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Abstract: AMP and oligonucleotides antiviral drugs have actively used in medicine, but the molecular mechanism of their action remains unclear. We studied the conformation changes of IFN with ligands, the quenching and lifetime of fluorescence, and isothermal titration calorimetry (ITC).

The most active quenching and decrease lifetime of fluorescence INF, when titrated with AMPs and ORN, was obtained using acid forms in combination with mannitol. And when titrated INF saline forms slightly different from control. Spectra of circular dichroism show the decrease in the structure of the number of secondary elements when interacting between INF and acidic forms nucleotides. An increase in the content of secondary structure in the interaction between INF and salt forms ligands. The ITC curves titration indicate that the reaction between protein and acidic ligands is exothermic. And between INF with saline ligands endothermically. Exothermic protein-ligand interaction increases the conformational mobility of the protein and endothermic decrease.

The ORNs have the advantage of interacting with proteins, unlike salt ORNs and AMPs, because they have a stronger binding. Thus, we assume the same compound in various forms may act as an inhibitor and activator for the protein.

Keywords: Oligonucleotides; Interferon α2b; lifetime of fluorescence; isothermal titration calorimetry

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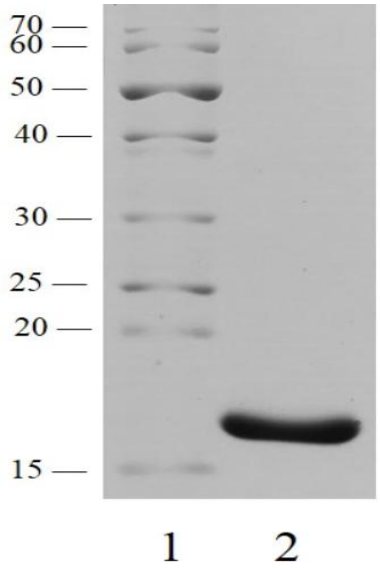
Introduction

Gel electrophoresis of interferon α-2b: **1** - a mixture of marker proteins "PageRuler Unstained Protein Ladder"; **2** - interferon α-2b ~ 18.2 kDa



Interactions of protein nucleic acids play a decisive role in many biological processes. RNA-based drugs that can bind and affect the work of epigenetic regulators and transcriptional proteins through interaction, with regulatory domains, can be used as safe analogs. In the previous work we showed, the combination of oligonucleotides with alcohol sugar D-mannitol leads to changes in their biological activity and efficiency.
ORNs increase interferon production and stimulate non-specific antivirus protection, but the molecular mechanism of its action is still unclear. We studied the interactions between Interferon α2b and mononucleotides (AMP), yeast oligoribonucleotides (ORNs), their Na+ salts (ORNsNa), and ORNs with D--mannitol (ORNs:D-M). We study the interaction and conformational changes of IFN with ligands, the quenching and lifetime of fluorescence, and isothermal titration calorimetry (ITC).

