overexpressed in *E. coli* Tuner (DE3) pLacI cells grown at 310 K. Purification of protein samples used three chromatographic steps including DEAE-TSK, Phenyl-TSK and G25 columns (Yang, Miron, Duchambon *et al.*,

Table 1
Data statistics.

Values in parentheses are for the highest resolution shell.

Data set	Native	Strontium λ_1	Strontium λ_2
Wavelength (Å)	0.9756	0.76933 (peak)	0.76957 (inflection)
Space group	*C*2		
Unit-cell parameters (Å, °)	a = 60.3, b = 59.4, c = 105.1, β = 94.67	a = 59.7, b = 59.1, c = 104.4, β = 95	a = 59.9, b = 59.2, c = 104.5, β = 94.9
Resolution range (Å)	52.41–1.80 (1.90–1.80)	52.20–2.46 (2.59–2.46)	42.14–2.46 (2.60–2.46)
No. of observations	199 958	41 812	33 733
No. of unique reflections	34 221	12 375	11 658
R_{sym} (%)	7.8 (39.5)	4.1 (6.0)	4.0 (6.0)
Completeness (%)	99.3 (95.5)	92.2 (51.3)	86.6 (41.6)
$\langle I\rangle/\langle\sigma(I)\rangle$	17.4 (4.1)	23.6 (10.9)	21.8 (9.0)
Phasing power		1.46	1.58

2006). The P1-XPC peptide (95% purity) with sequence NWKLLAKGLLIRERLKR, derived from the human XPC protein (Asn847–Arg863), was purchased from Biofidal (Vaulx-en-Velin, France). The stock solution of the complex contains 1 m*M* (16.9 mg ml^{-1}) ΔN25-HsCen2, 1.2 m*M* P1-XPC, 3 m*M* $CaCl_2$ and 200 m*M* NaCl in 20 m*M* Tris buffer pH 6.7.

2.2. Preparation of native crystals

Initial crystals were obtained from the stock solution by the hanging-drop vapour-diffusion method at 290 K by mixing 1.5 µl complex solution with 1.5 µl of a 1 ml reservoir consisting of 30% monomethyl polyethylene glycol (MPEG) 500, 100 m*M* NaCl and 100 m*M* Bicine pH 9.0. Twinned crystals were obtained after 5 d, which is typical of problematic spontaneous nucleation but fast uncontrolled subsequent growth. The crystallization was evaluated according to the principles outlined in reverse screening (Stura *et al.*, 1994). To avoid spontaneous nucleation, as well as to optimize the pH and the precipitant concentration for defect-free growth, the drops were streak-seeded (Stura & Wilson, 1992). Crystals grew rapidly after streak-seeding freshly mixed drops and large single crystals (about 300 × 100 × 50 µm) suitable for X-ray diffraction analysis were obtained after macroseeding and reach maximum size in 2–3 d (Fig. 1).

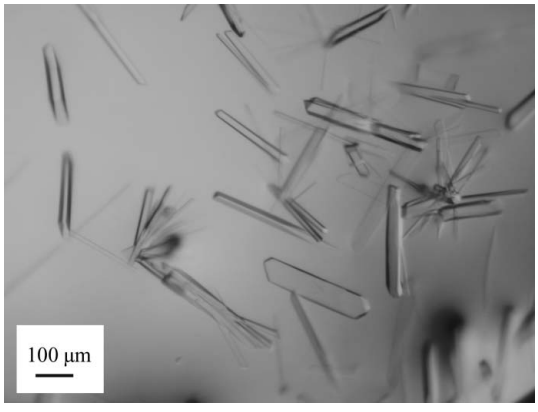

Figure 1
Native crystals of ΔN25-HsCen2 in complex with the peptide P1-XPC obtained in 30% MPEG 500, 100 m*M* NaCl and 100 m*M* bicine pH 9.0. The crystals were obtained by transfer of seeds in order to limit spontaneous nucleation.

2.3. Data collection

After transferring to a cryoprotectant solution (mother liquor with an additional 10% ethylene glycol), crystals were flash-frozen in either nitrogen gas or liquid ethane. The native data set was collected under cryogenic conditions at beamline ID29 of ESRF using X-rays with a wavelength of 0.9756 Å. A 300° data set was collected to 1.65 Å resolution (1° oscillation frames, each exposed for 1 s). All data were indexed and integrated using *MOSFLM* and merged and scaled using *SCALA* (Collaborative Computational Project, Number 4, 1994) (Table 1). The native crystals belong to space group *C*2 and contain two molecules in the asymmetric unit, giving a V_M of 2.46 Å^3 Da^{-1} and an approximate solvent content of 41% as estimated by the program *TRUNCATE* (French & Wilson, 1978).

2.4. Initial phasing

The structure was solved by molecular replacement using the program *Phaser* implemented in the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994; McCoy *et al.*, 2005). The crystallographic structure of the N-terminal (1–90) and C-terminal (91–162) domains of troponin C were used separately as input models (PDB code 1top) in *Phaser* (Satyshur *et al.*, 1994). The best solution presented a correlation coefficient of 36% with two full troponin C molecules in the asymmetric unit. The refined structure of ΔN25-HsCen2 in complex with the P1-XPC peptide will be presented elsewhere.

