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Original article

FLUORESCENCE-BASED STUDY OF OLIGONUCLEOTIDE INTERACTIONS WITH RECOMBINANT PROTEINS: INSULIN, INTERFERON α2-β, SOMATOTROPIN, AND THEIR RECEPTORS

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Background. Oligonucleotides (OLNs) can participate in a wide range of protein-ligand interactions and perform numerous cellular functions by forming structures that enable specific interactions with DNA, RNA, and proteins, what is crucial for many biological processes. Advances in understanding these interactions could lead to the development of new technologies for treating various diseases. However, the mechanism of interaction between proteins and OLNs is complex and still requires detailed study. More research is needed to fully elucidate this process and enhance our understanding of these biomolecular interactions.
Objective. The aim of this study was to synthesize, purify, and investigate the interaction of OLNs with recombinant signaling proteins interferon α2-β and insulin with their receptors and somatropin by assessing binding strength using fluorescence spectroscopy.
Methods. The interactions were analyzed using the Stern–Volmer equation in both general and modified forms, as well as the Hill equation. OLNs were synthesized via the solid-phase phosphoramidite method, purified through solid-phase extraction, and subsequently verified with a spectrophotometer.
Results. Fluorometric titration revealed that OLNs bind to proteins within the medium affinity range, forming non-fluorescent complexes, with the most active interactions observed with shorter OLN. Positive cooperative binding of interferon to G20 and T20, and negative cooperative binding of insulin to C20 and A20, were identified. Additionally, negative cooperative binding of somatropin to C20 was observed.
Conclusions. The study demonstrated the interaction between OLNs and recombinant signaling proteins and receptors through various binding mechanisms, which could potentially affect their conformation and biological activity. These findings have implications for the therapeutic use of OLNs in the context of signaling proteins and receptors.

Keywords: interferon; interferon receptor; insulin; insulin receptor; somatotropin; oligonucleotide; protein-ligand interaction; spectroscopy.

Introduction

The study of interactions between biopolymers, recombinant signalling proteins, and their receptors have attracted considerable attention in the field of molecular biology [1]. Oligonucleotides (OLNs) that bind to proteins and their receptors play an important role in various biological processes. These interactions provide valuable information about molecular recognition and are used in numerous biotechnology and medical processes. Oligoribonucleotides have been the subject of much research for a long time, with a particular emphasis in recent years. They have attracted considerable attention as potential candidates for the development of new drugs against viruses, inflammation, and tumors. These molecules play a key role in the mechanisms of antiviral cell defense and contribute significantly to essential cellular processes, including growth, differentiation, apoptosis, and pathogenesis.

Previous studies in our laboratory have shown that complexes of acidic forms of yeast RNA oligoribonucleotides with D-mannitol (ORNs-D-M) have a broad spectrum of antiviral activity [2]. ORNs-D-M preparations have shown highanti-influenza activity both in the prevention and treatment of influenza infections by inhibiting neuraminidase activity [3]. It has been shown that while a similar RNA preparation in its salt form produces only immunomodulatory activity, its acidic form induces anti-inflammatory activity. Moreover, in combination with D-mannitol, ORNs-D-M has a pronounced antiviral effect. In previous studies, fluorescence spectroscopy was used to study the interaction of 2′-5′-oligoadenylates and their analogs with albumin and interferon. The mechanisms of antiviral activity of 2-5A were related to the activity of interferon. Mass spectrometry revealed stable interactions between these proteins and OLNs. In particular, the results showed that 2′-5′-A3, it's epoxy analog 2′-5′-A3-epo, and 3′-5′-triadenylate are not able to bind to insulin, but all studied 2′-5′ and 3′-5′-oligoadenylates can bind to α-interferon in a ratio of one to five ligand molecules per pro-

tein molecule, forming stable complexes [4]. Studies of biomolecular reactions using DNA oligonucleotide aptamers for real-time quantification of input protein concentration are known. Due to the spontaneous interaction of DNA aptamers with specific proteins and their interaction with complementary DNA strands, exchange reactions rapidly produce output DNA strands that reflect the concentration of input proteins. An important feature is that the sequence of the output strands can be selected independently of the choice of sensor proteins by using a series of chain-shift reactions. Moreover, this system allows for the detection of not only an increase but also a decrease in protein concentration. The control of the dynamic range of the output signal depends on the initial concentrations of the sensor components as well as on the location of the quencher and fluorophore molecules on the DNA strands [5].

Homopolymeric OLNs, consisting of repeating units of a single nucleotide, have special properties with potential applications in various fields such as drug delivery, diagnostics, and gene therapy. The ability of these OLNs to interact with recombinant signalling proteins and their receptors opens up new avenues for understanding the underlying mechanisms of cellular processes and developing innovative therapeutic strategies.

Understanding the molecular details of the interaction of homopolymeric OLNs with recombinant signalling proteins and their receptors opens up broad prospects for the development of targeted therapeutics and precise control of cellular processes.

The nature of the interaction between proteins and OLNs remains poorly understood. Therefore, our research is aimed at studying the interaction of OLNs with recombinant signalling proteins, such as interferon α2-β, insulin, and somatropin, with their receptors. Our work aimed to synthesize and purify OLNs and study their interactions with recombinant signalling proteins, interferon α2-β and insulin with their receptors and somatropin, using fluorescence spectroscopy. These interactions were analyzed using the Stern–Volmer equation in its general and modified forms, as well as the Hill equation.