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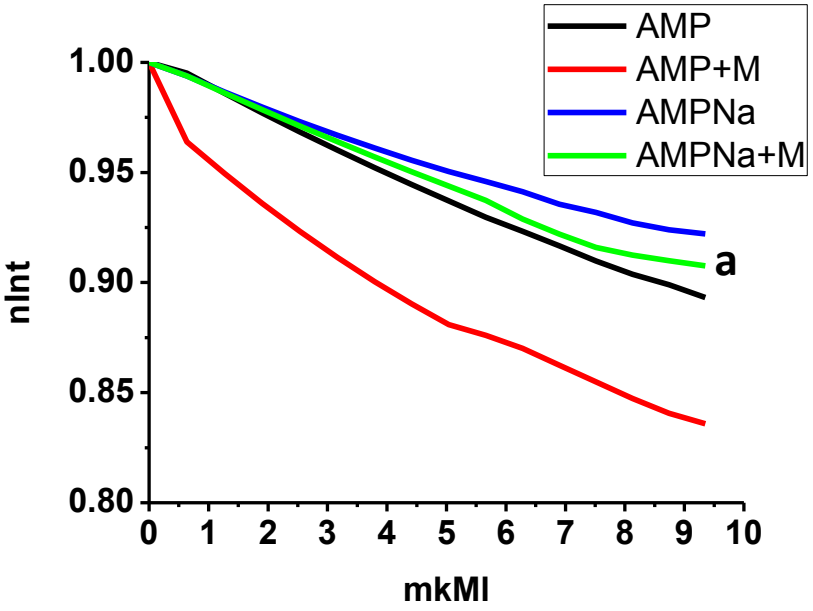
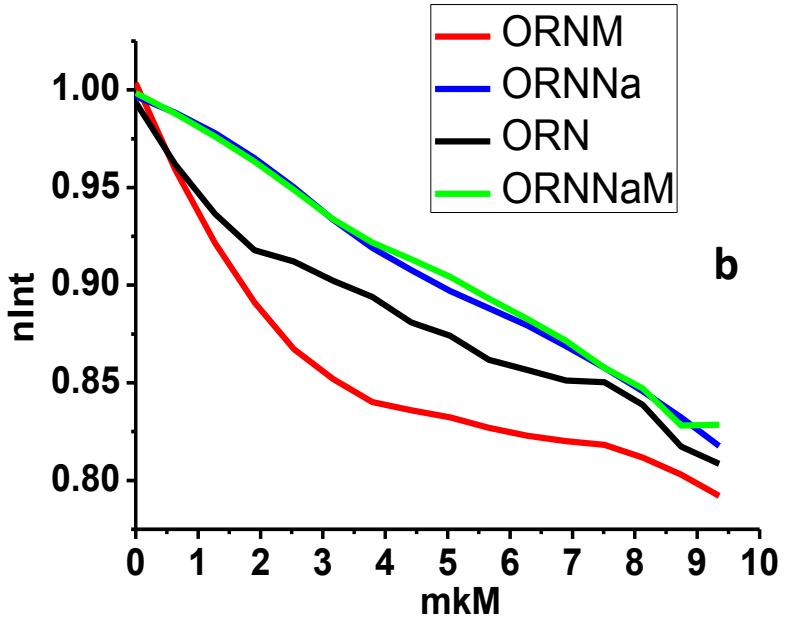
The dependence of the fluorescence intensity of IFN-α2b on the concentration of different forms of AMP and oligoribonucleotides





When using ORN and ORN:D-M quenching of the fluorescent INF were 25% and 28%, AMP and AMP: DM - 15 and 21%. Quenching INF fluorescence in the titration of ORNsNa and ORNsNa: DM was 16% and 17%, AMPNa and AMPNa: DM - 8% and 10%. The results obtained for quenching the IFN-α2b fluorescence intensity with the addition of ORN and AMP show that ligands of nucleic nature in acid form, and especially in combination with mannitol, are more strongly bound to interferon α-2b in comparison with saline analogs. That served as an impetus for further research.

