Hieronym J. Jakubowski

THE PLANT AMINOACYL-tRNA SYNTHETASES.

2'-DeoxyATP AND ATP IN REACTIONS CATALYSED BY YELLOW LUPIN AMINOACYL-tRNA SYNTHETASES *

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2'-Deoxyadenosine triphosphate (dATP) is not a substrate for valyl-tRNA, leucyl-tRNA and isoleucyl-tRNA synthetases from yellow lupin seeds. Yellow lupin seryl-tRNA, phenylalanyl-tRNA, tyrosyl-tRNA, arginyl-tRNA, lysyl-tRNA and methionyl-tRNA synthetases use dATP as a substrate both in aminoacyl deoxyadenylate formation and tRNA aminoacylation reactions. Generally, dATP is a poorer substrate, being 3-50% as effective as ATP. None of the other nucleoside triphosphates tested was shown to be a substrate for these enzymes.

Specificity (kcat/Km) towards ATP is greater in tRNA aminoacylation than in pyrophosphate exchange reaction catalysed by seryl-tRNA synthetase. Energy of activation is the same (Ea = 18.5 kcal) for dATP- and ATP-dependent pyrophosphate exchange. Both dATP- and ATP-dependent tRNA aminoacylation reactions exhibit the same temperature dependence with Ea = 9.0 kcal.

Half lives of the enzyme-bound seryl adenylate and deoxyadenylate are 2 min and 4 min, respectively (pH 8.0, 25°C). Both enzyme-bound phenylalanyl adenylate and deoxyadenylate exhibit the same stability (half lives 0.3 min), and enzyme-bound tyrosyl deoxyadenylate is hydrolysed faster (half life 0.6 min) than tyrosyl adenylate (half life 2.5 min).

Serine is transferred both from enzyme-bound seryl adenylate and deoxyadenylate to tRNA with the same 60% efficiency. One mol of ATP or 1.1 mol of dATP is hydrolysed per one mol of seryl-tRNA formed during aminoacylation in the complete ATP- or dATP-dependent system, respectively. AMP concentrations (up to 0.5 mm), which do not affect the equilibrium of the ATP-dependent tRNA esterification with serine, significantly change the equilibrium of the dATP-dependent reaction.

All aminoacyl-tRNA synthetases share a common substrate, ATP, which can be substituted in some systems by 2'-deoxyATP, but by no other natural nucleoside triphosphate (Mitra & Mehler, 1969; Loftfield, 1972). It is

* Supported by the Polish Academy of Sciences within the project 09.7.1.
reasonable to speculate that ATP binding sites of some aminoacyl-tRNA synthetases might be similar or even identical. It is likely that these enzymes may be grouped into several classes with respect to the structure of their ATP binding sites. Similarities, if any, of the ATP binding sites of different aminoacyl-tRNA synthetases could shed some light on the evolutionary origin of these enzymes.

The structure of the ATP binding sites can be probed with ATP derivatives. Here, dATP has been employed as the probe and the dATP- and ATP-dependent reactions of several plant aminoacyl-tRNA synthetases are described. Previously we have shown that dATP cannot serve as a substrate for some lupin aminoacyl-tRNA synthetases (Jakubowski & Pawelkiewicz, 1975a).

MATERIALS

Seryl-tRNA and valyl-tRNA synthetases were purified to apparent homogeneity from yellow lupin seeds as described previously (Jakubowski & Pawelkiewicz, 1975a,b). Homogeneous lupin methionyl-tRNA (Joachimiak et al., 1978) and arginyl-tRNA synthetases were a kind gift of Dr A. Joachimiak. Phenylalanyl-tRNA and tyrosyl-tRNA synthetases, 66% and 90% pure, respectively, were prepared by ammonium sulphate fractionation, aminoethyl-Sepharose chromatography (Jakubowski & Pawelkiewicz, 1973), Sephadex G-200 gel filtration and hydroxylapatite chromatography (Jakubowski, H., in preparation). Lupin phenylalanyl-tRNA synthetase, $M_r = 275,000$, is a double dimer of two 75,000 and 68,000 dalton subunits. Lupin tyrosyl-tRNA synthetase, $M_r = 90,000$, is a dimer of two 44,000 dalton subunits.

