Isolation and characterization of a nitric oxide synthase (NOS)-like protein of pea (Pisum sativum L.)

ABSTRACT

Nitric oxide synthase (NOS) activity based on citrulline formation assay, which was used in mammalian system, was detected in Pisum Sativum L. (pea) extracts. The pea NOS-like protein was most efficiently extracted with the addition of protease inhibitors (ethylene bis (oxyethylenenitrilo)tetraacetic acid (EGTA) and leupeptin) in the extraction buffer and under alkaline condition (pH 8.5ï 9.0) as compared to neutral condition in mammalian system. The precipitation of this protein with various concentrations of ammonium sulfate, sodium citrate and sodium chloride caused rapid loss of NOS activity, in contrast to that in the mammalian system, and the protein was not precipitated by organic solvents (acetone or polyethylene glycol, PEG). The pea NOS-like protein was successfully isolated using ion-exchange column, but did not bind to ß-nicotinamide adenine dinucleotide phosphate (NADPH) and calmodulin affinity columns suggesting that it lacked binding sites for the cofactors NADPH and calmodulin that were required for NOS activity in mammalian cells. The results indicated that the pea NOS like protein was significantly different in structure from mammalian NOS.

Keyword: Nitric oxide synthase; NOS; Pea