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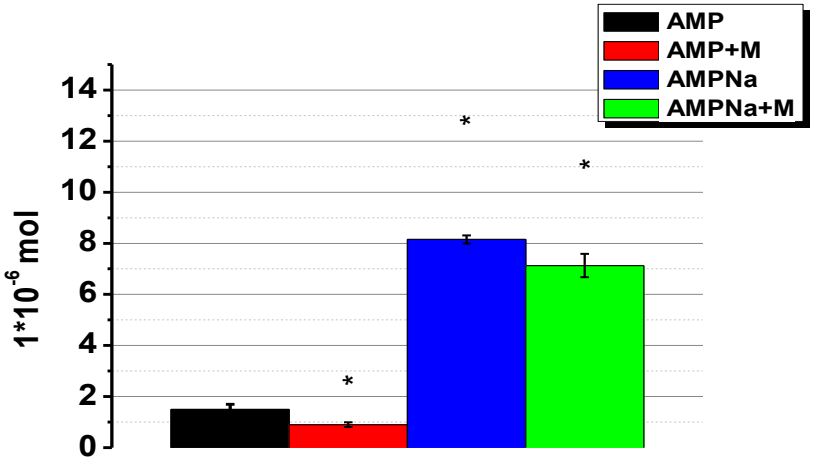
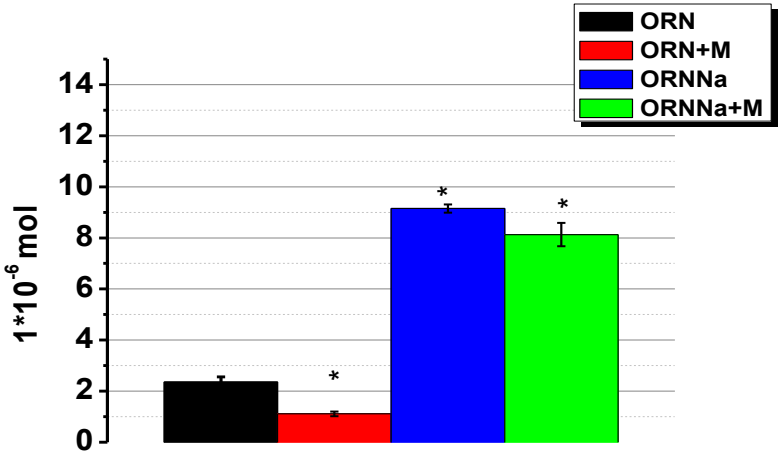
The binding constant of interferon α-2b to AMP and oligonucleotides





The dissociation constant Kd=1,11±0.09μM μM in the fluorescence quenching interaction between INF and ORNs-D-M. The dissociation constant between IFN and ORNs is Kd=2,36±0.47μM, between INF and ORNsNa - Kd=9,15±0.16μM and INF and ORNsNa-D-M Kd=8.13±0.46μM. The dissociation constant Kd=0,94 ±0.09μM μM in the fluorescence quenching interaction between INF and AMP-D-M. The dissociation constant between IFN and AMP is Kd=1,53±0.41μM, between INF and AMPNa - Kd=7,1 ±0.19μM and INF and AMPNa-D-M Kd=8.14±0.73μM.

* - *statistically significant difference compared to control (interferon), P≤0,05.*

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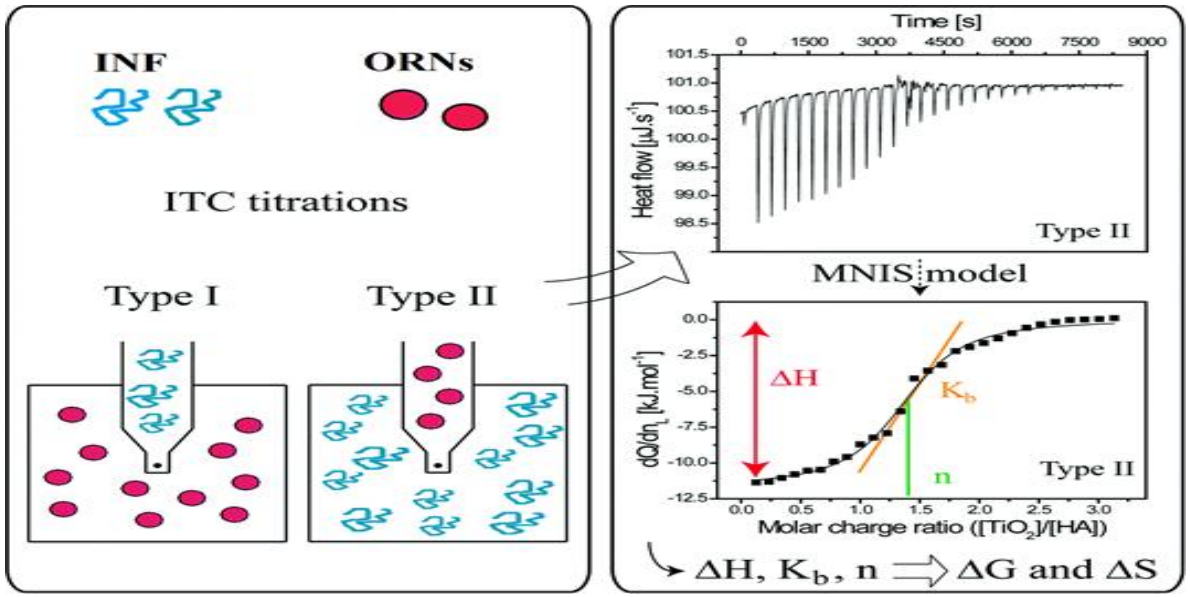
Scheme of the experiment on nanocalorimetric titration of INF by RNA preparations