Leucyl-tRNA, isoleucyl-tRNA and lysyl-tRNA synthetases were partially purified from yellow lupin seeds as described previously (Jakubowski & Pawelkiewicz, 1977).

Transfer RNA was prepared from yellow lupin seed meal according to Vanderhoef et al. (1970). Uniformly labelled $[{}^{14}C]$amino acids and $[{}^{14}C]$ATP (550 Ci/mol) were obtained from the Institute for Research, Production and Application of Radioisotopes (Prague, Czechoslovakia). $[{}^{14}C]2'$-DeoxyATP (546 Ci/mol) and $[{}^3H]$serine (5000 Ci/mol) were from The Radiochemical Centre (Amersham, England). Unlabelled amino acids were from Sigma (St. Louis, Mo., U.S.A.). ATP was purchased from Reanal (Budapest, Hungary) and 2'-deoxyATP was from Boehringer (Mannheim, F.R.G.). $[{}^{32}P]$Pyrophosphate was prepared by pyrolysis of $[{}^{32}P]$phosphate (Instytut Badań Jądrowych, Świerk near Otwock) as described by Loftfield & Eigner (1966).

Reeve Angel (Clifton, N. J., U.S.A.) SB-2 ion exchange paper was kindly supplied by Dr Robert B. Loftfield. Nitrocellulose filters (type BA 85) were from Schleicher and Schüll (Dassel, F.R.G.).
METHODS

The experiments were carried out at 25°C in a standard buffer containing 100 mM-Hepes, pH 8.0, 10 mM-MgCl₂, 10 mM-2-mercaptoethanol, 0.2 mM-EDTA and 0.2 mg/ml bovine serum albumin.

Pyrophosphate exchange assay. The initial rate of [³²P]pyrophosphate exchange into either ATP or dATP was measured by adding appropriate enzyme to a solution containing either ATP or dATP at 1 mM concentration, amino acid and [³²P]pyrophosphate in the standard buffer. Samples for arginine enzyme contained additionally 4 mg/ml lupin tRNA. Aliquots were spotted onto the origin line of SB-2 ion exchange paper, which was then developed in 0.1 M-pyrophosphate (pH 8.0). The [³²P]ATP spots remaining at the origin were cut out, dried and counted (Eigner & Loftfield, 1974).

tRNA aminoacylation assay. The initial rate of tRNA aminoacylation was measured by adding appropriate enzyme to a solution containing 1 mM either ATP or dATP, 20-40 μM-[¹⁴C]amino acid and 4 mg/ml lupin tRNA in the standard buffer. Aliquots were withdrawn and subjected to the standard trichloroacetic-acid-precipitation procedure (Eigner & Loftfield, 1974).

Seryl adenylate and deoxyadenylate assays. Enzyme-bound seryl adenylate or deoxyadenylate was assayed in the standard buffer containing 0.34 μM-seryl-tRNA synthetase, 8 units/ml yeast inorganic pyrophosphatase (Boehringer, Mannheim, F.R.G.), 2.5 μM either [¹⁴C]ATP or [¹⁴C]dATP and 1 mM-serine. Aliquots were filtered through nitrocellulose discs, which were then washed with three 1 ml portions of ice-cold buffer containing 20 mM-Hepes, pH 8.0, 5 mM-MgCl₂ and 30 mM-2-mercaptoethanol.

Decay of the enzyme-bound seryl adenylate or deoxyadenylate was followed after adding 1 mM-ATP to the reaction mixture containing seryl adenylate or deoxyadenylate formed in situ as described above. In some experiments seryl adenylate and deoxyadenylate were labelled with [³⁵S]serine.