Materials and Methods

Reagents

1. The following reagents were used for the synthesis: Cap Mix A, (80% Tetrahydrofuran, 10% Acetic Anhydride, 10% Pyridine) (Applied Biosystems, USA); Cap B solution containing 80% acetonitrile (ACN), 10% N-methylimidazole (NMI) and 10% pyridine (Sigma-Aldrich, USA); TCA Deblock, Trichloroacetic acid/Dichloromethane 3/100 (v/v) (Sigma-Aldrich, USA); ETT Activator (Sigma-Aldrich, USA); Oxidizing Reagent for DNA Synthesis, 0.05M Iodine in Pyridine (Sigma-Aldrich, USA). DNA Phosphoramidite (Sigma-Aldrich, USA) was also used for the synthesis of the corresponding OLNs: DMT-dA(bz), DMT-dC(bz), DMT-dG(ib) and DMT-dT.

2. For solid phase extraction (SPE) purification were used: for the aqueous solutions, a degassed aqueous bistillate with a conductivity of 18.2 mΩ was used, obtained using an Arium Pro bistillator (Sartorius, Germany) and a DEGASSING STATION degasser (TA Instruments, USA) (it was also used for the preparation of solutions); 1 M TEAA (Triethylammonium acetate buffer) (Sigma-Aldrich, USA), pH 7.2; 3% Ammonia solution (Supelco, USA); 97% 1M TEAA + 3% ACN (Sigma-Aldrich, USA); 2% TFA Trifluoroacetic acid (Sigma-Aldrich, USA).

3. To study the kinetics of protein-ligand interaction, the following were used: insulin (Farmak, Ukraine), interferon α2-β (SPC InterpharmBiotech, Ukraine), insulin and interferon receptors (CLOUD-CLONE Corp. (CCC), USA) and somatotropin (S.A. Balkan Pharmaceuticals, Moldova).

Phosphoamide synthesis

Solid-phase phosphoamidite synthesis was performed on RNA/DNA Synthesizer H-8 (K&A Laborgeraete, Germany). Standard 200 nmol, 1000 Å columns were used for synthesis:

- 5′-DMT-dA(Bz) Synthesis Column; (Biosearchtech, UK);
- 5′-DMT-T Synthesis Column (Biosearchtech, UK);
- 5′-DMT-dC(Ac) Synthesis Column; (Biosearchtech, UK);
- 5′-DMT-dG(iBu) Synthesis Column; (Biosearchtech, UK).

Detection was performed using the online trityl monitor for all columns (all trityl collected). Using the licensed software for the instrument, the protocol was run on a 200 nmol column and an ACGT column. Also, at the end of the synthesis, the 5′-end of the DMT protecting group was left for further purification.

We obtained samples of homopolymeric oligodeoxynucleotides A20, C20, G20, T20.

The obtained OLNs were placed on controlled porous glass (CPG) columns. Therefore, using 1 ml of ammonia solution 25% (Supelco, USA) in a VORTEMP thermostat (Uniequip, Germany) at 55 °C and 16 hours, our final products were isolated.

Purification of oligonucleotides

OLNs were purified by SPE. The device used was a P-8 (K&A Laborgeraete, Germany) with a polymerized dextran MicroPure II Column (Biosearchtech, UK) and a protocol for deoxyoligonucleotides.

Sample Preparation: The crude oligonucleotide sample was dissolved in a 1.0 M TEAA (Triethylammonium acetate) buffer, pH 7.2 (Sigma-Aldrich, USA).

Column Conditioning: The SPE column was conditioned with TEAA to activate the stationary phase and prepare it for sample loading.

Sample Loading: The prepared oligonucleotide sample was loaded onto the conditioned column.

Washing: Impurities were removed from the column by sequential washing with:

- Ammonium Hydroxide 3% (Sigma-Aldrich, USA);
- Deionized water;
- 97% 1 M TEAA and 3% acetonitrile (ACN) (Sigma-Aldrich, USA);
- Deionized water;
- Trifluoroacetic Acid (20 ml of 100% TFA in 980 ml deionized water, Sigma-Aldrich, USA);
- Deionized water.

Elution: The purified oligonucleotide was eluted from the column using 30% acetonitrile (ACN).

The NanoDrop One C (Thermo Fisher Scientific, USA) spectrophotometer, an innovative instrument for sample analysis in biological research, was used to verify the purity of the OLNs. After purification of the OLNs by SPE, the samples were prepared for measurement.

First, the spectrophotometer was calibrated using pure solvent (30% ACN) as a zero standard. Then 1-2 μL of purified oligonucleotide was applied to the special optical platform of the spectrophotometer. The instrument measures the absorbance of light by the sample at 260 nm, which is the standard wavelength for assessing the purity of nucleic acids.

The optical density (OD) value obtained from the measurement allows you to determine the concentration of OLNs and their purity. A pure oligonucleotide sample has an OD260/OD280 ratio in the range of 1.8–2.0, indicating high purity.

Fluorescence spectroscopy

The fluorescence analysis was performed on a FluoroMax-4+ spectrofluorimeter (HORIBA Jobin Yvon SAS, USA) at an excitation wavelength of $E_x = 295$ nm, emission range of $E_m = 305–405$ nm with a 5 nm band gap. In the experiment, 50 pM protein was used in Tris-HCl buffer (50 mM/L, pH 7.4) with a volume of 200 μL, and 20 μL of OLNs at a concentration of 2 μM/ml were added to a final volume of 400 μL. The fluorescence quenching value Q was determined as the relative decrease in the intensity of protein emission in the presence of OLN compared to the control (the intensity of protein emission).