[modified from https://doi.org/10.1039/c5en00139k]. We used type II when the ligand added to the protein. ITC measurements record the change in the heat for 3 min for each of the 25 injections. The fitting function calculated the results according to the experimental curves in the nanoanalysis program. ITC measurements made it possible to obtain the values of ΔH and Kb and calculate the entropy of ΔS and the total free energy of ΔG during the interaction of INF-RNA

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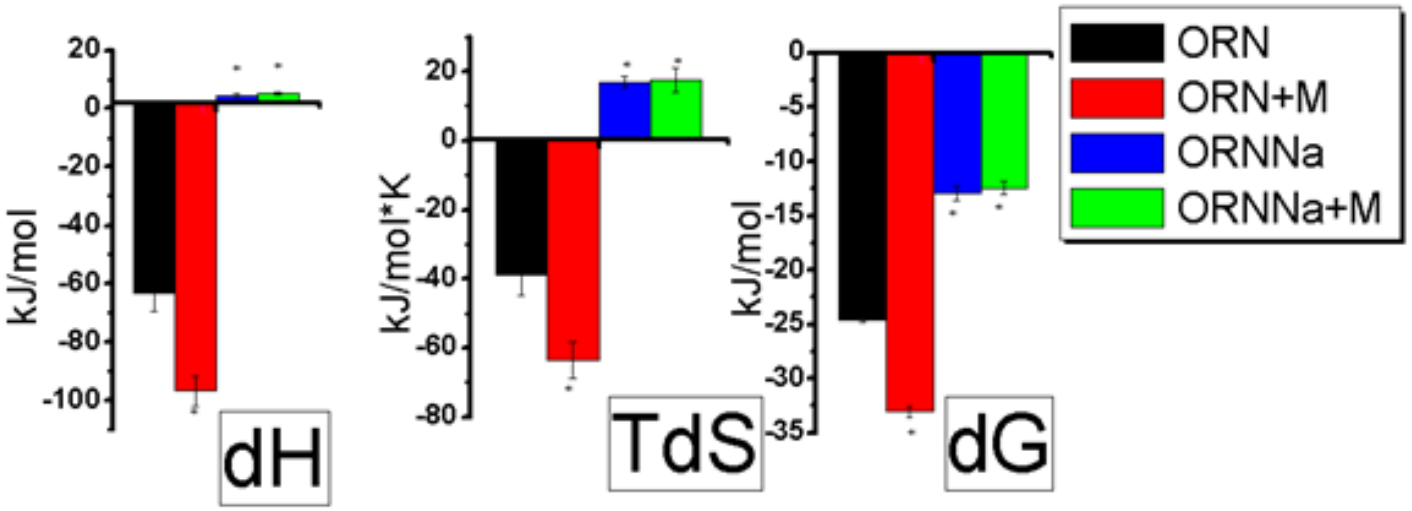
Thermodynamics of interaction of interferon α-2b with different forms of ORN



a **b** **c**

Changes in energy parameters in the interaction of interferon α-2b with ORN: a) enthalpy; b) entropy; c) Gibbs energy

In the interaction of interferon and ORN and AMP in acidic form, the reaction is exothermic. The ITC curve is endothermic between interferon and ORN and AMP in salt form.

