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High-Level Expression of Soluble Recombinant RNase P Protein from *Escherichia coli*

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We have expressed recombinant RNase P protein from *Escherichia coli* in high yield. A hexahistidine sequence at the amino terminus allowed protein purification in a single step. Mass spectrometry confirmed the molecular weight of the purified protein and indicated a purity of >95%. Protein functionality was demonstrated by reconstitution of active holoenzyme.

RNase P is a ribonucleoprotein that cleaves the 5′ leader sequence of pre-tRNA molecules to produce the mature 5′ end. *Escherichia coli* RNase P is composed of a 119-amino-acid protein component (10) and an RNA component of 377 nucleotides (16). The RNA component provides the catalytic activity (2). The RNA alone is catalytic in vitro under high-ionic-strength conditions but is an extremely poor catalyst under low-ionic-strength conditions (8). However, complementation with the protein component restores catalytic activity at low ionic strength. The protein component has an influence on both cleavage rate and substrate specificity (7, 9, 13) and may facilitate product release by means of ionic shielding (15).

Studies on the protein component and the holoenzyme have been greatly hampered by difficulties in purifying high concentrations of functional RNase P protein (18, 19). Herein we describe a procedure to purify soluble, active RNase P protein in high yield under native conditions by a single-step procedure that involves metal chelate affinity chromatography. We demonstrate that the purified, recombinant protein is functional by reconstitution of the RNase P holoenzyme followed by measuring processing of a *Bacillus subtilis* pre-tRNA^{His} substrate.

Cloning of *E. coli* RNase P protein. The *E. coli rnpA* gene coding for the protein component of RNase P was obtained by PCR from bacterial genomic DNA by using oligonucleotides complementary to the 5′ and 3′ regions of the *rnpA* gene plus added restriction sites (TTTGGATCCATGGTTAAGCTCGC ATTTCCC and TTTGAGCTCCAGGACCCGCGAGCCAG, respectively; The Midland Certified Reagent Company). The amplified DNA was sequenced (5) and cloned into vector pQE30 (Qiagen Inc.), which adds 12 residues (MRGSHHHHHHGS) at the amino terminus of the RNase P protein, including a series of six histidines specifically engineered into this construct. This QIA Express (Qiagen) pQE expression vector combines the phage T5 promoter with a repression system to allow tight regulation of protein production by means of the *lac* operator/*lac* repressor and is induced by isopropylthiogalactopyranoside (IPTG).

Protein purification. *E. coli* cultures were grown at 37°C in LB broth (containing 100 μg of ampicillin and 25 μg of kanamycin per ml) to an approximate A_{600} of 1.5. Protein expression was induced by addition of 2 mM IPTG (Sigma), and the cultures were further incubated for 2 h at 25°C. Cells were harvested at 4°C by centrifugation at 8,000 × *g*. The cell pellet was resuspended in 2× volumes of lysis buffer [50 mM Tris-HCl, 10 mM $Mg(C_2H_3O_2)_2 \cdot 4H_2O$, 1 M NH_4Cl, 0.25% Tween 20 (pH 7.9)] with protease inhibitors (pepstatin [1 μg/ml] and antipain [2 μg/ml]; Sigma). Cells were lysed by two cycles of freeze-thawing in dry ice-ethanol followed by six sonication pulses of 1 min each. The cell lysate was centrifuged at 14,000 × *g* for 20 min at 4°C and the supernatant was collected and loaded onto a Ni^{2+}-nitrilotriacetic acid column (Qiagen) preequilibrated with lysis buffer. The column was extensively washed with lysis buffer, and the protein was eluted with 0.5 M imidazole in 100 mM Tris-HCl (pH 8). Protein concentration was determined both by the bicinchoninic acid method (Pierce) and by the Lowry protein assay.

