Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Structure and oligomeric state of the mammalian tumour-associated antigen UK114

Daniela Deriu,[a] Christophe Briand,[a]* Edita Mistiniene,[b] Vytautas Naktinis[c] and Markus G. Grütter[a]

[a]Institute of Biochemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland, [b] Institute of Biotechnology, V. Graiciuno 8, Vilnius 2028, Lithuania, and [c]SICOR Biotech UAB, V. Graiciuno 8, Vilnius 2028, Lithuania

Correspondence e-mail: cbriand@bioc.unizh.ch

Received 27 May 2003
Accepted 25 June 2003

PDB Reference: UK114, 1nq3, r1nq3sf.

The tumour-associated antigen UK114, isolated from goat liver, belongs to the YER057c/YIL051c/YjgF protein family, which has members in both the prokaryotes and eukaryotes. The crystal structure of a mammalian representative, goat UK114, was determined, revealing a trimeric arrangement in the crystal. It was confirmed by ultracentrifugation that UK114 is a trimer in solution. These results are in agreement with the published structures of homologues from unicellular organisms, but contrast with those reported for the rat homologue of UK114, for which a dimeric quaternary structure was proposed.

1. Introduction

UK114, a small protein of 14.3 kDa, was first identified and isolated in 1994 from goat liver by perchloric acid extraction (Bartorelli *et al.*, 1994). UK114 is a tumour-associated antigen that is expressed at low levels in the cytosol of normal cells but is highly expressed at the cell surface of neoplastic cells (Bartorelli *et al.*, 1996). Since tumour cells expressing UK114 are susceptible to antibody-mediated cytolysis, UK114 is a potential target for cancer immunotherapy and chemotherapy (Bartorelli *et al.*, 1996). UK114 belongs to the large YER057c/YIL051c/YjgF protein family. The oligomeric state of these proteins is still a matter of debate (Melloni *et al.*, 1998; Oka *et al.*, 1995; Schmiedeknecht *et al.*, 1996; Sinha *et al.*, 1999).

The YabJ protein from *Bacillus subtilis* (PDB code 1qd9), the *Escherichia coli* homologue (PDB code 1qu9) and protein HI079 from *Haemophilus influenzae* all revealed a trimeric arrangement during structural studies (Parsons *et al.*, 2003; Sinha *et al.*, 1999; Volz, 1999). In contrast, a monomeric state was reported for the human mononuclear monocyte p14.5 (Schmiedeknecht *et al.*, 1996). The perchloric acid-soluble protein (PSP) from rat liver (Oka *et al.*, 1995) has been described to be a homodimer in solution, resulting from a disulfide bridge between two subunits. This result was supported by preliminary crystallographic data from a $P2_13$ crystal form of PSP, which hinted at the presence of two molecules per asymmetric unit (Djinovic Carugo *et al.*, 1999). A bovine brain μ-calpain activator almost identical to goat liver UK114 (99% of pairwise identity) was proposed to have a dimeric oligomerization state based on size-exclusion chromatography studies. UK114 was also shown to be a μ-calpain activator able to bind calcium (Melloni *et al.*, 1998). In this study, we present the crystal structure of UK114. Sedimentation-velocity experiments and evaluation of inter-subunit contacts clearly support a trimeric oligomeric state of the protein in solution.

2. Experimental

UK114 was expressed in *E. coli* strain K802 (pT7a-UK114). The bacterial pellet was resuspended in 10 m*M* Tris–HCl pH 7.5, 5 m*M* EDTA and 2 m*M* PMSF and sonicated. Major contaminants were removed by precipitation with ethylene imine polymer and UK114 was purified in two steps using a Q-Sepharose Fast Flow and an SP Sepharose Fast Flow column. The pooled protein peaks were subjected to buffer-exchange chromatography into 10 m*M* sodium phosphate buffer pH 7.5. Purified recombinant UK114 was crystallized at 291 K by hanging-drop vapour diffusion. 2 µl of protein solution (11 mg ml^{-1}) was mixed with 2 µl of precipitant solution containing 22%(*w*/*v*) PEG 8000, 50 m*M* potassium phosphate pH 5.6. Crystals grew within 4–5 d. Data were collected at 100 K to 2.2 Å using a MAR Research imaging plate (Hamburg, Germany) at the Swiss Light Source, Paul Scherrer Institut, Villigen, Switzerland. Data were reduced with *DENZO* (Otwinowski & Minor, 1997) and programs from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). The structure was solved by molecular replacement with *AMoRe* (Navaza, 1994) using a polyserine model based on the structure of the trimeric purine regulatory protein YabJ (Sinha *et al.*, 1999; PDB code 1qd9). The structure was refined with *CNS* (Brünger *et al.*, 1998), alternating cycles of torsion-angle simulated annealing and individual *B*-factor refinement with manual rebuilding using the program *O* (Jones *et al.*, 1991). Data between 20 and 2.2 Å were used

© 2003 International Union of Crystallography
Printed in Denmark – all rights reserved

Table 1
X-ray diffraction data-collection and refinement statistics.

