VALYL-tRNA SYNTHETASE FROM YELLOW LUPIN SEEDS

Instability of enzyme-bound noncognate adenylates versus cognate adenylate

Hieronim JAKUBOWSKI
Institute of Biochemistry, Agricultural University, ul.Wołyńska 35, 60-637 Poznań, Poland

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1. Introduction

The error frequency in protein biosynthesis was estimated to be 1 in 3000 [1,2]. Since the misactivated amino acid (in the form of aminoacyl-tRNA) is incorporated on ribosomes into polypeptide chains at the position of the correct amino acid [3], the precision of synthesis of aminoacyl-tRNA esters must be of the level of 1 misacylation/3000 correct acylations or better.

Generally, the aminoacyl-adenylate formation reaction is less specific than the overall aminoacylation reaction. As early as 1961 Berg et al. [4,5] showed that an aminoacyl-tRNA synthetase activates several amino acids whereas only the cognate amino acid is finally esterified onto tRNA. Transfer RNA apparently induces increase in the specificity of aminoacyl-tRNA synthetases. Recently, several theories have been presented which could account for the high fidelity of aminoacyl-tRNA formation: kinetic proofreading [6–8], hydrolytic editing [9–11], and chemical proof-reading [12,13]. All these share a common feature: there is a path for rejection of an error in addition to a main path leading to the final product. The rejection path is either not specified [8] or rejection may possibly occur at the level of aminoacyl adenylate [10] or occurs at the level of aminoacyl-tRNA [9,12,13]. In the last two cases tRNA is apparently required for the rejection.

The first experimental evidence for the existence of rejection mechanisms in protein biosynthesis came from work on isoleucyl-tRNA synthetase from *E. coli* [14,15]: it was shown that $tRNA^{Ile}$ induces breakdown of isoleucyl-tRNA synthetase-bound valyl-adenylate. Subsequent work indicated that $Val\text{-}tRNA^{Ile}$ could be a transient intermediate in the $tRNA^{Ile}$-induced hydrolysis of valyl-adenylate [16].

Here I report the results of studies on lupin valyl-tRNA synthetase-bound cognate and noncognate adenylates. The enzyme-bound valyl adenylate is much more stable than the enzyme-bound noncognate (threonyl and α-aminobutyryl)-adenylates. The enzyme rejects noncognate adenylates in the absence of tRNA.

2. Materials and methods

Valyl-tRNA synthetase from yellow lupin seeds was prepared as in [17].

Radioactively labeled amino acids and radioactive ATP were obtained from the Institute for Research, Production, and Application of Radioisotopes, Prague. Unlabeled valine and threonine were from Sigma. DL-α-Aminobutyric acid was from Chemapol, Prague. ATP was purchased from Reanal, Budapest. Nitrocellulose filters (type BA 85) were a product of Schleicher and Schüll, and polyethylene-imine-cellulose (PEI-cellulose) was from Merck. Yeast inorganic pyrophosphatase was purchased from Boehringer, and bovine pancreatic ribonuclease was from Worthington.

Experiments were performed at 25°C in a medium containing 100 mM Hepes (pH 8.0), 10 mM $MgCl_2$, 30 mM 2-mercaptoethanol, 9.4 units/ml inorganic pyrophosphatase and 0.13 μM lupin valyl-tRNA synthetase.

Time course of enzyme-bound aminoacyl-adenylate formation with lupin valyl-tRNA synthetase was followed after addition of 2 μM [^{14}C]ATP (550 Ci/mol) and 30 mM unlabeled amino acid to the medium. Aliquots (60 μl) were removed at appropriate time intervals, added to an ice-cold solution of 20 mM Hepes, 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol and 10% glycerol, immediately filtered through nitrocellulose discs (i.d. 2.4 cm) and washed with three 1 ml aliquots of the buffer. The discs were oven-dried, immersed in toluene-based scintillation fluid and counted in a Beckmann LS-100 scintillation counter. 1 pmol corresponds to 880 cpm retained radioactivity on the discs. Controls were run without amino acid and amounted to 0.25 pmol which was subtracted from the results.

Hydrolysis of the enzyme-bound aminoacyl adenylates was followed after addition of 1 mM ATP to the medium containing the appropriate [^{14}C]-adenylate. Aliquots (60 μl) were quenched in ice-cold buffer and the nitrocellulose-bound radioactivity was determined as above.

The assay for ATP pyrophosphatase activity of lupin valyl-tRNA synthetase was carried out in the medium containing 4 μM [^{14}C]ATP and 22 mM amino acid. At appropriate time intervals 5 μl aliquots were spotted onto the origin line of PEI-cellulose plates which were then processed as in [18].

3. Results and discussion

Lupin valyl-tRNA synthetase forms a stable complex with valyl adenylate which may be isolated by gel filtration or retained on nitrocellulose discs [19]. The amount of the complex increases with time (fig.1). The half life for this increase is ~10 min. The result is the same when the experiment is carried out with either [^{14}C]ATP or [^{14}C]valine and could indicate the presence of a second binding site for valyl adenylate. Similarly, a second binding site for valine but not for valyl-adenylate was observed with *E. coli* valyl-tRNA synthetase [20].

Quite different results were obtained with noncognate adenylates. The amount of lupin valyl-tRNA synthetase-bound α-aminobutyryl [^{14}C]adenylate retained on nitrocellulose discs was significantly lower than that observed for the cognate adenylate.

