Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Expression, purification, crystallization and preliminary X-ray diffraction analysis of the human calcium-binding protein MRP14 (S100A9)

Hiroshi Itou,[a] Ikuko Fujita,[a] Kohtaro Ishikawa,[a] Min Yao,[a] Nobuhisa Watanabe,[a] Masaki Suzuki,[b] Jun Nishihira[b] and Isao Tanaka[a]*

[a]Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan, and [b]Central Research Institute, School of Medicine, Hokkaido University, Sapporo 060-0810, Japan

Correspondence e-mail:
tanaka@castor.sci.hokudai.ac.jp

Received 5 April 2001
Accepted 8 June 2001

MRP14 is a protein that is specifically expressed in myeloid and epithelial cells during the stages of acute or chronic inflammatory states such as rheumatoid arthritis or sarcoidosis. MRP14 has EF-hand motifs as Ca^{2+}-binding sites and belongs to the S100 family of proteins. This paper deals with the sample preparation (cloning, overexpression and purification), crystallization and preliminary crystallographic analysis of recombinant human MRP14. Crystals of MRP14 were obtained by the hanging-drop vapour-diffusion method. MRP14 crystals belong to space group $P2_1$, with unit-cell parameters $a = 57.59$, $b = 178.44$, $c = 61.23$ Å, $\beta = 113.17^\circ$, and diffract to 2.1 Å resolution.

© 2001 International Union of Crystallography
Printed in Denmark – all rights reserved

1. Introduction

Infiltrating macrophages express specific proteins MRP14 and MRP8 in the event of acute or chronic inflammation (Odnik *et al.*, 1987). These proteins were originally isolated from human peripheral blood mononuclear cell cultures as part of a complex using a monoclonal antibody directed against human macrophage migration inhibitory factor (MIF) and were thus named MIF-related proteins (Burmeister *et al.*, 1986). These proteins have two EF-hand motifs as Ca^{2+}-binding sites and belong to the S100 family, members of which have similar amino-acid sequences (Schäfer & Heizmann, 1996). Members of the S100 family of proteins have a strong tendency to dimerize and have been implicated in the Ca^{2+}-dependent regulation of a variety of intracellular activities (Donato, 1999). The S100 family of proteins were found to be expressed in a cell- and tissue-specific manner. Subsequent studies showed that they were associated with different human pathological states such as cancer, neurodegeneration and inflammatory disease (Donato, 1999).

MRP14 and MRP8 are expressed with tight regulation in granulocytes, monocytes, neutrophils and keratinocytes during their differentiation (Lagasse & Clerc, 1988; Zwadlo *et al.*, 1988). Phagocytes expressing these proteins are found in a variety of inflammatory conditions including rheumatoid arthritis and allograft rejections (Goebeler *et al.*, 1994). Inflammatory disorders such as chronic bronchitis and cystic fibrosis are associated with elevated plasma levels of MRP14 and MRP8 (Brun *et al.*, 1994; Roth *et al.*, 1992). These proteins are co-expressed and it has been shown that these proteins associate in a Ca^{2+}-dependent manner to form a non-covalent heterodimer (Teigelkamp *et al.*, 1991). The MRP8/14 heterocomplex exhibits specific biological functions such as inhibition of casein kinase (Murao *et al.*, 1989), fatty-acid binding (Roulin *et al.*, 1999; Siegenthaler *et al.*, 1997) and translocation to cytoskeleton and plasma membrane (Roth *et al.*, 1993; van den Bos *et al.*, 1996) in phagocytes under the inflammatory process. In this complex, MRP14 plays some indispensable roles (Murao *et al.*, 1989; Roth *et al.*, 1993; van den Bos *et al.*, 1996).

MRP14 has a relative molecular mass of 14 kDa and is composed of 114 amino acids. A distinguishing feature of MRP14 is its extended C-terminus, rendering it considerably larger than other S100-family members (with molecular masses of about 10 kDa; Kligman & Hilt, 1988). This C-terminal tail region of MRP14 is homologous in sequence and displays a function similar to that of a region of the plasma glycoprotein high molecular weight kininogen (HMWK; Hessian *et al.*, 1995) and has also been reported to possess complete identity with the N-terminus of the neutrophil immobilizing factor (NIF; Freemont *et al.*, 1989; Watt *et al.*, 1983). A recent report has also revealed that MRP14 selectively activates the β_2 integrin Mac-1 (Newton & Hogg, 1998). These facts suggest that MRP14 plays important roles in immobilizing leukocytes at the endothelial surface and in extravasation of leukocytes into tissues. Independent expression of MRP14 and its functions have also been reported (Aguiar-Passeti *et al.*, 1997; Bhardwaj *et al.*, 1992; Delabie *et al.*, 1990; Goebeler *et al.*, 1994).

