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Optical Response of the Polynucleotides-Proteins Interaction

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The optical absorption, fluorescence, and phosphorescence spectra of RNAs and oligonucleotides of different origin, as well as their mixtures with human albumin are investigated. It is confirmed that the energy structures of DNA, RNA, and complex protein macromolecules are determined mainly by the individual properties of their π-electron systems. The positions of the RNA excited singlet and triplet energy levels obtained by authors' previous work are determined more precisely. It is shown that mainly adenine bases are traps for mobile triplet excitons in RNA (contrary to DNA, in which AT complexes are the triplet traps). The spectral manifestation of the RNA/oligonucleotides-albumin interaction is studied. It turns out that namely the phosphorescence spectra of these compounds due to their sharp structure at 4.2 K are the most suitable for the study of the RNA-albumin interaction. The phosphorescence spectra of albumin-2′5′A_3 solvents manifest the penetrative binding of 2′5′A_3 to an albumin macromolecule. The obtained data show that at least a weak non-penetrative binding of RNA to human albumin can exist.

Keywords Human albumin; oligoadenylate; phosphorescence; RNA; triplet excitations

1. Introduction

The detection of poly- and oligonucleotides by spectroscopic methods are usually realized using the fluorescent probes, the binding of which to biological molecules leads to some spectral manifestation of this event (see, e.g., [1]). However, the usage of molecular probes can cause the disturbance of biological systems. Moreover, the

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molecular probes in a number of cases manifest the toxic effect [2,3]. One of the possible ways to avoid these undesirable effects is the usage of the autofluorescence of biological objects. On the other hand, the autofluorescence of biological objects is very sensitive to the surrounding [4] and the interaction between them. The interaction between oligonucleotides and proteins is especially important. It was shown [5–11] that oligonucleotides of the RNA type in a number of cases manifest the biological activity that is not caused by their specific bases sequences but by the possibility of their binding to some proteins. The latter is very important in genic therapy for cancer viruses' treatment and other contagious diseases. The aims of this paper are as follows: to check the RNA and proteins energy levels positions; to study the possibility of the spectral detection of RNA in a biological surrounding (e.g., proteins); and to answer the question is there spectral manifestation of the RNA-protein interaction, particularly for the RNA-human albumin.

2. Experimental

The protein human albumin, π-electron-containing amino acids (tyrosine, tryptophan, and phenylalanine), oligoRNA, tRNA, oligoadenylate $2'5'A_3$, and the model compounds such as monophosphates of 5′-adenosine (rAMP), 5′-uridine (rUMP), 5′-guanosine (rGMP), and 5′-cytidine (rCMP) were provided by the Institute of Molecular Biology and Genetics (IMBG) of the NAS of Ukraine. The steady state fluorescence measurements were performed with a Hitachi MPF-4 spectrofluorometer; and the absorption spectra were recorded on a Specord UV-VIS spectrophotometer. The phosphorescence spectra were obtained using a laboratory-designed equipment. The spectral measurements were carried out at 4.2 K, 77 K, and ambient temperatures.

3. Results and Discussion

3.1. *The Spectral Properties of RNA and Synthetic Ribonucleotides (Yeast RNA, the Model Compound of the RNA Elementary Links, OligoRNA, the RNA's Fragment that Contains 25 Links, Ribonucleotide Trimer – Oligoadenylate $2'5'A_3$)*

a) The Absorption Spectra. The structure of the model compound of the RNA elementary links and ribonucleotide trimer – oligoadenylate $2'5'A_3$ are presented in Figure 1.

Figure 2 shows the absorption spectra of the RNA elementary fragments. The absorption spectra of the oligoRNA and the additive sum of the absorption spectra

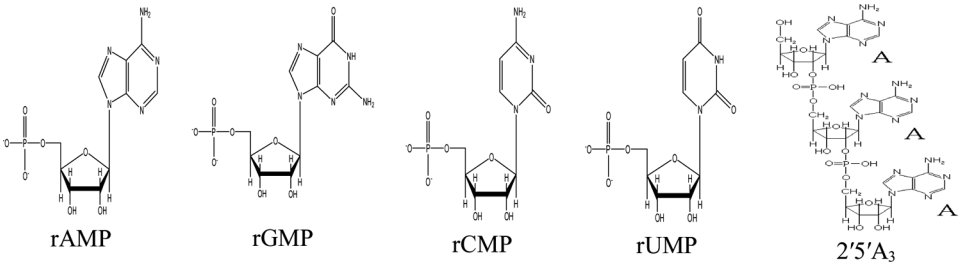

Figure 1. The structure of ribonucleotide containing compounds.