Analysis of the quenching mechanism

The Stern–Volmer constant is a value used to characterize the effect of external factors (e.g., binding to a partner molecule) on the fluorescence intensity of a fluorescent probe (e.g., fluorescent marker or fluorescent molecular probe). It is determined for a fluorophore and a partner (e.g., protein) and indicates the ability of the fluorophore to lose its fluorescent intensity when bound to the partner. An increase in the Stern–Volmer constant indicates an increase in the binding efficiency of the fluorophore, which can be used to study the interaction between molecules such as OLNs and proteins. [6]

The Hill equation is used to describe cooperative interactions between biomolecules such as ligands and their receptors. With the help of fluorescence spectroscopy, the Hill equation can be used to analyze the kinetics of the fluorescence signal, which depends on the interaction of an oligonucleotide (ligand) with a protein (receptor). [7]

Statistical processing of the results

The experimental data obtained from four independent replicates were subjected to statistical analysis, including the calculation of the mean value (M), standard deviation (δ or ±SD), and standard error (m or ±SE). One-way analysis of variance (ANOVA) was used to assess the statistical significance of differences between experiments. The data were then processed and graphically displayed using OriginPro 8.0 software.

Results

Abbreviations: Insulin (INS), Interferon α2-β (INF), Insulin receptor (INSR), Interferon-α/β receptor 1 (INFR), Somatotropin (STP), Homopolymeric OLNs of different lengths and compositions (A20 (Poly(dA)20); G20 (Poly(dG)20); T20 (Poly(dT)20); C20 (Poly(dC)20); A5 (Poly(dA)5); G5 (Poly(dG)5); rA5 (Poly(A)5); rG5 (Poly(dG)5).

We studied the interaction of OLN with recombinant signalling proteins, such as INF α2-β, insulin, and somatropin, with their receptors. The strength of protein-ligand binding was assessed by fluorescence spectroscopy. The interaction was analyzed using the Stern–Volmer equation in its general and modified forms, as well as the Hill equation.

We computed the second-order Stern–Volmer constants (K_{sv2}) for the cases where K_{sv1} did not follow a linear plot ($R < 0.9$) (Fig. 1). Each of these K_{sv2} constants reflects the effect of one or more ROS molecules on the fluorescence intensity when interacting with a protein. In some cases, such as when G20 and T20 are added to INF, negative cooperative binding is observed, meaning that the interaction of one OLN molecule can lead to a decrease in the interaction with a second OLN molecule or protein.

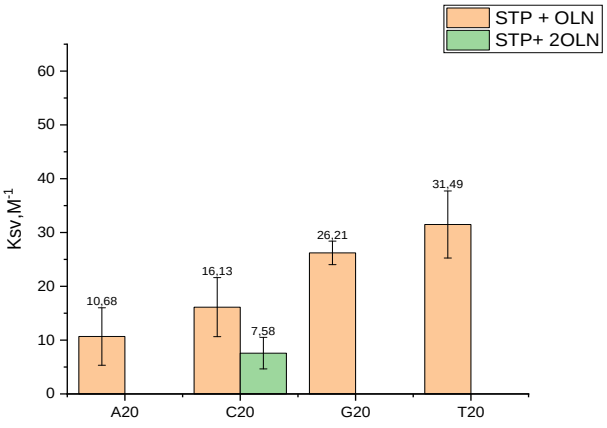

Figure 1: K_{sv} constants of oligonucleotides (OLN) in binding to somatotropin (STP)

Based on the data obtained (Stern–Volmer constants), the values of Ksv in relation to somatotropin are statistically insignificant (in some cases several times). In the interaction at different OLNs with STP, an increased affinity of adenine deoxynucleotide A20 to the protein $K_{sv1}(A20) = 10.68 M^{-1}$ can be noted. There is also a negative cooperative binding in the case of interaction between C20 and STP, which reflects the effect on the fluorescence of the first oligonucleotide molecule $K_{sv1}(C20) = 16.13$ and the second molecule $K_{sv2}(C20) = 7.58$.

In Fig. 2 we can see a strong interaction between the group of twenty-mer OLNs A20 – $K_{sv1}(A20) = 9.65 M^{-1}$, T20 – $K_{sv1}(T20) = 8.79 M^{-1}$, and G20 – $K_{sv1}(G20) = 8.47 M^{-1}$ with the insulin receptor (Fig. 2a), but at the same time, increased affinity is observed primarily in the interaction with T20 ($K_{sv} = 9.18 M^{-1}$). We can also see a decrease in K_{sv} when the 2nd ORNs molecule is attached to the protein (Fig. 2b) (positive cooperative binding): A20 ($K_{sv1}(A20) = 59.63 M^{-1}$ and $K_{sv2}(A20) = 36.34 M^{-1}$) and C20 ($K_{sv1}(C20) = 28.2 M^{-1}$ and $K_{sv2}(C20) = 15.88 M^{-1}$).

According to the calculation presented in Fig. 3, it is immediately apparent that deoxynucleotides have a strong interaction with INFR (Fig. 3a), regardless of size and composition, and in general, this is very atypical.

In the interaction of ORNs with INF, the negative cooperative interaction of proteins with G20 (Fig. 3b) ($K_{sv1}(G20) = 1.97 M^{-1}$ and $K_{sv2}(G20) = 16.35 M^{-1}$) and T20 ($K_{sv1}(T20) = 7.92 M^{-1}$ and $K_{sv2}(T20) = 15.9 M^{-1}$), which are statistically similar to each other in both constants, is noteworthy.

Analyzing the data obtained in transposed form from the standpoint of analyzing individual oligonucleotides, we see a significant difference in the activity of oligonucleotide interaction due to their different compositions (Fig. 4). Considering the group of substances with a length of 20 nucleotides, we would like to point out the high degree of interaction of the oligonucleotide with INSR and INFR (except for C20 with INSR (Fig. 4c).

