

Structural Analysis of Human Pancreatic-Type RNases

*Hidenori Yamada 7468, Shinya Fujiki 7613, Kohei Miyata 8374,
Takashi Maeda 7619, Midori Kitazoe 8135, Megumi Kosaka 8347, and
Mamoru Yamanishi 1799

Okayama University

Although crystallization of a protein is a key step to determine the structure of the protein, there are many proteins that are hardly crystallized. Thus, the development of new method to crystallize a protein is urgently required. Human pancreatic-type RNases constitute a group of proteins with ribonucleolytic activity differing specific activity, substrate preferences and optimal conditions of enzymatic reaction. So far, eight RNases in human have been found in a gene level, and five (RNase 1–RNase 5) of them have been actually detected in a protein level. Using recombinant proteins, crystal structures of RNase 2–RNase 5 have been determined. All of these RNases were found to have similar folds to that of bovine RNase A, a prototype of this protein-family. Nevertheless, RNase 1 has not been crystallized yet. Thus, this protein is considered to be a good model for the development of a new method to crystallize a hardly crystallizable protein.

In this regard, we prepared two mutant

proteins, 3L-RNase 1 and 4L-RNase 1, which contained leucine zipper-like hydrophobic packing surface with three and four Leu residues on the site corresponding to the exposed side of the second helix of RNase A, respectively. Excitingly, both 3L-RNase 1 and 4L-RNase 1 were crystallized. We collected the diffraction data of these crystals up to about 1.75 Å resolutions. We are trying to solve their structures by molecular replacement using RNase A as a model. Both crystals were found to have the same space group of F222. So far, the structures of both mutants have been determined to 2.8–2.9 Å resolution, revealing that each asymmetric unit contains two molecules facing each other's introduced hydrophobic surface. These results suggest that an introduction of a hydrophobic surface into a hardly crystallizable protein help its packing in crystallization.

Local structure around In atoms in $\text{In}_x\text{Ga}_{1-x}\text{N}$ ($x=0.3, 0.4, 0.5$) single-quantum-wells by XAFS

T. Miyanaga*/3319, T. Azuhata/6266, S. Matsuda/7568, Y. Ishikawa/8156, T. Uruga¹/182,
H. Tanida¹/1275, S.F. Chichibu², T. Sota³, and T. Mukai⁴

Department of Materials Science and Technology, Faculty of Science and Technology, Hirosaki University,
Hirosaki, Aomori 036-8561, Japan

¹SPRING8, 1-1-1 Koto, Sayo-gun, Hyogo 679-5198, Japan

²Institute of Applied Physics, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8573, Japan

³Department of Electrical, Electronics, and Computer Engineering, Waseda University, Shinjuku, Tokyo 169-8555, Japan

⁴Nitride Semiconductor Research Laboratory, Nichia Corporation, Oka, Kaminaka, Anan, Tokushima 774-8601, Japan

InGaN is a key material in high-brightness blue/green light emitting diodes and purplish-blue laser diodes. Although such devices have very high densities of threading dislocations, they show high quantum efficiency. In mole fluctuation in InGaN active layers is proposed as its origin.¹⁾ In this work, EXAFS measurements were carried out to study local structures around In atoms in InGaN.

The samples used are $\text{In}_x\text{Ga}_{1-x}\text{N}$ ($x=0.3, 0.4, 0.5$) single-quantum-wells (SQWs) of 3 nm thickness, and were grown by metalorganic chemical vapor deposition on a sapphire (0001) substrate.²⁾ XAFS data were collected with a Si(111) double-crystal monochromator and focused beam at BL38B1. In K_{α} -fluorescence emission was measured using a 19-element Ge detector. The samples were set in both horizontal and vertical directions to electric field of incident X-ray.

Figures 1 and 2 show the EXAFS $k\chi(k)$ oscillation functions and their Fourier transforms of In K -edge for $\text{In}_{0.4}\text{Ga}_{0.6}\text{N}$ in horizontal and vertical directions analyzed by XANADU code.³⁾ We find clear differences between them. By non-linear least square fitting method, we preliminarily obtained the structure parameters from the EXAFS data for horizontal (vertical) direction: $r_{\text{In-N}}$ is 2.09 Å (2.10 Å), $r_{\text{In-Ga}}$ is 3.22 Å (3.26 Å) and $r_{\text{In-In}}$ is 3.23 Å (3.29 Å). These results suggest that InGaN SQW is biaxially compressed in a - b -plane by the effect of

adjacent layers. More detailed analyses are in progress.

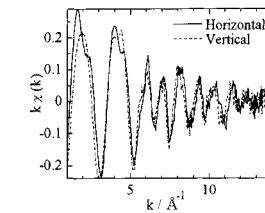


Fig. 1 The EXAFS $k\chi(k)$ spectra of In K -edge for $\text{In}_{0.4}\text{Ga}_{0.6}\text{N}$ SQW in horizontal (solid line) and vertical (dashed line) directions.

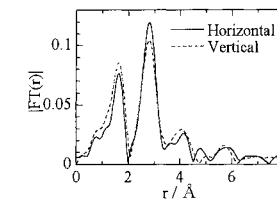


Fig. 2 The Fourier transforms of the EXAFS shown in Fig. 1.

References

- 1) S.Chichibu, T.Azuhata, T.Sota, and S.Nakamura, Appl. Phys.Lett. 69, 4188 (1996).
- 2) S.Nakamura, M.Senoh, N.Iwasa, S.Nagahama, T.Yamada, and T.Mukai, Jpn. J.Appl.Phys. 34, L1332 (1995).
- 3) H.Sakane, T.Miyanaga, I.Watanabe, N.Matsubayashi, S.Ikeda and Y.Yokoyama, Jpn. J.Appl.Phys. 32, 4641 (1993).