Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary crystallographic analysis of a new crystal form of arylsulfatase A isolated from human placenta

Krzysztof Lewinski,[a]* Maksymilian Chruszcz,[a] Dorota Książek[b] and Piotr Laidler[b]

[a]Faculty of Chemistry, Jagiellonian University, 30-060 Kraków, Poland, and [b]Institute of Medical Biochemistry, Collegium Medicum, Jagiellonian University, 31-034 Kraków, Poland

Correspondence e-mail: lewinski@chemia.uj.edu.pl

Received 12 October 1999
Accepted 28 February 2000

Depending on pH, arylsulfatase A exists in solution as a dimer or as an octamer. The enzyme isolated from human placenta was crystallized at pH 5.4 in a new crystal form with space group *C*2, unit-cell parameters $a = 154.0$, $b = 190.3$, $c = 112.5$ Å, $\beta = 122.4^\circ$ and four subunits in the asymmetric unit. At pH 6.5–6.7, tetragonal crystals are obtained that are isomorphous to the known crystals of recombinant arylsulfatase A obtained at pH 5.0–5.4. The crystal structure of both forms was determined by the molecular-replacement method. The monoclinic crystals contain octamers of the same type as found in the tetragonal form.

1. Introduction

Arylsulfatase A (ASA; E.C. 3.1.6.1) is one of the most intensely studied sulfatases present in the human organism. Its major physiological function is the hydrolysis of sulfate–ester bonds in sulfated glycolipids. ASA, like other members of the sulfatase family, requires post-translational modification of a conserved cysteine residue to a formylglycine in order to achieve catalytic activity (Schmidt *et al.*, 1995; Waldow *et al.*, 1999). The absent or reduced activity of this lysosomal enzyme causes various onsets of a neurological disease, metachromatic leukodystrophy (Kolodny & Fluharty, 1995). On the other hand, an increase of its concentration and/or enzymatic activity in body fluids has been observed in patients with some types of cancer (Laidler *et al.*, 1991; Honke *et al.*, 1995). Mature ASA is a 489 amino-acid N-glycoprotein. The enzyme from human placenta has recently been shown to possess almost exclusively high mannose-type glycans (Laidler & Lityńska, 1997) attached to two or three asparagines.

The ASA chain is, presumably, non-intentionally cleaved proteolytically between residues 444–445 and/or 447–448. The 7 kDa C-terminal part dissociates at reducing conditions (Fujii *et al.*, 1992); therefore, on a polyacrylamide gel, two non-identical subunits of molecular masses around 59 and 54 kDa are observed (Laidler *et al.*, 1994).

Using the equilibrium ultracentrifugation method, Nichol & Roy (1965) showed that in 0.10 ionic strength buffer ASA from ox liver exists as a dimer of molecular weight 107 kDa at pH 7.5 or as an octamer of molecular weight 411 kDa at pH 5.0. At intermediate pH values, the different oligomeric forms of ASA coexist. The equilibrium depends both on pH and ionic strength, as an increase of ionic strength to 2.0 resulted in the formation of tetramers even at pH 7.5. Similar behaviour was also observed for ASA from human liver, for which pH-dependent oligomerization at pH 8.1 and 5.0 was reported (Draper *et al.*, 1976).

The crystal structure of recombinant ASA expressed in BHK cells was determined recently (Lukatela *et al.*, 1998). The Fourier maps at the active site in the vicinity of formylglycine showed density which was interpreted as disorder of the aldehyde group with a possible contribution from aldehyde hydrate. The postulated mechanism of ester hydrolysis in which formylglycine in the active site first forms aldehyde hydrate was different to that proposed for arylsulfatase B (Bond *et al.*, 1997).

