Trial for peptide bond formation using model molecules based on the interactions between the CCA sequence of tRNA and 23S rRNA

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ABSTRACT
The peptidyl transfer reaction catalyzed by the ribosome is a sophisticated product of evolution. The molecular mechanism of peptide bond formation has not been fully elucidated although the essential involvement of 23S rRNA has been established. The universal CCA sequence at the 3'-end of tRNA plays an important role in this process, by interacting with specific nucleotides in 23S rRNA. However, reconstitution of peptidyl transferase activity by a naked 23S rRNA (without the help of any of the ribosomal proteins) has not been reported. To investigate the possible evolutionary development of the peptidyl transfer reaction, we tried to obtain peptide bond formation using a piece of tRNA — an aminoacyl-minihelix — mixed with sequence-specific oligonucleotides that contained puromycin. This system reproduced conceptually the equivalent interactions between the CCA trinucleotide of tRNA and 23S rRNA. Peptide bond formation was detected by gel electrophoresis, TLC and mass spectrometry. These results have implications for the evolution of the peptidyl transfer reaction in biological system.

INTRODUCTION
The ribosome plays a crucial role in protein biosynthesis. It is composed of proteins and RNAs organized into two subunits. In E. coli, the large (50S) subunit consists of 23S and 5S rRNAs, and 34 proteins. The small (30S) subunit contains 16S rRNA and 21 proteins, and functions as the site for decoding mRNA (1). The large subunit is able to carry out the peptidyl transfer reaction in the absence of the small subunit in a template-independent manner (2). Thus, the large subunit may have preceded the small subunit during the course of evolution.

The L-shaped three dimensional structure of tRNA is composed of two domains. One is the 12 bp minihelix that ends in the 3'-CCA trinucleotide common to all tRNAs. The other domain is at right angles to the minihelix and contains the anticodon triplet. Recently, we showed that an aminoacyl-minihelix was an efficient substrate for the 50S subunit in the peptidyl transfer reaction (3). This result is consistent with modern peptide bond formation being derived from the interaction between an aminoacyl-minihelix and the primitive ribosome.

The universal CCA sequence of tRNA has an important role in the peptidyl transfer reaction. Point mutations at any of the CCA nucleotides in tRNA abolished the peptidyltransfer activity (4,5). G2252, G2253 and U2585 in 23S rRNA are proposed to interact with CCA (5). This interaction is thought to be essential because these 3 nucleotides of 23S rRNA and the CCA sequence of tRNA are highly conserved (6,7).

To investigate one possible evolutionary development of the peptidyl transfer reaction, we tried to obtain peptide bond formation using a system comprised of an aminoacyl-minihelix and oligonucleotide substrates containing puromycin. The system was based on the interaction between the CCA trinucleotide of the minihelix and specific bases in 23S rRNA.

RESULTS AND DISCUSSION
The interaction of the conserved CCA terminus of tRNA with rRNA in the peptidyl transferase P site is thought to position two aminoacyl-tRNAs in close proximity. With this in mind, our model molecules were designed with the following characteristics: 1) The CCA terminus of the aminoacyl-tRNA interacts with a second esterified RNA through complementary base pairing. 2) The 3'-ends of the two aminoacyl-RNAs come in close proximity. In order to satisfy these criteria, puromycin-containing substrates were designed to have characteristics of aminoacyl-tRNA and of rRNA.

We made two puromycin (Pm)-containing molecules —
Pm-TTGGT and Pm-TGGT. For each, either TTGGT or TGGT was linked to puromycin through a 5'-5' phosphodiester bond. (For technical reasons, T (deoxythymidine) was used instead of U (uridine).) These molecules were incubated with N-acetylalanyl-minihelix in the absence of ribosomes. The mixture was treated with phosphodiesterase I (to liberate any N-acetylalanyl-puromycin that might form) and product formation was monitored by TLC analysis. Reactions containing either Pm-TTGGT or Pm-TGGT resulted in a new species having the same mobility as N-acetylalanyl-puromycin (generated in a ribosome-catalyzed fragment reaction that served as a control). In addition, mass spectrometry of the final product (after phosphodiesterase I digestion) gave a peak of 585 Da, which is the expected mass of the protonated form of N-acetylalanyl-puromycin. These results show that the reaction produced a peptide bond in the absence of the ribosome.

Interestingly, imidazole was required for peptide bond formation. Possibly the lone pair electrons of the imidazole nitrogen abstract a proton from the protonated α-amino group on puromycin. In the ribosome, tertiary nitrogens on bases are candidates for proton abstraction, particularly if they are activated by a metal ion (8). In primordial peptide bond synthesis, imidazole (known to be synthesized in prebiotic conditions (9)) may have been necessary to catalyze peptide bond formation.

In conclusion, a peptide bond can be formed between the amino acids attached to an aminoacyl-minihelix and a small RNA that bears puromycin. This reaction is dependent on interactions between the CCA sequence of tRNA and the small RNA. Because the first peptidyl transfer reactions were probably accomplished in the absence of protein, essential RNA-RNA interactions such as those described here probably occurred. Possibly the CCA sequence of tRNA has been conserved during evolution mainly for this purpose.

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