RED LIGHT SIGNAL TRANSDUCTION THROUGH NUCLEOSIDE DIPHOSPHATE KINASES (NDP KINASES) IN *PISUM SATIVUM* ALASKA

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To analyze light signal transduction in *Pisum sativum* Alska, *in vitro* system to analyze molecular bases of red light signal transduction was established. The third internodes of etiolated pea seedlings was cut and irradiated by red light. After red light irradiation, it was homogenized and crude membrane fraction and soluble fraction were prepared. From the crude membrane fraction, plasma membrane was further purified. By use of the plasma membrane, red light irradiation stimulated the binding of [α-^{32}P]ATP to 92 kDa protein, the binding of [α-^{32}P]GTP to 16 kDa, 21 kDa, 32 kDa, and 37 kDa proteins and the binding of [α-^{32}P] UTP to 21 kDa and 79 kDa proteins.

By use of the crude membrane fraction and the soluble fraction, the phosphorylation of proteins at 0 ℃ for 15 sec with 4×10^{-8} M [γ-^{32}P]ATP was analyzed. Red light stimulated the phosphorylation of 18 kDa proteins, but successive irradiation with red-far red irradiation suppressed the phosphorylation of the 18 kDa proteins in the crude membrane fraction. In the soluble fraction the successive irradiation with red and far-red light partly suppressed the phosphorylation of the 18 kDa proteins.

The 18 kDa proteins were purified and the partial amino acid sequence revealed that the protein was nucleoside diphosphate kinases (NDP kinase). The cDNA for the NDP kinase designated as PNDKN1 was isolated from the cDNA library prepared from mRNA from the third internodes. The deduced amino acid sequence of the cDNA showed 100 % match with the partial amino acid sequence. The purified NDP kinase was autophosphorylated in the gel and also on the PVDF membrane. The phosphoamino acid analysis of these phosphorylated and autophosphorylated proteins identified P-Ser. The cDNA, PNDKN1 contained consensus sequence for histidine, which was reported to be phosphorylated.

The purified NDP kinase from rat showed protein kinase activity phosphorylating histone H1 and also had the activity to produce GTP from GDP and ATP. Thus the NDP kinase may function as a transducer of light signal by initiating phosphorylation cascade or may provide high concentration of GTP at the vicinity of GTP-binding proteins.

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