Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and preliminary X-ray analysis of the small subunit (R2F) of native ribonucleotide reductase from *Corynebacterium ammoniagenes*

Hideaki Ogata,[a] Patrick Stolle,[b] Matthias Stehr,[b] Georg Auling[b] and Wolfgang Lubitz[a]*

[a]Max-Planck-Institut für Bioanorganische Chemie, Stiftstrasse 34-36, D-45470 Mülheim an der Ruhr, Germany, and [b]Institut für Mikrobiologie, Leibniz Universität Hannover, Schneiderberg 50, D-30167 Hannover, Germany

Correspondence e-mail: lubitz@mpi-muelheim.mpg.de

Received 22 June 2009
Accepted 22 July 2009

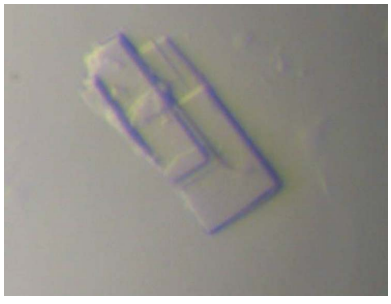
Ribonucleotide reduction, the unique step in DNA-precursor biosynthesis, involves radical-dependent redox chemistry and diverse metallo-cofactors. The metallo-cofactor (R2F) encoded by the *nrdF* (*n*ucleotide *red*uction) gene in *Corynebacterium ammoniagenes* ATCC 6872 was isolated after homologous expression and a new crystal form of ribonucleotide reductase R2F was obtained. R2F was crystallized at 277 K using the vapour-diffusion method with PEG as the precipitating agent. A data set was collected to 1.36 Å resolution from a single crystal at 100 K using synchrotron radiation. The crystal belonged to space group *C*2, with unit-cell parameters $a = 96.21$, $b = 87.68$, $c = 83.25$ Å, $\beta = 99.29^\circ$. The crystal contained two molecules per asymmetric unit, with a Matthews coefficient (V_M) of 2.69 Å^3 Da^{-1}; the solvent content was estimated to be 54.3%. X-ray fluorescence spectroscopy and MAD diffraction data indicated the presence of manganese in the molecule and the absence of iron.

1. Introduction

DNA synthesis requires a continuous supply of deoxyribonucleotides. These are provided by ribonucleotide reductase (RNR), which catalyzes the reduction of ribonucleotides to 2′-deoxyribonucleotides (Follmann, 2004). Three classes of RNR (EC 1.17.4) have been described, which differ in their cofactor or metal requirements (Eklund *et al.*, 2001). While the redox chemistry in class I appears to be heterogeneous, class II enzymes use cobalamin-dependent radical generation (Licht *et al.*, 1996) and a stable glycyl radical is formed by class III enzymes with the help of an iron–sulfur protein and *S*-adenosylmethionine (Logan *et al.*, 1999). Based on the discovery of a new pattern of allosteric regulation (Eliasson *et al.*, 1996) and differences in metal-site structure and radical identity (Högbom *et al.*, 2004), class I has been further divided into class Ia enzymes, with the *Escherichia coli* prototype encoded by *nrdAB* genes, class Ib enzymes, with the *Salmonella typhimurium* prototype encoded by *nrdEF* genes, and class Ic, which consists of *Chlamydia trachomatis* RNR and related enzymes.

The nature of the Mn-RNR from the Gram-positive bacterium *Corynebacterium ammoniagenes* ATCC 6872 (Willing *et al.*, 1988; Griepenburg *et al.*, 1998) as a distinct class IV enzyme (Follmann, 2004) has been disputed by its allocation to class Ib from heterologous expression in *E. coli* (Fieschi *et al.*, 1998; Huque *et al.*, 2000). The *C. ammoniagenes* Mn-RNR consists of a monomeric catalytic R1E subunit (81 kDa) and a dimeric metallo-cofactor (R2F; 38 kDa) which contains manganese and a tyrosyl radical (Griepenburg *et al.*, 1996).

To date, several crystal structures of *E. coli* and mouse R2 or *Mycobacterium tuberculosis* and *C. ammoniagenes* R2F expressed in *E. coli* have been studied in various oxidation states and forms obtained by site-directed mutagenesis or by metal substitution (Nordlund *et al.*, 1990; Logan *et al.*, 1996; Voegtli *et al.*, 2000; Högbom



et al., 2002, 2003; Uppsten *et al.*, 2004; Strand *et al.*, 2004; Lendzian, 2005). Recently, the structure of the Mn/Fe metallo-cofactor of the chlamydial RNR has been studied by extended X-ray absorption fine-structure spectroscopy (Younker *et al.*, 2008).