In the diagrams on Fig. 4, you can see the display of binding constants, where red indicates the binding of A + B = AB, and green – A + 2B = AB2. This means that the protein binds to several oligonucleotide molecules. In other cases, when it is possible to bind the second oligonucleotide molecule, it is more efficient than with the first molecule. This means that different effects can be observed under different conditions of protein-oligonucleotide interaction, and this can be important for understanding the molecular mechanisms that occur in the system.

In our fluorescence studies, when an OLN binds to a protein, there is a change in the intensity of the fluorescent signal. After that, we calculate the dissociation constant, which characterizes the strength of the interaction between the oligonucleotide and its receptor (Fig. 5).

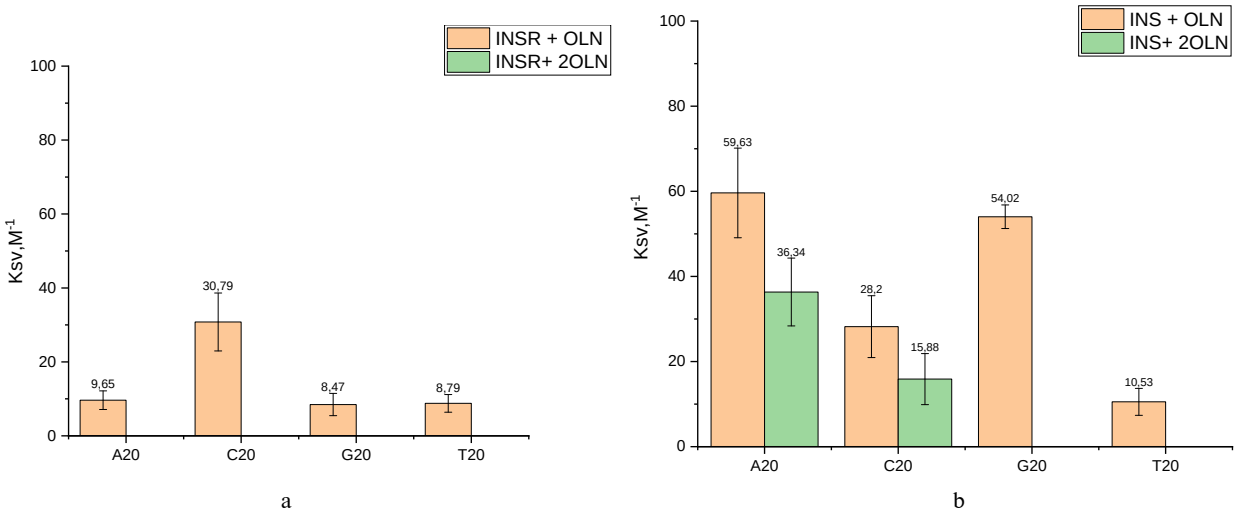

Figure 2: K_{sv} constants of oligonucleotides in binding to the (a) insulin receptor (INSR) and (b) insulin (INS)

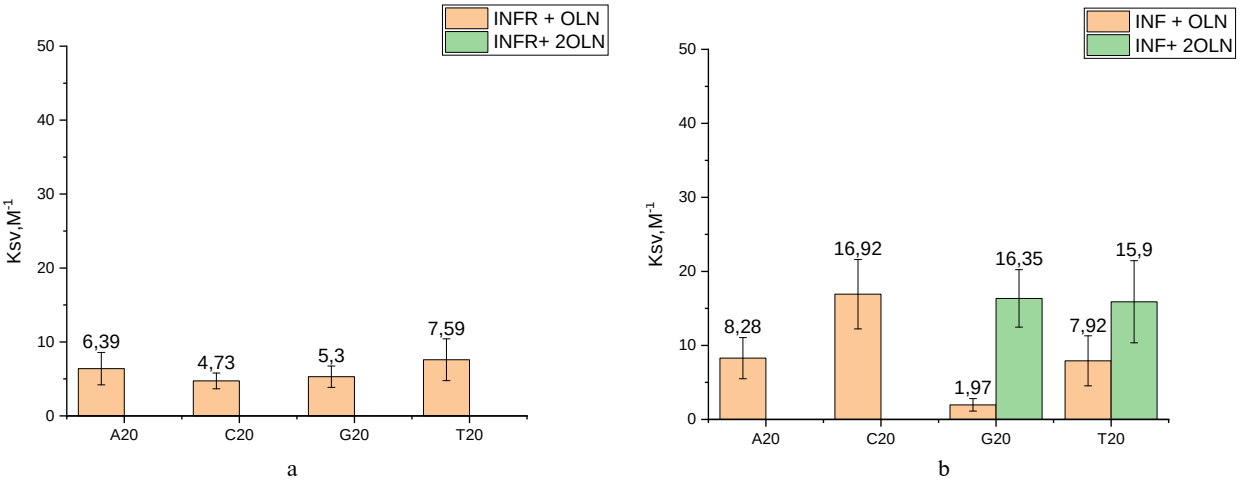

Figure 3: Ksv constants of oligonucleotides in binding to the (a) interferon-α/β receptor 1 (INFR) and (b) interferon α2-b (INF)

When evaluating the binding constants to somatropin, we can note that A20, C20, and T20 bind to somatropin with an average of 10^{-6} M, with a positive cooperative binding in the case of C20, with a very large difference between $K_{d1}(C20) = 5.75 \cdot 10^{-7}$M and $K_{d2}(C20) = 0.16 \cdot 10^{-7}$M, indicating a weak interaction with the first nucleotide but a very strong interaction with the second (Fig. 5).

In Fig. 6a, we can see a strong interaction between oligonucleotide C20 and INSR, and A20, T20, and G20 make up the 2nd statistical group of weaker interaction.

