A preliminary neutron diffraction analysis of Achromobacter protease I

Yuki Ohnishi¹, Takeharu Masaki², Taro Yamada³, Kazuo Kurihara⁴, Ichiro Tanaka¹, Nobuo Niimura*¹

¹Faculty of Engineering, Ibaraki University, 4-12-1, Nakanarusawa, Hitachi, Ibaraki 316-8511, Japan.
²Faculty of Agriculture, Ibaraki University, 3-21-1 Chuo, Ami, Ibaraki 300-0393, Japan.
³Frontier Research Center of Applied Atomic Sciences, Ibaraki University, 162-1 Shirakata, Tokai, Naka, Ibaraki 319-1106, Japan.
⁴Quantum Beam Science Director, Japan Atomic Energy Agency, 2-4 Shirakatashirane, Tokai, Naka, Ibaraki 319-1195.

E-mail: niimura@mx.ibaraki.ac.jp

Abstract. Achromobacter protease I (API, E.C. 3.4.21.50) is one of the serine proteases produced by Achromobacter lyticus M497-1. API is distinct from the other tripsin type protease in its lysine specificity. The neutron structure analysis of catalytic triad with Trp169 and His210 was presented. His57 was double protonated and formed hydrogen bonds to Ser194Oγ and Asp113Oδ¹, Oδ².

1. Introduction
Achromobacter protease I (API, E.C. 3.4.21.50) is one of the serine proteases isolated from soil bacterium, Achromobacter lyticus M487-1 [1, 2]. API specifically hydrolyzes the peptide bonds at the carboxyl side of lysine residues. When API is compared with tripsin type protease, API has several characteristics such as lysine specificity, a higher peptidase activity, a wide pH optimum ranging from 8.5 to 10.7 and stability against denaturation with 4M urea and 0.1% sodium dodecyl sulfate, respectively [3]. From the X-ray structure analysis of API (protein data bank ID: 1arh), imidazole-indole stacks between Trp169 and His210, which is closed to catalytic triad residues, contributes to reduces a space at the active site [4]. This structure seems to be a high catalytic potentiality of lysine specificity. To elucidate these results in detail by observing protons, hydrogen bonds and hydration structure of the protein, we have carried out neutron diffraction experiment. In this study, structure of API was determined by neutron diffraction data. The preliminary results of neutron structure analysis of the catalytic triad with Trp169-His210 will be presented.

* To whom any correspondence should be addressed.
2. Materials and Methods

API was purified as reported previously [2]. Purified API was stored at 277K and concentrated to 15.3 mg/mL before crystallization.

Crystallization was performed by vapor diffusion method followed by modified macro-seeding technique which was used as a large crystal growth [5]. The neutron diffraction experiment was carried out at room temperature by the single-crystal diffractometer BIX-4, which is installed at the JRR-3 reactor of the JAEA [6]. A step scanning method with an interval of 0.3° was used to collect data and the exposure time was 2 hours per frame. The intensities of Bragg reflections were integrated and scaled by the program DENZO and SCALEPACK [7]. The neutron crystal structure refinement was performed by using PHENIX for joint XN refinement [8] and Coot [9] for model building. In order to obtain the initial model for neutron structure analysis, an X-ray structure analysis was carried out by using another crystal which was grown under the same conditions and the X-ray diffraction data were collected by MacScience DIP2000 equipped with x-ray generator M06CEXG-SRA using Cu rotating anode. The neutron and X-ray data collection and joint XN refinement statistics were summarized in Table 1.

3. Results

Catalytic triad with Trp169 and His210

Nuclear density map of the catalytic triad with Trp168 and His210 is shown in Figure 1. His57 was doubly protonated as deuterium atom, and hydrogen bonds were observed between His57D^ε2 and Ser194O^γ, His57D^δ1 and Asp113O^δ1, and His57D^δ1 and Asp113O^δ2. The distances of these hydrogen bonds are 2.1, 2.0 and 2.6 Å, respectively.