MRP14 is a protein that has an indispensable role in Ca^{2+}-dependent functions during inflammation. Understanding of the structure of MRP14 is required in order to understand these functions on the molecular level.

We have previously analysed the crystal structure of human MRP8 (Ishikawa *et al.*, 2000). In this report, we present the first crystallization and X-ray diffraction analysis of human MRP14 (hMRP14) protein.

2. Methods and results

2.1. Cloning, overexpression and purification

The gene encoding human MRP14 has already been identified (Lagasse & Clerc, 1988). The hMRP14 gene was amplified by PCR using human cDNA prepared from human peripheral blood as a template DNA and inserted into a pET-3a vector (Novagen) *via* T-vector sub-cloning (pGEM-T Easy vector, Promega). The vector pET/hMRP14 was transformed into *Escherichia coli* B834 (DE3), the expression host cell. The cells were grown at 310 K. Overexpression of recombinant hMRP14 was induced by 1 m*M* IPTG. After the IPTG induction, the medium was incubated at 295 K for 14 h with shaking in order to prevent the formation of inclusion bodies of MRP14. The cells were harvested by centrifugation at 3500*g* for 20 min at 277 K and resuspended in buffer *A* (50 m*M* Tris–HCl pH 7.5 containing 0.1 m*M* PMSF, 1 m*M* DTT, 5 m*M* $MgSO_4$ and 1 mg DNaseI). The cells were disrupted by a French press (Amico Inc.) at 8.3 MPa and the homogenate was clarified by centrifugation at 14 000*g* for 1 h at 277 K. Ammonium sulfate was slowly added to the supernatant of the cell extract to 60% saturation and the solution was centrifuged at 8000*g* for 20 min at 277 K. The supernatant was then brought to 90% saturation and centrifuged. The protein pellet was resuspended in buffer *B* (50 m*M* Tris–HCl pH 7.5 containing 10 m*M* EDTA, 0.1 m*M* PMSF and 1 m*M* DTT) and dialyzed against the same buffer. MRP14 was purified by anion-exchange chromatography using the Bio-CAD60 system equipped with a QE/L column (Applied Biosystems). The protein was eluted using a linear gradient of 0–1.0 *M* NaCl in a 20 m*M* Tris/bis-tris anion-exchange standard buffer at pH 6.0. The fractions containing the target protein were dialyzed against buffer *C* (20 m*M* MOPS pH 6.8 containing 150 m*M* NaCl, 1 m*M* EDTA and 1 m*M* DTT), concentrated to 10 ml and loaded on a Hi-Load 26/60 Superdex75pg size-exclusion chromatography (SEC) column (Amersham Pharmacia Biotech). MRP14 was eluted as a single peak and the molecular weight estimated from the elution volume suggested that this protein exists as a dimer. The fraction containing MRP14 was pooled and dialyzed against MilliQ water containing 10 m*M* $CaCl_2$ and 1 m*M* DTT. After dialysis, the sample solution was concentrated by ultrafiltration using CentriPlus-3 and Centricon-3 (Amicon Inc.) to a final concentration of 10 mg ml^{-1}. The purity of the purified sample was analysed by MALDI-TOF mass spectrometry (Voyager DE-PRO, Applied Biosystems) and dynamic light scattering (Dyna-Pro, Protein Solutions Inc.).

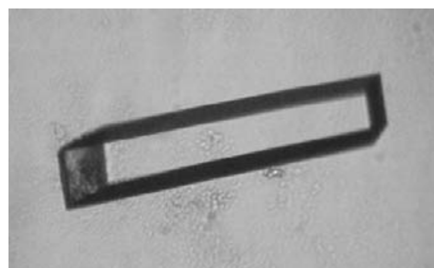

Figure 1
Crystals of hMRP14 as grown by the hanging-drop method under the improved conditions. The dimensions of this crystal are 0.15 × 1.0 × 0.2 mm.

2.2. Crystallization

All crystallization experiments were performed using the hanging-drop vapour-diffusion method in a 24-well tissue-culture Linbro plate (ICN Inc.) at 293 K. The initial crystallization condition screening was carried out using a reservoir consisting of 0.5 ml of Hampton Research Crystal Screens I and II. Each drop contained 2 µl reservoir solution and an equal volume of the protein solution. Crystals were obtained within 72 h under condition number 36 of Crystal Screen I (0.1 *M* Tris–HCl pH 8.5, 8% PEG 8K); these initial crystals were multi-crystal clusters. Further optimization of the conditions to improve crystals was performed. Optimization of the protein concentration and the mixing ratio of the reservoir solution and the protein solution were carried out. Screenings of additives to improve the quality of crystals were also performed using Additive Screens (Hampton Research); some amphiphiles improved MRP14 crystals. Further screening of detergents was carried out and the best single crystals were finally obtained with 0.1 *M* Tris–HCl pH 7.8–8.2, 1–2%(*w*/*v*) PEG 6K and 0.25–1.75%(*w*/*v*) CHAPS (Fig. 1).