Also on the right diagram (Fig. 6b) we can see a decrease in Kd when the 2nd ORNs molecule is attached to the protein in positive cooperative binding: $K_{d1}(A20) = 5.38 \cdot 10^{-7}$M and $K_{d2}(A20) = 1.58 \cdot 10^{-7}$M and $K_{d1}(C20) = 4.19 \cdot 10^{-7}$M and $Kd2(C20) = 0.48 \cdot 10^{-7}$M.

According to the calculation presented in Fig. 7a, a strong affinity in the interaction of INFR and A20, C20, G20 molecules is noteworthy.

In the interaction of ORNs with INF (Fig. 7b), the negative cooperative interaction of proteins with G20 ($K_{d1}(G20) = 7 \cdot 10^{-7}$M and $K_{d2}(G20) = 41.55 \cdot 10^{-7}$M) and T20 ($K_{d1}(G20) = 0.02 \cdot 10^{-7}$M and $K_{d2}(G20) = 5.78 \cdot 10^{-7}$M) is noteworthy, the remaining nucleotides constitute the second statistical binding group. We can see the strong interaction of OLNs of 5 nucleotides in length, A20 with INF, which constitute one statistical group, and C20 and T20 constitute the 2nd statistical group of weaker interaction. G20 is a single molecule in this case.

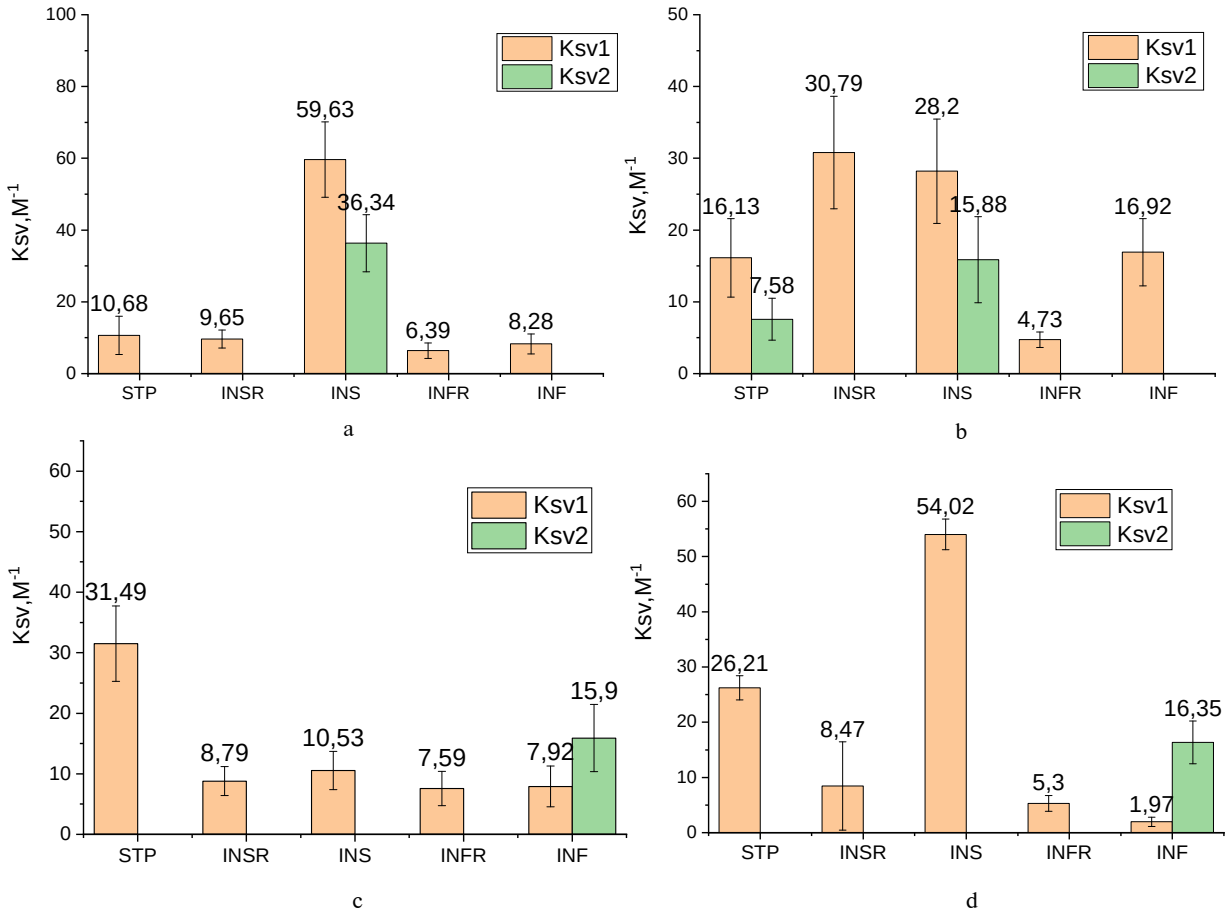

Figure 4: Stern–Volmer constants of oligonucleotides in binding to recombinant signalling proteins (insulin (INS), interferon α2-b (INF), insulin receptor (INSR), interferon-α/β receptor 1 (INFR), and somatotropin (STP)): (a) A20 with signaling proteins; (b) C20 with signaling proteins; (c) T20 with signaling proteins; (d) G20 with signaling proteins

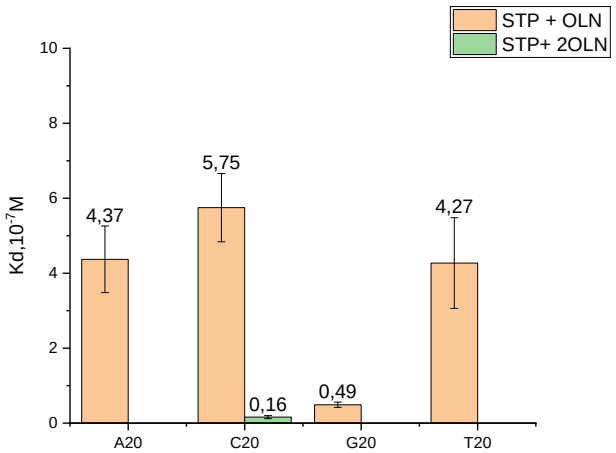

Figure 5: Dissociation constants of oligonucleotides in binding to somatotropin (STP)

Analyzing the data obtained in transposed form from the point of view of analyzing individual oligonucleotides, we can note that there are two statistically similar groups when binding to A20 (Fig. 8a): A20 with INF and its receptor and insulin receptor, and the group A20 + somatotropin and insulin.

