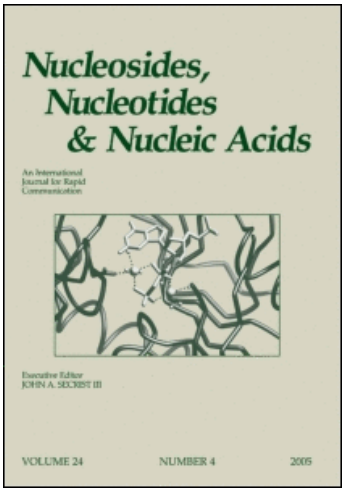

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INHIBITION OF THE FIRST STAGE OF RNA LIGASE REACTION BY ATP ANALOGUES

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Abstract. It was shown that most ATP anologues which are modified with respect to polyphosphate part of the molecule are competitive inhibitors of the first stage of the RNA ligase reaction. All investigated compounds, with the exception of AMP and ADP, form a covalent AMP-RNA ligase complex.

T4 RNA ligase (polynucleotidyl synthetase, EC 6.5.1.3) has been found to catalyze the ATP-dependent circularization and intermolecular joining of oligonucleotides. This catalysis is found to occur in three stages[1,2] :

1. $E + ATP \overset{Mg^{2+}}{\rightleftharpoons} E - pA + PP_i$
2. $E - pA + pX \rightleftharpoons E \cdot [A(5')ppX]$
3. $E \cdot [A(5')ppX] + Y \rightleftharpoons YpX + AMP + E$

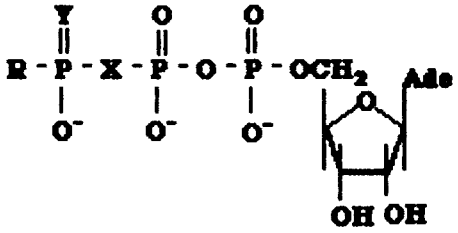
In the first stage of the reaction the RNA ligase reacts with ATP to form the covalent intermediate - AMP-RNA ligase (E-pA) complex. This reaction is well-characterized, as the optimal conditions for the reaction[3], the inhibition of RNA ligase by several compounds[4,5], and the nature of the covalent bond between AMP and the enzyme[6] have been previously determined. We have investigated the influence of the modification of the polyphosphate part of ATP upon the first stage of the T4 RNA ligase reaction. In order to determine the effect of the phosphate moiety of ATP upon the reaction, the following compounds were studied : adenosine (A), AMP, ADP, γ-thio-ATP (D), β,γ-imine-ATP

TABLE 1. Half inhibition (I_{50}) of the T4 RNA ligase by A, AMP, ADP and ATP analogues (I - VI)

Inhibitors	A	AMP	ADP	I	II	III	IV	V	VI
I_{50} *), μM	—	20.0	12.0	0.2	2.0	0.75	11.0	4.0	0.2

*) I_{50} - the inhibitor concentration which is required for 50% inhibition of the T4 RNA ligase reaction at the substrate (ATP) concentration of 2.5 μM, RNA ligase - 0.2 μg.

(II),β,γ-methilene-ATP (III), P^1,P^3-di(adenosine-5') triphosphate (IV), P^1,P^4-di(adenosine-5') tetraphosphate (V) and adenosine 5'-tetraphosphate (VI) :



I. R = O⁻; Y = S; X = O; II. R = O⁻; Y = O; X = NH;

III. R = O⁻; Y = O; X = CH_2; IV. R = Ado; Y = X = O;

V. R = AMP; Y = X = O; VI. R = OPO_3^{2-}; Y = X = O;

The preliminary information about efficiency of the inhibition was received from half inhibition (I_{50}) data (Table 1).

From data presented in Table 1 it is concluded that all compounds, with the exception of adenosine, are inhibitors of the T4 RNA ligase. The addition of one (AMP), two (ADP) or four phosphates (adenosine 5'-tetraphosphate) to the adenosine increases the efficiency of inhibition in direct proportion to the number of phosphate residues. These experiments led us to conclude that the polyphosphate part of ATP is very important in the reaction of ATP with the enzyme. The best inhibitors are γ-thio-ATP and adenosine 5'-tetraphosphate

In order to further characterize the kinetics of the reaction, the K_i for the inhibitors of RNA ligase were determined. The kinetics of labeled AMP-RNA ligase complex formation was investigated in the presence of different concentrations of [^{14}C] ATP and inhibitors (Table 2).

TABLE 2. Inhibition constants (K_i) of AMP,ADP, and ATP analogues*)

Inhibitors	AMP	ADP	I	II	III	IV	V	VI
K_i ,μM	250 ± 22	60 ± 5	1.5 ± 0.07	12.9 ± 1.3	5.5 ± 0.4	41 ± 4	23 ± 2	0.8 ± 0.05

*)Under the conditions of our experiments in the absence of inhibitors, the K_m of the reactions of ATP with RNA ligase was found to be 1.0± 0.08 μM and V_{max} = 0.05± 0.004 μM / min·mg protein

TABLE 3. The yields of the covalent AMP-RNA ligase complex formation during the reaction of ATP analogues with RNA ligase.

Inhibitors	I	II	III	IV	V	VI
*) Yields of the covalent AMP-RNA ligase complex formation (%)	62	38	32	10	10	70

*) Under the conditions of the reaction (25°C, 5 min) ATP with RNA ligase yields 65% of covalent AMP-RNA ligase complex.

We have found that all ATP analogues are competitive inhibitors of the first stage of the RNA ligase reaction. The best inhibitors are γ-thio-ATP (I) and adenosine 5'-tetraphosphate (VI), which led us to conclude, that the electrostatic interaction of ATP and RNA ligase is very important in the first stage of the reaction. However, compounds (IV) and (V) which are joined to an additional adenosine group exhibited much higher values for K_i in comparison with compounds (I) and (VI), respectively. This would indicate, that other factors besides electrostatic forces play a role in the inhibition as both sets of compounds (I and IV, V and VI) contain the same number of phosphate residues. We propose that this effect is determined by the steric influence of the additional adenosine residue in the case of compounds (IV) and (V). The efficiency of RNA ligase inhibition by ATP analogues depends therefore not only upon the quantity of phosphate groups in the inhibitor molecule but also upon the substitution at the γ-phosphorus atom of ATP.

It is known that RNA ligase and its adenylated analogue may be separated by SDS electrophoresis[7]. This method was used for the estimation of the type of binding of AMP, ADP and ATP analogues with RNA ligase. It was shown that all investigated compounds,

with the exception of AMP and ADP, form a covalent AMP-RNA ligase complex. The yields of reaction are represented in Table 3.

These experiments show that compounds (IV,V) are poor substrate of the first stage of the RNA ligase reaction. It is very interesting to mention that adenosine 5'-tetraphosphate is a somewhat better substrate than ATP itself. This fact shows once more that the quantity of phosphate groups in the adenosine nucleotide is very important for the covalent AMP-RNA ligase complex formation.

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