Stoichiometry of ATP- and dATP-dependent seryl-tRNA formation. The rationale and procedure used were those described by Mulvey & Fersht (1977). The aminoacylation reactions were initiated by adding a solution of either ATP or dATP to the reaction mixtures (150 μl) containing 90 μM-[¹⁴C]serine (105 Ci/mol), 23 μM-tRNA₆₅₅ as 8 mg/ml solution of unfractionated lupin tRNA, and 0.75 μM lupin seryl-tRNA synthetase in the standard buffer. Aliquots of 15 μl were treated with ice-cold 5% trichloroacetic acid solution on Whatman 3 MM filter paper discs 2.4 cm in diameter. The discs were washed three times with the trichloroacetic acid solution, once with ice-cold ethanol, oven-dried and counted on a Beckman LS 100 scintillation counter.

This assay procedure is reliable and accurate. Application of 15 - 100 pmol of [¹⁴C]Ser-tRNA on 3 MM filter paper discs followed by ice-cold trichloroacetic
acid and ethanol washing, drying and scintillation counting gives 105% of
the number of counts found on drying a solution of the same sample on
the disc. This figure is independent of the time of washing up to two
hours. On the other hand, when the washing was carried out at room
temperature (22°C) the efficiency was time-dependent, being 83% after 1 h
washing. Therefore, care was taken to use ice-cold solutions for washing
and the washing was done in an ice-bath.

ATP and dATP concentrations were determined spectrophotometrically
using an excitation coefficient of 15.4 mm⁻¹cm⁻¹ at 260 nm in distilled water
(P-L Biochemicals Specifications). It has been verified by thin-layer chro-
matography on polyethyleneimine cellulose plates that at least 99% of the
UV-absorbing material in ATP and dATP preparations were in fact nucleoside
triphosphates. Only traces of mono- and diphosphates were seen on chro-
matograms developed in 2 M-formate, pH 3.4.

RESULTS

Is 2'-deoxyATP a substrate for lupin aminoacyl-tRNA synthetases? Lupin
valyl-tRNA synthetase does not utilize any nucleoside triphosphate other
than ATP as an energy source in the aminoacylation reaction. On the
other hand, lupin seryl-tRNA synthetase utilizes also dATP, but no other
nucleoside triphosphate (Table 1). These results show that, contrary to earlier

Table 1

Effect of ATP substitution by other nucleoside 5'-triphosphates (NTP) on
activity of seryl-tRNA and valyl-tRNA synthetase from yellow lupin seeds
in tRNA aminoacylation

The initial velocities of aminoacylation were measured with the indicated nucleoside
triphosphate (1 mM) instead of ATP as described in Methods. Reactions with ATP
are taken as 100%.

<table>
<thead>
<tr>
<th>Nucleoside 5'-triphosphate</th>
<th>Relative velocity (%) with seryl-tRNA synthetase</th>
<th>Relative velocity (%) with valyl-tRNA synthetase</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2'-dATP</td>
<td>30.3</td>
<td>0.3</td>
</tr>
<tr>
<td>GTP</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>ITP</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>XTP</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>CTP</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>UTP*</td>
<td>10.0*</td>
<td>3.0*</td>
</tr>
<tr>
<td>TTP</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>control, NTP omitted</td>
<td>0.4</td>
<td>0.13</td>
</tr>
</tbody>
</table>

* This activity is due to about 1% ATP contamination present in the UTP (Serva)
preparation.
suggestions (Mitra & Mehler, 1969), dATP cannot serve as a substrate for all aminoacyl-tRNA synthetases. Therefore, several other lupin aminoacyl-tRNA synthetases were examined using both pyrophosphate exchange and tRNA aminoacylation assays (Table 2). In addition to valyl-tRNA synthetase, also leucyl-tRNA and isoleucyl-tRNA synthetases do not utilize dATP as the energy source. Other aminoacyl-tRNA synthetases tested use dATP as a substrate in both reactions. In all cases except for the serine enzyme in pyrophosphate exchange, dATP is a poorer substrate than ATP. Doubling the concentrations of ATP and dATP does not increase the rates of reactions shown in Table 2.