Values in parentheses are for the highest resolution shell.

Resolution range (Å)	20.0–2.2
Unique reflections	39547
Multiplicity	4.4
Completeness (%)	98.8 (96)
$I/\sigma(I)$	20.14 (4.45)
Space group	$P2_12_12_1$
Unit-cell parameters (Å, °)	a = 51.0, b = 78.0, c = 192.5, $\alpha = \beta = \gamma$ = 90
No. of molecules per AU	6 (2 trimers)
R_{merge}† (%)	7.8 (27)
R_{cryst}‡ (%)	18.8
R_{free} (%) (5% of the data)	22.6
No. of protein atoms	5859
No. of water molecules	259
Mean overall B factor (Å^2)	27.1
R.m.s.d. from ideal geometry	
Bond angles (°)	1.2
Bond lengths (Å)	0.005
Ramachandran plot regions (%)	
Most favoured	92.2
Additionally allowed	6.9
Generously allowed	0.0
Disallowed	0.9
Asp28 (average values, °)	φ = 67.7, ψ = −101.9

† $R_{merge} = \sum_{hkl}\sum_j |I_{j,hkl} - \langle I_{hkl}\rangle| / \sum_{hkl}\sum_j I_{j,hkl}$. ‡ $R_{cryst} = (\sum_{hkl} ||F_o| - |F_c||)/\sum_{hkl} F_o$.

with no σ cutoff. The final model was validated by *PROCHECK* (Laskowski *et al.*, 1993). Crystallographic statistics are given in Table 1.

The shape complementarity of the interfaces between UK114 monomers was calculated using the program *SC* from *CCP*4 (Lawrence & Colman, 1993), following the procedure described by Micheau *et al.* (2002). Electrostatic calculations were carried out with *GRASP* (Nicholls *et al.*, 1991). The desolvation and bound-state potential were calculated as described by Lee & Tidor (2001). Surface hydrophobicity was calculated with *HYDROMAP* (Scarsi *et al.*, 1999).

Sedimentation-velocity experiments were performed using a Beckman Optima XL-I analytical ultracentrifuge with Rayleigh Interference Optics. UK114 was diluted in water to a concentration of 5.4 µ*M* and Epon double-sector centrepieces were filled with 400 µl of the protein sample and water, respectively. Using an An50-Ti rotor, the samples were centrifuged with a rotor speed of 42 000 rev min^{-1} at 293 K. 100 scans were acquired in time intervals of 5 min. A partial specific volume of 0.746 cm^3 g^{-1} for a UK114 trimer was calculated based on the protein structure using the program *HYDROPRO* (Garcia De La Torre *et al.*, 2000). Analysis of the run was performed using the analysis program *SEDFIT* (Schuck, 2000).

3. Results and discussion

3.1. Structure of UK114

The UK114 monomer consists of a six-stranded β-sheet packed against two α-helices. The β-sheets of each monomer build up the internal part of the trimer, while α-helices form the outer shell of the resulting triangular β-barrel (Fig. 1*a*). The surface buried per monomer owing to the trimer contacts is 1950 Å^2. This value is in the range of a specific biological interaction between subunits and is definitely too large to represent only crystal contacts (Janin, 1997). Analysis of monomer–monomer surface complementarity performed with *SC* (Lawrence & Colman, 1993) generates a value of 0.72, thus indicating a good complementarity. Shape and electrostatic complementarity further confirm that the contacts observed between subunits are specific for the protein oligomeric state and are not artefacts resulting from crystal packing (Figs. 1*b* and 1*c*).

Trimeric UK114 possesses a polar central cavity, filled with 21 water molecules, which is divided into two parts by the side chains of the Tyr32 from each monomer as observed also in the *B. subtilis* and *E. coli* homologue structures (Sinha *et al.*, 1999; Volz, 1999). The OH group of each tyrosine interacts with a well defined water molecule in the centre of the cavity (Fig. 1*d*).