It was postulated that the tetragonal crystals of recombinant ASA contain the octameric form of the enzyme. This hypothesis is consistent with the fact that the crystals were obtained at pH 5.0–5.4, where the octameric form of ASA is expected. Using different crystallization conditions, we have obtained isomorphous crystals at pH 6.5–6.7, where the dimeric form should be present in solution. It is likely that the crystallization process selects for the octamers; however, it cannot be excluded that the octamers observed in the tetragonal crystals do not correspond to those existing in solution, especially as we have obtained a new monoclinic crystal form at acidic pH. Here, we report the crystallization conditions of both crystal forms and the preliminary structure of the monoclinic crystals of arylsulfatase A isolated from human placenta.

2. Materials and methods

ASA from human placenta was purified to homogeneity, immunochemically identified and assayed using procedures described previously (Laidler *et al.*, 1985, 1988). The

protein concentration was determined according to Lowry *et al.* (1951). The solution used for crystallization experiments contained 5.8 mg ml^{-1} ASA in 10 m*M* Tris–HCl buffer pH 7.3. Crystallization experiments were performed by the hanging-drop vapor-diffusion method using standard 24-well crystallization plates. Typically, 3–5 µl of the protein solution was mixed with an equal volume of reservoir solution. Preliminary crystallization trials were conducted by the sparse-matrix sampling method (Jancarik & Kim, 1991) using a Hampton Crystal Screen kit. Two distinct forms of crystals suitable for X-ray diffraction were obtained after a period of 4–6 weeks. The first crystal form, tetragonal bipyramids of edges up to 0.5 mm, grew from 7% PEG 8000 and 0.1 *M* $CaAc_2$ in 0.1 *M* cacodylate buffer pH 6.5–6.7. The second crystal form, monoclinic plates of dimensions up to 0.5 × 0.2 × 0.2 mm, was obtained from 7% PEG 8000 and 0.2 *M* $CaCl_2$ in 0.1 *M* cacodylate buffer pH 5.4.

X-ray diffraction data for both crystal forms were measured at room temperature with a MAR 300 mm image-plate detector and Cu *Kα* radiation from a rotating-anode generator. Frames of 1° oscillation angle were collected with an exposure time of 15 min per frame. The *DENZO* and *SCALEPACK* programs from the *HKL* package (Otwinowski & Minor, 1997) were used to determine the unit-cell parameters and process the data.

The tetragonal crystals diffracted to 2.7 Å resolution and belonged to the space group *I*422, with unit-cell parameters a = 131.5, c = 192.1 Å. They were isomorphous to those obtained under different conditions by Lukatela *et al.* (1998) and their structure was determined by the molecular-replacement method.

Fresh monoclinic crystals diffracted to 2.7 Å resolution but decayed very rapidly during data collection and only a partial data set was collected. In the resolution range 40.0–3.20 Å, 56 667 reflections were observed, which reduced to 30 268 unique reflections with an $R_{\rm merge}$ of 0.147 and a completeness of 67.4%. The space group is *C*2 and the unit-cell parameters are a = 154.0, b = 190.3, c = 112.5 Å, β = 122.4°. Assuming four subunits of molecular weight 59 kDa in the asymmetric part of the unit cell, the volume-to-mass ratio is 2.9 Å^3 Da^{-1}.

The monoclinic structure was solved by the molecular-replacement method using *AMoRe* (Navaza, 1994). A dimer of ASA was generated by applying twofold symmetry to the protomer from the tetragonal structure (PDB code 1auk; Lukatela *et al.*, 1998) and was used as a search model. The rotation function was calculated with diffraction data in the resolution range 8.0–4.5 Å using a Patterson radius of 40 Å. The rotation function gave eight solutions with correlation coefficients (CC) in the range 21.1–23.5%, with the next best solution having a CC of 7.3%. A translation search performed over the same resolution range using the Crowther & Blow (1967) translation function combined with analysis of packing revealed that an asymmetric part of the unit cell contains four ASA chains. Two of them form a dimer in a general position and the others are halves of two dimers positioned on a crystallographic twofold axis. The model was subjected to further refinement using *X-PLOR* (Brünger, 1992). One cycle of rigid-body, simulated-annealing, positional and *B*-factor refinement using data in the resolution range 8.0–3.2 Å and NCS restraints yielded an R value of 0.233 and $R_{\rm free}$ of 0.299 (for 5% of data), confirming the correctness of the solution.