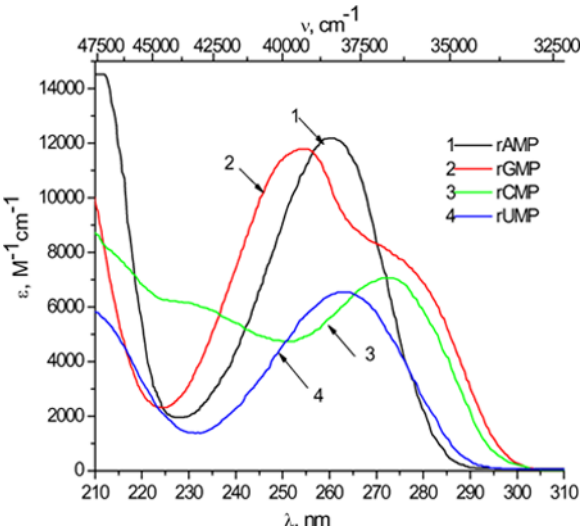

Figure 2. The absorption spectra of ribonucleotides rAMP (1), rGMP (2), rCMP (3), and rUMP (4). Aqueous solution, at $T = 293$ K.

of ribonucleotides are presented in Figure 3 (we take into account that the average quantities of ribonucleotides of the different types are approximately equal for this type of RNA). It is evident that the absorption spectra of RNA is very close to the additive sum of the absorption spectra of model compounds, as it was shown in our previous work [13], including DNA [1,12,14,15].

The similar situation is observed for oligoadenylate $2'5'A_3$ and rAMP (Fig. 4) that models elementary fragment of $2'5'A_3$. The results presented above, as in the case with DNA [1,12,14,15], give the ground to consider that photons in poly- and

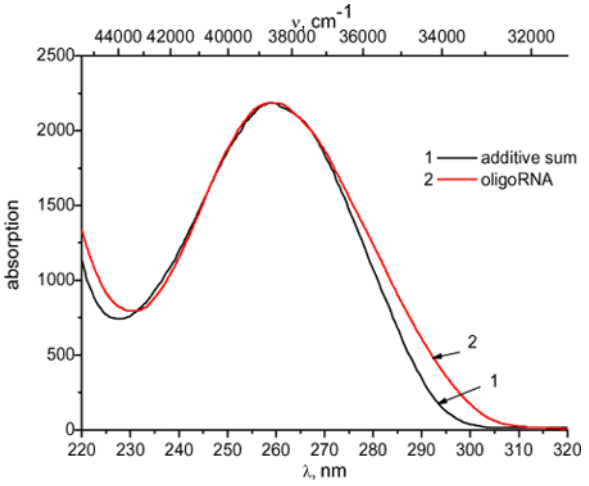

Figure 3. The absorption spectra of oligoRNA (1) and the additive sum of the absorption spectra of ribonucleotides (2) in the ratio: rAMP-29%, rCMP-21%, rGMP-20%, and rUMP-30%.

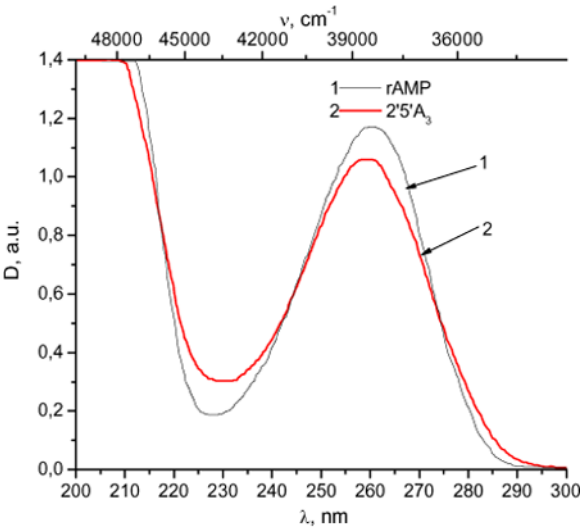

Figure 4. The absorption spectra of rAMP (1) and $2'5'A_3$ (2). Aqueous solutions.

