

Crystal structure analysis of nuclear receptors complexed with novel synthetic ligands

Nobutaka NUMOTO^{1,*}

¹ Medical Research Institute, Tokyo Medical and Dental University (TMDU),
1-5-45 Yushima Bunkyo-ku, Tokyo, 113-8510, Japan

1 Introduction

The nuclear receptors bind lipophilic small molecules called hormones and regulate transcription of genes. The functions of nuclear receptors and their specific ligands are closely involved in the onset and treatment of cancer, autoimmune diseases, so that ligand molecules that regulate signaling of nuclear receptors are actively studied to apply for pharmaceuticals.

Retinoid X receptor (RXR) has been studied as a major nuclear receptor, and various agonists, antagonists, and partial agonists have been developed followed by the structural studies of its complexes. However, the detailed model of the complex of RXR and partial agonists, which show intermediate activities, is unclear. We have previously reported the structure of the partial agonist bound RXR [1], but the structure shows the inactive tetramer. In this study, we aimed to obtain the structure of the active form in complex with the partial agonists and the full agonists.

Vitamin D receptor (VDR) plays an essential role in regulating calcium and phosphate homeostasis in the body. It has been reported that active form of vitamin D₃ promotes bone anabolic activity by activating VDR, so that VDR is recognized as an effective target for the treatment of osteoporosis. However, the use of the activated vitamin D₃ as a therapeutic agent is limited due to hypercalcemia. Continuous efforts to develop the various derivatives of the activated vitamin D₃ are performed, and we have intended to obtain the structural information of VDR complexed with various novel synthesized ligands.

2 Experiment

Ligand binding domain of human RXR and rat VDR were overexpressed in *Escherichia coli* and purified as described previously. The synthesized ligands and co-activator derived peptides are added before crystallization to form the ternary complexes and shift to their active forms. Crystallizations were performed by the sitting-drop vapor-diffusion method at 20°C. Prior to data collection, the crystals were soaked in cryo-protectant solutions containing 20% (v/v) glycerol or ethylene glycol.

X-ray diffraction experiments were performed at the beamlines BL-1A, -17A, and NE3A at PF and PF-AR, KEK. Data collection was partly performed by their automatic data-collection system [2] and the

data was automatically processed by PReMo [3]. Alternatively, the data were manually processed and scaled using XDS [4]. The initial phases were determined by the molecular replacement method using the program PHASER [5]. Several cycles of manual model rebuilding by using COOT [6] and refinement by using PHENIX [7] were performed.

3 Results and Discussion

We obtained the crystals of the ternary complex of RXR-ligand-peptide, which diffracted up to 1.8 Å resolution. The crystal structure shows canonical activated form as the dimer of the ternary complexes. The partial agonist is accommodated at the same ligand binding pockets with the same conformation as that of full agonist. By integrating the previous results of the structure of the inactivated tetramer and that of the activated dimer in this study, we suggest the molecular mechanism of the partial agonist for RXR via conformational diversity in the same ligand binding site.

We also obtained various crystals (Fig. 1) of the ternary complexes of VDR and determined the structures of VDR complexed with novel synthesized ligands, which are derivatives of lithocholic acid. The structures reveals that the substituents added to the A-ring of the ligands impact on the conformation of L400 of VDR and entry of solvent into the ligand binding pocket. On the other hand, the structures elucidate that VDR can accommodate various length of substituents added to the D-ring. Accumulation of these structural information will be useful for future drug design.

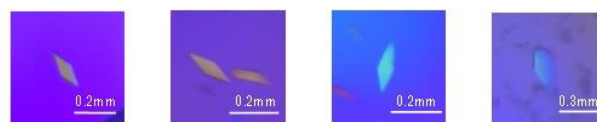


Fig. 1: Crystals of the ternary complexes of VDR.

References

- [1] Y. Miyashita *et al.*, *FEBS Lett.* **593**, 242 (2018).
- [2] Y. Yamada *et al.*, *AIP Conf Proc.* **1234**, 415 (2009).
- [3] Y. Yamada *et al.*, *J. Phys Conf Ser.* **425**, 012017 (2013).
- [4] W. Kabsh, *Acta Cryst.* **D66**, 125 (2010).
- [5] A. J. McCoy *et al.*, *J. Appl. Crystallogr.* **40**, 658 (2007).

- [6] P. Emsley *et al.*, *Acta Cryst.* **D66**, 486 (2010).
[7] P. D. Adams *et al.*, *Acta Cryst.* **D66**, 213 (2010).

* numoto.str@mri.tmd.ac.jp