BL-1A, BL-5A, BL-17A, AR-NE3A, AR-NW12A/2016G508

# Structural biology of human TRPV1 channel intracellular domain toward elucidation of redox mechanism

Miki Tanaka[1,2], Kaori HAYAKAWA[1,2], Nozomi OGAWA[3], Tatsuki KUROKAWA[4], Yasuo MORI[3] and Masaki UNNO[1,2,*]

[1] Graduate School of Science and Engineering, Ibaraki University, 4-1-12 Nakanarusawa, Hitachi, 316-8511, Japan

[2] Frontier Research Center for Applied Atomic Sciences, 162-1 Shirakata, Tokai, Naka 319-1106, Japan

[3] Graduate School of Engieering, Kyoto University, 8-13 Katsura, Kyoto 615-8510

[4] Faculty of Medicine, Oita University, 1-1 Idaigaoka, Hazamacho, Yufu 879-5593, Japan

## 1 Introduction

Transient Receptor Potential (TRP) cation channel exists as a tetramer and it is involved in various biological functions as sensors of stimulus reception. One of them, TRPV1, is activated by capsaicin - main component of pepper -, heat higher than 42°C, and oxidation. TRPV1 has a very characteristic structure called Ankyrin Repeat Domain (ARD) at the N-terminus. Human TRPV1 (hTRPV1) forms an inter-subunit disulfide bond (Cys258- Cys742), suggesting that it is involved in redox-sensing [1]. Therefore, in this study, we aimed to determine the structure of hTRPV1-ARD containing Cys258, the structure of hTRPV1-Ctrem containing Cys742 and their complex under non-reducing condition to elucidate the binding mode of the disulfide bond at atomic level.

## 2 Experiment

hTRPV1-ARD (101-365) fused with GST-tag was expressed in *E.coli* Rosetta (DE3). After GST affinity chromatography was performed to collect the GST-hTRPV1-ARD, the GST-tag was cleaved by Thrombin. Thereafter, GST affinity chromatography was performed again to separate GST-tag and succeeded obtaining hTRPV1-ARD. Crystallization of the purified hTRPV1-ARD was performed with the similar conditions as rTRPV1-ARD [2]. Because hTRPV1-Ctrem was not able to be obtained with sufficient quantity, a peptide of small region of hTRPV1-Ctrem was prepared and co-crystallization with hTRPV1-ARD was attempted. X-ray diffraction experiments and the structural analysis were performed.

## 3 Results and Discussion

Good quality crystals were grown in the presence of 0.125 M sodium citrate (pH 5.0), 5% (w/v) PEG 8000 and 2.5% (v/v) glycerol as a reservoir solution. In the X-ray diffraction experiments, crystals did not diffract X-ray well at the initial stage and data suitable for structure analysis could not be obtained, however 4.5 Å resolution data could be obtained under the condition that the cryoprotectant was changed to glucose from glycerol. Molecular replacement was performed using the structure of rTRPV1-ARD as an initial model, and the structure of hTRPV1-ARD could be determined at the main chain level (Fig. 1). This crystal belonged to the space group *C*2, has a high solvent content of 72%, and has a very unique structure containing six molecules of hTRPV1-ARD in the asymmetric unit. When compared with the structure of rTRPV1-ARD, some differences in the structure of the ligand binding pocket were found near Cys258 which was suggested to form a disulfide bond with the C-terminal Cys742. This difference between structures of hTRPV1-ARD and rTRPV1-ARD can be considered to relate to the high oxidation sensitivity of Cys258 in hTRPV1 [3].

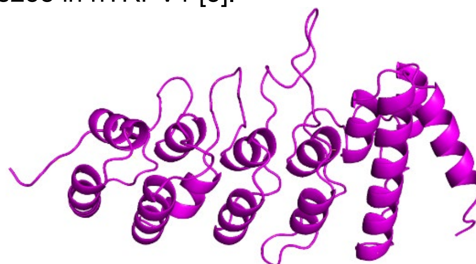

Fig. 1: Overall Structure of hTRPV1-ARD [3].

Acknowledgement

The X-ray data collection was also conducted at the Swiss Light Source in the summer 2019 (Proposal numbers 20191094 and 20191134 at PSI). This work was partly supported by the Ibaraki Prefecture Sendouteki Kenkyu. This work was also performed under the Cooperative Research Program of "Network Joint Research Center for Materials and Devices.".

References

[1] N. Ogawa *et al*., *J. Biol. Chem.* **291**, 4197 (2016).
[2] P. V. Lishko *et al*,, *Neuron* **54**, 905 (2007).
[3] M. Tanaka *et al*., *Acta Crystallogr.* **F76**, 130 (2020)

Research Achievements

1. Poster Award in the 46th Symposium on Biomolecular Science (2019), Tsukuba

2. Flash and Poster Presentations Award, AsCA2019, Singapore

* masaki.unno.19@vc.ibaraki.ac.jp