**2'-5'-Linked Triadenylates Act as Protein Kinase Activity Modulators**

**Skorobogatov OY, Kukharenko AP, Kozlov OV, Dubey IY and Tkachuk ZY***

*Department of Protein Synthesis Enzymology, Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Ukraine*

**Abstract**

2'-5'-linked triadenylates present the class of low molecular compounds, capable of regulating various cell functions. Their main function is the activation of ribonuclease L – the main enzyme of the innate immunity. Their dephosphorylation results in loss of this function, which, on the other hand grants them a handful of new, yet undiscovered abilities.

**Aim:** Study the concentration-dependent influence of dephosphorylated 2'-5'-linked tradenylates on the protein kinases activity.

**Methods:** Protein kinases were titrated with radioactively labeled ATP. After that the radioactivity value was determined with the use of scintillation counter.

**Results:** We have found out that 2'-5'-linked are capable of changing the biological activity of various protein kinases by altering the amount of ATP they can potentially cleave.

**Conclusions:** We suggest that this effect could occur to the specific changes in protein kinases structure.

**Keywords:** 2'-5'-Linked oligoadenylates; Protein kinase

**Introduction**

Triphosphorylated 2'-5'-linked triadenilates (ppp2'-5'А3) activate ribonuclease L (RNase L) and trigger interferon-induced antiviral protection of the cell [1]. Interaction of ppp2'-5'А3 with the enzyme is the essential condition for the induction of ribonuclease activity [2]. RNase L binding to the 5'-end of 2'-5'-oligonucleotide requires the presence of at least one phosphate group, while the activation of enzyme requires at least two phosphate groups [3]. Three active domains of the RNase L have been discovered so far. The first one is responsible for direct binding to ppp2'-5'А3, the second one has nuclease activity, and the third one possesses protein kinase (PK) activity [4]. In the absence of ppp2'-5'А3, the inhibitory sequence within the third domain of RNase L inhibits RNase domain. Interaction of ppp2'-5'А3 with RNase L induces structural alterations within protein’s structure, causing the formation of a dimeric form. As a result, the ribonuclease domain acquires the capacity of viral RNA disintegration. Dephosphorylated 2'-5'-tryadenilates (2'-5'А3) do not bind to RNase L. Their role in cell metabolism is not completely clear. PKs are the key mediators of various cellular reactions (Table 1). In many diseases, including cancer pathology and hypertension, down regulation of PK activity is observed. That is why many protein kinase inhibitors exhibit antitumor activity. Previously we have shown that the 2'-5'А3 and their analogues have specific biological effects, affecting the voltage-dependent calcium channels, activity of which is associated with the protein kinase A [5]. It was shown that 2'-5'А3 and their analogues affect the contraction of smooth muscle of aorta and femoral artery in rats. The action of the 2'-5'А3 and their analogues can be eliminated by using the inhibitors of calcium-dependent potassium channels of high conductivity or PK A inhibitors [6-13]. We’ve demonstrated the capability of 2'-5'А3 binding to several biologically relevant proteins [14], as well as its capability of altering protein’s secondary structure [15]. We have suggested a possible concept of 2'-5'А3 involvement in various biological processes, which includes its unspecific binding to cell proteins, including protein kinases, followed by conformational changes within target proteins, which, in the end, leads to activity value shifts, both negative and positive. In this paper we demonstrate the direct evidence for the capability of 2'-5'А3 and its epoxy-modified analogue to modulate the activity of protein kinase Aurora. Our previous data demonstrate that 2'-5'А3 are capable of affecting the value of L-currents of Ca2+ channels [16]. Considering the fact that this process is regulated via the specific protein kinase’s activity, current study is focused on the investigation of possible direct 2'-5'А3 influence on protein kinase activity.

**Materials and Methods**

We used protein kinases produced by Millipore (USA), CK2 and peptide substrate RRREEETEEE (CK-tide) by New England Biolabs, (USA).

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*Corresponding author: Tkachuk ZY, Group of Molecular Pharmacology, Department of Protein Synthesis Enzymology, Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Ukraine, Tel: +380(44) 526-20-16; Fax: +380(44) 526-07-59; E-mail: ztkachuk@yahoo.com

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**Table 1:** Biological activities of different protein kinases.

<table>
<thead>
<tr>
<th>Protein Kinase Name</th>
<th>Biological Effect</th>
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<tbody>
<tr>
<td>Casein kinase 2 (CK2)</td>
<td>Increased activity associated with inflammatory reactions and cell differentiation and tumor growth [7].</td>
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<tr>
<td>Fibroblast growth factor receptor 1 (FGFR1)</td>
<td>Regulation of angiogenesis and cell proliferation [8].</td>
</tr>
<tr>
<td>Tyrosine-protein kinase (TIE2)</td>
<td>Angiogenesis, intercellular interactions, the activity of endothelial cells, oncology [9].</td>
</tr>
<tr>
<td>Rho-associated protein kinase (ROCK)</td>
<td>Activates myosin. Its content changes during the development of the heart in pathological myocardial hypertrophy and dilated cardiomyopathy [10].</td>
</tr>
<tr>
<td>Aurora protein kinase</td>
<td>Proliferation, regulation of mitosis [12].</td>
</tr>
<tr>
<td>Apoptosis signal-regulating kinase 1 (ASK1)</td>
<td>Apoptosis, cell division [13].</td>
</tr>
</tbody>
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**Keywords:** 2'-5'-Linked oligoadenylates; Protein kinase
Synthesis of oligoadenylates

Natural 2’-5’ triadenilate and its epoxy modified analogue, 2’-5’ epoxetriadenilate (2’-5’A3-epo), were synthesized as described previously (Figure 1 for oligoadenylates’ chemical formulae) [5].

