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addenda and errata

Crystallization and preliminary X-ray structural studies of human prouroguanylin. Corrigendum

Len Ito,[a] Yuji Hidaka,[b]* Masaki Okumura,[a] Hironori Konishi,[b] Knut Adermann[c] and Hiroshi Yamaguchi[a]*

[a]School of Science and Technology, Kwansei Gakuin University, Sanda, Hyogo 669-1337, Japan, [b]School of Science and Engineering, Kinki University, Higashi-Osaka, Osaka 577-8502, Japan, and [c]IPF PharmaCeuticals GmbH, Feodor-Lynen-Strasse 31, D-30625 Hannover, Germany

Correspondence e-mail: yuji@life.kindai.ac.jp, hiroshi@kwansei.ac.jp

A correction is made to the list of authors for Ito *et al.* [*Acta Cryst.* (2008). F**64**, 531–532].

In the article by Ito *et al.* (2008) one of the authors names was not listed. The correct list of authors is Len Ito, Yuji Hidaka, Masaki Okumura, Hironori Konishi, Knut Adermann and Hiroshi Yamaguchi, as given above.

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Crystallization and preliminary X-ray structural studies of human prouroguanylin

Len Ito,[a] Yuji Hidaka,[b] Masaki Okumura,[a] Hironori Konishi[b] and Hiroshi Yamaguchi[a]*

[a]School of Science and Technology, Kwansei Gakuin University, Sanda, Hyogo 669-1337, Japan, and [b]School of Science and Engineering, Kinki University, Higashi-Osaka, Osaka 577-8502, Japan

Correspondence e-mail: hiroshi@kwansei.ac.jp

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Uroguanylin, which serves as an endogenous ligand of guanylyl cyclase C, is initially secreted in the form of a precursor, prouroguanylin. The N-terminal region of prouroguanylin interacts with the mature portion of prouroguanylin during the folding pathway. Here, a preliminary X-ray crystallographic study of prouroguanylin is presented. Prouroguanylin was refolded, purified and crystallized using the hanging-drop vapour-diffusion method. Prouroguanylin crystals were cryocooled and used for data collection. The diffraction data showed that the crystals belonged to space group $P6_122$, with unit-cell parameters $a = b = 55.6$, $c = 157.7$ Å, and diffracted to 2.5 Å resolution. The structure is currently being analyzed.

1. Introduction

Many peptide hormones are expressed *in vivo* in the form of precursor proteins, *i.e.* prohormones, which are subsequently processed into a biologically active form. However, the detailed role of the propeptides in the precursor proteins is still unclear. Uroguanylin has been identified as an endogenous ligand of guanylyl cyclase C (GC-C; Hamra *et al.*, 1993; Miyazato *et al.*, 1996), which plays a role in the regulation of water in the intestine and kidney by affecting the cystic fibrosis transmembrane conductance regulator chloride channel (CFTR; Chao *et al.*, 1994). Uroguanylin consists of 16 amino-acid residues, contains two intramolecular disulfide bonds and is secreted as its precursor protein prouroguanylin, which has 86 amino-acid residues (Miyazato *et al.*, 1996; Fig. 1). The mature form of uroguanylin does not possess sufficient information to permit the adaptation of its native conformation and to assume the correct disulfide pairing during folding. Uroguanylin requires the assistance of a propeptide from prouroguanylin for correct folding with native disulfide pairing (Hidaka *et al.*, 1998, 2000; Schulz *et al.*, 1999). Little information is available about the functional sites/regions of the propeptide that are involved in this chaperone function.

To further investigate the chaperone function of the propeptide, we conducted X-ray crystallographic analysis of a prouroguanylin mutant.

2. Materials and methods

2.1. Expression of prouroguanylin

Wild-type human prouroguanylin was produced as inclusion bodies and the solubility of the refolding intermediates was very low; therefore, the wild-type protein could not be prepared in good yield. The Q51K/A55K mutant (2K mutant) was designed to increase the solubility of prouroguanylin. *Escherichia coli* BL21 (DE3) cells transformed with pET25b containing the 2K mutant cDNA were grown at 310 K in 2×YT medium (1 l) supplemented with ampicillin (50 mg ml^{-1}), harvested by centrifugation after 18 h and lysed by sonication after resuspension in buffer *A* (100 m*M* Tris–HCl pH 7.4 and 300 m*M* NaCl). The mixture was centrifuged (10 000*g* for 20 min) and the 2K mutant was obtained as an insoluble material. The yield of the 2K mutant was approximately 12–15 mg from 1 l culture medium.

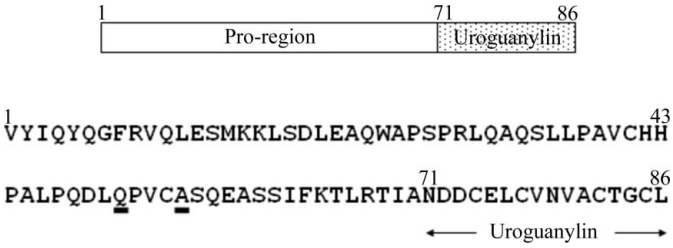

Figure 1
Primary structure of prouroguanylin, a precursor of uroguanylin. The 2K mutant (a Q51K/A55K double mutant) was used for crystallization. Q51 and A55 are underlined.