Expression levels of the recombinant protein were high in IPTG-induced cells, as determined by silver staining, in contrast to uninduced cells, in which the levels of recombinant protein were undetectable (not shown). Typical yields were of the order of 17 mg of pure, recombinant protein per liter of culture. Since the basis for the Ni^{2+}-nitrilotriacetic acid column purification requires the histidine-tagged terminal sequence, endogenous C5 protein or holoenzyme would not stick to that column and would not be copurified with the recombinant C5 protein. In any case, levels of endogenous C5 protein or holoenzyme, as estimated from the work of Vioque and Altman (19), are very low. They state that C5 is a very minor protein in *E. coli*, being present as about 250 copies per cell. Their one-step affinity chromatography procedure resulted in the purification of approximately 5 to 30 μg/20 g of cells for the endogenous protein and about sixfold amplification with their recombinant protein. Our recombinant protein represents over a 1,000-fold amplification above this endogenous level. In addition to purified RNase P protein from *E. coli*, we have also isolated *B. subtilis* RNase P protein in soluble form by using the approach described herein (not shown) and are currently characterizing the functional activity of this recombinant protein.

Physical characterization. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Coomassie blue staining of the purified, recombinant protein revealed a single band of the expected size (not shown). The molecular weight of the recombinant protein was further verified by electrospray ionization-mass spectroscopy (ESI-MS; Fig. 1). A single peak at 15,188.3 Da was observed, in agreement with a calculated molecular weight of 15,188.64 based on the expected amino acid composition of the recombinant protein including the histidine tag. From the ESI-MS data, we estimated a purity of greater than 95%.

Functional characterization. The recombinant protein was assayed for its ability to allow the catalytic RNA to complete

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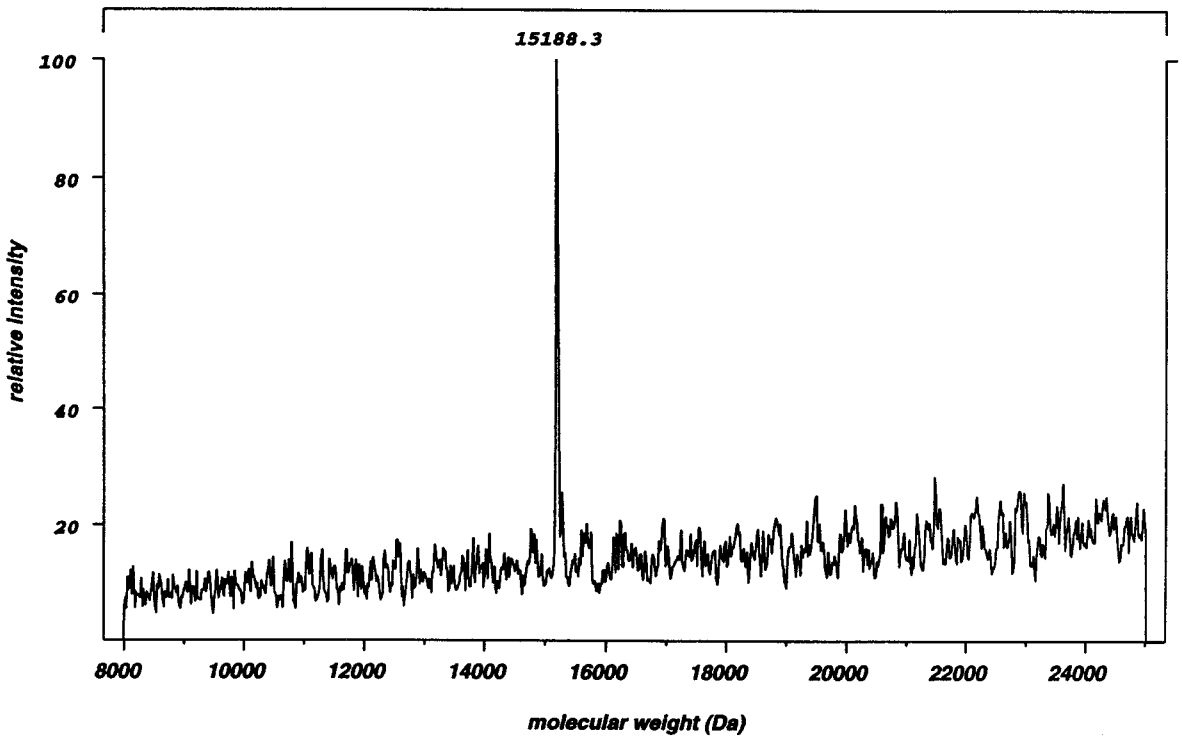

FIG. 1. ESI spectrum of purified, recombinant RNase P protein. Spectra were run by Kenneth Straub of Syntex Discovery Research. The purified protein was extensively dialyzed against 10 mM CH_3COONH_4 and analyzed in a Finnigan-MAT TSQ700 instrument with an electrospray ion source. A deconvoluted spectrum obtained on the purified, recombinant protein is shown.

