Subsites for substrate recognition by bacterial ribonuclease P

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ABSTRACT

We have prepared series of shape variant RNAs of a tRNA precursor and analyzed the substrate shape recognition by bacterial ribonuclease P ribozyme and holoenzyme. The results showed the evidence for the presence of subsites for the recognition of the tRNA shape. We will discuss a new model for substrate recognition and the role of the protein component.

INTRODUCTION

Ribonuclease P (RNase P) is a ubiquitous ribonucleoprotein enzyme which catalyzes the 5'-end processing reaction of a pre-tRNA. In bacteria, the catalytic RNA subunit alone can perform the enzymatic activity without the protein component as a ribozyme in the presence of essential magnesium ions (1,2). Studies on bacterial enzymes from Escherichia coli and Bacillus subtilis have revealed that the interactions at the 5' and 3' termini play important roles in the substrate recognition (3-8). The 3'-CCA is thought to take part in cleavage site selection, and the 5'-leader sequence of a pre-tRNA is for high affinity (Fig.1). Till now, we have many exceptions that the cleavage site of RNAs is not always determined by the relative position of the 3'-CCA: the cleavage site locates near the expected position but not always on the position expected. Besides, the cleavage site of hairpin RNA shifts depending on the concentration of magnesium ions (9-11). These facts demonstrate that something important is missed in the present recognition model. On the other hand, the protein component acts as an acceptor for the 5'-leader sequence of a pre-tRNA on the surface of the RNA subunit, stabilizing the enzyme-substrate complex by countervailing the surface charge of RNA molecules. The substrate recognition mechanism, however, is complicated. The ribozyme itself has strict shape specificity toward RNAs. The ribozyme cannot cleave a hairpin RNA tagged with 3'-CCA sequence at the low concentrations of magnesium ions, however, the holoenzyme cleaves a hairpin RNA as well as a canonical pre-tRNA (12,13). The protein subunit causes the enzyme to be of low shape specificity (14).

In this study, we focused on the substrate shape specificity of the E. coli ribozyme and holoenzyme, and analyzed the reaction by the enzyme of series of shape variant RNAs.

RESULTS AND DISCUSSION

We have prepared series of shape variant RNAs: a hairpin RNA, the bottom-half variant RNAs, the acceptor variant RNAs, and the T-stem variant RNAs. The hairpin RNA is mimicking the top-half part of a pre-tRNA. The comparison of the hairpin RNA and the pre-tRNA shows the effects of the bottom-half part in the reactions. In the bottom-half variant RNAs, the constant bottom-half region is engineered to move on the top-half helix of a pre-tRNA. The comparison of this series of RNAs shows the effects of the position of the bottom-half. In the acceptor variant RNAs, the position of the 3'-ACCA sequence was engineered. The comparison of this series of RNAs shows the effect of the position of the 3'-CCA and the length of the acceptor-stem. In the T-stem variant RNAs, the length of the T-stem is engineered. The comparison of this series of RNAs shows the effects of the position of T-arm and the length of the T-stem. Reactions were done by the ribozyme at low and high Mg²⁺ concentrations, and by the holoenzyme at low Mg²⁺ concentrations. The results showed the presence of two new subsites for substrate recognition in the substrate binding site. The detail of the subsites and the recognition model will be discussed in the meeting.
REFERENCES


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