Table 2
Utilization of 2'-dATP in tRNA aminoacylation and pyrophosphate exchange reactions catalysed by lupin aminoacyl-tRNA synthetases

The assays were done as described in Methods. In each case the initial velocity of ATP-dependent reaction is taken as 100%.

<table>
<thead>
<tr>
<th>Aminoacyl-tRNA ligase for</th>
<th>tRNA aminoacylation (%)</th>
<th>Pyrophosphate exchange (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Leu</td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Ile</td>
<td>0.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Met</td>
<td>3.3</td>
<td>10.0</td>
</tr>
<tr>
<td>Lys</td>
<td>10.4</td>
<td>24.1</td>
</tr>
<tr>
<td>Arg</td>
<td>20.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Tyr</td>
<td>17.0</td>
<td>24.0</td>
</tr>
<tr>
<td>Phe</td>
<td>49.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Ser</td>
<td>49.0</td>
<td>150.0</td>
</tr>
</tbody>
</table>

dATP- and ATP-dependent reactions of seryl-tRNA synthetase: catalytic indices and temperature effects. Catalytic indices for ATP and dATP in pyrophosphate exchange and tRNA aminoacylation catalysed by lupin seryl-tRNA synthetase are given in Table 3. \( K_m \) values for dATP are higher than those for ATP in both reactions, whereas the changes in \( k_{cat} \) are in opposite directions.

Table 3
Catalytic indices for ATP and 2'-dATP in pyrophosphate exchange and tRNA aminoacylation reactions catalysed by lupin seryl-tRNA synthetase (pH 8.0, 25°C)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Pyrophosphate exchange</th>
<th>tRNA aminoacylation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( K_m (\mu M) )</td>
<td>( k_{cat} (s^{-1}) )</td>
</tr>
<tr>
<td>ATP</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>2'-dATP</td>
<td>65</td>
<td>6</td>
</tr>
</tbody>
</table>
Both ATP- and dATP-dependent pyrophosphate exchange exhibit the same temperature dependence. Arrhenius energy of activation for both reactions is 18.5 kcal (Fig. 1A). Also ATP- and dATP-dependent tRNA aminoacylation exhibits the same temperature dependence with $E_a = 9.0$ kcal (Fig. 1B).

![Fig. 1. Arrhenius plots of seryl-tRNA synthetase activity in ATP- and dATP-dependent reactions. A, Pyrophosphate exchange; B, tRNA aminoacylation. The initial velocities of the reactions were determined at indicated temperatures in the range of 0 - 30°C as described in Methods. The scale on the ordinate axis, denoted ln V, is in arbitrary units. (●) dATP- and (○) ATP-dependent reactions.](image)

**Enzyme-bound seryl deoxyadenylate.** In the absence of ATP, seryl-tRNA synthetase catalyses serine-dependent incorporation of pyrophosphate into dATP. This could indicate that the serine enzyme forms seryl deoxyadenylate. To test this more directly, seryl-tRNA synthetase was mixed with [$^{14}$C]serine and dATP in the standard buffer and filtered through Sephadex G-100 column (Jakubowski, 1978). The radioactivity which emerged bound to the enzyme was identified as the enzyme-bound [$^{14}$C]seryl deoxyadenylate. Serine from this complex was transferred to tRNA with a 60% efficiency and a rate constant $k > 4$ min$^{-1}$, which is the same as with the enzyme-bound seryl adenylate (Jakubowski, 1978).

Addition of inorganic pyrophosphatase raises the amount of the enzyme-bound seryl adenylate 6 times, whereas the effect is over 10 times greater for the enzyme-bound seryl deoxyadenylate (Table 4). The amounts of seryl adenylate and seryl deoxyadenylate formed with a given amount of seryl-tRNA synthetase in the presence of inorganic pyrophosphatase are equal, which indicates that the same number of binding sites on the enzyme is occupied by either seryl adenylate or seryl deoxyadenylate.

