Purification and Partial Characterization of Barley Oxalate Oxidase

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Introduction: Oxalate oxidase (EC.1, 2, 3, 4), an acute phase protein of plants, is used widely in agriculture, food industries, medical diagnosis of kidney stones and oxaluria. In this study oxalate oxidase from barley was purified and partially characterized.

Materials & Methods: Roots and sprouts of barley cultured in hydroponic states were separated and extracted. Ion exchange resins of the enzyme were done through heat treatment (80°C, 3min), ammonium sulphate fractionation and two-step chromatography. Purification and weight-determining of the enzyme were done by DEAE-Cellulose and DEAE-Sepharose fast flow, respectively. The enzyme activity was measured by spectrophotometric and specific gel staining methods. Purity and molecular mass of the product was estimated using SDS-PAGE. Isoelectric points (pIs) of the separated enzymes were estimated using chromatofocusing and isoelectric focusing. In addition, the effect of different concentrations of urea and denaturing agents were tested on the enzyme activity.

Results: Oxalate oxidase was purified at least 688-fold with purity of more than 90 percent. The molecular mass of the enzyme was estimated to be 25-26 and 115-120 KDa, respectively in reducing and non-reducing SDS-PAGE. The enzyme sub-units showed no activity in the specific gel staining method. At least two isoenzymes with pIs of 6.8 and 6-6.2 were identified. The purified enzyme was resistant to many denaturing agents such as heat, urea and detergents. These characteristics make oxalate oxidase a suitable enzyme for oxalate assays.

Conclusion: So in accordance with the obtained data from this study, it can be concluded that we can utilize the purification method of this enzyme for measuring oxalate.

Key Words: Oxalate Oxidase, Purification, Characterization, Barley.

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Reference