Table 1
Summary of crystallographic data.

Values in parentheses refer to the outer resolution shell.

Beamline	BL44B2
Wavelength (Å)	0.70000
Resolution (Å)	40–2.1 (2.21–2.1)
Space group	$P2_1$
Unit-cell parameters	a = 57.59, b = 178.44, c = 61.23, β = 113.17
No. of reflections	251869 (37031)
Unique reflections	65554 (9583)
Completeness (%)	99.7 (99.7)
Redundancy	3.8 (3.9)
$I/\sigma(I)$	6.5 (2.5)
$R_{\rm meas}$ (%)	6.6 (33.9)

† $R_{\rm meas} = \sum_h [N/(N-1)]^{1/2} \sum_j |\langle I(h)\rangle - I(h)_j| / \sum_h \sum_j \langle I(h)\rangle$, where N is the redundancy.

2.3. Data collection and preliminary analysis

X-ray diffraction data were collected to 2.1 Å resolution at 100 K by flash-cooling

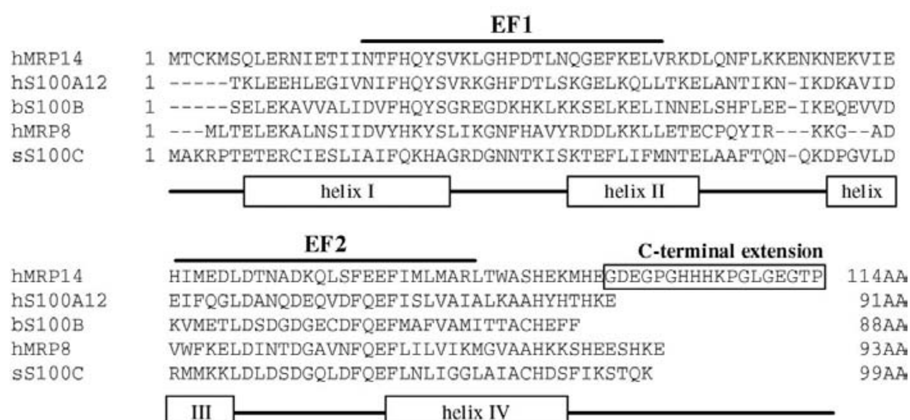

Figure 2
Amino-acid sequence alignment of hMRP14 and search models for the MR structure analysis. The hMRP14 is larger than other proteins because of its unique long C-terminal extension.

after soaking in the cryoprotectant solution with 30%(*v*/*v*) glycerol on the SPring-8 beamline BL44B2. Data were processed using *MOSFLM* (Leslie, 1993) and *SCALA* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). A summary of the data is shown in Table 1. The space group of this crystal was assigned as $P2_1$, with unit-cell parameters $a = 57.59$, $b = 178.44$, $c = 61.23$ Å, $\beta = 113.17°$. The asymmetric unit contains four dimers of MRP14, giving a crystal volume per protein mass (V_M; Matthews, 1968) of 2.7 $Å^3\,Da^{-1}$ and a solvent content of 54.9%.

We subsequently attempted to solve the MRP14 structure by the molecular-replacement (MR) method using other S100-family proteins as a search model with the program *AMoRe* (Navaza, 1994) from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). Search models were selected from the X-ray crystal structures of the S100 family of proteins based on their sequence similarity to hMRP14. The models used were S100C (PDB code 1qls; Réty *et al.*, 2000), MRP8 (S100A8, PDB code 1mr8; Ishikawa *et al.*, 2000), S100B (1mho; Matsumura *et al.*, 1998) and S100A12 (1e8a; Moroz *et al.*, 2001) (27, 30, 34 and 43% identity, respectively, excluding C-terminal extension). However, these attempts did not yield any reasonable results. In comparison with other members of the S100 family (Fig. 2), MRP14 has a long C-terminal extension, which may in part explain the difficulty in analysing the structure of this protein by the MR method. Preparation of Se-Met MRP14 crystals for use with the multiple-wavelength anomalous dispersion (MAD) method is now in progress.

