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Novel extracellular ribonuclease from Bacillus intermedius - binase II: Purification and some properties of the enzyme

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Abstract

The recombinant enzyme binase II was isolated from the culture liquid of Bacillus subtilis 3922 transformed with the pJF28 plasmid bearing the birB gene. The procedure of the enzyme purification included precipitation by polyethylene glycol with subsequent chromatography on DEAE-cellulose, heparin-Sepharose, and Toyopearl TSK-gel. The enzyme was purified 142-fold yielding a preparation with specific activity 1633 U/mg. The molecular weight of binase II is 30 kD. The enzyme is activated by Mg2+ and virtually completely inhibited by EDTA. The pH optimum for the reaction of RNA hydrolysis is 8.5. The properties of the enzyme are close to those of RNase Bsn from B. subtilis. The character of cleaving of synthetic single- and double-stranded polyribonucleotides by binase II suggests that the enzyme binds the substrate in the helix conformation, and its catalytic mechanism is close to that of RNase VI from cobra venom.

Keywords

Bacillus intermedius, Bacillus subtilis, Binase II, Biosynthesis, Enzyme purification, RNase Bsn