oligonucleotides are absorbed by π-electron-containing systems of the individual links, and their energy structure (i.e., the system of energy levels) is determined mainly by the individual properties of its elementary links. So, the system of energy levels of the RNA elementary fragments and oligoRNA can be regarded as that of these more complex compounds.

b) The Fluorescence. The fluorescence spectra of model compound are presented in Figure 5 (at $T = 77$ K and $T = 4.2$ K).

The positions of the first excited electronic singlet energy levels are evaluated by the intersection of the long-wave edge of absorption and the blue edge of fluorescence spectra curves normalized on the first absorption maxima. The energy values of these levels are presented in Table 1.

The data presented below give the reason to suggest that the energy diagram of RNA singlet levels should be built based on the values presented in Table 1. So, the RNA macromolecule has the sequences of singlet energy levels $S_{rAMP} > S_{rUMP} > S_{rCMP} > S_{rGMP}$. That is why the emission of rGMP should dominate in the RNA fluorescence. However, the comparison of the fluorescence spectra of RNA and ribonucleotides shows that the fluorescence spectra of RNA differ from the rGMP spectra. Therefore, we can suppose that, due to the singlet excitation energy transfer in RNA macromolecules, some singlet traps work, and their emission dominates in the RNA fluorescence.

The situation similar to the previous one was observed for oligoRNA (contain 25 links) and tRNA. As for a trimer $2'5'A_3$, its fluorescence spectrum is very close to that of the model compound rAMP (Figs. 7 and 8), as it was expected

c) The Phosphorescence. The phosphorescence spectra of the model compound of RNA elementary links (Fig. 9) give opportunity to evaluate the position of triplet levels using blue edges of the phosphorescence spectra. The edge of the phosphorescence spectra is especially well-defined at 4.2 K. The positions of the triplet levels of model compounds (obtained from phosphorescence spectra) are

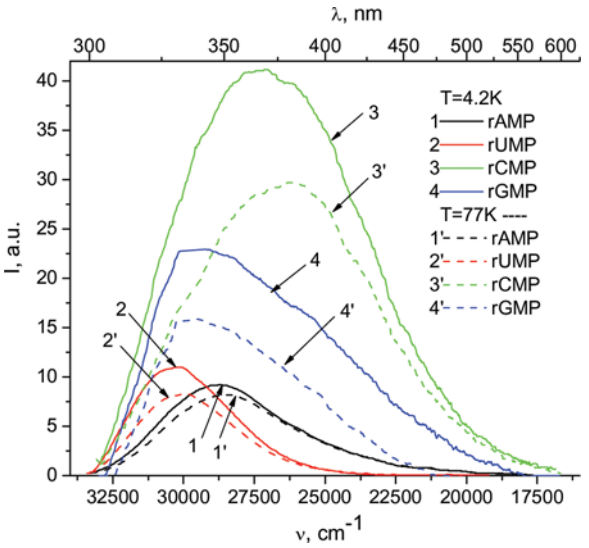

Figure 5. The fluorescence spectra of the ribonucleotides at $T = 4.2$ K (1–4) and $T = 77$ K (1′–4′). Aqueous solution, excitation at 260 nm, $C_{rAMP,rCMP,rGMP,rUMP} = 0.34$ mM.

presented in Table 2. It is easy to see that the lowest triplet level, as in the case of DNA [1,15], belongs to an adenine link. Contrary to DNA, whose phosphorescence spectra are related to an AT trap, the RNA phosphorescence is mainly determined by the emission of adenine links, as it follows from Figure 10.

It appears that the ribonucleic model compounds are less stable than deoxyribonucleic ones. Unfortunately, this fact was the main cause of the not accurate estimation of the positions of triplet levels in [13], where the lowest triplet level was identified as the rUMP level. So, the lowest electronic triplet level of RNA belongs to adenine.

