Studies on the bioluminescent mechanism of symplectin photoprotein

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Tobiika (Symplectoteuthis oualaniensis L.) is a flying squid that emits blue light (470 nm). We have studied its bioluminescent mechanisms and confirmed that the light comes from its photoprotein ‘symplectin’. Symplectin is a 60 kDa protein and possesses dehydrocoelenterazine (DCT) as an organic substance. DCT exists as thiol bound form with symplectin active site cysteine to construct the chromophore for bioluminescence. The structural analysis of symplectin active site is our main research focus now.

Form the sequence analysis of symplectin with LC-Q-TOF-MS, MS/MS and cDNA, symplectin has 501 amino acids sequence. Partial degradation of symplectin with trypsin afforded 40 kDa protein symplectin A', which is the C-terminal part of symplectin and has also bioluminescent activity. We suppose that the active site of symplectin must exist in the 40 kDa symplectin A', therefore, we synthesized fluorinated dehydrocoelenterazine (F-DCT) as a probe to investigate the active site of symplectin. We found that F-DCT strongly binds to sulfhydryl residue of cysteine to afford stable chromophore, which was proved with NMR and MS methods. But unfortunately, we could not perfectly deduce the active site cysteine residue of symplectin.

Then, we synthesized a photoprobe to label the active site of symplectin. As a model chromophore of symplectin, azide-fluoro-coelenterazin was photo-irradiated in a solution. Hexafluoroisopropanol was the best solvent for converting the azide to nitrene from the data MS analysis of photo-irradiated products.

Now we investigate the active site by using photoaffinity labeling and nano-LC-Q-TOF-MS analysis, and also study the relationships between DCT structures and symplectin bioluminescent activities.