2.5. Isothermal titration calorimetry of Sr^{2+} binding

Two Ca^{2+} sites, a strong (EF-hand IV) and a moderate site (EF-hand III), were observed in HsCen2 by flow dialysis (Durussel *et al.*, 2000; Matei *et al.*, 2003); both were localized in the C-terminal domain of the protein. Isothermal titration calorimetry (ITC) using centrin concentrations in the range 10–35 µ*M* could only detect the strong binding site, which shows an affinity of 5 × 10^4 *M*$^{-1}$ (C. T. Craescu, unpublished results). The capacity of other divalent metal ions, such as Sr^{2+}, to replace Ca^{2+} was analyzed by ITC at 303 K in a

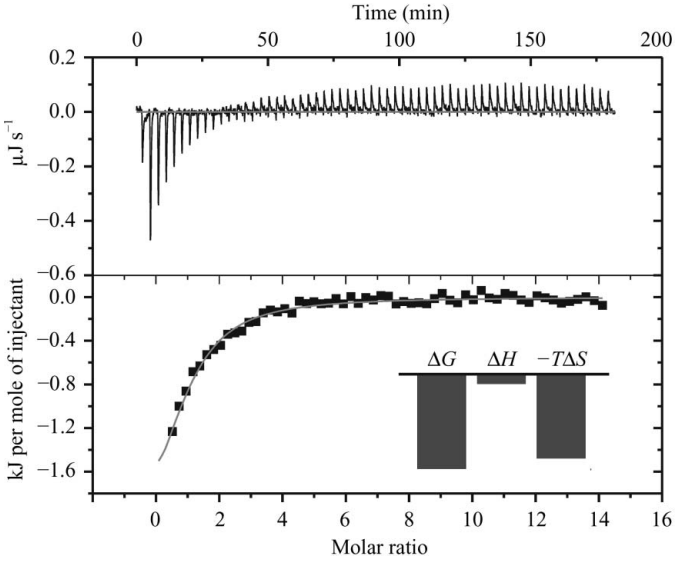

Figure 2
Titration of HsCen2 with Sr^{2+} ions. The isotherm, fitted to a single-site binding model, gives stoichiometry n = 1, binding constant K_a = 4.0 (0.3) × 10^4 *M*$^{-1}$ and reaction enthalpy ΔH = −2720 (84) J mol^{-1}. The lower panel schematically shows the balance of the energetic terms contributing to the binding process.

working buffer containing 50 m*M* MOPS pH 7.4 and 100 m*M* NaCl using a MicroCal MCS instrument (MicroCal Inc., Northampton, MA, USA). The buffer and the protein solutions were first decalcified using a Chelex-100 resin (BioRad). In a typical experiment, the $SrCl_2$ solution (2 m*M*) was titrated into the HsCen2 solution (1.337 ml at 35 µ*M*), equilibrated in the calorimeter cell, by automatic injections of 5 µl.

The experimental data (Fig. 2) could only be fitted to a one-site binding model with a stoichiometry of 1:1, indicating that only one EF-hand in the integral protein binds the metal ion with significant affinity ($>10^4\ M^{-1}$). The binding constant was found to be 4.0 (0.3) $\times$ $10^4\ M^{-1}$, a value which is very similar to those observed for Ca^{2+} binding to human centrins (Cox *et al.*, 2005). The reaction is driven by the entropy contribution, probably by a large increase in water entropy (Fig. 2).

2.6. Heavy-atom derivatives

The ITC results on Sr^{2+} binding encouraged us to use Sr^{2+} to obtain isomorphous heavy-atom derivatives (Blundell & Johnson, 1976). We focused on replacing Ca^{2+} ions by alkaline earth ions such as Sr^{2+} or by trivalent lanthanide ions such as Sm^{3+} or Dy^{3+}. Crystals were prepared from a protein stock solution containing ΔN25-HsCen2 and P1-XPC with no additional $CaCl_2$. The crystals were soaked in three different cryoprotectant solutions with 10 m*M* $SrCl_2$, 2 m*M* $DyCl_3$ or 2 m*M* $SmCl_3$. An isomorphous derivative was obtained with strontium soaking. The presence of the strontium in the crystal was detected prior to data collection by measuring the fluorescence signal on beamline FIP-BM30A. The absorption edge for Sr was scanned between 16 080 and 16 150 eV and two data sets were collected at wavelengths $\lambda_1 = 0.76933$ Å (peak, $f''_{\rm max}$) and $\lambda_2 = 0.76957$ Å (inflection point, $f'_{\rm min}$). These data were processed with *MOSFLM* and *SCALEIT* (Collaborative Computational Project, Number 4, 1994). The presence of Sr^{2+} was assessed by calculating isomorphous difference Fourier maps from partial phases obtained by molecular replacement (data not shown).

