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CHEMICAL ENZYMATIC SYNTHESIS OF (2′-5′)-OLIGOADENYLATES USING NUCLEASE FROM *Spicaria violacea* MYCELIAL FUNGUS

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(2′-5′)-Oligoadenylates [ppp5′A(2′p5′A)$_n$, **1**] are oligomers of adenylic acid with (2′-5′)-phosphodiester bonds that are synthesized in cells of mammals from 5′-ATP in the presence of interferon and/or double-helix RNA. They are activators of latent endonuclease L, which hydrolyzes mRNA of viruses and is in several instances responsible for the antiviral effect of interferon [1-3]. Furthermore, (2′-5′)-oligoadenylates affect cell growth, differentiation, and proliferation and apoptosis [4, 5], are active toward plant viruses [6, 7], and induce cytokinic activity in plant tissues [8]. Various types of biological activity can appear not only for **1** but also for dephosphorylated derivatives [A(2′p5′A)$_n$, **2**] [9, 10].

Existing chemical methods for synthesizing these compounds are complicated and multi-step. Methods for preparing (2′-5′)-oligoadenylates that include polymerization of AMP derivatives to form both (2′-5′)- and (3′-5′)-internucleotide bonds and subsequent treatment of the resulting oligomers with several highly purified enzymes that selectively hydrolyze (3′-5′)-internucleotide bonds and terminal phosphates have been described [11-13]. The drawbacks of these methods are that they are labor-intensive due to the need to separate and purify several individual enzymes and the duration of the enzymatic steps.

Herein we report the ability to use the filtrate of culture liquid (CL) of the mycelial fungus *Spicaria violacea* to produce (2′-5′)-oligoadenylates. It contains phosphatase and nuclease, which can hydrolyze (3′-5′)- but not (2′-5′)-internucleotide phosphodiester bonds in the chemically synthesized polynucleotides with mixed (2′-5′)-(3′-5′)-internucleotide bonds.

We used *S. violacea* BIM F-329 strain from the Belorussian collection of nonpathogenic microorganisms of the Institute of Microbiology of the National Academy of Sciences of Belarus. The conditions for cultivating the fungus and the preparation of the CL filtrate containing phosphatase and nuclease have been described by us [14].

Mixed (2′-5′)-(3′-5′)-polyadenylates were synthesized by polymerization of 2′(3′)-AMP in dioxane using diphenylchlorophosphate and tributylamine [11, 12].

Enzymatic hydrolysis of oligo- and polynucleotides by the enzyme complex in CL filtrate from *S. violacea* was carried out in Tris-HCl buffer (50 mM, pH 6.0) containing $MgCl_2$ (10 mM) at 60°C.

The compositions of mixtures produced by enzymatic hydrolysis of polyadenylates were analyzed by HPLC on a Waters chromatograph using a Nova-Pak C18 column (30 × 3.9 mm) with elution by CH_3CN (7%) in aqueous KH_2PO_4 (0.1 M, pH 4.38) for 30 min. The chromatographic mobility of individual compounds in the mixture was compared with those of oligoadenylates of known phosphodiester bonding and chain length that were prepared by the triester synthesis method [15].

The presence in *S. violacea* CL of phosphatase and specific nuclease that can hydrolyze only natural (3′-5′)-phosphodiester bonds was proved by experiments on the action of CL filtrate on polyadenylic acid that contained only (3′-5′)-internucleotide bonds and (2′-5′)-oligoadenylates. Reaction mixtures containing poly-A (2 mg) or trimer A(2′p5′A)$_2$ (2 mg) in Tris-HCl buffer (1 mL, 50 mM, pH 6.0) and *S. violacea* CL (250 μL) was incubated for 5 h. It was found that the enzymes of *S. violacea* CL filtrate hydrolyzed completely polyadenylic acid to adeonosine in 2 h whereas trimer A(2′p5′A)$_2$ was stable to this enzyme preparation.

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Treatment of mixed (2′-5′)-(3′-5′)-polyadenylates with *S. violacea* CL filtrate under analogous conditions led to their hydrolysis and formed short-chain (2′-5′)-oligomers, as a result of which mixtures of adenosine and dephosphorylated (2′-5′)-oligoadenylates containing about 25% dimer (**2**, n = 1), 10% trimer (**2**, n = 2), and 4% tetramer (**2**, n = 3) were formed. HPLC showed that these mixtures did not contain isomeric (3′-5′)-oligoadenylates. This indicated that nuclease in *S. violacea* CL hydrolyzes specifically (3′-5′)-phosphodiester bonds in mixed (2′-5′)-(3′-5′)-polyadenylates.

The total pool of (2′-5′)-oligoadenylates and individual oligomers **2** (n = 1-3) from enzymatic hydrolysis reactions was separated preparatively using anion-exchange column chromatography over Sephadex DEAE A-25 in the HCO_3^- form with elution by triethylammonium bicarbonate (TEAB, 0.5 M) or a TEAB gradient (0 → 0.5 M), respectively. The structures of the resulting short dephosphorylated (2′-5′)-oligoadenylates **2** (n = 1-3) were confirmed by comparing their UV and PMR spectra with those of compounds with known structures.

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