In order to determine the metal composition and the coordination of its metal centre, we purified the metallo-cofactor of the native Mn-RNR from *C. ammoniagenes*. The R2F subunit obtained from homologous expression from its original source, *C. ammoniagenes* ATCC 6872 (Schimpff-Weiland *et al.*, 1981; Willing *et al.*, 1988), showed a high RNR activity in a biochemical complementation assay with the large catalytic R1E subunit (Stolle *et al.*, in preparation). Here, we report the crystallization and preliminary X-ray analysis of *C. ammoniagenes* R2F. X-ray fluorescence spectroscopy (XFS) and MAD diffraction data indicated the presence of manganese in the molecule.

2. Materials and methods

2.1. Purification

The *nrdF* gene of *C. ammoniagenes* strain ATCC 6872 was homologously expressed using the vector pOCA2 and the R2F protein was prepared as described elsewhere (Stolle *et al.*, in preparation). *C. ammoniagenes* pOCA2 was grown aerobically in LB medium in the presence of chloramphenicol (15 µg ml^{-1}) in a 10 l bioreactor (Biostat V, B-Braun) at 303 K with aeration at 8 l min^{-1} and agitation at 350 rev min^{-1} until an OD_{600} of 6 was reached. After a 4 h incubation in the presence of 1 m*M* IPTG and 25 µ*M* Mn^{2+}, the biomass was harvested and resuspended in 85 m*M* potassium phosphate buffer containing 2 m*M* DTT pH 6.6 as a standard buffer supplemented with 10 m*M* KCl. The homogenate resulting from disruption in a French pressure-cell press was precipitated with ammonium sulfate (472 mg ml^{-1}), dissolved in the standard buffer as above and desalted on a HiTrap desalting column (GE Healthcare). A 20 ml UNOsphere Q anion-exchange column (GE Healthcare) was used for stepwise gradient elution at 350 m*M* KCl in standard buffer with 2 m*M* magnesium acetate and the eluate was applied onto a Superdex 200 column (GE Healthcare) using the standard buffer. The resulting 30–45 kDa eluate was dialyzed and applied onto a final MonoQ HR 5-5 anion-exchange chromatography column (GE Healthcare) and eluted at 490 m*M* KCl, 50 m*M* Tris–HCl, 2 m*M* DTT, 15% glycerol pH 7.5. The metallo-cofactor was assessed for purity by SDS–PAGE and confirmed by Western blotting using an anti-R2F antibody. The protein was quantified using the Bradford protein assay (Roth). Amicon Ultra-4 devices (10 kDa molecular-weight cutoff, Millipore) were used for buffer exchange and concentration prior to crystallization. The native R2F protein, purified to homogeneity, was stored in standard buffer with 15%(*v*/*v*) glycerol in the gaseous phase of liquid nitrogen.

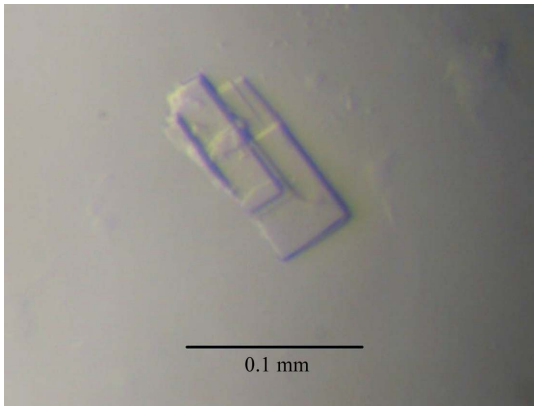


Figure 1
Crystal of ribonucleotide reductase R2F from *C. ammoniagenes*.

Table 1
X-ray data-collection statistics.

Values in parentheses are for the highest resolution shell.

