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The partial purification of *Methanococcus jannaschii* RNase P holoenzyme

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ABSTRACT

The RNase P RNA from the archaeon *Methanococcus jannaschii* lacks essential secondary structures required for the recognition of pre-tRNA, and as a result is not catalytically-active *in vitro* (1). This is in contrast to *Methanobacterium thermoautotrophicum*, whose RNase P RNA is catalytically active in the absence of any protein in the appropriate ionic conditions (1). In order to better understand how the *Methanococcus jannaschii* RNase P holoenzyme compensates for missing structural elements, the enzyme has been partialy purified. *Methanococcus jannaschii* RNase P has a buoyant density in $CsSO_4$ of 1.39 g/ml and an apparently high molecular weight of greater than 400kDa indicating a significant protein fraction. The holoenzyme has a Km of 32 nM and tolerates a wide range of ionic conditions. The profficiency of the holoenzyme suggests that the protein components of the *Methanococcus jannaschii* RNase P most likely provide both structural stability and substrate recognition to the holoenzyme.

INTRODUCTION

It is well established that the RNA component of RNase P in Bacteria is catalytically active *in vitro* (2). However, the RNA from certain archaeal RNase P RNAs was not determined to be active until fairly recently (1). The few catalytic RNase P RNAs from the Archaea require 3M NH_4OAc and 300mM $MgCl_2$ to carry out the reaction *in vitro*, and even then the reaction is very inefficient compared to that of *E. coli* (1). In addition to being catalytically active at high ionic concentrations certain archaeal RNase P RNAs can be reconstituted with *Bacillus subtilis* protein in moderate ionic conditions (1). Nevertheless, the RNase P RNA from *Methanococcus jannaschii* is not active *in vitro,* nor can its activity be reconstituted in the presence of Bacillus *subtilis* protein (1). This lack of catalytic activity in the RNA alone reaction is presumed to be due to the absence of P8 and L15 in the secondary structure (1). Both of these structures play an essential role in the binding of substrate in Bacteria (3,4).

There are only two published characterizations of RNase P holoenzymes from Archaea; those of *Haloferax volcanii* (5) and *Sulfolobus acidcaldarius* (6,7). The density of *Sulfolobus acidcaldarius* is 1.27g/ml in $CsSO_4$, close to that of protein alone, (6,7) and the density of *Haloferax volcanii* is 1.61g/ml, close to that of RNA alone (5). *Methanobacterium thermoautotrophicum* whose RNA is catalytically active *in vitro* and can be reconstituted with the *Bacillus subtilis* protein has a density of 1.41g/ml between that of protein and RNA alone (Tom Hall, personal communication). The comparison of both the *Methanococcus jannaschii* and *Methanobacterium thermoautotrophicum* RNase P RNA, protein, and their functional contibutions will give us more insight to the ability of protein to compensate for the missing RNA components.

METHODS

Cell Extracts

Methanococcus jannaschii was grown in ATCC 1343 media under the appropriate conditions and harvested by centrifugation. Cells were resuspended in TMG-100 and lysed by French press and DNase I digestion (10ug/ml final concentration) (5-6), after which, debris was removed by centrifugation.

Partial Purification

Two consecutive density gradients were run for 48 hours at 130,000 x g in 1.39 g/ml $CsSO_4$. This was followed by loading the active fractions on to a DEAE column that was eluted with a NH_4Cl gradient from 20-500mM (6,7). The remaining active fractions were then loaded onto Q Sepharose column and eluted with a NH_4Cl gradient from 0.1- 1M. The final step in purification used a Sepharose CL-4B-200 size exclusion column. RNase P activity was monitored through cleavage of α^{32}-P labeled pre-$tRNA^{Asp}$.

RNase P Reactions

RNase P reactions were performed using the pre-$tRNA^{Asp}$ from *Bacillus subtilis* transcribed *in vitro* by run off transcription in the presence of α^{32}-P GTP from pDW128. The ionic conditions of all RNase P reactions were 100 mM. NH_4OAc and 25mM $MgCl_2$ unless otherwise stated. The reaction products were separated on 12% acrylamide urea gels and developed by phosphoimagery.