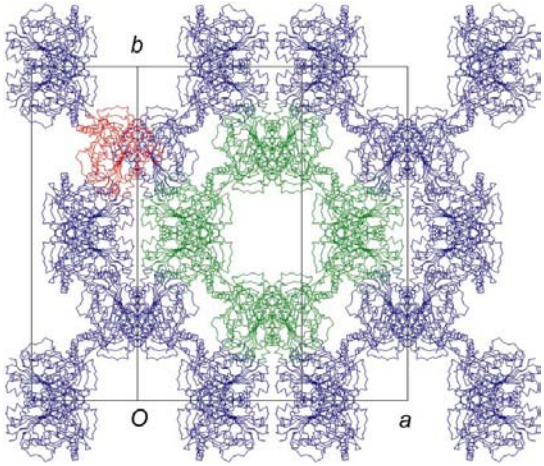

Figure 1
Molecular packing in one layer of the unit cell projected along the c* axis. An octamer of the first type is shown in green and one monomer of the dimer positioned on the twofold axis is shown in red.

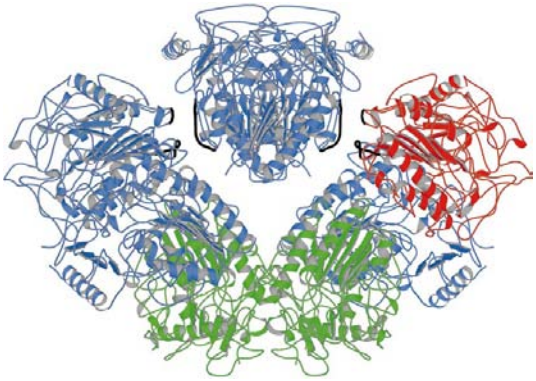

Figure 2
Packing of dimers in the octameric assembly of the second type centred at (0, 0.5, 0). The subunits are coloured as in Fig. 1. Loops involved in intermolecular contacts between dimers are shown in black. The viewing direction is approximately perpendicular to the (01$\bar{1}$) plane.

3. Results and discussion

In the monoclinic structure, the ASA dimers are positioned in layers parallel to the *ab* plane (Fig. 1). Analysis of the molecular packing revealed two types of octameric assemblies. The first type is consistent with the octamers proposed in the tetragonal structure (Lukatela *et al.*, 1998). In this assembly, the contact regions between the dimers are relatively small and involve some hydrophobic interactions, three pairs of hydrogen bonds between amino acids and several hydrogen bonds mediated by water molecules. In the monoclinic structure, the first type of octamer is centred at (0.5, 0.5, 0) and has approximate non-crystallographic symmetry 422. Contacts between dimers are consistent with those proposed by Lukatela *et al.* (1998); however, their details may be different owing to the absence of the strict crystallographic symmetry present in the tetragonal structure. The average calculated buried surface area between monomers is approximately 400 Å^2.

The second octameric assembly of non-crystallographic symmetry 222 centred at (0, 0.5, 0) could be formed from four dimers, each of them belonging to a separate octamer of the first type. Intermolecular contacts involving hydrogen bonds between dimers in this assembly are possible only for a few residues in loops 319–324 and 363–370. The large distance between dimers excludes other direct interactions except those mediated by water molecules (Fig. 2). The average buried surface area calculated for this type of octamer is approximately 140 Å^2 per monomer, significantly less than for the first type.