Assaying protein kinase activity in the presence of oligoadenylates

The study of the protein kinase activity in the presence of 2’-5’A3 was conducted in test system by directly determining the product of PK activity using [32P]-ATP in vitro [17]. The radioactivity was determined with the use of scintillation counter TriSarb-7000 (Perkin-Elmer, USA). Protein kinase activity level was determined by the ratio of the radioactivity of reaction products in the presence and absence of 2’-5’A3. Protein kinase was titrated with 2’-5’A3 and 2’-5’A3-epo at concentrations from 5 μM to 160 μM in the presence of 25 and 100 μM ATP. To determine the IC50 value, oligoadenylates were used at the concentration range from 50 nM to 50 μM.

Statistical analysis

Each protein kinase activity experiments were conducted at least 3 times. The data were probed with the use of one-way analysis of variance (Anova), followed by the Student t-test. Results are expressed as mean ±SD (standard deviation). Obtained data has been shown to be statistically significant (n=3, p<0.05).

Computer modeling

Docking experiments were carried out using the Gold Suite software. Considering the absence of any information within literature sources, binding site was set by single atom selection (c-gamma atom of Leucin 194) deep in the protein’s cavity and spread to 15 angstrom diameter sphere. Visualization of docking results was performed with the use of PyMol software.

Results and Discussion

We have investigated the influence of 2’-5’A3 and 2’-5’A3-epo (Figure 1) on the activity of several PKs: Aurora, FGFR, TIE2, ROCK1, JNK1, CK2 and ASK. For clarity sake, we have only provided the titration curves for protein kinase Aurora in the presence of 2’-5’A3 and 2’-5’A3-epo within this study, since we believe that this particular kinase is the most relevant to suggested biological function of oligoadenylates. As it follows from the obtained results, these PKs behave differently upon binding to oligoadenylates. In most cases, the bot 2’-5’A3 and 2’-5’A3-epo stimulate the activity of protein kinases. It turned out that 2’-5’A3 mainly activates studied protein kinases and particularly strongly (84%) increases the activity of protein kinase CK2. JNK1 and ROCK1 kinases are up regulated moderately (31% and 40% respectively) by 2’-5’A3. The protein kinases TIE2, ASK, and FGFR activity is increased by 18-25% upon binding of 2’-5’A3. On the contrary, in case of Aurora kinase in particular, 2’-5’A3 demonstrates opposite effect – 35% inhibition. A similar to 2’-5’A3, yet more pronounced effect was demonstrated by the 2’-5’A3-epo: its stimulating activity was higher than 2’-5’A3. On the contrary to 2’-5’A3, 2’-5’A3-epo inhibits the ASK activity by 21%, and increases the inhibition of the Aurora kinase by 5% (40% inhibition vs. 35%). It also significantly inhibits the ASK (21%) and CK2 (12%) kinases. It is worth mentioning, that 2’-5’A3 and especially its epoxy modified analogue had pronounced stimulatory effect on protein kinases, specifically ASK. However, 2’-5’A3 had little stimulatory effect on the protein kinase TIE2, in contrast to the inhibitory action of 2’-5’A3-epo. At the next stage of our research, we determined the dependence of Aurora activity on the concentration of oligoadenylates. The enzymatic reaction was performed in the presence of 2’-5’A3 and 2’-5’A3-epo at different concentrations – from 5 to 160 μM, at two ATP concentrations – 25 μM and 100 μM. Titration curves were constructed by plotting the activity of protein kinase versus the logarithm of concentration of particular oligoadenylate in μM (lg C). It was found that at both ATP concentrations obtained curves are not linear. At the ATP concentration of 100 μM, the curves for both 2’-5’A3 and 2’-5’A3-epo are V-shaped (Figures 2 and 3). Titration of Aurora with 2’-5’A3 resulted in slight rise of its enzymatic activity within the lgC 0.7-1.0 concentration range. Further increase in lgC to 1.3 lead to sharp activity drop, while lgC rise to 1.9 led to the restoration of Aurora enzymatic activity of TIE2, 2’-5’epoА 3 inhibits this protein kinase by 24%, and 35%). It also significantly inhibits the ASK (21%) and CK2 (12%) kinases.
Let us examine some possible mechanisms of the outlined above effects. There are many protein kinases, however, all of them possess the ATP binding site, target protein binding site, and a site for transferring effects. There are many protein kinases, however, all of them possess the ATP binding site, target protein binding site, and a site for transferring effects. There are many protein kinases, however, all of them possess the ATP binding site, target protein binding site, and a site for transferring effects. There are many protein kinases, however, all of them possess the ATP binding site, target protein binding site, and a site for transferring effects. There are many protein kinases, however, all of them possess the ATP binding site, target protein binding site, and a site for transferring effects. There are many protein kinases, however, all of them possess the ATP binding site, target protein binding site, and a site for transferring effects. There are many protein kinases, however, all of them possess the ATP binding site, target protein binding site, and a site for transferring effects. There are many protein kinases, however, all of them poss