The enzyme-bound seryl deoxyadenylate, as well as seryl adenylate (Jakubowski, 1978), can be retained on nitrocellulose discs. With this assay it was shown that the serine to dAMP ratio in the enzyme-bound seryl deoxyadenylate is 1 : 1; it was also found (Fig. 2) that the enzyme-bound seryl deoxyadenylate is more stable (half life 4 min) than seryl adenylate (half life 2 min). Thus the 50% drop in the rate of dATP-dependent charging of tRNA with serine cannot be due to relative instability of the
Table 4

Effect of inorganic pyrophosphatase on formation of enzyme-bound seryl adenylate and deoxyadenylate by lupin seryl-tRNA synthetase

The experiments were carried out in the standard buffer (see Methods). Seryl-tRNA synthetase, 0.33 μm, was incubated with 64 μm-[14C]serine (105 Ci/mol) and additions listed in the Table in a final volume of 75 μl for 2 min at 0 C. Then excess of unlabelled serine (1 mm) and tRNA (1 mg/ml) were added and [14C]seryl-tRNA formed during subsequent 1 min incubation at 0 C was determined as described in Methods.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Seryl adenylate or deoxyadenylate (pmol/sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. dATP (1 mm)</td>
<td>1.2</td>
</tr>
<tr>
<td>2. dATP (1 mm)+ pyrophosphatase (40 μg/ml)</td>
<td>80.</td>
</tr>
<tr>
<td>3. ATP (1 mm)</td>
<td>13.</td>
</tr>
<tr>
<td>4. ATP (1 mm)+ pyrophosphatase (40 μg/ml)</td>
<td>80.</td>
</tr>
</tbody>
</table>

enzyme-bound seryl deoxyadenylate. The same efficiency of serine transfer from adenylate and deoxyadenylate to tRNA also indicates that the stability of either seryl adenylate or deoxyadenylate does not contribute to the rate of transfer.

Stoichiometry of [14C]seryl-tRNA formation. During the charging of tRNA with [14C]serine, 1.00 ± 0.02 mol of ATP is hydrolysed per 1 mol of [14C]Ser-tRNA formed. When dATP is used as an energy source, 1.11 ± 0.02 mol of dATP is hydrolysed per 1 mol of [14C]Ser-tRNA (Fig. 3).

Equilibrium of ATP- and dATP-dependent seryl-tRNA formation: effect of AMP and PPi. In the absence of added AMP and PPi, both ATP- and dATP-dependent tRNA aminoacylation reaches the same final level.

![Fig. 2. Hydrolysis of enzyme-bound seryl adenylate (O) and seryl 2-deoxyadenylate (●). Enzyme-bound seryl [14C]adenylate and seryl [14C]deoxyadenylate were formed in situ. The hydrolysis was followed after addition of 1 mm-ATP to the [14C]adenylate or [14C]deoxyadenylate solution. After indicated time intervals aliquots were filtered through nitrocellulose discs as described in Methods.](image-url)
Fig. 3. Time-course of the aminoacylation of tRNA by seryl-tRNA synthetase. The aminoacylation was followed in the standard buffer with 0.75 μM lupin seryl-tRNA synthetase, 90 μM-[¹⁴C]serine (1 pmol = 124.3 c.p.m.), 23 μM-tRNA₅₀ and either ATP (○) or dATP (●) at concentrations (μM) indicated in the figure. Other details are given in Methods.

corresponding to almost complete charging. When AMP+PPᵢ (final conc. 0.09 M) were added to the reaction mixtures, the ATP-dependent level of charging dropped by 15% whereas the level of dATP-dependent charging dropped by 50%. This effect, as well as the effects of subsequent increasing of AMP+PPᵢ concentrations to 0.22 mM and finally to 0.43 mM are presented in Fig. 4. The new plateaus of tRNA charging established after additions of AMP+PPᵢ correspond to equilibrium constant $K_{eq} = 1.4\pm0.3$ for ATP-dependent reaction and $K_{eq} = 0.24\pm0.06$ for dATP-dependent reaction.

