STUDY OF PROTON TRANSFER IN *E. COLI* PHOTOLYASE

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Photolyase is a flavoprotein which utilizes blue-light energy to repair UV-light damaged DNA. The catalytic cofactor of photolyase, flavin adenine dinucleotide (FAD), has five redox states. Conversions between these redox states involve intraprotein electron transfer and proton transfer, which play important role in protein function. Here we systematically studied proton transfer in *E. coli* photolyase *in vitro* by site-directed mutagenesis and steady-state UV-vis spectroscopy, and proposed the proton channel in photolyase. We found that in the mutant N378C/E363L, proton channel was completely eliminated when DNA substrate was bound to the protein. Proton is suggested to be transported from protein surface to FAD by two pathways: the proton relay pathway through E363 and surface water to N378 and then to FAD; and the proton diffusion pathway through the substrate binding pocket. In addition, reaction kinetics of conversions between the redox states was then solved and redox potentials of the redox states were determined. These results described a complete picture of FAD redox changes, which are fundamental to the functions of all flavoenzymes.