

# Purification, Characterization and Thermostability of Ribulose 1,5-Bisphosphate Carboxylase-Oxygenase from Barley Leaves

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The enzyme ribulose-1,5-bisphosphate carboxylase-oxygenase (rubisco) and its functional subunits from barley (*Hordeum vulgare* L.) leaves were purified to homogeneity by activity-directed sequential steps of chromatography. Based on the molecular mass estimation by SDS-PAGE, the large subunit (LS) had an apparent molecular weight of *ca.* 55 kDa, whereas the small subunit (SS) was *ca.* a 14 kDa polypeptide chain. The N-terminal sequences, established by automated Edman degradation analysis of the purified subunits, showed very close sequence homologies (52–92%) with the subunits of other rubisco enzymes reported from several photosynthetic species. In order to establish the chemical heterogeneity in the rubisco from barley, the amino acid composition of purified native enzyme was analyzed and the results systematically compared with other known type-I rubisco enzymes from spinach, maize, tobacco and pea. Major differences have been observed in the amino acid composition of barley rubisco, the concentration of cysteine, serine, threonine, isoleucine, leucine, arginine and tryptophan residues were found quite variable as compared to other higher plants. The thermostability of the native rubisco was also investigated using circular dichroism and fluorescence spectroscopy. The critical ( $T_c$ ) and melting ( $T_m$ ) temperatures were determined to be 60 °C and 57 °C, respectively, and at this temperature the enzyme not only retains its structural integrity but also its enzymatic activity. Results of these studies were discussed in the light of structural and functional adaptation of this bifunctional enzyme in  $C_3$  and  $C_4$  plants to their environments.