Electrostatic calculations show that the central cavity of the protein is negatively charged, which may enable the binding of cations such as calcium. Interestingly, at the entrance to the cavity, Asp28 of each monomer adopts peculiar dihedral torsion angles corresponding to the disallowed region of the Ramachandran plot (Table 1). By manually forcing these angles into allowed regions, the side chain of the Asp28, normally pointing towards the trimer axis, turns aside, thus enlarging the opening of the hydrophilic cavity. Asp28 is exclusively

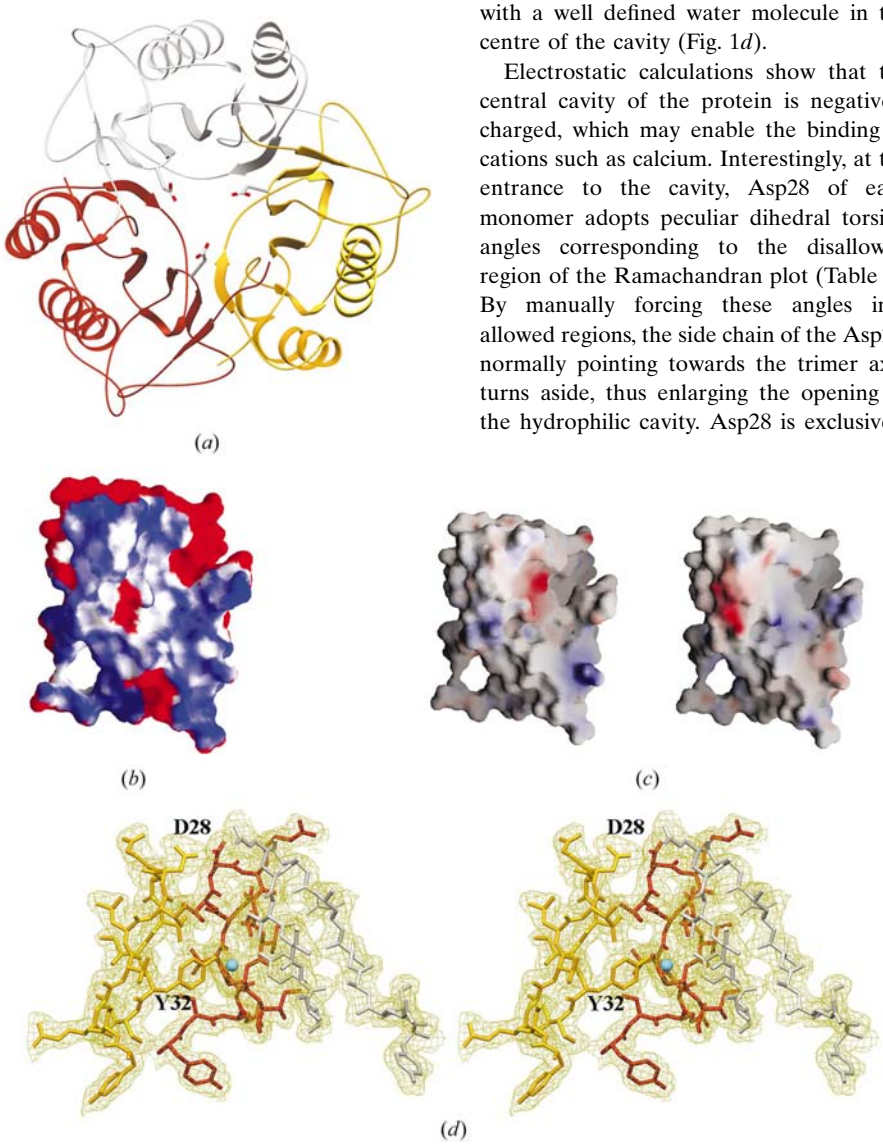

Figure 1
(*a*) Cartoon representation of the UK114 trimer; Asp28 is represented by sticks. (*b*) Analysis of intersubunit contacts in UK114. The molecular surface of a monomer is depicted as follows: red for areas which are not buried by intersubunit contacts and a colour gradient from blue to white for buried areas exhibiting a surface complementarity, with values ranging from 1 (perfect complementarity, blue) to 0 (white). (*c*) Desolvation potential (left) and bound-state subunit–subunit potential (right) mapped on the same UK114 monomer surface. (*d*) Stereo representation of residues 21–33. The $2F_o - F_c$ map is contoured at 1.0σ. Figures were prepared using *SETOR* (Evans, 1993) and *GRASP* (Nicholls *et al.*, 1991).

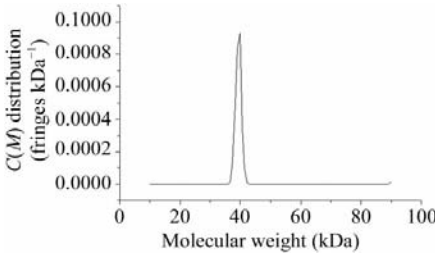

Figure 2
Analytical ultracentrifugation results (sedimentation velocity). The derived $c(M)$ molar-weight distribution for UK114 shows only one species of molecular weight approximately 40 kDa.

conserved in the mammalian members of the YER057c/YIL051c/YjgF protein family. An Asn is found in the *B. subtilis* as well as in the yeast structure, whilst in the homologous *E. coli* this position is occupied by a Gly. These residues adopt similar (φ, ψ) torsion angles as observed in the UK114 structure. Whether UK114 triggers the activation of μ-calpain by releasing calcium in its proximity or alters its trimeric arrangement as a consequence of calcium binding needs to be elucidated.