This analysis supports the structure of octamers proposed by Lukatela *et al.* (1998). The fact that the octamers of the first type

are observed in two different molecular-packing systems strongly argues that they are the correct quaternary structure occurring in solution. As this structure was also observed in crystals obtained at pH 6.7, it indicates that at this pH the octamers are more stable in solution than was considered previously or that the presence of the precipitant and salt shifts the equilibrium toward the aggregates of higher molecular weight. This is in agreement with the observation that the net charge preventing ASA from oligomerization can be damped either by lowering the pH or by increasing the ionic strength of the solution (Nichol & Roy, 1965).

The determination of the detailed structure of the monoclinic crystals, especially with better than present resolution, is still highly desirable. Low symmetry and different crystallization conditions may help to determine the state of the post-translationally modified cysteine. This form should also allow us to determine the asymmetric features of ASA molecules, especially in the contact region where structure disorder, probably resulting from the imposed crystallographic symmetry, was observed in the tetragonal form. It may also help to resolve the differences between the different glycoforms present in the native enzyme which were absent in recombinant ASA.

We are grateful to Lukasz Lebioda for critical reading of the manuscript and valuable suggestions, Maria Łabędź for help with protein isolation and purification and to Mariusz Jaskólski for making possible data collection at the Center for Biocrystallographic Research, Polish Academy of Sciences, Poznan. This work was supported by the Polish State Committee on Research (KBN), Jagiellonian University, Collegium Medicum, No. 501/Pk/3/L.

References

Bond, C. S., Clements, P. R., Ashby, S. J., Collyer, C. A., Harrop, S. J., Hopwood, J. J. & Guss, J. M. (1997). *Structure*, **5**, 277–289.

Brünger, A. T. (1992). *X-PLOR Version* 3.1. *A System for X-ray Crystallography and NMR.* Yale University, Connecticut, USA.

Crowther, R. A. & Blow, D. M. (1967). *Acta Cryst.* **23**, 544–549.

Draper, R. K., Fiskum, G. M. & Edmond, J. (1976). *Arch. Biochem. Biophys.* **177**, 525–538.

Fujii, T., Kobayashi, T., Honke, K., Gasa, S., Ishikawa, M., Shimitzu, T. & Makita, A. (1992). *Biochim. Biophys. Acta*, **1122**, 93–98.

Honke, K., Fujii, T. & Makita, A. (1995). *Cancer J.* **8**, 139–143.

Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.

Kolodny, E. H. & Fluharty, A. L. (1995). *The Metabolic and Molecular Bases of Inherited Disease*, Vol. 2, edited by C. R. Scriver, A. L. Beaudet, W. S. Sly & D. Valle, pp. 2693–2739. New York: McGraw–Hill.

Laidler, P., Kowalski, D. & Silberring, J. (1991). *Clin. Chim. Acta*, **204**, 69–78.

Laidler, P. & Lityńska, A. (1997). *Int. J. Biochem. Cell Biol.* **23**, 475–483.

Laidler, P., Lityńska, A., Gałka-Walczak, M. & Wojczyk, B. (1994). *Int. J. Biochem.* **26**, 1395–1401.

Laidler, P., Waheed, A. & van Etten, R. L. (1985). *Biochim. Biophys. Acta*, **827**, 73–83.

Laidler, P., Waheed, A. & van Etten, R. L. (1988). *Acta Biochem. Pol.* **35**, 343–356.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. Biol. Chem.* **193**, 265–275.

Lukatela, G., Krauss, N., Theis, K., Selmer, T., Gieselmann, V., von Figura, K. & Saenger, W. (1998). *Biochemistry*, **37**, 3654–3664.

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

Nichol, L. W. & Roy, A. B. (1965). *Biochemistry*, **4**, 386–395.

Otwinowski Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.

Schmidt, B., Selmer, T., Ingendoh, A. & von Figura, K. (1995). *Cell*, **82**, 271–278.

Waldow, A., Schmidt, B., Dierks, T., von Bülow, R. & von Figura, K. (1999). *J. Biol. Chem.* **274**, 12284–12288.