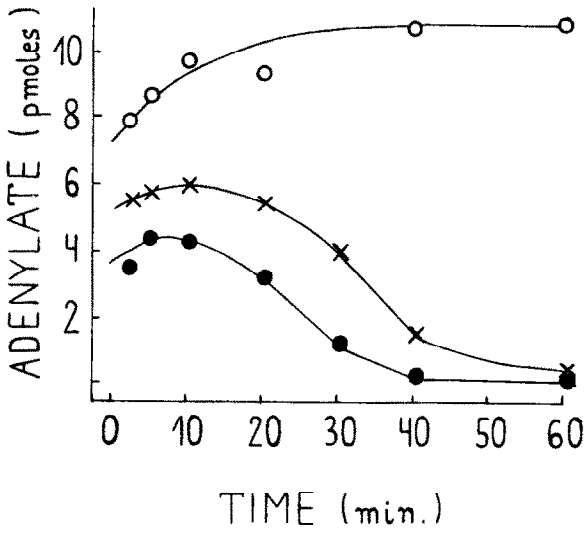

Fig.1. Time course of enzyme-bound aminoacyl-adenylate formation with lupin valyl-tRNA synthetase. Valyl (○), α-aminobutyryl (×) and threonyl (●) adenylates were assayed as in section 2. 7.8 pmol adenylate correspond to 1:1 enzyme–adenylate complex.

Moreover, its amount decreased with time and it was almost undetectable after 1 h at 25°C. The same results were obtained with threonyl-[^{14}C]adenylate except that its amount was lower and the complex disappeared after 40 min at 25°C (fig.1). This behaviour of noncognate adenylates is due to their instability. The half lives of the enzyme-bound α-aminobutyryl and threonyl-adenylates measured in the presence of free ATP and corresponding amino acid are ~1 min and 20 s, respectively. These figures are considerably below the half life of the enzyme-bound valyl-adenylate, which is 40 min (fig.2). Since tRNA induces the hydrolysis of the enzyme-bound noncognate aminoacyl adenylate [15], the above experiments were repeated with pancreatic ribonuclease-treated (10 min, 25°C, 0.24 mg/ml) ribonuclease valyl-tRNA synthetase. The half lives of noncognate adenylates were about 1.5-times greater with ribonuclease-treated enzyme, which is within the accuracy of the determination and indicates that the observed instability of the enzyme-bound noncognate adenylates is not caused by tRNA impurities in the valyl-tRNA synthetase preparation. Thus, lupin valyl-tRNA synthetase acts as an ATP pyrophosphatase in the

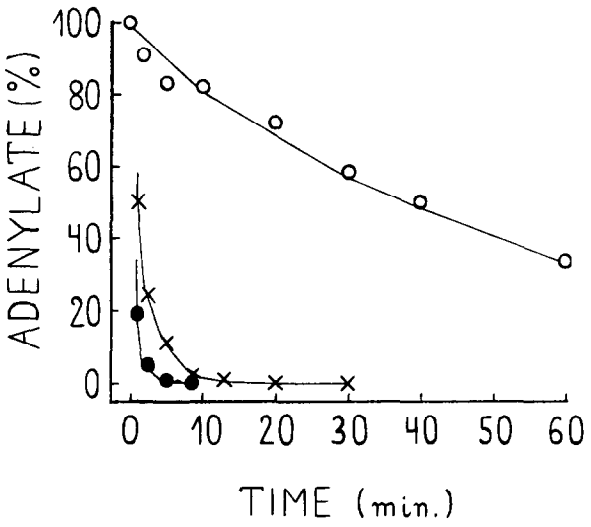

Fig.2. Hydrolysis of enzyme-bound aminoacyl-adenylates. Hydrolysis of valyl (○), α-aminobutyryl (×) and threonyl (●) adenylates was followed as in section 2.

presence of either α-aminobutyric acid or threonine and in absence of tRNA (fig.3).

The above phenomenon is interesting as a possible additional mechanism of increasing specificity of the

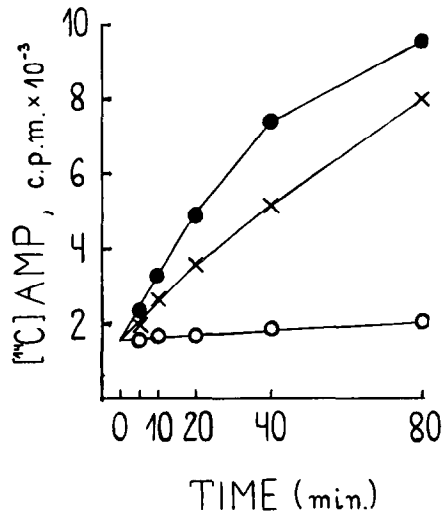

Fig.3. ATP pyrophosphatase activity of lupin valyl-tRNA synthetase in the presence of valine (○), α-aminobutyric acid (×) and threonine (●). The experiment was carried out as in section 2.

aminoacyl-tRNA synthetase. One can argue that the hydrolysis of enzyme-bound noncognate adenylates, despite it is ~100-times faster than that of cognate adenylate, is too slow to allow any additional discrimination. More precisely, noncognate adenylates survive on the enzyme long enough (half lives are 0.3–1 min) to be transfered to tRNA (half life of the transfer for valine is 0.0035 min [19], that for threonine and α-aminobutyric acid might be at least 0.028 min as determined by ATP pyrophosphatase assay). However, it is conceivable that under some physiological conditions (low temperature (?); excess of aminoacyl-tRNA synthetase over tRNA; low concentration of ATP) the instability of enzyme-bound noncognate adenylates might contribute to overall specificity of the aminoacyl-tRNA synthetase. My preliminary experiments indicate that threonine-dependent ATP pyrophosphatase activity of lupin valyl-tRNA synthetase increases only by a factor of 2 in the presence of saturating concentrations of tRNA.

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