AN OAT PROTEIN KINASE PREFERENTIALLY PHOSPHORYLATE THE Pfr FORM OF OAT PHYTOCHROME A IN VITRO

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Phytochrome phosphorylation attracted much attention regarding its functional roles in the light signaling mechanism. There are two *in vivo* phosphorylation sites of Ser-7 and Ser-598 that are regarded to have functional roles. A protein kinase that preferentially phosphorylated Ser-598 of the Pfr form of oat phytochrome A over the Pr form *in vitro* was purified from the etiolated oat seedlings. It had a single band of 32 kDa on a SDS gel, and also phosphorylated casein and phosvitin using ATP as a phosphate doner but did not phosphorylated itself. The internal amino acid sequences and inhibitory characteristics of the purified kinase indicated that it was a CKI-like protein kinase.

Introduction

Protein phosphorylation/dephosphorylation is regarded to play a crucial role in the signal transmission of phytochrome as in other cellular signaling processes. Oat phytochrome A (phyA) has two *in vivo* phosphorylating sites of Ser-7 in the N-terminal extension region (NTE) and Ser-598 in the hinge region between the N- and C-domains (1, 2). Although many reports support the Lagariass group hypothesis that phytochrome is an autophosphorylating kinase (3), no one provided a crucial evidence that the autophosphorylating kinase activity is modulated by red light and far-red light. On the other hand, Ser-598 of oat phyA is phosphorylated in the red/far-red light dependent manner and is likely to be involved in phyA signaling (2).

In the present study, we isolated and characterized a CKI-like protein that preferentially phosphorylated Ser-598 of the Pfr form to the Pr form of oat phyA *in vitro*.

Materials and Methods

Phytochrome and proteine kinase: Oat phyA was purified from the etiolated oat seedlings by a procedure of back extraction and a kinase phosphorylating phyA was purified to be a single band on a SDS gel from the etiolated oat seedlings by successive chromatographies on on a hydroxyapatite, phenyl-Sepharose CL-4B, CM-Cellulose and phosphocellulose columns. The protein kinase activity was assayed