Fig. 4. Effect of AMP+PPᵢ on the equilibrium of ATP- (○) and dATP-dependent (●) seryl-tRNA formation. Reactions were carried out at 25°C in solutions (300 μl) containing the standard buffer, 1 mM-ATP (○) or dATP (●), 6.7 μM-tRNA₅₀, 51 μM-[¹⁴C]serine and lupin seryl-tRNA synthetase; 0.09 mM, 0.22 mM and 0.43 mM-AMP+PPᵢ were added at time 0, 30, and 60 min, respectively; 15 μl aliquots were quenched on filter paper discs by the trichloroacetic acid procedure.
As expected, in the case of ATP-dependent tRNA aminoacylation the addition of either 0.5 mM-AMP or 0.5 mM-pyrophosphate has no effect on the equilibrium. Contrary to this the level of the dATP-dependent reaction is lowered by 70% in the presence of 0.5 mM-AMP, whereas 0.5 mM-pyrophosphate is without effect (Fig. 5).

![Graph showing time-course of tRNA aminoacylation](image)

**Fig. 5.** Time-course of the aminoacylation of tRNA by seryl-tRNA synthetase. ATP. (○) and dATP-dependent (●) aminoacylation was followed under the conditions described in the legend to Fig. 4. AMP (final concentration 0.5 mM) was added at 30 min.

**DISCUSSION**

We have previously shown that dATP is not a substrate for lupin valyl-tRNA and tryptophanyl-tRNA synthetases (Jakubowski & Pawelkiewicz, 1975a) and these were the first enzymes of this class which were shown to be unable to use dATP as an energy source in tRNA aminoacylation. Here several other lupin aminoacyl-tRNA synthetases were surveyed with respect to utilization of dATP both in pyrophosphate exchange and tRNA aminoacylation reactions. It is striking that valyl-tRNA, leucyl-tRNA and isoleucyl-tRNA synthetases, which belong (at least in bacteria) to the single polypeptide class of aminoacyl-tRNA synthetases, do not use dATP as a substrate, either in pyrophosphate exchange or in tRNA aminoacylation. In a similar study (Friest et al., 1976b) it was shown that baker’s yeast valyl-tRNA and isoleucyl-tRNA synthetases do not utilize dATP in the tRNA aminoacylation reaction. This may be considered to be a reflection of likenesses between ATP binding sites of these enzymes in yeast and lupin. Contrary to our results with lupin tyrosyl-tRNA synthetase which utilizes dATP, the same authors (Friest et al., 1976b) have found that dATP is not a substrate for yeast tyrosyl-tRNA synthetase. This may reflect known differences in quaternary structure of yeast (x) (Ofengand, 1977) and lupin (x2) enzymes.
On the other hand, *E. coli* tyrosyl-tRNA synthetase, which is an $\alpha_2$ dimer (Ofengand, 1977), utilizes dATP in aminoacylation (Mitra & Mehler, 1969), but not in pyrophosphate exchange (Mitra & Mehler, 1969; Santi & Pena, 1973). In contrast with lupin lysyl-tRNA synthetase which utilizes dATP in pyrophosphate exchange and tRNA aminoacylation, baker's yeast lysine enzyme does not use dATP in tRNA aminoacylation (Friest et al., 1978), and *E. coli* lysine enzyme hardly uses dATP in pyrophosphate exchange and uses it almost as well as ATP in tRNA aminoacylation (Mitra & Mehler, 1969). The results presented in Table 2 are in accordance with the earlier reports that dATP is a substrate for phenylalanyl-tRNA synthetase of *E. coli* (Santi et al., 1971) and yeast (Friest et al., 1976a), seryl-tRNA synthetase of yeast (Friest et al., 1976a), and arginyl-tRNA synthetases of *E. coli* (Hirshfield & Bloemers, 1969) and yeast (Friest et al., 1978). Utilization (or not) of dATP by aminoacyl-tRNA synthetases may serve as criterion in classifying ATP binding sites of these enzymes. Inhibition by PP$_i$ (Santi & Pena, 1973) and AMP may also serve this purpose. Lupin valyl-tRNA synthetase is inhibited by PP$_i$ to a greater extent than seryl-tRNA synthetase (Jakubowski, 1978), whereas AMP is more inhibitory with seryl-tRNA synthetase (Jakubowski, unpublished).