* - *statistically significant difference compared to control (acid form), $P \leq 0,05$.*

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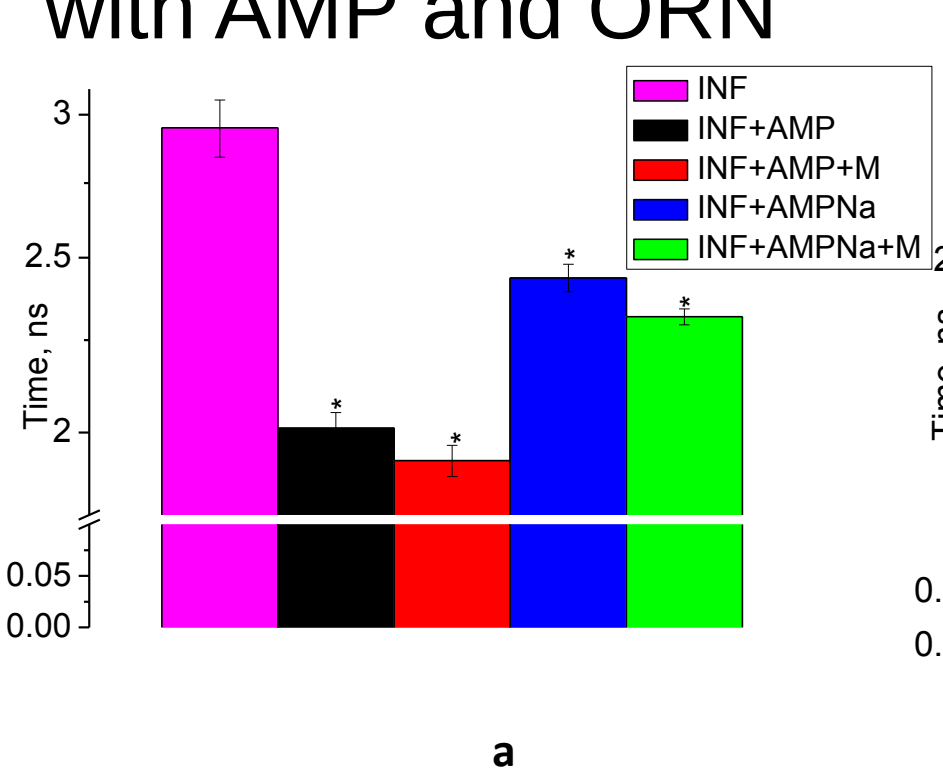
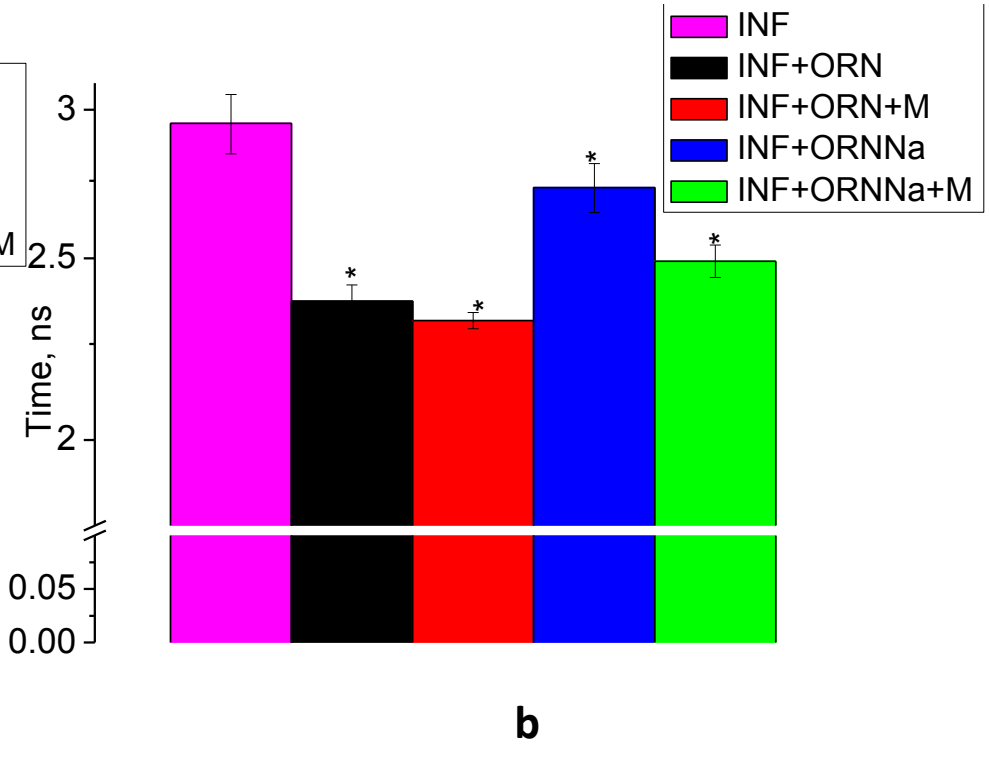
The fluorescence lifetime of interferon α-2b with AMP and ORN



