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Non-enzymatic aminoacylation of an RNA minihelix with an aminoacyl phosphate oligonucleotide

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ABSTRACT

Efficient aminoacylation of an RNA minihelix (a proposed precursor of tRNA) was achieved without any ribozymes or protein enzymes. The reaction was promoted with a template-like bridging oligonucleotide and a 5'-aminoacyl-phosphate-oligonucleotide. Aminoacylation was template-dependent and showed high preference for a particular ribose hydroxyl. The 5'-aminoacyl-phosphate-oligonucleotides have the same adenylate linkage as in aminoacyl-AMP — the universal intermediate used in modern aminoacylation systems with aminoacyl-tRNA synthetases. Thus, the results raise the possibility that this kind of system could have played a role in the transition from the RNA world to the theatre of proteins.

INTRODUCTION

Aminoacylation of transfer RNA (tRNA) is the biochemical step that establishes the rules of the genetic code, and has long been considered to be a key event making possible the transition from a predominantly RNA-based world to one that broadly used proteins. In contemporary aminoacylation, most aminoacyl-tRNA synthetases catalyze the overall reaction in two-steps. First, the carbonyl groups of amino acids are activated by formation of 5'-aminoacyl-AMP adenylates. The enzyme-bound adenylates then react with the 2' or 3' ribose hydroxyls of specific tRNAs, and thereby transfer the aminoacyl groups (1-3). In these reactions, each amino acid is paired with the cognate anticodon triplet that is imbedded in its tRNA. With this perspective, we wondered whether a system that used some of the key features of the adenylate chemistry could be used in an early, oligonucleotide-based system of aminoacylation, prior to the existence of protein catalysts. For these reasons, we investigated the possibility of using aminoacyl phosphate oligonucleotides (and template-like bridging oligonucleotides) for aminoacylation of tRNA.

RESULTS AND DISCUSSION

The free energy of hydrolysis of the aminoacyl phosphate bond (in an aminoacyl adenylate) is greater than that of the aminoacyl-ester bond (in aminoacyl-tRNA), by approximately 3 kcal mol^{-1} at pH 7.0 (4). Thus, the transfer of the activated aminoacyl group from the adenylate to the 3'-end of RNA is a 'downhill' reaction. With this in mind, we designed a system composed of a template-like bridging oligonucleotide and a 5'-aminoacyl-phosphoate-oligonucleotide that could be combined with a tRNA-like substrate. For our substrate, we used the minihelix domain of the two-domain tRNA structure.

The minihelix is a 12 bp stem-loop domain that terminates at the 3'-end in the universal CCA trinucleotide, with the site of aminoacylation being the terminal A. The minihelix portion of tRNA is a substrate for specific aminoacylation by many aminoacyl tRNA synthetases (5,6), and is thought to be an ancient part of tRNA (7-12). Indeed, the genetic code may have arisen from a 'second', more primitive code imbedded in the minihelix domain (5,6,8,9,11,12).

For the preparation of aminoacyl phosphate oligonucleotides, amino acids were coupled (using 1,3-dicyclohexylcarbodiimide (DCC)) through mixed phosphoanhydride linkages to the 5'-phosphates of dT_6dA_2 oligonucleotides. (Deoxynucleotides were used for reasons of technical convenience, particularly to avoid byproducts in the synthesis.) The bridging oligonucleotide was designed to be complementary to both the ACCA of the minihelix and to the amino acid-bearing aminoacyl phosphate oligonucleotide. Thus, the sequence of the bridging oligonucleotide was $U_2A_6UG_2U$. Incubation of minihelix, bridging oligonucleotide and 5'-Ala-p-dT_6dA_2 resulted in aminoacylation of the minihelix substrate. Denatured PAGE analysis under acidic conditions (pH 5) demonstrated formation of aminoacyl-minihelix with a yield of 15%. The reaction was dependent on a template-like bridging oligonucleotide. To specify the aminoacylation site, the cis-hydroxyls of the minihelix substrate were oxidized (treated with $NaIO_4$) to create cis aldehydes at the terminal adenosine.

No aminoacylation was detected with the oxidized substrate, consistent with the idea that aminoacylation occurred at either the 2'- or 3'- position (or both) of the terminal nucleotide.

To establish the positional specificity, 2'-deoxyadenosine and 3'-deoxyadenosine, respectively, were placed at the terminus of the minihelix substrate. The 2'-deoxyadenosine derivative was aminoacylated. In contrast, a minihelix with a 3'-deoxyadenosine was not. These results are consistent with aminoacylation being at the 3'OH of the terminal adenosine. This result suggests that the ternary complex (minihelix, bridging oligonucleotide, and aminoacyl phosphate oligonucleotide) forms an extended Watson-Crick duplex.

Thus, aminoacylation of RNA using aminoacyl-p-oligonucleotides could in principal provide a route to making the transition from an RNA world to the theater of proteins and, in addition, could have been an early step in the establishment of the genetic code. Notably, aminoacyl adenylates can be formed under prebiotic conditions (13). A system of this sort could also have combined with or evolved side-by-side with ribozyme-based catalysis of aminoacylation (14-17). Significantly, the peptide bond is lower in energy (by ~3 kcal mol^{-1} (4)) than the aminoacyl-ester linkage. Thus, when two aminoacyl RNAs are brought into close proximity, peptide bond formation occurs spontaneously (18-21). So, an RNA-directed aminoacylation system, such as the one described, here could rapidly lead to a world where peptides and eventually proteins played an increasingly significant role.

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