On the basis of initial velocity and $K_m$ measurements it was argued that dATP cannot be an effective energy source for the aminoacyl-tRNA synthesis in vivo (Mitra & Mehler, 1969). However, Santi et al. (1971) suggested that dATP may be involved in the synthesis of phenylalanyl-tRNA in vivo. The kinetic indices of ATP- and dATP-dependent reactions catalysed by lupin seryl-tRNA synthetase (Table 3) indicate that dATP is four times less effective than ATP in seryl-tRNA synthesis. Data of Table 2 and preliminary values of $K_m$ for ATP and dATP determined for other lupin aminoacyl-tRNA synthetases indicate that dATP may be even less effective with other synthetases. Moreover, the equilibrium of dATP-dependent seryl-tRNA formation is affected by AMP + PP$_i$ and AMP alone to a much greater extent than that of ATP-dependent reaction (fig. 4 and 5). Thus, taking into account relative concentrations of ATP and dATP in vivo, it follows that dATP has only a negligible role as an energy source in the aminoacyl-tRNA formation in vivo.

In pyrophosphate exchange (Table 3) ATP ($k_{\text{cat}}/K_m = 0.13$ s$^{-1}$ $\mu$m$^{-1}$) is only a slightly better substrate than dATP ($k_{\text{cat}}/K_m = 0.09$ s$^{-1}$ $\mu$m$^{-1}$) for lupin seryl-tRNA synthetase. However, in tRNA aminoacylation reaction, this enzyme clearly discriminates dATP. The lower specificity of aminoacyl-tRNA synthetases towards non-cognate amino acids in the pyrophosphate exchange than in tRNA aminoacylation is well established (Loftfield, 1972). The present observation extends this to an ATP analogue: dATP is almost as good as ATP in pyrophosphate exchange whereas in tRNA aminoacylation ATP is a better substrate than dATP.
Since the $k_{cat}$ values differ for ATP- and dATP-dependent reactions and Arrhenius energy of activation is identical for both, it follows that the difference is due to the entropy of activation term. Thermodynamic activation parameters calculated from data of Fig. 1 and Table 3, are given in Table 5. Substitution of ATP by dATP results in the greatest effect (18% increase) on entropy of activation ($\Delta S^-$) for pyrophosphate exchange. It is remarkable that the change of $\Delta S^-$ for tRNA aminoacylation is in the opposite direction (5.1% decrease). This may be an experimental manifestation of different orientational requirements for the reactants (most likely the orientation of ATP with respect to its microenvironment) in the transition state during pyrophosphate exchange and tRNA aminoacylation reactions.

**Table 5**

*Thermodynamic activation parameters for ATP- and dATP-dependent pyrophosphate exchange and tRNA aminoacylation catalysed by lupin seryl-tRNA synthetase (pH 8.0, 25°C)*

<table>
<thead>
<tr>
<th></th>
<th>Pyrophosphate exchange</th>
<th>tRNA aminoacylation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATP</td>
<td>dATP</td>
</tr>
<tr>
<td>$\Delta F^*$</td>
<td>16.58</td>
<td>16.34</td>
</tr>
<tr>
<td>$\Delta H^*$</td>
<td>17.9</td>
<td>17.9</td>
</tr>
<tr>
<td>$\Delta S^*$</td>
<td>4.4</td>
<td>5.2</td>
</tr>
</tbody>
</table>

* Values in parentheses indicate the change of an activation parameter of dATP-dependent reaction relative to that of ATP-dependent reaction.

