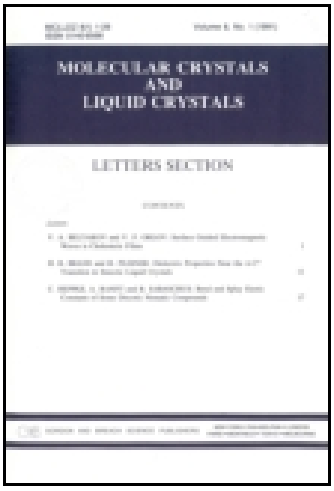

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The Detection of Interaction Between Oligonucleotides and Interferon, A Key Protein of Antiviral Cell Defence System

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The Detection of Interaction Between Oligonucleotides and Interferon, A Key Protein of Antiviral Cell Defence System

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The interaction between 2′-5′A_3 oligonucleotides and recombinant α-interferon was studied by MALDI-TOF mass spectrometry and fluorescence spectroscopy methods. The ability of this key protein of antiviral cell defense system to bind up to 5 molecules of oligoadenylates depending on their structure was established. The presence of interaction in oligoadenylate-interferon system was also confirmed by fluorescence studies. Investigated compounds quenched the emission of α-interferon from 40 to 63%. The efficiency of quenching of the fluorescence of α-interferon also depends on the oligoadenylates structure.

Keywords Oligoadenylates; α-interferon; MALDI-TOF mass spectrometry; fluorescence spectroscopy

1. Introduction

Short 2′-5′-oligoadenylates together with α-interferon play an important role in the antiviral interferon-induced cells defense mechanism [1,2]. It is well known that antiviral properties of 2′-5′A_3 oligoadenylates are directly connected with 2′-5′ oligoadenylate synthetase (2′-5′OAS) system that activates ribonuclease L (RNaseL) which destroys viral mRNA [1,3]. OAS can be activated by viral double stranded RNA [4,5]. Synthesis of 2′-5′-oligoadenylates begins after the activation of 2′-5′OAS. Also it was shown in clinical tests that artificially synthesized oligonucleotides which contain three or more elementary links - adenosine or its derivatives and analogs, can be used as a treatment for blood and eyes diseases, as immunomodulators, antiviral and anticancer drugs [6]. But the exact mechanism of oligoadenylates antiviral activity is still unknown. From the other hand, as the key protein of this system is α-interferon it's very important to know if 2′,5′-oligonucleotides and their

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modified analogues can directly bind to interferon. Therefore, the detailed investigation of this class of oligonucleotides and their ability to bind to interferon would give the opportunity not only to clarify the mechanism of their unique biological action but also to develop the treatment based on 2′-5′A_3 able to effectively protect cells against different viruses.

MALDI-TOF mass spectrometry (matrix assisted laser desorbtion and ionisation time-of-flight mass spectrometry) and fluorescence spectroscopy are among the methods that allow getting insights into this problem. MALDI-TOF mass spectrometry method was successfully applied for investigation of biological objects: amino acids [9], peptides and proteins [10,11], oligonucleotides and nucleic acids [12–15]. For example, mass spectrometry was successfully used for identification of noncovalent interaction in different biomolecular complexes such as protein-protein, protein-nucleic acid etc. [11,15,16–20].

2. Experimental

2′-5′-triadenylate 2′-5′-A_3 and its cordycepin (2′-5′-A_3cord), inosine (2′-5′-A_3ino) and epoxy (2′-5′-A_3epo) analogues were synthesized by solution phosphotriester method as described in [7,8].

Recombinant interferon α-2b was provided by the "Interpharmbiotech" (Ukraine).

Samples were dissolved in water and heated at 37°C for 10minutes. Concentration of oligonucleotides were 10^{-4} M; interferon - 10^{-5} M.