When binding to G20 (Fig. 8d), only one group is statistically similar: insulin and its receptor and IGF receptor and somatropin. When binding to T20 (Fig. 8c), one group is statistically similar: insulin receptor and INF with its receptor and somatropin. When binding to C20, one group is statistically similar: insulin, IGF and somatropin.

The analysis showed that oligonucleotides A20 (Fig. 8a) and C20 (Fig. 8b) showed a significant positive cooperative interaction with insulin. In particular, it was observed that pairs of C20 and somatotropin, as well as rG5 and insulin, also showed positive cooperative binding. A common property of these oligonucleotides is their ability to promote protein binding, which may indicate their potential to influence the regulation of biological processes.

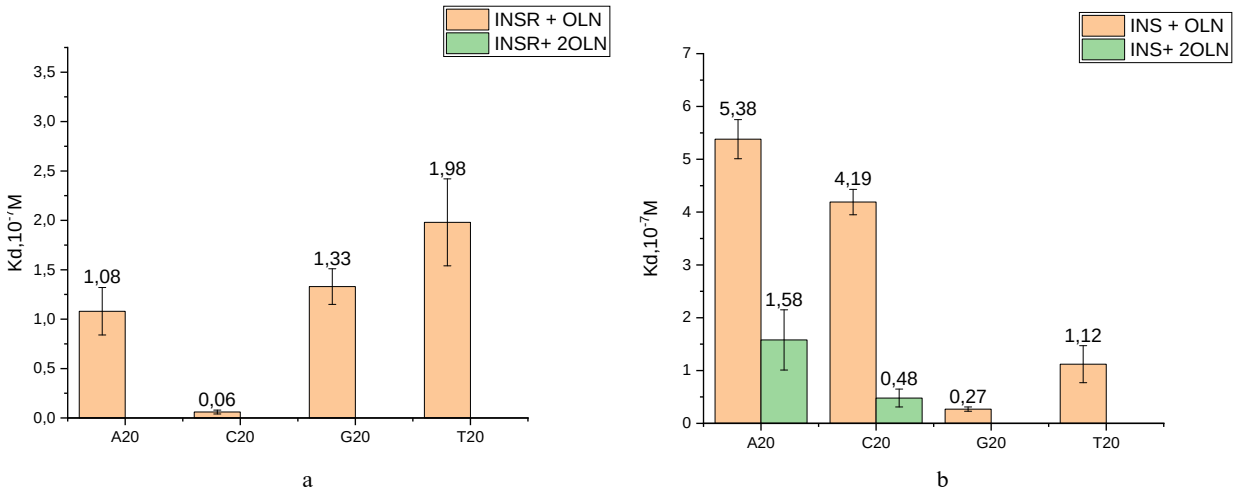

Figure 6: Dissociation constants of oligonucleotides in binding (a) insulin receptor (INSR) and (b) insulin (INS)

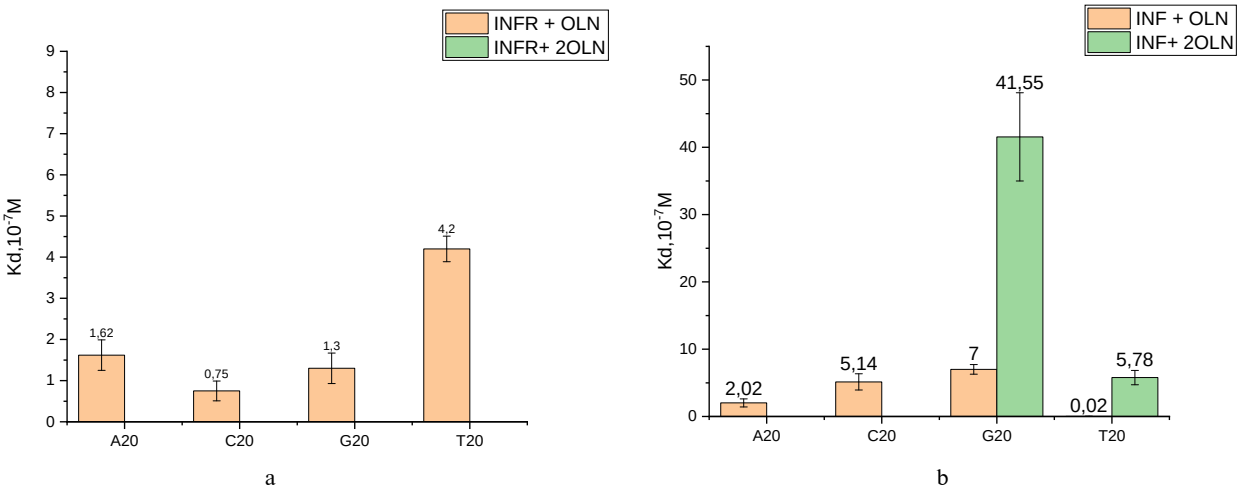

Figure 7: Kd constants of oligonucleotides in binding to the (a) interferon-α/β receptor 1 (INFR) and (b) interferon α2-b (INF)

