Crystal Structures Restriction Endonuclease *Eco*O109I DNA Bound to Divalent Metal

Tsuyoshi Imasaki[a], Hiroshi Hashimoto[a], Toshiyuki Shimizu[a], Keiko Kita[b], Mamoru Sato[a], [a]*Graduate School of Integrated Science, Yokohama City University.* [b]*Graduate School of Agiculture, Kyoto University.* E-mail: ima@tsurumi.yokohama-cu.ac.jp

Over 3,000 type II restriction endonucleases have been discovered. They require divalent metals (such as Mg^{2+} or Mn^{2+}) as cofactors with their activity. Most type II restriction endonucleases have activity under the existence of Mg^{2+} or occasionally Mn^{2+}. The restriction endonuclease does not support catalysis with Ca^{2+} and Ba^{2+}, however, it forms a stable protein-metal-DNA complex without cleaving DNA.

*Eco*O109I is a type II restriction endonuclease and recognizes a palindromic sequence RGGNCCY (R = A,G: Y = T,C) and the enzyme cleaves the sequence between the second and third base, and produces leaving 5'-overhanging ends under the existence of Mg^{2+} or Mn^{2+}. In contrast, Ba^{2+} does not support catalysis. The structures of *Eco*O109I DNA-free and *Eco*O109I DNA-complex have been determined [1]. The structures of *Eco*O109I DNA-complex have one metal ion per the active site. To explore how *Eco*O109I uses divalent metal ions, we determined the crystal structure of *Eco*O109I with its cognate DNA substrate containing Ba^{2+} or Mn^{2+} at 1.6 Å resolution. In the Ba^{2+} bound structure, DNA stays intact and one Ba^{2+} was found per active site, whereas in the Mn^{2+} structure, DNA was cleaved and three Mn^{2+} were found per active site.

[1] Hashimoto H., Shimizu T., Imasaki T., Kato M., Shichijo N., Kita K., Sato M., *J. Biol. Chem*., 2005, **280**, 5605.

Keywords: protein structures, DNA-protein complexes, endonucleases