Measurements were performed using MALDI-TOF instrument Voyager DE PRO (Applied Biosystems, USA). 3,5-Dimethoxy-4-hydroxycinnamic acid (sinapic acid, Sigma-Aldrich, USA) was used as a matrix. Exact molecular weights were obtained via the subtracting the mass of a single hydrogen (1.0079) from m/z value of any mass shown on a spectrum. Calibration of mass spectrometer was held using standard mixture Calmix3. Matrix solution: solution of sinapic acid (10 mg/ml) was made in a mixture of acetonitrile and 0.1% water solution of trifluoroacetic acid in the ratio 1:1 (Sigma-Aldrich). For the deposition on the samples plate 1.5–2 μL solution of the sample and matrix in the ratio (1:1) was taken. The linear mode of time of flight detector was used [21]. The deviation of molecular weight for the same substance in different spectra can be explained by the change of the device resolution depending on the analysis parameters. The MALDI-MS spectral data were processed using the Data Explorer 4.0 (Applied Biosystems).

Fluorescence measurements were performed using Cary Eclipse spectroflurimeter (Australia). The value of fluorescence quenching was calculated from the spectra using the following formula:

$$Q = [(F_0 - F)/F_0] \times 100\%$$

F – fluorescence intensity of protein in the presence of 2′-5′-oligoA,
F_0 – fluorescence intensity of protein under the same condition.

3. Results and Discussion

Four types of biopolymers, including the unmodified trinucleotide 2′-5′-A_3 and its inosine, cordycepin and epoxy-modified analogues which contain 3 nucleoside elementary links but differ in the structure of 3′-terminal nucleoside or ribose fragment, and their complexes with recombinant protein α-interferon were investigated by MALDI-TOF mass spectrometry and fluorescence spectroscopy. Oligonucleotide structures are shown in Fig. 1. At first sight

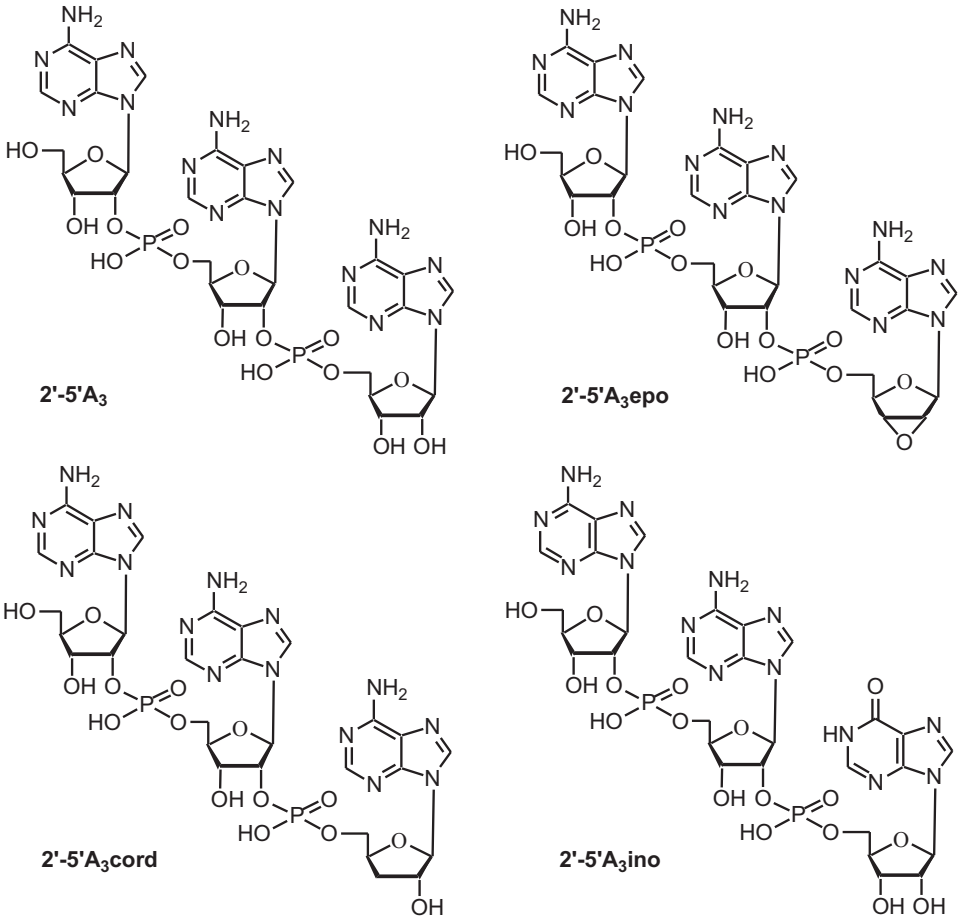

Figure 1. The chemical structure of oligoadenylates.

they don't have significant difference in chemical structure, however, they have different biological activity [6].