In this report, we have demonstrated the preliminary neutron diffraction analysis of API. The structure of catalytic triad involving Trp169 and His210 has determined. The appropriate resolution of the data was 2.0 Å, because the completeness of 20-4.0 Å, 4.0-2.0 Å and 2.0-1.86 Å shells are 96.4%, 69.0% and 20.1%, respectively, after the recent re-processing of data. For this refinement, however, 1.86 Å neutron data was used to obtain the fine Fourier map. The further refinement is under way.

Acknowledgments

This work was supported in part by a Grant of Regional Consortium by Kanto Economical and Industry, a “Grant-in-aid for scientific research” from the Ministry of Education, Culture, Sports, Science and Technology of Japan, a common-use facility of Japan Atomic Energy Agency, and a research grant funded by the Human Frontier Science Program.
Table 1. Data collection and refinement statistics

|                         | Neutron | X-ray |
|-------------------------|---------|-------|
| Space group             | $P1$    | $P1$  |
| Unit cell (Å)           | $a = 39.755, b = 40.792, c = 43.991$ | $a = 39.755, b = 40.792, c = 43.991$ |
|                         | $\alpha = 64.83, \beta = 66.07, \gamma = 73.41$ | $\alpha = 64.83, \beta = 66.07, \gamma = 73.41$ |
| Beam port/radiation     | 1G, JRR-3 | MacScience M06XCEXG-SRA |
| Wavelength (Å)          | 2.60    | 1.54  |
| Diffractometer          | BIX-4   | DIP2020 |
| Exposure time (min/fr)  | 118     | 5     |
| Total frames            | 823     | 390   |
| Resolution (Å)          | 80 - 1.86 | 80 - 1.90 |
| Observed reflection     | 18557   | 58252 |
| Unique reflection       | 11068   | 15308 |
| $I/\sigma (I)$          | 3.9 (2.2) | 21.6 (5.7) |
| Completeness (%)        | 58.9 (19.5) | 88.4 (79.8) |
| $^aR_{\text{merge}}$    | 16.7 (27.3) | 6.1 (16.3) |
| $^bR_{\text{crysta/Rfree}}$ (%) | 22.90 / 28.34 | 14.71 / 20.56 |

$^aR_{\text{merge}} = \frac{\sum |I - <I>|}{\sum I}$, where I is the intensity of a reflection and $<I>$ is the average intensity.

$^bR_{\text{crysta/Rfree}} = \frac{\sum ||F_o|| - ||F_c||}{\sum ||F_o||}$ calculated for the reflections of working and test sets, respectively.

Values in parentheses are for the highest resolution shell.

Figure 1. Nuclear density map for catalytic triad of API with $\pi$-$\pi$ stacking Trp168 and His210. Map colour of $2|F_o|-|F_c|$ for model is grey.
References

[1] Masaki T, Nakamura K, Isono M, Soejima M 1978 Agric. Biol. Chem. 42, 1443-1445.
[2] Masaki T, Fujihashi T, Nakamura K, Soejima M 1981 Biochim. Biophys. Acta. 660, 51-55.
[3] Masaki T, Tanabe M, Nakamura K, Soejima M 1981 Biosci. Biotechnol. Biochem. 56, 1604-1607.
[4] Shiraki K, Norioka S, Li S, Sakiyama F 2002 J. Biochem. 131. 213-218.
[5] Ohnishi Y, Masaki T, Yamada T, Tanaka I, Niimura N (To be published)
[6] Kurihara K, Tanaka I, Refai-Muslih M, Ostermann A, Niimura N 2004 J. Synchr. Rad. 11, 68-71.
[7] Otwinowski, Z. & Minor, W. (1997). Methods Enzymol. 276, 307–326.
[8] Adams P D, Mustyakimov M, Afonine P V, Langan P  2009 Acta Cryst. D65, 567-573.
[9] Emsley  P, Cowtan K, 2004 Acta Crystallogr. D. 60, 2126-2132.