5′-end processing of a *B. subtilis* synthetic pre-tRNA^{His} (1, 6). The *E. coli* M1 RNA was transcribed from T7 polymerase vectors (Promega Biotec) as previously described (4). Duplicate processing reactions were carried out for 3 min essentially as described previously (19) in low-ionic-strength buffer (50 mM Tris-HCl [pH 8.0], 10 mM $MgCl_2$, 100 mM NH_4Cl) or high-ionic-strength buffer (50 mM Tris-HCl [pH 8.0], 100 mM $MgCl_2$, 100 mM NH_4Cl, 5% polyethylene glycol). The 3-min time point was well within the linear range of the reaction. The holoenzyme was reconstituted by a 5-min preincubation at 37°C of the RNA and protein components followed by substrate addition. The fraction of active protein was determined by titration versus a constant amount of *E. coli* catalytic RNA (1 nM) (4) and substrate (200 nM; 1.29×10^{16} cpm/mol). Reaction products were separated by electrophoresis, and gel slices corresponding to the 5′ processed and unprocessed tRNA^{His} were cut out and quantitated by scintillation counting to determine the extent of processing. As Table 1 shows, very similar levels of substrate processing were observed for the holoenzyme whether the protein component was in molar excess or in equimolar amounts with the RNA component. An approximately 46% decrease in product formation was observed for the holoenzyme when the molar ratio between protein and catalytic RNA was reduced from 1:1 to 0.5:1. No processed pre-tRNA^{His} was observed when the recombinant protein or catalytic RNA was assayed alone in low-ionic-strength buffer or when the catalytic RNA alone was assayed in high-ionic-strength buffer. At this short incubation time, no processing was observed by the RNA alone because the rate of M1 alone is not sufficient to achieve processing in the absence of the protein during the time of the assay (14). Average variation among duplicates was approximately ±1% of product.

Implications. Previous methods for the purification of recombinant RNase P protein resulted in low yields of ≤2 mg/l (20) or in protein preparations with as low as 25% active protein (18). In contrast, herein we report yields of 17 mg of pure, recombinant RNase P protein per liter of culture and high protein activity as determined by enzymatic assays. It is unlikely that the observed solubility of the recombinant protein is the result of the extraneous residues incorporated during the cloning procedure. Little effect on protein function or structure has been reported for the hexahistidine sequence when this sequence is used with other protein constructs (3, 12, 17). Our results indicate that the presence of these extraneous residues at the amino terminus of the recombinant protein does not interfere with protein function in the holoenzyme;

TABLE 1. Reconstitution of RNase P holoenzyme

Condition	% Pre-tRNA^{His} processed[a]
Protein/RNA molar ratio	
0.5:1	6.3
1:1	11.6
2:1	13.5
4:1	13.8
RNA alone	
High ionic strength	<0.1
Low ionic strength	<0.1
Protein alone, low ionic strength	0.2

[a] A background value of 1.3 has been subtracted from all values. This background (1.3% ± 0.2%) was due to some tailing of the substrate and did not represent a true band. The percent processed values are low because the experiment was done at a short time point to keep within the linear range of the reaction.

however, precise studies need to be performed to determine what effect the histidine modification may have on other aspects of protein function. We have developed antibodies against the recombinant protein for use in the purification of RNase P protein that has no added histidines.

The purification of RNase P protein in native, soluble form is of substantial importance as it now becomes possible to carry out more detailed analysis on the interaction of the protein and RNA components in the holoenzyme. In addition, soluble, native RNase P protein should be amenable to physical studies involving nuclear magnetic resonance spectroscopy, X-ray crystallography, and other techniques. These analyses, combined with available three-dimensional models of the catalytic RNA (11, 21), should provide a detailed picture of the three-dimensional structure of the RNase P holoenzyme and of its possible modes of interaction with pre-tRNAs and other substrates.

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