	Native	Peak	Remote
Wavelength (Å)	1.00000	1.89000	1.89900
Space group	*C*2	*C*2	*C*2
Unit-cell parameters			
a (Å)	96.21	95.97	96.08
b (Å)	87.68	87.55	87.64
c (Å)	83.25	83.29	83.32
β (°)	99.29	99.36	99.36
Resolution (Å)	31.17–1.36 (1.41–1.36)	33.01–1.84 (1.91–1.84)	33.03–1.85 (1.92–1.85)
No. of observed reflections	502993	206100	204123
No. of unique reflections	140012	58827	58039
R_{merge}†	0.073 (0.477)	0.077 (0.341)	0.073 (0.382)
Completeness (%)	96.1 (72.7)	99.7 (100.0)	99.8 (99.8)
$\langle I/\sigma(I)\rangle$	13.4 (2.1)	28.4 (4.1)	13.6 (3.7)
Redundancy	3.6	3.5	3.5

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the *i*th observation and $\langle I(hkl)\rangle$ is the mean intensity of the reflections.

2.2. Crystallization

Crystallization of the native RNR metallo-cofactor from *C. ammoniagenes* was carried out using the sitting-drop vapour-diffusion method at 277 K under aerobic conditions. The protein droplets were prepared by mixing 10 µl RNR solution and 10 µl reservoir buffer solution and were set up in a Cryschem plate (Hampton Research, California, USA) with 1 ml reservoir solution. Crystals were obtained after three weeks. Crystals suitable for diffraction experiments were obtained under the following conditions: 0.1 *M* sodium citrate, 27.5% PEG 4000, 0.05 *M* ammonium acetate pH 6.0 and 0.1 *M* ammonium acetate pH 7.0 with 0.05 *M* Tris–HCl pH 7.5 buffer. The crystal dimensions were typically 0.1 × 0.05 × 0.01 mm (Fig. 1).

2.3. Data collection and analysis

In order to collect the data at cryogenic temperature, the crystal was frozen in liquid nitrogen and mounted on the goniostat under a nitrogen-gas stream at 100 K. All data were collected on beamline BL41XU at SPring-8 (Hyogo, Japan). An MX225HE detector (MAR Research, Germany) was used. A complete native data set was collected to 1.36 Å resolution at an X-ray wavelength of 1.00000 Å. For the native data set, 180 frames of 1 s exposure time and 1.0° oscillation were collected. The distance between the crystal and the detector was maintained at 190 mm. In addition, two-wavelength Mn-MAD data sets were collected to 1.84 and 1.85 Å resolution, respectively. The wavelength of the X-rays was chosen as 1.89000 Å (peak) and 1.89900 Å (remote), respectively. For the Mn-MAD data set, 180 frames of 1 s exposure time each and 1.0° oscillation were collected. The distance between the crystal and the detector was maintained at 85 mm. Diffraction images were indexed, integrated and scaled using the program *HKL*-2000 (Otwinowski & Minor, 1997). The molecular replacement and the initial refinement were carried out using the programs *MOLREP* (Vagin & Isupov, 2001) and *REFMAC* (Vagin *et al.*, 2004), respectively, from the *CCP*4 program package (Collaborative Computational Project, Number 4, 1994). The conditions of the data collection and the results obtained are

summarized in Table 1. XFS experiments for the determination of the metal components were carried out on beamline BL14.2 at BESSY II (Berlin, Germany).

3. Results and discussion

The new crystal form of RNR R2F from *C. ammoniagenes* was obtained at 277 K using the sitting-drop vapour-diffusion method with PEG as a precipitating agent. The crystals diffracted to 1.36 Å resolution and belonged to space group *C*2, with unit-cell parameters $a = 96.21$, $b = 87.6$, $c = 83.25$ Å, $\beta = 99.29^{\circ}$. The calculated Matthews coefficient (V_M) of 2.69 Å^3 Da^{-1} with a solvent content of 54.3% indicates the presence of two molecules in the asymmetric unit.

XFS experiments were carried out in order to determine the metal components of the RNR R2F subunit. No fluorescence was observed near the energy range of the metals Fe, Co, Ni and Cu (data not shown). However, fluorescence corresponding to Mn was observed. Furthermore, the Mn-MAD method was applied to a crystal of RNR R2F at X-ray wavelengths of 1.89000 and 1.89900 Å. The anomalous difference Patterson map showed peaks corresponding to the Mn atoms. The results showed that Mn is the metal component of *C. ammoniagenes* RNR R2F.