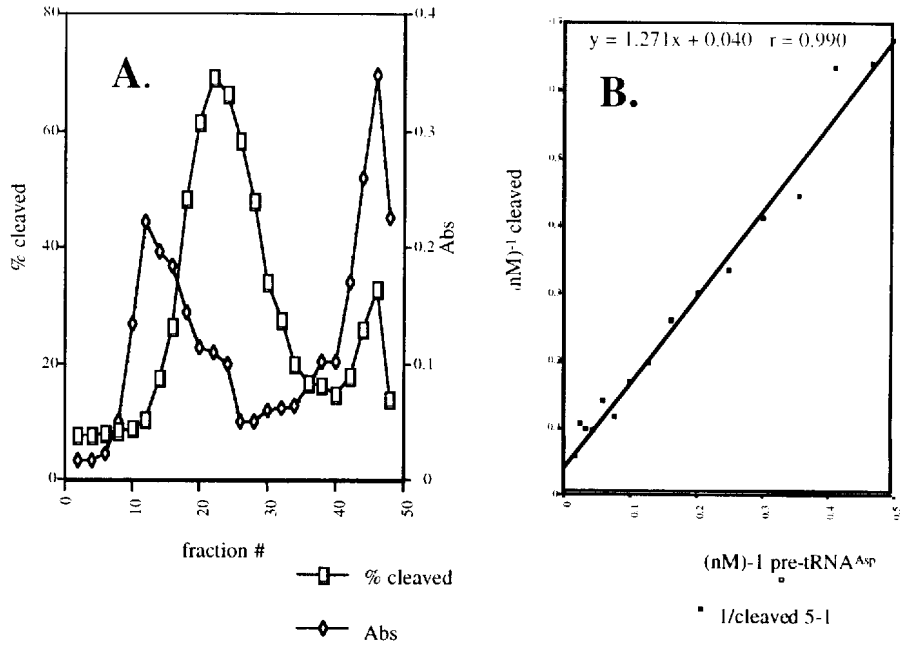

Figure 1. A. Activity of $CsSO_4$ fraction against the absorbance at 540nm using the Perice BCA kit. Fractions were collected from most to least dense. B. Lineweaver-Burk plot, My = 1.271x + 0.040 r = 0.990

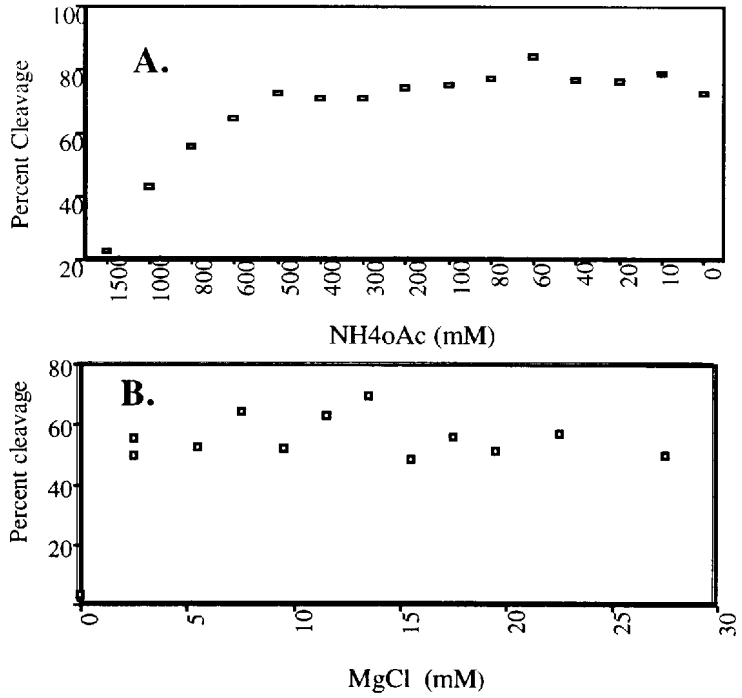

Figure 2. A. The percent cleaved of fractions with a given amount of NH_4Cl added to reactions already containing 20mM salt. B. The percent cleaved with the given amount of $MgCl_2$.

