（学位第３号様式）

学　位　論　文　要　旨

氏　名	Funahashi Aki
題　目	Studies on a novel fluorescent protein from eel muscle (ウナギ筋肉に存在する新奇蛍光タンパク質に関する研究)

A novel Green fluorescent protein (eel GFP) derived from muscle of *Anguilla japonica* was firstly reported for the molecular weight, fluorescent properties and partial amino acid sequences by Hayashi and Toda. Thereafter, eel GFP requires the binding of bilirubin as ligand to fluoresce. In order to examine the distributions and characteristics of eel GFP, the complementary DNAs were isolated from six genus *Anguilla, A. japonica*, *A. australis, A. anguill, A. bicolor bicolor*, *A.bicolor pacifica*, and *A. mossambica* based on partial amino acid sequences, and the proteins were expressed by *E. coli* with their recombinant DNAs. Moreover, the culture cells expressing eel GFP were generated and used to investigate an effect of the eel GFP-bilirubin complex on oxidative stress tolerance.

In fluorescence observation of the eel GFP, the fluorescence was confirmed in muscle from all of genus *Anguilla,* albeit with different intensity among species. The full-length cDNAs of eel GFP were 631–644-bp long and contained an open reading frame of 417 bp encoding 139 amino-acid residues, which showed similarity to fatty acid binding protein (FABP). Exon- intron structures of eel GFP are consistent with the ORF starting at 1st exon and ending at 4th exon, and conserved for exon number and length of many FABP family.

The proteins of eel GFP expressed in *E. coli* with their recombinant DNAs were fluoresced by the addition of bilirubin, and the excitation and emission spectra had maximum wavelengths of 490–496 and 527–530 nm, respectively. In all six genus *anguilla*, the deduced amino-acid sequences were conserved with eight amino acids which were recognized as ligand binding sites, and tripeptide, Gly58-Pro59-Pro60 which may play a role as chromophore. The fluorescent intensities were stronger in the order of *A. japonica* = *A. bicolor* > *A. mossambica* >> *A. australis* >> *A. anguilla*, assuming that the differences of fluorescent intensity were affected by the combination of two amino acids replacement, Leu63 - Tyr110 or Phe63 - Leu110.

To clarify the function of eel GFP, the investigation was made on growth rate and oxidative stress test using cultured cells of HEK293-eel GFP, HEK293-CV and HEK293-jf GFP, that *A. japonica* eel GFP – vector, only control vector and *Aequrorea victoria* GFP – vector were transfected to human embryonic kidney (HEK) 293 cells. As compared to the three cultured cells containing phenol red with antioxidation under the general cell culture condition, the growth rates were decreased to 52% and 31% in HEK293-CV and HEK293-jf GFP without phenol red, respectively. On the other hand, HEK293-eel GFP had a growth rate of approximately 70%. The eel GFP-expressing cells were approximately 2-fold resistant to oxidative stress such as H_2O_2 exposure. The fluorescence intensity partially decreased or disappeared after exposure to H_2O_2, and these phenomena were suggested to release the oxidized biliverdin from GFP-bilirubin. These results suggested that eel GFP coupled with bilirubin provided the antioxidant activity to the cells as compared to bilirubin free GFP.