Synthesis of hybrid bisnucleoside 5', 5''-P1, P4-tetraphosphates by aminoacyl-tRNA synthetases

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Abstract

Aminoacyl tRNA synthetases, by means of a back reaction, are able to synthesize certain 5', 5''-P1, P4-bisnucleoside tetraphosphates of biological importance, such as Ap4A. Here it is shown that HisRS and TrpRS (Bacillus stearothermophilus) and AlaRS (E. coli) also synthesize the hybrid compounds Ap4G, Ap4C, and Ap4U. GlnRS (E. coli) is unable to synthesize any of the above compounds.


Abbreviations: HPLC, high pressure liquid chromatography; PEI, polyethyleneimine; RS, aminoacyl tRNA synthetase.

Introduction

The enzymatic synthesis of 5', 5''-bisadenosine-P1-P4-tetraphosphate (Ap4A) was first demonstrated by Zamecnik et al., (1) using E. coli lysyl-tRNA aminoacyl synthetase (LysRS). It has since been shown that at least 13 different synthetases are competent, to some extent, in this synthesis (2). The formation of bisnucleoside tetraphosphates is generally considered to proceed according to the scheme:

1. amino acid + ATP → amino acyl-AMP + PPi
2. amino acyl-AMP + ATP → Ap4A + amino acid

where reaction 2 is the reverse of reaction 1, with ATP replacing pyrophosphate. Except for studies with LysRS from rat liver (3), the cognate amino acid is always required, implying the formation of an amino acyl-adenylate as an obligate intermediate in this synthesis.

From all sources examined so far, the enzymes with the highest rates of Ap4A synthesis are LysRS (E. coli, yeast, sheep liver, rat liver) and PheRS (E. coli, yeast, Physarum polycephalum, sheep liver), as well as the E. coli AlaRS (4–9). These are also the only 3 enzymes that show activation by Zn2+. Enzymes unable to synthesize Ap4A are ArgRS from yeast and TrpRS from beef pancreas (5). Enzymes with low rates of Ap4A synthesis (none of these is stimulated by Zn2+) include AspRS (yeast) CysRS (E. coli), HisRS (E. coli), IleRS (E. coli).
LeuRS (yeast), MetRS (E. coli, sheep liver), and ValRS (yeast) (5, 8).

Since AP4A was found as a normal constituent of cells (10), studies have focused predominantly on the synthesis of this compound. Additional bisnucleoside oligophosphate compounds have since been identified in various cells or tissues: Gp4G, Gp3G, and Gp2G in brine shrimp embryos (11), as well as the hybrid compounds Ap4G and Ap3G in E. coli and Salmonella typhimurium (12, 13). Physiological roles for these compounds are possible since Ap4A concentrations increase in replicating cells (10) and increased concentrations of Ap4A, Ap3G, AP4G are associated with stress in bacteria (12, 13).

The synthesis of hybrid bisnucleoside tetraphosphates (Ap4X) or triphosphates (Ap3X) has been reported by 2 laboratories, both using the E. coli LysRS (7, 14). In the present report it is shown that several different synthetases from E. coli or Bacillus stearothermophilus synthesize hybrid compounds, and that these enzymes show different preferences for nucleoside triphosphates to replace pyrophosphate in reaction 2.

Experimental procedures

Materials

Amino acids, nucleotides, alkaline phosphatase, pyrophosphatase, and snake venom phosphodiesterase were purchased from Sigma Chemical Co. Ap3A, AP4A, and Ap5A were from PL Biochemicals. Tritiated ATP, GTP, CTP, UTP, and UDP were from ICN or Amersham. Polyethyleneimine (PEI) cellulose sheets for thin layer chromatography were obtained from Brinkmann, and all other chemicals were from Fisher.

Methods

The different synthetases were supplied by colleagues (see Acknowledgement) as prepared in these laboratories (15–17). The enzymes were all extensively purified, and two were almost homogeneous (Table 1).

The synthesis of Ap4A or Ap4X was performed at 37°C in a 50 µl reaction volume containing: Tris-HCl (pH 7.4 at 37°C), 50 mM; dithiothreitol, 0.2 mM; MgCl2, 10 mM; ATP, 2 mM; other nucleotides, when included, at 2 mM; the cognate amino acid, when included, at 2 mM; ZnCl2, when included, at 20 µM or 80 µM; 40 ng pyrophosphatase; and 1 µCi of the appropriate tritiated nucleotide, as indicated in the figure or tables. Reactions were started by the addition of enzyme. To ascertain that reaction rates were linear for the conditions used, enzyme activity was measured as a function of both time and protein concentration for the synthesis of Ap4A and Ap4U. The synthesis of other compounds was usually measured for a fixed time of 100 or 200 min. For each experiment reactions were performed in duplicate; rate values were determined from 2–4 experiments.

At appropriate times a 5 µl aliquot was spotted at the origin of a lane on the PEI cellulose sheet. These sheets (20 × 20 cm) were prewashed by ascending chromatography in water, and scored with a paper clip to produce lanes 1 cm wide. Radiactive standards, mixed with 5% glycerol and bo-

<table>
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<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Purity</th>
<th>PPl-ATP exchange</th>
<th>Ap4A synthesis</th>
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<td>HisRS</td>
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