RESULTS and DISCUSSSION

The RNase P holoenzyme of *Methanococcus jannaschii* was found to have an apperent buoyant density in $CsSO_4$ of 1.39 g/ml close to the density of *Methanobacterium thermoautotrophicum* RNase P. The *Methanococcus jannaschii* RNase P was eluted from the DEAE column between 200-300mM NH_4Cl, and the activity from the Q sephrose column was eluted between 500-600mM NH_4Cl.

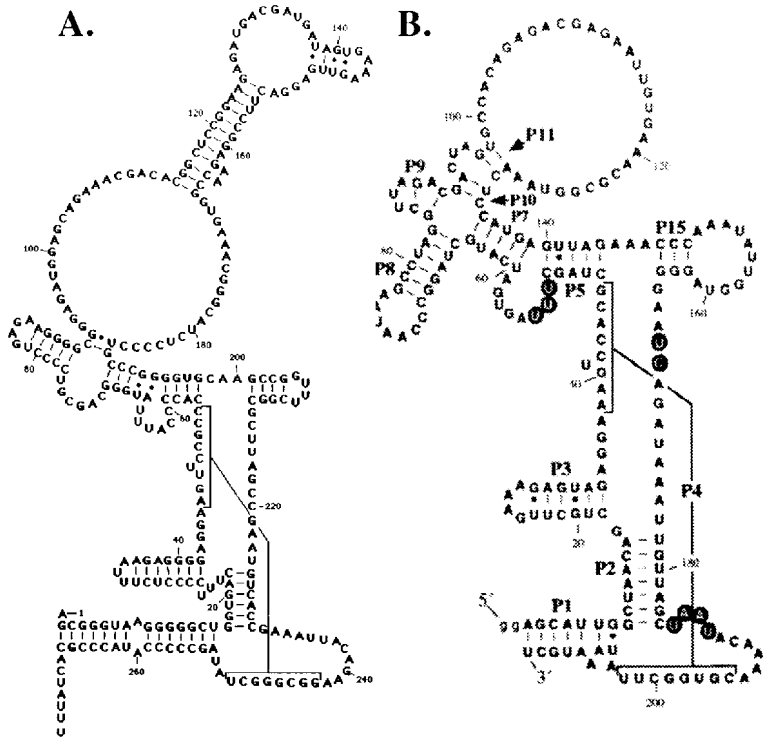

Figure 3. A. The secondary structure of *Methanococcus jannaschii* B. The secondary structure of Micro P, representing the minimum conserved sequence for RNA alone activity (8)

The molecular weight of the enzyme is greater than 400kDa, estimated by protein column markers (Sigma). The extreme size of the RNase P from *Methanococcus jannaschii* and how bouyant density compared to bacteral RNase Ps is consistent with a larger protein fraction and the need for the protein to compensate for the missing RNA structures.

The *Methanococcus jannaschii* holoenzyme has a Km of 32mM ±12 and remains maximally active between 2.5-30mM $MgCl_2$ and from 20-500mM NH_2OAc. The absence of P8 and L15 in the *Methanococcus jannaschii* RNase P RNA appears to have little affect on the catalytic activity of the holoenzyme. The range and level of activity at varying ionic conditions suggest that the enzyme is extremely robust. This is in contrast with the *Methanobacterium thermoautotrophicum* RNase P holoenzyme, whose peak ionic conditions are vary narrow at 8mM Mg_2Cl and 500mM NH_4Cl (Tom Hall, personal communication). The final purification and the identification of subunits of both RNase P holoenzymes from *Methanococcus jannaschii* and *Methanobacterium hermoautotrophicum* will provide greater insight into the ablity of protein to take over the function once carried out by RNA in other RNase Ps.

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