a



b

Dependence on IFNα-2b fluorescence life when given a) AMP and b) oligoribonucleotides

Energy Transfer Efficiency - The ratio of the number of energy transfer events to the number of donor excitation events

* - *statistically significant difference compared to control (interferon), P≤0,05.*

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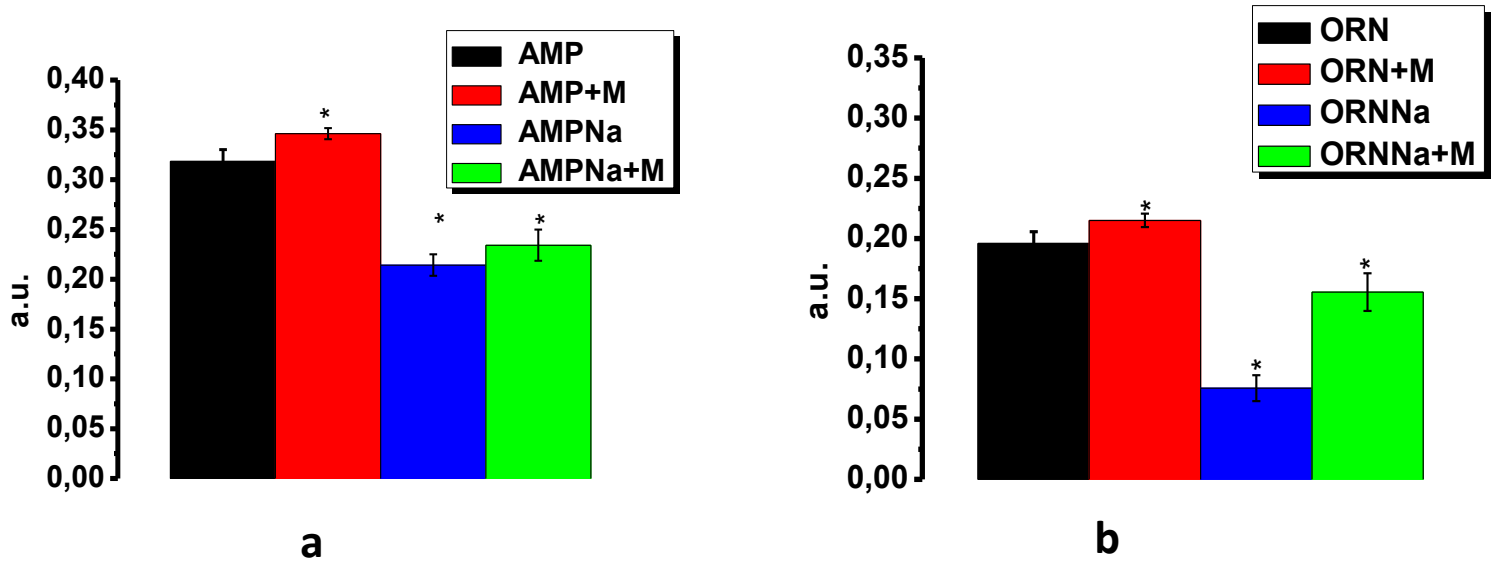
Efficiency of Foster energy transfer between interferon and AMP and ORN