3. Conclusion

Structural studies on human centrins are hampered by the high internal flexibility (Yang, Miron, Mouawad *et al.*, 2006) and the tendency to form heterogeneous self-assemblies in a Ca^{2+}-dependent manner (Tourbez *et al.*, 2004). The variable N-terminal fragment of HsCen2, including the first 25 residues, plays an important role in the homo-molecular interactions responsible for these assemblies (Tourbez *et al.*, 2004; Yang, Miron, Duchambon *et al.*, 2006). Nevertheless, solution NMR structures could be obtained for isolated constructs representing HsCen2 domains (Matei *et al.*, 2003; Yang, Miron, Duchambon *et al.*, 2006) or for the C-terminal domain in complex with P1-XPC (Yang, Miron, Mouawad *et al.*, 2006). Crystallization of a mouse centrin construct including an additional 25 N-terminal non-native residues (Park *et al.*, 2005) or of a ciliate centrin (He *et al.*, 2005) produced diffracting crystals, but no X-ray structure has been reported to date.

The use of a 17-mer peptide derived from the XPC sequence allowed us to grow crystals of the centrin complex that diffracted to 1.80 Å. Strontium binding to HsCen2 was evaluated during heavy-ion screening and the substitution proved to be effective as judged from the electron-density map obtained by molecular replacement. This was correlated with ITC measurements, which show similar binding affinity for Ca^{2+} and Sr^{2+}. Therefore, ITC has proved to be a simple and efficient method to quantify the replacement of Ca^{2+} or other metal ions by another alkaline ion.

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References

Araki, M., Masutani, C., Takemura, M., Uchida, A., Sugasawa, K., Kondoh, J., Ohkuma, Y. & Hanaoka, F. (2001). *J. Biol. Chem.* **276**, 18665–18672.
Blundell, T. L. & Johnson, L. N. (1976). *Preparation of Heavy-Atom Derivatives*, pp. 183–239. New York: Academic Press.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Cox, J. A., Durussel, I., Firanescu, C., Blouquit, Y., Duchambon, P. & Craescu, C. T. (2005). *Biochemistry*, **44**, 840–850.
Durussel, I., Blouquit, Y., Middendorp, S., Craescu, C. T. & Cox, J. A. (2000). *FEBS Lett.* **472**, 208–212.
French, G. S. & Wilson, K. S. (1978). *Acta Cryst.* A**34**, 517–525.
He, X.-J., Li, L., Hou, H., Zhao, Y., Liang, A. & Rao, Z. (2005). *Protein Pept. Lett.* **12**, 609–611.
McCoy, A. J., Grosse-Kunstleve, R. W., Storoni, L. C. & Read, R. J. (2005). *Acta Cryst.* D**61**, 458–464.
Matei, E., Miron, S., Blouquit, Y., Duchambon, P., Durussel, I., Cox, J. A. & Craescu, C. T. (2003). *Biochemistry*, **42**, 1439–1450.
Nishi, R., Okuda, Y., Watanabe, E., Mori, T., Iwai, S., Masutani, C., Sugasawa, K. & Hanaoka, F. (2005). *Mol. Cell. Biol.* **25**, 5664–5674.
Park, J. H., Krauss, N., Pulvermüller, A., Scheerer, P., Höhne, W., Giessl, A., Wolfrum, U., Hofmann, K. P., Ernst, O. P. & Choe, H.-W. (2005). *Acta Cryst.* F**61**, 510–513.
Popescu, A., Miron, S., Blouquit, Y., Duchambon, P. & Craescu, C. T. (2003). *J. Biol. Chem.* **278**, 40252–40261.
Satyshur, K. A., Pyzalska, D., Greaser, M., Rao, S. T. & Sundaralingam, M. (1994). *Acta Cryst.* D**50**, 40–49.
Stura, E. A., Satterthwait, A. C., Calvo, J. C., Kaslow, D. C. & Wilson, I. A. (1994). *Acta Cryst.* D**50**, 448–455.
Stura, E. A. & Wilson, I. A. (1992). *Crystallization of Nucleic Acids and Proteins: A Practical Approach*, pp. 177–208. Oxford University Press.
Tourbez, M., Firanescu, C., Yang, A., Unipan, L., Duchambon, P., Blouquit, Y. & Craescu, C. T. (2004). *J. Biol. Chem.* **279**, 47672–47680.
Volker, M., Monè, M. J., Karmakar, P., van Hoffen, A., Schul, W., Vermeulen, W., Hoeijmakers, J. H. J., van Driel, R., van Zeeland, A. A. & Mullenders, L. H. F. (2001). *Mol. Cell*, **8**, 213–224.
Yang, A., Miron, S., Duchambon, P., Assairi, L., Blouquit, Y. & Craescu, C. T. (2006). *Biochemistry*, **45**, 880–889.
Yang, A., Miron, S., Mouawad, L., Duchambon, P., Blouquit, Y. & Craescu, C. T. (2006). *Biochemistry*, **45**, 3653–3663.