The emission of adenine links is also dominating in the $2'5'A_3$ phosphorescence spectra (see Fig. 10).

3.2. *The Spectral Properties of Human Albumin Protein*

a) The Optical Absorption Spectra. It is known [16] that the main centers of the absorption and emission in the optical range are π-electron systems of tryptophan, tyrosine, and phenylalanine amino acids, the optical absorption of which are given

Table 1. Positions of electronic excited singlet energy levels of ribonucleotides rAMP, rUMP, rCMP, and rGMP evaluated at 77 K and 4.2 K

	S_1 (4.2 K), cm^{-1}	S_1 (77 K), cm^{-1}
rAMP	33600	33450
rUMP	33350	33350
rCMP	33150	33050
rGMP	32750	32450

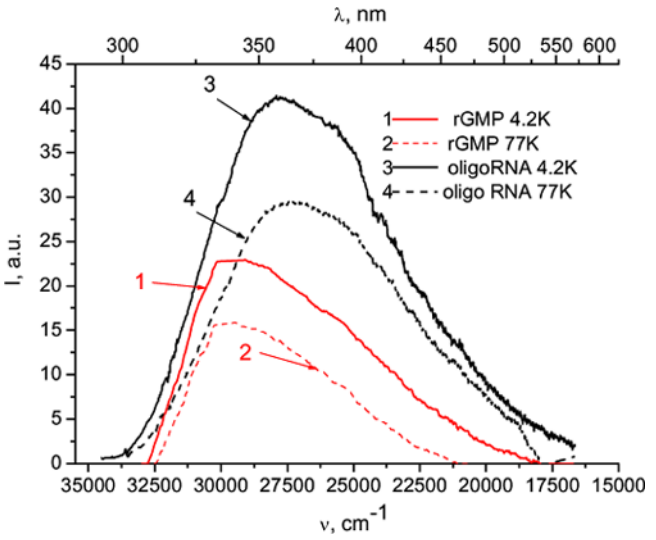

Figure 6. The fluorescence spectra of rGMP and oligoRNA. Aqueous solution, excitation at 260 nm, $C_{RNA} = 10$ mM, $C_{rGMP} = 0.34$ mM.

in Figure 11. The spectra presented in this paper are close to the corresponding spectra obtained in [4].

The absorption spectrum of human albumin (HA) protein, the combination of the tryptophan, tyrosine, and phenylalanine spectra, is given in Figure 12. This combination is constructed, by using the extinction coefficients of absorbing centers and the known ratios of their quantities (Trp:Tyr:Phe = 1:18:31 [17]). As is shown in

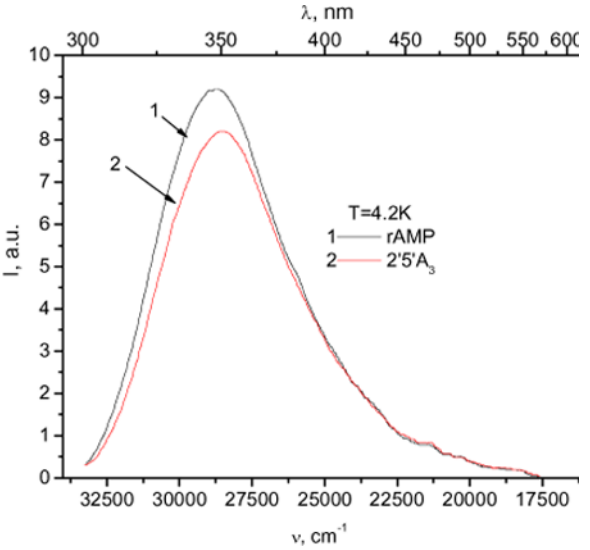

Figure 7. The fluorescence spectra of rAMP (1) and $2'5'A_3$ (2) at $T = 4.2$ K. Aqueous solution, excitation at 260 nm, $C_{2'5'A3} = 1$ mM, $C_{rAMP} = 0.34$ mM.

