Abstract

A novel proteolytic activity integrally associated with barley thylakoid membranes has been discovered and characterized. This enzymatic activity mediates senescence-dependent degradation of Lhcb3, one of the apoproteins of the major light-harvesting chlorophyll a/b protein complex of photosystem II. Once senescence of barley leaves is initiated by detachment and dark incubation, the degradation of Lhcb3 can proceed and be followed in vitro in an experimental system composed of thylakoids isolated from senescing leaves incubated in darkness in suitable medium at 25°C. The protease involved is present in its active form and Lhcb3 is susceptible for proteolytic attack already in fresh leaves, although Lhcb3 degradation does not take place unless undefined extrinsic membrane proteins protecting Lhcb3 are removed in a senescence-dependent manner. It is thus concluded that senescence-dependent Lhcb3 degradation is regulated at the substrate availability level. The protease involved is ATP stimulated, has an optimum activity at pH 7.8, and requires 3 mM added Mg^{2+} (replicable by micromolar doses of Zn^{2+}) for its proper activity. Studies using typical inhibitors of various classes of proteases indicate that the enzyme is a metalloprotease with disulfide linkage essential for its activity. Micromolar doses of Zn^{2+} were demonstrated to restore the activity of Lhcb3-degrading enzymes abolished by an ethylenediaminetetraacetic acid pretreatment of the thylakoids and it is inferred that the protease involved is a zinc-binding metalloprotease. Mg^{2+} was shown to be able to partially replace zinc as the bound ion.