Cloning of the gene encoding RNase binase from *Bacillus intermedius* 7P

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Submitted March 23, 1992

EMBL accession no. X53697

Binase is the extracellular alkaline ribonuclease produced by *Bacillus intermedius*. The enzyme (EC 3.1.4.23) belongs to cyclizing ribonucleoside-3'-transferases and is mainly specific towards the purine bases of RNA molecules.

Using the gene for analogous RNase barnase from *Bacillus amyloliquefaciens* as a specific probe we have cloned 5kb genomic fragment from *Bacillus intermedius* containing the complete gene for the binase (1,2). The cloning was succeeded only in *E.coli* strain producing the protein barstar, natural inhibitor of barnase (3).

In Fig.1 there is an alignment of amino acid sequences of RNases: the barnase (a), the binase deduced from the nucleotide sequence (b) and determined by direct sequencing of the protein according to the Edman method (c) (4). The positions of mismatches between the two last structures are framed. Easily seen that the most of the mismatches are the rearrangements of the adjacent amino acids in the structures. Indeed the pairs N21,D22 and S66,G67 in our structure are inverted in the other one. Moreover there is a D to N substitution at position 40.

Surprisingly though the RNases binase and barnase have a strong degree of sequence identity (83%) (Fig.1) their signal peptides differ drastically (Fig.2).

ACKNOWLEDGEMENTS

We thank Dr.Hartley for kindly providing the plasmids pMT416 and pMT316, Dr.Karpeysky for helpful discussions and advice.

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Figure 1. The alignment of amino acid sequences of the barnase a) and binase deduced by translation of nucleotide sequence of the gene b) and determined by the sequencing of the protein c).

a) MMKMEGIALKKRLSWISVCLLVLSAAGMLFSTAATETSSHKAHTE
 b) MKKISSVFTMFALIAAILFSGFIPQQAETPLTQTATNETATIQLTSDVHTL

Figure 2. Amino acid sequences of signal peptides barnase a) and binase b).