

A complex containing both trypsin inhibitor and dehydroascorbate reductase activities isolated from mitochondria of etiolated mungbean seedlings

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Abstract

A complex containing trypsin inhibitor (TI) activity was extracted with 0.1 M TRIS buffer (pH 7.9) from trypsin-treated mitochondria of etiolated mung bean seedlings, and further purified with a Superdex 200 FPLC column. This partially purified complex with an Mr about 820 kDa exhibited additional dehydroascorbate (DHA) reductase [glutathione dehydrogenase (ascorbate)] activity with specific activities of 0.21, 1.53 and 1.54 $\mu\text{mol ascorbate formed min}^{-1} \text{ mg}^{-1}$ protein at pH 6.0, 6.5 and 7.0, respectively, when glutathione was added. Much lower DHA reductase activity (0.013 and 0.026 $\mu\text{mol ascorbate formed min}^{-1} \text{ mg}^{-1}$ protein at pH 6.5 and 7.0, respectively) was found when glutathione was omitted. The isolated complex gave positive results when it was tested by TI activity staining after SDS-PAGE, and could be recognized by a polyclonal antibody which was raised against 38 kDa sweet potato Kunitz-type TI, one of the root storage proteins of sweet potato. The possible physiological functions of this complex with both TI and DHA reductase activities were discussed.