As described above, the enzyme-bound seryl deoxyadenylate may be isolated in the same way as seryl adenylate and their properties are similar with the exception of greater stability of the former. It should be noted, however, that the increased stability of the enzyme-bound seryl deoxyadenylate is not a general property of enzyme-bound deoxyadenylates. This point is illustrated in Table 6, which shows that enzyme-bound phenylalanyl adenylate

**Table 6**

*Stabilities of enzyme-bound aminoacyl-adenylates and deoxyadenylates formed in situ with seryl-tRNA, phenylalanyl-tRNA and tyrosyl-tRNA synthetases (pH 8.0, 25°C)*

<table>
<thead>
<tr>
<th></th>
<th>Half life of enzyme-bound adenylate</th>
<th>deoxyadenylate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seryl</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Phenylalanyl</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Tyrosyl</td>
<td>2.5</td>
<td>0.6</td>
</tr>
</tbody>
</table>
and deoxyadenylate formed with lupin phenylalanyl-tRNA synthetase exhibit the same stability, whereas the enzyme-bound tyrosyl adenylate formed by lupin tyrosyl-tRNA synthetase is more stable than tyrosyl deoxyadenylate.

The stoichiometry data (Fig. 3) confirm that dATP is less effective than ATP as an energy source in seryl-tRNA formation catalysed by lupin seryl-tRNA synthetase. The 10% non-effective breakdown of dATP may in part account for the decrease in $k_{cat}$ observed for dATP-dependent seryl-tRNA formation.

REFERENCES


SYNTETAZY AMINOACYLO-tRNA Z ROŚLIN. 2'-DezoksyATP I ATP W REAKCJACH KATALIZOWANYCH PRZEZ SYNTETAZY AMINOACYLO-tRNA Z NASION ŁUBINU ŻÓLTEGO

Streszczenie

Trójfosforan 2'-dezoksyadenozyny (dATP) nie jest substratem dla syntetaz waliło-, leucyno- i izoleucyno-tRNA z nasion lubinu żółtego. Łubinowe syntetazy serylo-, fenylalanynylo-, tyrozyno-, argynio-, lizyno- i metionyno-tRNA wykorzystują dATP jako substrat zarówno w reakcjach tworzenia aminocytolodzoksyadenilanów jak i aminocytolacji tRNA. 2'-Dezoksy-ATP jest od 3 do 50% tak efektywny jak ATP. Żaden inny badany trójfosforan nukleozydu nie jest substratem dla tych enzymów.

Specyficzność (k_{cat}/K_m) względem ATP jest większa w reakcji aminocytolacji tRNA niż w reakcji wymiany pirofosforanu katalizowanych przez syntetazy serylo-tRNA. Energia aktywacji Arrheniusa jest taka sama (E_a = 18.5 kcal) dla wymiany pirofosforanu z dATP jak i ATP. Również reakcje aminocytolacji tRNA zarówno w obec dATP jak i ATP wykazują taką samą zależność od temperatury (E_a = 9.0 kcal).

Okres półtrwania związku z enzymem adenilanu serynowego wynosi 2 min, a dezoksyadenilanu serynowego 4 min (pH 8.0, 25°C). Zarówno związany z enzymem adenilan jak i dezoksyadenilan fenylalaninowy wykazują jednakową trwałość (t_{0.5} = 0.3 min), a dezoksyadenilan tyrozynowy rozpada się szybciej (t_{0.5} = 0.6 min) niż adenilan tyrozynowy (t_{0.5} = 2.5 min).

Seryna jest przenoszona z dezoksyadenilanu, jak i z adenilanu, na tRNA z tą samą 60% wydajnością. Jeden mol ATP lub 1.1 mol dATP jest hydrolizowany na każdy mol serylo-tRNA. AMP zmienia równowagę esteryfikacji tRNA seryną wobec dATP, lecz nie wobec ATP.

Received 22 October, 1979.