First of all, MALDI-TOF mass spectra of oligoadenylate allowed to establish their molecular weight with high accuracy (<1%) (Table 1).

Table 1. Calculated molecular weight of oligoadenylates and *m*/*z* values obtained from mass spectra

Name of substance	Molecular formula	Theoretical value of molecular weight	*m*/*z* [(M+H)$^+$]
2′-5′-A_3	$C_{30}H_{37}N_{15}O_{16}P_2$	925.65	928.12
2′-5′-A_3epo	$C_{30}H_{35}N_{15}O_{15}P_2$	907.64	913,27
2′-5′-A_3 ino	$C_{30}H_{36}N_{14}O_{17}P_2$	926.64	927.66
2′-5′-A_3cord	$C_{30}H_{36}N_{15}O_{15}P_2$	909.65	910.78

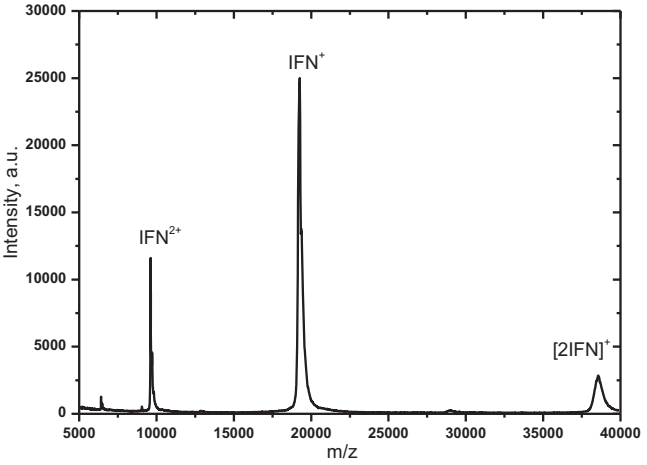

Figure 2. MALDI mass spectrum of interferon.

As it was mentioned above, in our investigation we studied protein α-interferon (IFN). This protein is widely used for the treatment of viral diseases e.g. hepatitis C. Mass spectrum of α-interferon contains peak at $m/z = 19265$ that belong to singly charged protein molecule (Fig. 2).

Theoretical molecular weight of interferon α-2b that was calculated from amino acid sequence is 19271Da [22]. So deviation of the experimental value is only 0.04%. These values are in a good agreement with results from other works [23,24].

The next stage was to investigate the interferon-2′-5′A_3 mixture. Its mass spectrum shows that this protein is capable to interact with several molecules of oligonucleotide. Addition of interferon to 2′-5′A_3 led to significant changes in mass spectra. The appearance of peaks at m/z 20179, 21112, 22044, 22998 and 23899 was observed (Fig. 3). These peaks belong to complexes consisting of one molecule of interferon and up to 5 molecules

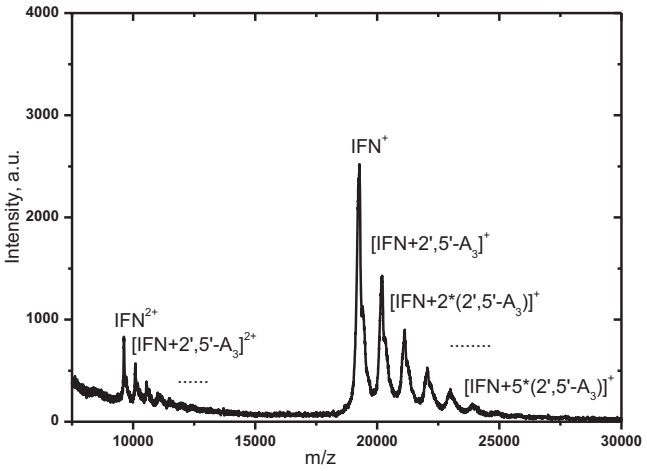

Figure 3. MALDI mass spectrum of interferon-2′-5′A_3 system.