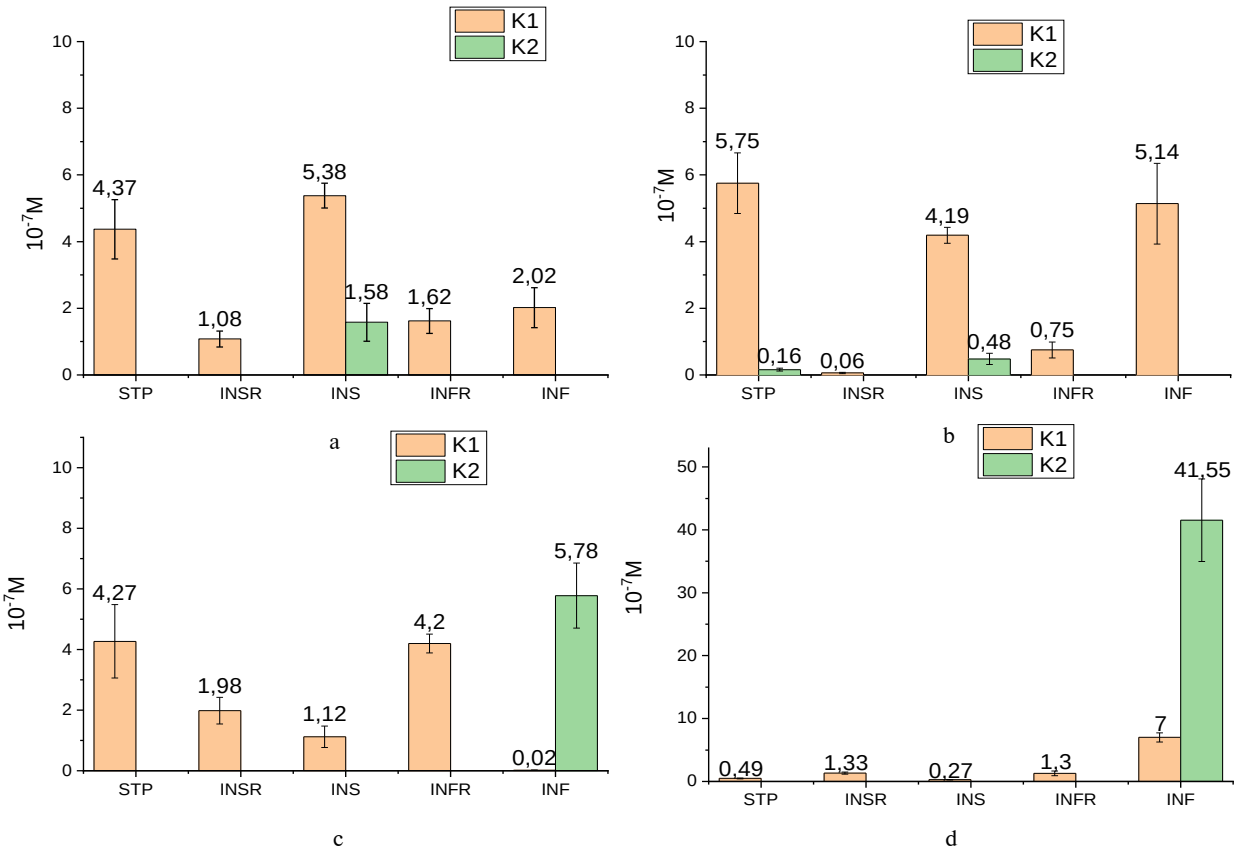

Figure 8: Binding constants of oligonucleotides to recombinant signalling proteins (insulin (INS), interferon α2-b (INF), insulin receptor (INSR), interferon-α/β receptor 1 (INFR), somatotropin (STP)): (a) A20 with signaling proteins; (b) C20 with signaling proteins; (c) T20 with signaling proteins; (d) G20 with signaling proteins

Discussion

Potential applications of these OLNs may also extend to the field of oncology, where they can be used to target signalling pathways associated with the growth and spread of cancer cells. In addition, they may hold promise in the treatment of immunodeficiency states or autoimmune diseases, where modulating signalling proteins may be key to restoring normal immune system function.

OLN drugs with low affinity ($Kd \sim 10^{-6}$ M) with carrier proteins in the circulatory system, which allows them to retain compounds in the blood. They are reversibly dissociated, and the free fraction can bind to the extracellular domain of surface proteins on target cells [8]. But if we consider our particular case of interaction with signal OLNs, in cases with short OLNs, the interaction activity is stronger ($Kd \sim 10^{-8}$ M). This observation lends support to the proposition that the length of the oligonucleotide plays a significant role in determining the nature of the interaction, as evidenced by the principal component analysis. Furthermore, the nature of the substance plays an equally important role, we can note the significantly different properties of G20 compared to other OLNs. At the same time, we can note that the separation by purines/pyrimidines and sugars has a weak correlation with the results obtained. Analyzing the work of our colleagues, we can see that such a change in interaction activity by changing the oligonucleotide length is not constant for all proteins and it strongly depends on the nature of the protein we analyze [9].

In oligonucleotide-binding proteins, allosteric or cooperative interactions between monomers can determine the affinity of binding to the oligonucleotide. This regulation can occur through various molecular mechanisms, including ligand-induced structural changes, variable heterodimer formation, or cooperative interactions between proteins after binding to the target oligonucleotide. It has been

shown in the literature that oligonucleotide-protein interactions can also occur through protein multimers with OLNs [8].

