

Overexpression, purification and characterization of chicken cryptochrome 4

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Cryptochromes (CRYs) are flavoproteins constituting a cryptochrome/photolyase family, and some CRYs function as blue light-photosensor to regulate growth, development, and circadian clock. Recent observations strongly suggest that CRYs may also be involved in light-dependent magnetoreception mediated by a blue light-induced radical reaction.

We have previously identified a novel class of vertebrate CRY in chick (cCRY4), and revealed its high expression in photosensitive neural tissues such as the pineal gland and retina. In order to perform functional characterization of cCRY4, we developed the overexpression and purification system of cCRY4 in this study. Since we failed to obtain cCRY4 having FAD chromophore by using some expression systems such as *E. coli* and HEK293 cell, we further screened another expression systems. Here, we report the protein extraction system using yeast, *Saccharomyces cerevisiae*. We also developed a modified protein extraction method using sonication but omitting Zymolyase treatment. We obtained a large amount (~mg) of partially purified GST-tagged cCRY4, and it was subjected to chromophore analysis and spectral analysis of the photoreaction. We present the binding of FAD chromophore and a putative photocycle of cCRY4.