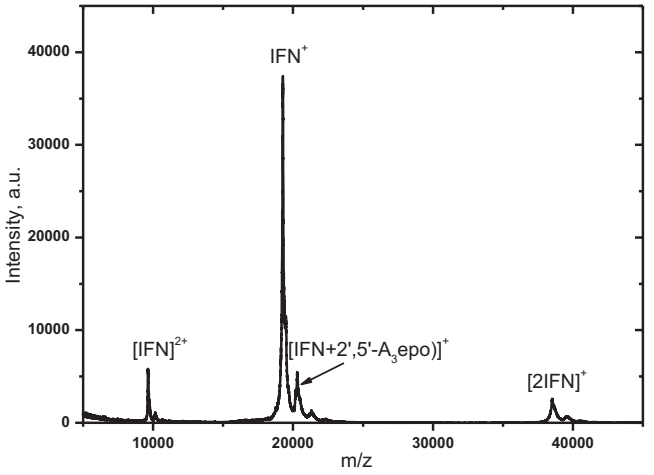

Figure 4. MALDI mass spectrum of interferon-2′-5′A_3epo system.

of 2′-5′A_3. Corresponding molecular weights are in a good agreement with theoretically calculated values. The deviation of experimental m/z value from theoretical for protein complexes is below 0.5%.

Then α-interferon-2′-5′A_3epo mixture was investigated. As seen from Fig. 4, mass spectrum of α-interferon-2′-5′A_3-epo mixture contains, in addition to the molecular ion peak of protonated of interferon (19271Da), an intensive peak at $m/z = 20177$ and two less intensive peaks. The molecular weights for these peaks correspond to the weights of protein/oligoadenylate adducts containing one interferon molecule and up to five 2′-5′A_3 molecules. Therefore the first peak belongs to α-interferon-2′-5′A_3-epo complex, whereas two less intensive peaks belong to proteins complexes with two and three oligonucleotide molecules. Thus 2′-5′A_3epo can bind to α-interferon too in an amount from 1 to 3 molecules.

The similar situation was observed for inosine-containing oligomer - 2′-5′A_3ino (Fig. 5). According to the data obtained from mass spectra, interferon molecule can bind only two molecules of 2′-5′A_3ino.

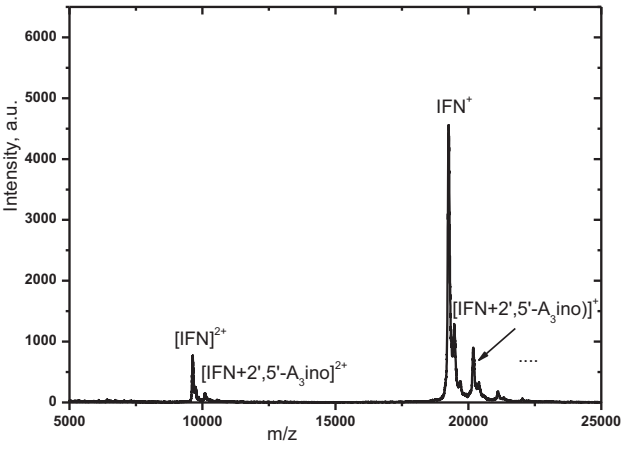

Figure 5. MALDI mass spectrum of interferon-2′-5′A_3ino system.