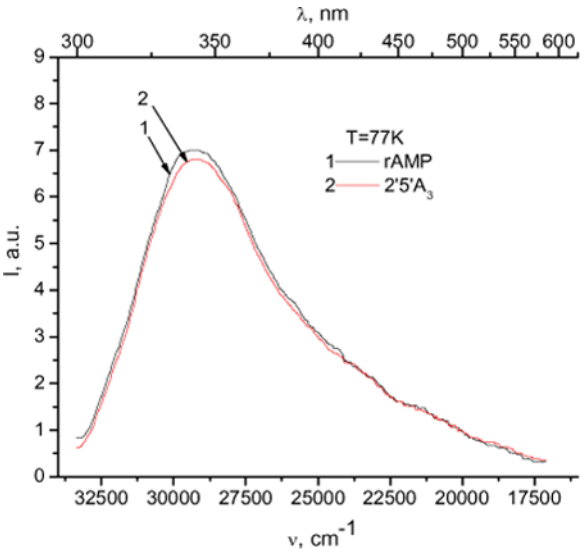

Figure 8. The fluorescence spectra of rAMP (1) and $2'5'A_3$ (2) at $T = 77$ K. Aqueous solution, excitation at 260 nm, $C_{2'5'A3} = 1$ mM, $C_{rAMP} = 0.34$ mM.

Figure 12, the spectral combination constructed in such a method is close to the HA absorption spectrum, but the 5-nm shift is observed. This shift is related (in our opinion) to the effect of the protein surrounding on absorbing centers [4]. The closeness of the constructed spectral combination to the real HA absorption spectrum confirms the known ratio between absorbing centers. Thus, the HA macromolecule really contains 1 tryptophan, 18 tyrosine, and 31 phenylalanine groups.

B) Fluorescence of HA. The fluorescence spectra of tryptophan, tyrosine, and phenylalanine at low temperatures are given in Figure 13 and close to the

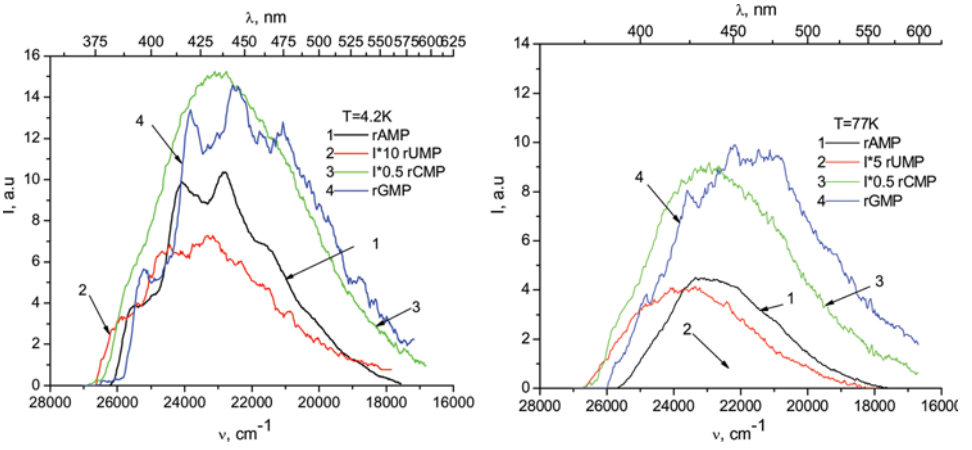

Figure 9. The phosphorescence spectra of the ribonucleotides at $T = 4.2$ K (1–4) and 77 K (1′–4′). Aqueous solution, excitation at 260 nm, $C_{rAMP,rCMP,rGMP,rUMP} = 0.34$ mM.

Table 2. Positions of electronic excited triplet energy levels of ribonucleotides rAMP, rUMP, rCMP, and rGMP evaluated at 77 K and 4.2 K

	T_1 (4.2 K), cm^{-1}	T_1 (77 K), cm^{-1}
rAMP	26300	25750
rUMP	26600	26700
rGMP	26700	25950
rCMP	26900	26750

corresponding spectra at room temperature cited in [4]. The fluorescence spectra of amino acids at $T = 4.2$ K was obtained for the first time.

The positions of singlet S_1 energy levels are estimated using the crossing of the optical absorption and fluorescence spectra curves. They are given in Table 3 and resemble the data obtained in [15].