Our study has shown that OLNs can interact with recombinant signalling proteins such as interferon α2-β, insulin, and somatropin, as well as their receptors. This confirms the results of previous studies that also revealed specific connections between OLNs and proteins. It is known from the literature that OLNs can bind to proteins and affect their functional activity [10, 11], which opens up new opportunities for the therapeutic use of OLNs in medicine.

Our data on the interaction of OLNs with interferon α2-β correlates with the results of other studies that have shown that interferons can modulate the immune response by binding to nucleic acids [12]. These results support the hypothesis that OLNs can be used to enhance or suppress the immune response, which is of great importance for the treatment of viral infections and other immune diseases.

The interaction of OLNs with insulin and its receptors is also an interesting aspect of our research. Insulin is a key hormone in the regulation of glucose metabolism, and its interaction with OLNs can affect its biological activity. Our results are in line with previous studies that have shown that OLNs can affect the functional activity of hormones by altering their binding to receptors [13]. OLNs can bind to specific sites of insulin, affect its conformation or interaction with the receptor, or block receptors, which prevents insulin binding. This opens up new prospects for the development of oligonucleotide therapies for the treatment of diabetes and other metabolic diseases.

OLNs can specifically bind to somatropin and its receptors, which can affect its biological activity. These results are supported by other studies that have shown that modifying the interactions between hormones and their receptors can be an effective approach to regulating physiological processes [14]. The research methods used, such as fluorescence spectroscopy and binding analysis using the Stern–Volmer equation and the Hill equation, are important for studying the interaction between OLNs and proteins. Fluorescence spectroscopy allows for the measurement of changes in the fluorescence signal upon interaction between proteins and OLNs, which allows to determine the binding strength and cooperativity. Limitations of the study include the fact that it was conducted in vitro, which does not fully reflect the processes in the body, the use of recombinant proteins that have certain differences in conformation from native proteins, and the use of fluorescence spectroscopy as a non-destructive but indirect method of investigation. In summary, our study adds new data to the understanding of the mechanisms of interaction between OLNs and recombinant signalling proteins and their receptors. The results confirm and extend the existing knowledge in this area, which is of great importance for the further development of therapeutic and diagnostic approaches in medicine. Given the results of our study, the investigated OLNs demonstrate high binding activity to recombinant signalling proteins and receptors, and we can consider their use as molecular antiviral agents or immune response modulators. OLNs with higher polymericity in salt form have higher binding constants than monomers and can have three binding mechanisms: static, positively, and negatively cooperative. OLNs bind differently to different proteins, and different OLNs can have different ways of binding to the same protein.

Conclusions

The results of our experiments indicate medium-affinity interactions of OLNs with recombinant signalling proteins and their receptors, which, in turn, may be important for their therapeutic use. Using fluorescence analysis and the Stern–Volmer equation in general and modified form and the Hill equation, we found that OLNs exhibit cooperative binding in some cases. We found positive cooperative binding of INF with G20 and T20, negative cooperative binding of INS with C20 and A20, and STP with C20.Thus, this study opens up prospects for the development of new therapeutic strategies based on the use of OLNs and requires further research to reveal their potential in clinical practice.

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Interests disclosure

The authors don't have a conflict of interest to declare.

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ФЛУОРЕСЦЕНТНЕ ДОСЛІДЖЕННЯ ВЗАЄМОДІЇ ОЛІГОНУКЛЕОТИДІВ ІЗ РЕКОМБІНАНТНИМИ БІЛКАМИ: ІНСУЛІНОМ, ІНТЕРФЕРОНОМ α2-β, СОМАТОТРОПІНОМ ТА ЇХНІМИ РЕЦЕПТОРАМИ

Проблематика. Олігонуклеотиди (OLNs) здатні брати участь у широкому спектрі різноманітних білок-лігандних взаємодій і виконують значну кількість клітинних функцій, утворюючи структури, які дають їм змогу специфічно взаємодіяти з ДНК, РНК і білками, що є важливим аспектом біологічних процесів. Успіхи у вивченні цих різноманітних взаємодій можуть сприяти розробці нових технологій лікування різних захворювань. Механізм взаємодії між білками та OLNs є складним і все ще потребує детального вивчення. Необхідні подальші дослідження, щоб повністю розкрити цей процес і забезпечити краще розуміння взаємодії між цими біомолекулами.

Мета. Синтезувати, очистити та дослідити взаємодію OLNs із рекомбінантними сигнальними білками інтерфероном α2-β та інсуліном з їхніми рецепторами і соматропіном, оцінивши міцність зв'язку за допомогою флуоресцентної спектроскопії.

Методика реалізації. Взаємодію аналізували за допомогою рівняння Штерна–Вольмера в загальній і модифікованій формах і рівняння Хілла. OLNs синтезували методом твердофазового фосфорамідиту з очищенням твердофазовою екстракцією та подальшою перевіркою на спектрофотометрі.

Результати. Флуорометричне титрування показало, що OLNs зв'язуються з білками в середній зоні афінності, утворюючи нефлуоресцентні комплекси, з найбільш активною взаємодією з короткими OLNs. Виявлено позитивне кооперативне зв'язування інтерферону з G20 і T20 та негативне кооперативне зв'язування інсуліну з C20 і A20. Також було показано негативне кооперативне зв'язування STP із C20.

Висновки. Дослідження продемонструвало взаємодію між OLNs і рекомбінантними сигнальними білками й рецепторами за допомогою різних механізмів зв'язування, що потенційно може впливати на їх конформацію та біологічну активність. Ці результати мають значення для терапевтичного використання OLNs у контексті сигнальних білків і рецепторів.

Ключові слова: інтерферон; інтерфероновий рецептор; інсулін; інсуліновий рецептор; соматотропін; олігонуклеотид; білок-лігандна взаємодія; спектроскопія.