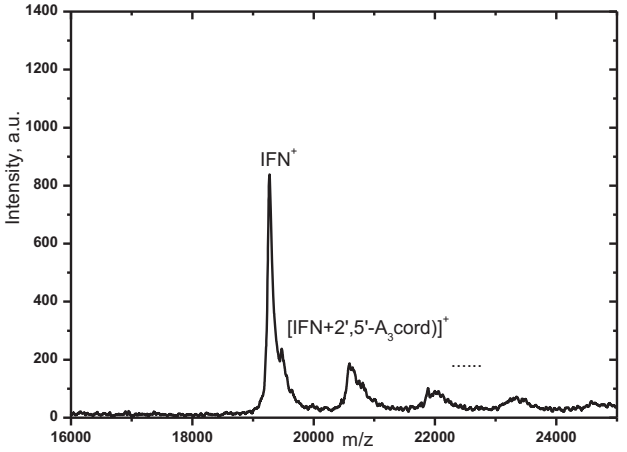

Figure 6. MALDI mass spectrum of interferon-2′-5′A_3cord system.

The experimental results were slightly different in the case of interferon-2′-5′A_3cord mixture (Fig. 6). Besides the peak of monocharged protein, its mass spectrum contains three additional peaks. These peaks belong to the complexes forming by interferon molecule with one, two and three molecules of 2′-5′A_3ino.

Thus, according to the results obtained from MALDI-TOF mass spectra, all investigated 2′,5′-oligoadenylates demonstrate the ability to bind to interferon at various ratios.

The presence of intermolecular interaction in oligonucleotide/interferon systems also was confirmed by the fluorescence spectroscopy methods. The fluorescence spectra of free α-interferon and in the presence of investigated 2′,5′-oligoadenylates were obtained and analyzed (Fig. 7). The spectral data were used for the calculation of fluorescence quenching of the protein emission by oligonucleotides, as described in experimental part (Table 2).

We can see from the table that all investigated compounds quenched the emission of α-interferon in the range 40–63%. The efficiency of quenching of α-interferon fluorescence

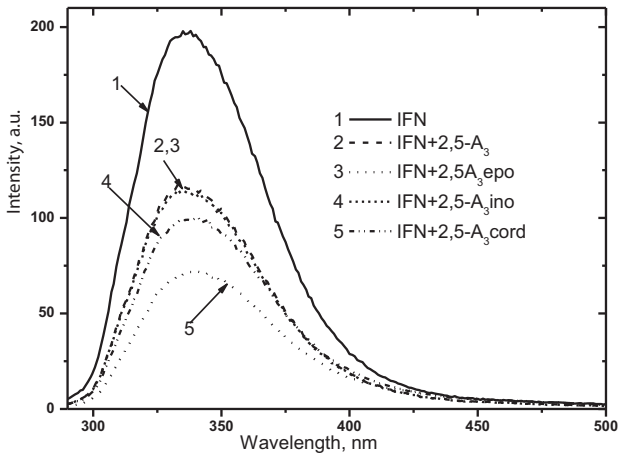

Figure 7. Fluorescence spectra of interferon-oligoadenylates systems ($\lambda_{ex} = 280$ nm).

Table 2. The fluorescence quenching of interferon emission by oligoadenylates

Oligoadenylate	Fluorescence quenching Q, %
2,5-A_3	41
2,5-A_3epo	63
2,5-A_3ino	40
2,5-A_3cord	58

also depends on the oligoadenylate structure. These data are in a good agreement with mass spectrometry results.

4. Conclusions

Mass spectra of 2′-5′A_3 triadenylate and its base-modified (2′-5′A_3ino) and sugar-modified (2′-5′A_3epo, and 2′-5′A_3cord) analogues and their mixtures with α-interferon were obtained and analyzed. The obtained results demonstrate that all investigated oligonucleotides directly interact with α-interferon. Depending on their molecular structure, they are capable to bind to the interferon in an amount from 1 to 5 molecules per protein molecule.

The presence of intermolecular interaction in 2′-5′A-oligonucleotide/α-interferon systems was confirmed by the fluorescence spectroscopy via the protein emission quenching by oligoadenylates.

We can thus conclude that the use of combination of mass spectrometry and fluorescence spectroscopy methods in protein-oligonucleotide interaction studies provides the possibility to identify proteins associated with diverse biological activity of oligoadenylates.

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