References

Aguiar-Passeti, T., Postol, E., Sorg, C. & Mariano, M. (1997). *J. Leukoc. Biol.* **62**, 852–858.

Bhardwaj, R. S., Zotz, C., Zwadlo, G., Roth, J., Goebeler, M., Mahnke, K., Falk, M., Meinardus-Hager, G. & Sorg, C. (1992). *Eur. J. Immunol.* **22**, 1891–1897.

Bos, C. van den, Roth, J., Koch, H. G., Hartmann, M. & Sorg, C. (1996). *J. Immunol.* **156**, 1247–1254.

Brun, J. G., Jonsson, R. & Haga, H. J. (1994). *J. Rheumatol.* **21**, 733–738.

Burmeister, G., Tarcsay, L. & Sorg, C. (1986). *Immunobiology*, **171**, 461–474.

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

Delabie, J., de Wolf-Peeters, C., van den Oord, J. J. & Desmet, V. J. (1990). *Clin. Exp. Immunol.* **81**, 123–126.

Donato, R. (1999). *Biochim. Biophys. Acta*, **1450**, 191–231.

Freemont, P., Hogg, N. & Edgeworth, J. (1989). *Nature (London)*, **339**, 516.

Goebeler, M., Roth, J., Burwinkel, F., Vollmer, E., Bocker, W. & Sorg, C. (1994). *Transplantation*, **58**, 355–361.

Hessian, P. A., Wilkinson, L. & Hogg, N. (1995). *FEBS Lett.* **371**, 271–275.

Ishikawa, K., Nakagawa, A., Tanaka, I., Suzuki, M. & Nishihira, J. (2000). *Acta Cryst.* D**56**, 559–566.

Kligman, D. & Hilt, D. C. (1988). *Trends Biochem. Sci.* **13**, 437–443.

Lagasse, E. & Clerc, R. G. (1988). *Mol. Cell Biol.* **8**, 2402–2410.

Leslie, A. G. W. (1993). *Proceedings of the CCP4 Study Weekend. Data Collection and Processing*, edited by L. Sawyer, N. Isaacs & S. Bailey, pp. 44–51. Warrington: Daresbury Laboratory.

Matsumura, H., Shiba, T., Inoue, T., Harada, S. & Kai, Y. (1998). *Structure*, **6**, 233–241.

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

Moroz, O. V., Antson, A. A., Murshudov, G. N., Maitland, N. J., Dodson, G. G., Wilson, K. S., Skibshøj, I., Lukanidin, E. M. & Bronstein, I. B. (2001). *Acta Cryst.* D**57**, 20–29.

Murao, S., Collart, F. R. & Huberman, E. (1989). *J. Biol. Chem.* **264**, 8356–8360.

Newton, R. A. & Hogg, N. (1998). *J. Immunol.* **160**, 1427–1435.

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

Odnik, K., Cerletti, N., Brüggen, J., Clerc, R. G., Tarcsay, L., Zwadlo, G., Gerhards, G., Schlegel, R. & Sorg, C. (1987). *Nature (London)*, **330**, 80–82.

Réty, S., Osterloh, D., Arie, J., Tabaries, S., Seeman, J., Russo-Marie, F., Gerke, V. & Lewit-Bentley, A. (2000). *Structure*, **8**, 175–184.

Roth, J., Burwinkel, F., van den Bos, C., Goebeler, M., Vollmer, E. & Sorg, C. (1993). *Blood*, **82**, 1875–1883.

Roth, J., Teigelkamp, S., Wilke, M., Grun, L., Tummler, B. & Sorg, C. (1992). *Immunobiology*, **186**, 304–314.

Roulin, K., Hagens, G., Hotz, R., Saurat, J. H., Veerkamp, J. H. & Siegenthaler, G. (1999). *Exp. Cell Res.* **247**, 410–421.

Schäfer, B. W. & Heizmann, C. W. (1996). *Trends Biochem. Sci.* **21**, 134–140.

Siegenthaler, G., Roulin, K., Chatellard-Gruaz, D., Hotz, R., Saurat, J. H., Hellman, U. & Hagens, G. (1997). *J. Biol. Chem.* **272**, 9371–9377.

Teigelkamp, S., Bhardwaj, R. S., Roth, J., Meinardus-Hager, G., Karas, M. & Sorg, C. (1991). *J. Biol. Chem.* **266**, 13462–13467.

Watt, K. W., Brightman, I. L. & Goetzl, E. J. (1983). *Immunobiology*, **48**, 79–86.

Zwadlo, G., Brüggen, J., Gerhards, G., Schlegel, R. & Sorg, C. (1988). *Clin. Exp. Immunol.* **72**, 510–515.