2.2. Protein refolding and purification

The recombinant 2K mutant, which was obtained as insoluble material, was dissolved in 30 ml 0.1 *M* Tris–HCl pH 8.0 containing 8 *M* urea and 10 m*M* DTT and kept at 323 K for 1 h. After centrifugation, the supernatant was applied onto a column of Cosmosil 140C_{18}-OPN (10 ml; Nacarai Tesque, Kyoto, Japan) which was pre-equilibrated and washed with 50 ml 20% acetonitrile in 0.05% trifluoroacetic acid (TFA). The adsorbed proteins were eluted with 80% acetonitrile in 0.05% TFA and collected. The protein was further purified by HPLC using Cosmosil 5C_{18}-AR (8 × 250 mm; Nacalai Tesque Inc., Kyoto, Japan). The HPLC apparatus consisted of a Waters 600 multisolvent delivery system (Millipore) equipped with a Hitachi L-4000 UV detector and a D-7500 chromato-integrator (Tokyo, Japan). The proteins were eluted using a linear gradient of acetonitrile in 0.05% TFA at a flow rate of 2 ml min^{-1} increasing at a rate of 1% per minute from solvent *A* (0.05% TFA/H_2O) to solvent *B* (0.05% TFA/CH_3CN).

After vacuum drying, the dried material (2K mutant, 25 nmol) was dissolved in 150 ml 6 *M* urea, 0.05% TFA and diluted in 2.85 ml 50 m*M* Tris–HCl pH 8.0 in the presence of 2 m*M* GSH (glutathione, reduced form) and 1 m*M* GSSG (glutathione, oxidized form) at 293 K for 2 d. The protein was further purified by ion-exchange chromatography using a DE-52 column (Whatman International Ltd, England), concentrated to 10 mg ml^{-1} and dialyzed against 20 m*M* Tris–HCl pH 7.0.

2.3. Crystallization

Crystallization was performed at 293 K in sitting drops by the vapour-diffusion method using 24-well plates (TPP, Switzerland). Initial crystallization conditions were established using Wizard I and II screening kits (Emerald Biostructures). Hanging drops were obtained by mixing 2.5 µl protein solution (10 mg ml^{-1} in 20 m*M* Tris–HCl pH 7.0) and 2.5 µl reservoir solution and were equilibrated against 600 µl reservoir solution. Recently, we have reported that certain amino-acid derivatives such as glycine ethyl ester (GlyEE) promote protein crystallization with high reproducibility (Ito *et al.*, 2008). The crystallization condition was refined by varying the concentration of protein and additives using GlyEE as well as the pH of the buffer. The best condition was determined as a protein solution containing 10 mg ml^{-1} protein in 20 m*M* Tris–HCl pH 7.0 and a reservoir solution containing 1.2 *M* NaH_2PO_4, 0.8 *M* K_2HPO_4, 0.1 *M* NaCl and 0.2 *M* GlyEE.

2.4. Data collection and processing

All diffraction data for the 2K mutant crystal were collected under cryogenic conditions from crystals soaked in a cryoprotectant buffer containing 25%(*v*/*v*) glycerol and cooled to 100 K in a nitrogen-gas stream. Diffraction images were collected using an R-AXIS V (Rigaku) detector at the BL38B1 station at SPring-8. The diffraction data were autoindexed and integrated using the program *MOSFLM* (Leslie, 1992) and scaled, reduced and analyzed with *SCALA* from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). Crystal data and relevant statistics are given in Table 1.

Table 1
Crystal data and diffraction data statistics.

Values in parentheses are for the highest resolution shell.

Space group	$P6_122$
Unit-cell parameters (Å)	$a = b = 55.6$, $c = 157.7$
Wavelength (Å)	1.000
Resolution (Å)	41.1–2.65 (2.79–2.65)
Measured/unique reflections	90669/4689 (13079/648)
Completeness (%)	99.9 (100.0)
Multiplicity	19.3 (20.2)
$I/\sigma(I)$	41.5 (12.4)
R_{merge} (%)	6.2 (25.8)

2.5. Results and discussion

The prouroguanylin crystals were analyzed by X-ray diffraction and belonged to space group $P6_122$ or $P6_522$, as deduced from systematic absences, with unit-cell parameters $a = b = 55.6$, $c = 157.7$ Å (Table 1). One molecule can be accommodated per asymmetric unit, suggesting a V_M value of 3.7 $Å^3$ Da^{-1} and a solvent content of 66.4%. A full data set was collected to 2.65 Å resolution. A total of 90 669 reflections were measured, including 4689 unique reflections. The completeness was 99.9%, with a multiplicity of 19.3 and an R_{merge} of 6.2%.

Molecular-replacement calculations were performed with *AMoRe* (Navaza, 1994) as implemented in the *CCP*4 suite using the NMR structure of proguanylin (PDB code 1o8r), which is also the precursor protein of an endogenous ligand of GC-C and shows 38% identity to prouroguanylin, as a starting model. A distinct peak was found with a correlation coefficient of 34.8% and an *R* factor of 56.2% after translation-function calculations within the resolution range 15–3.5 Å. The space group was determined to be $P6_122$. Structure determination and refinement are currently under way.

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