The energy transfer efficiency of IFNα-2b when added
a) AMP and b) oligoribonucleotides

* - *statistically significant difference compared to control (acid form), P≤0,05.*

Energy transfer efficiency - the ratio of the number of energy transfer events to the number of donor excitation events:

$$E = 1 - \tau'_{\mathrm{D}} / \tau_{\mathrm{D}},$$

where τ'_{D} and τ_{D} the lifetime of the fluorescence donor, in the presence and absence of the acceptor, respectively

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Results and discussion

Interactions of protein and nucleic acids play a decisive role in many biological processes. RNA-based drugs that can bind and affect the work of epigenetic regulators and transcriptional proteins through interaction, with regulatory domains, can be used as safe analogs. We studied the ability of yeast RNA (ORN), yeast RNANa salt (ORN Na), and yeast ORN:D-mannitol complex (ORN:D-M) and AMP to effect on fluorescence quenching and conformational changes of Interferon α2b. To determine the energy parameters of protein ligand interactions, we used isothermal titration nanocalorimetry Nano ITC.

It is shown that when using ORN and ORN:D-M quenching of the fluorescent INF were 25% and 28%, AMP and AMP: DM - 15 and 21%. Quenching INF fluorescence in the titration of ORNsNa and ORNsNa: DM was 16% and 17%, AMPNa and AMPNa: DM - 8% and 10%. INF has a life-time of 2.95 ns. When interacting with ORN and ORN: D-M INF has fluorescence time of 2.37 and 2.32 ns, respectively, AMP and AMP: D-M 2.01 and 1.92 ns. When interacting with ORNsNa and ORNsNa: D-M INF has a fluorescence time of 2.73 and 2.49 ns, respectively, AMPNa and AMPNa: D-M 2.31 and 2.43 ns.

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Thus, ORN, and especially ORN:D-M and AMP:D-M leads to a change in the conformational mobility of interferon α-2b by increasing the content of disordered regions. At the same time, salt analogues increase the number of structured secondary elements, such as α-helices, β turns and β antiparallel sheets and probably increase the conformational stiffness of interferon α-2b. The results of the study of enthalpy changes in the titration of interferon α-2b acid form of ORN and ORN:D-M was -63.28 kJ/mol and -96.61 kJ/mol, respectively, and for the ORNNa and ORNNa:D-M respectively 4,516 and 5,139 kJ/mol. The change in entropy when adding the ORN to interferon α-2b was -38.72 and in the case of the ORN:D-M -63.53 kJ/mol*K, respectively. The change in entropy when adding the ORNNa to interferon α-2b was 17.05 kJ/mol*K, and the ORNNa:D-M, respectively, 17.58 kJ/mol*K. A similar pattern demonstrated when studying the change in Gibbs energy during titration of interferon α-2b with ORNORN and ORN:D-M and it was -24.56 and -33.07 kJ/mol, respectively. And when titrated with ORNNa and its ORNNa:D-M, respectively -12.9 and -12.43 kJ/mol.

These results of studying the effects of thermodynamics of different forms of RNA and their complexes with D-mannitol in the titration of interferon α-2b may indicate different sites of binding of different forms of ORN to protein, as well as other modes of binding and various types of conformational changes in the protein.

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Conclusions

• AMP and ORN in acidic form and combination with mannitol bind more strongly to interferon α-2b than to saline analogs;

•In the interaction of interferon and AMP and ORN in acidic form, the reaction is exothermic. The ITC curve is endothermic between interferon and ORN and AMP in salt form;

•AMP and acidic ORN, and especially in combination with mannitol, lead to a change in conformational mobility by increasing the content of disordered sites. At the same time, salt analogs increase the number of structured secondary elements and probably increase the conformational rigidity of interferon;

•Pulse-time spectroscopy shows a higher efficiency of non-radiative energy transfer from interferon to AMP and ORN in acidic form, due to the closer distance between molecules and the higher Foster transfer rate.

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