

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.

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Keywords

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