Initial phases were calculated from the Mn-MAD data set using the *CCP*4 program suite. The phase calculation confirmed the presence of two molecules in the asymmetric unit. Subsequently, the molecular-replacement method was applied using the program *MOLREP*. The data for the oxidized state of the Fe-containing RNR R2F from *C. ammoniagenes* (PDB code 1kgp) were used as coordinates for the search model. After the calculation of the electron-density map using the molecular-replacement solution, a homodimer in the asymmetric unit was observed. The electron-density map shows the Mn site to be near to the tyrosyl radical Tyr115. Model building and refinement are now in progress.

We thank the staff of beamline BL41XU at SPring-8 (Hyogo, Japan) and beamline BL14.2 at BESSY II (Berlin, Germany) for their assistance during data collection. This work was supported by the Max Planck Society.

References

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Eklund, H., Uhlin, U., Färnegårdh, M., Logan, D. T. & Nordlund, P. (2001). *Prog. Biophys. Mol. Biol.* **77**, 177–268.
Eliasson, R., Pontis, E., Jordan, A. & Reichard, P. (1996). *J. Biol. Chem.* **271**, 26582–26587.
Fieschi, F., Torrents, E., Toulokhonova, L., Jordan, A., Hellman, U., Barbe, J., Gibert, I., Karlsson, M. & Sjöberg, B. M. (1998). *J. Biol. Chem.* **273**, 4329–4337.
Follmann, H. (2004). *Chem. Soc. Rev.* **33**, 225–233.
Griepenburg, U., Blasczyk, K., Kappl, R., Hüttermann, J. & Auling, G. (1998). *Biochemistry*, **37**, 7992–7996.
Griepenburg, U., Lassmann, G. & Auling, G. (1996). *Free Radic. Res.* **24**, 473–481.
Högbom, M., Galander, M., Andersson, M., Kolberg, M., Hofbauer, W., Lassmann, G., Nordlund, P. & Lendzian, F. (2003). *Proc. Natl Acad. Sci. USA*, **100**, 3209–3214.
Högbom, M., Huque, Y., Sjöberg, B. M. & Nordlund, P. (2002). *Biochemistry*, **41**, 1381–1389.
Högbom, M., Stenmark, P., Voevodskaya, N., McClarty, G., Gräslund, A. & Nordlund, P. (2004). *Science*, **305**, 245–248.
Huque, Y., Fieschi, F., Torrents, E., Gibert, I., Eliasson, R., Reichard, P., Sahlin, M. & Sjoberg, B. M. (2000). *J. Biol. Chem.* **275**, 25365–25371.
Lendzian, F. (2005). *Biochim. Biophys. Acta*, **1707**, 67–90.
Licht, S., Gerfen, G. J. & Stubbe, J. (1996). *Science*, **271**, 477–481.
Logan, D. T., Andersson, J., Sjöberg, B. M. & Nordlund, P. (1999). *Science*, **283**, 1499–1504.
Logan, D. T., Su, X.-D., Åberg, A., Regnström, K., Hajdu, J., Eklund, H. & Nordlund, P. (1996). *Structure*, **4**, 1053–1064.
Nordlund, P., Sjöberg, B. M. & Eklund, H. (1990). *Nature (London)*, **345**, 593–598.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Schimpff-Weiland, G., Follmann, H. & Auling, G. (1981). *Biochem. Biophys. Res. Commun.* **102**, 1276–1282.
Strand, K. R., Karlsen, S., Kolberg, M., Rohr, A. K., Görbitz, C. H. & Andersson, K. K. (2004). *J. Biol. Chem.* **279**, 46794–46801.
Uppsten, M., Davis, J., Rubin, H. & Uhlin, U. (2004). *FEBS Lett.* **569**, 117–122.
Vagin, A. A. & Isupov, M. N. (2001). *Acta Cryst.* D**57**, 1451–1456.
Vagin, A. A., Steiner, R. A., Lebedev, A. A., Potterton, L., McNicholas, S., Long, F. & Murshudov, G. N. (2004). *Acta Cryst.* D**60**, 2184–2195.
Voegtli, W. C., Khidekel, N., Baldwin, J., Ley, B. A., Bollinger, J. M. Jr & Rosenzweig, A. C. (2000). *J. Am. Chem. Soc.* **122**, 3255–3261.
Willing, A., Follmann, H. & Auling, G. (1988). *Eur. J. Biochem.* **170**, 603–611.
Younker, J. M., Krest, C. M., Jiang, W., Krebs, C., Bollinger, J. M. Jr & Green, M. T. (2008). *J. Am. Chem. Soc.* **130**, 15022–15027.