3.2. Solution studies on UK114

To confirm that UK114 is a trimer in solution, sedimentation-velocity experiments were performed and data were analyzed using the continuous distribution $c(s)$ Lamm equation model (Schuck, 2000). Parameter fitting gave the best $c(s)$ distribution and Fig. 2 reports the $c(M)$ molar-weight distribution [directly derived from $c(s)$ distribution]. The calculated molecular weight for trimeric UK114 is 42 501.81 Da (after removal of the first Met). Our results indicate a unique molecular species in solution with a molecular weight of approximately 40 kDa. This result clearly indicates that goat UK114 exists as a unique species in solution, with a trimeric quaternary structure.

4. Conclusion

The tumour-associated antigen UK114 has proved to be extremely useful in the identification of a variety of human cancers. Our crystallographic and ultracentrifugation results provide strong evidence for a trimeric oligomeric state of the protein in solution and in the crystal structure. This contrasts with earlier suggestions of a dimeric arrangement of the mammalian members of the YER057c/YIL051c/YjgF family. Drug-design investigations performed using the UK114 structure could help to identify naturally occurring or chemically synthesized substances with potential for use in cancer chemotherapy.

We thank Dr G. Capitani for reading the manuscript. Financial support by the Swiss National Science foundation and from the Baugartenstiftung to MGG (CH-8022 Zurich) is gratefully acknowledged. We thank the staff of the SLS PX beamline (Villigen) for support and access to beamtime. We are indebted to Thomas Schalch (ETH, Zurich) for help with the ultracentrifugation measurements.

References

Bartorelli, A., Berra, B., Clemente, C., Liverani, A., Santi, C., Ronchi, S., Botturi, M., Corchia, P., Orefice, S. & Mor, C. (1994). *J. Tumor Marker Oncol.* **9**, 57–64.
Bartorelli, A., Biancardi, C., Cavalca, V., Ferrara, R., Botta, M., Arzani, C., Colombo, I., Berra, B., Ceciliani, F., Ronchi, S. & Bailo, M. (1996). *J. Tumor Marker Oncol.* **11**, 57–61.
Brünger, 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.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Djinovic Carugo, K., Saraste, M. & Oka, T. (1999). *Acta Cryst.* D**55**, 667–668.
Evans, S. V. (1993). *J. Mol. Graph.* **11**, 134–138.
Garcia De La Torre, J., Huertas, M. L. & Carrasco, B. (2000). *Biophys. J.* **78**, 719–730.
Janin, J. (1997). *Nature Struct. Biol.* **4**, 973–974.
Jones, T. A., Zou, J. Y., Cowan, S. W. & Kjeldgaard, M. (1991). *Acta Cryst.* A**47**, 110–119.
Laskowski, R. A., MacArthur, M. W., Moss, D. S. & Thornton, J. M. (1993). *J. Appl. Cryst.* **26**, 283–291.
Lawrence, M. C. & Colman, P. M. (1993). *J. Mol. Biol.* **234**, 946–950.
Lee, L. P. & Tidor, B. (2001). *Protein Sci.* **10**, 362–377.
Melloni, E., Michetti, M., Salamino, F. & Pontremoli, S. (1998). *J. Biol. Chem.* **273**, 12827–12831.
Micheau, O., Thome, M., Schneider, P., Holler, N., Tschopp, J., Nicholson, D., Briand, C. & Grutter, M. G. (2002). *J. Biol. Chem.* **277**, 45262–45171.
Navaza, J. (1994). *Acta Cryst.* A**50**, 157–163.
Nicholls, A., Sharp, K. A. & Honig, B. (1991). *Proteins*, **11**, 281–296.
Oka, T., Tsuji, H., Noda, C., Sakai, K., Hong, Y. M., Suzuki, I., Munoz, S. & Natori, Y. (1995). *J. Biol. Chem.* **270**, 30060–30067.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Parsons, L., Bonander, N., Eisenstein, E., Gilson, M., Kairys, V. & Orban, J. (2003). *Biochemistry*, **42**, 80–89.
Scarsi, M., Majeux, N. & Caflisch, A. (1999). *Proteins*, **37**, 565–575.
Schmiedeknecht, G., Kerkhoff, C., Orso, E., Stohr, J., Aslanidis, C., Nagy, G. M., Knuechel, R. & Schmitz, G. (1996). *Eur. J. Biochem.* **242**, 339–351.
Schuck, P. (2000). *Biophys. J.* **78**, 1606–1619.
Sinha, S., Rappu, P., Lange, S. C., Mantsala, P., Zalkin, H. & Smith, J. L. (1999). *Proc. Natl Acad. Sci. USA*, **96**, 13074–13079.
Volz, K. (1999). *Protein Sci.* **8**, 2428–2437.