Both the fluorescence spectra of native HA at 77 K and 4.2 K and the normalized spectra of tryptophan, tyrosine, and phenylalanine are showed in Figure 14.

The comparison of these spectra gives the possibility to conclude that the fluorescence of albumin macromolecules includes basically the emission of tyrosine (in the region 280–310 nm) and tryptophan groups (in the region 310–400 nm) (Fig. 14). Phenylalanine manifests itself very slightly due to a low quantum yield.

C) Phosphorescence of HA. Using the blue edges of the phosphorescence spectra of amino acids, we evaluated the positions of triplet T_1 energy levels (Table 4).

The phosphorescence of HA is observed mainly at low temperatures. In our opinion, the main reason for such a phenomenon is the capture of triplet excitations by molecular oxygen at room temperature. As is shown in Figure 15, the HA phosphorescence spectrum cannot be presented as a combination of the phosphorescence spectra of tryptophan, tyrosine, and phenylalanine (taking their relative content

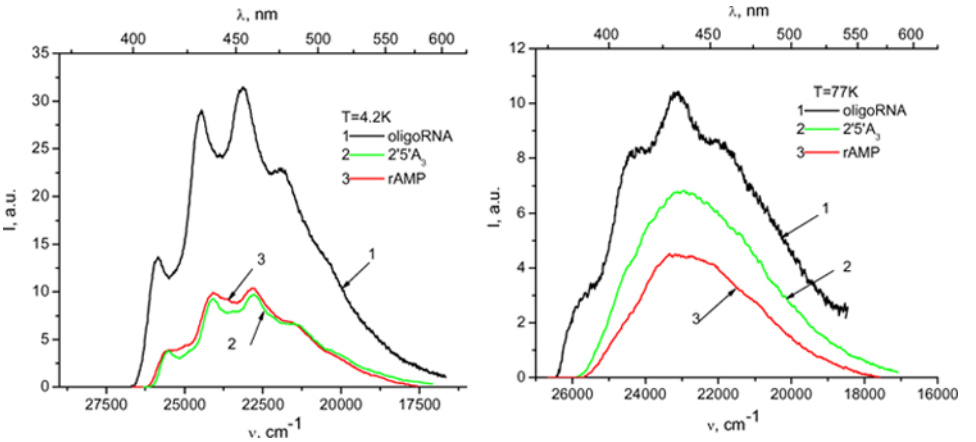

Figure 10. The phosphorescence spectra of the oligoRNA (1), $2'5'A_3$ (2) and rAMP (3). Aqueous solution, excitation at 260 nm, $C_{rAMP} = 0.34$ mM.

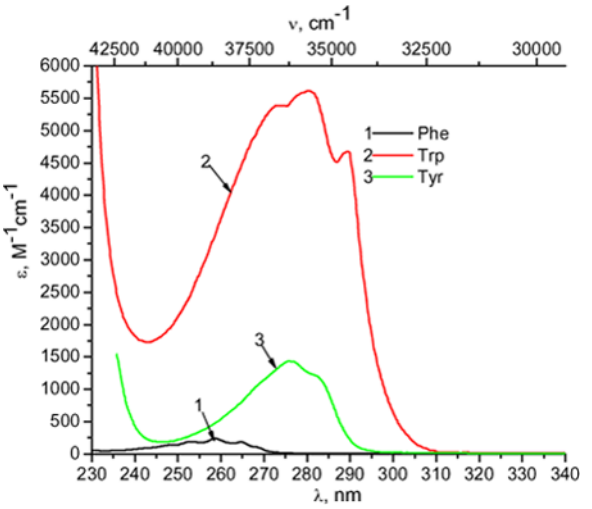

Figure 11. The optical absorption spectra of π–electron-containing amino acids: tyrosine (1), tryptophan (2) and phenylalanine (3). Aqueous solution, $T = 293$ K.

into account). This fact is the evidence of the energy transfer between π-electron-containing amino acids, which is in agreement with the data cited in [4] for azurin and interpheron proteins. The comparison of the spectra of HA and amino acids shows that (in a different extent) the emissions of all three π-electron-containing amino acids contained in the HA macromolecule are present in the protein phosphorescence (Fig. 15). Phenylalanine emits very slightly due to a low quantum yield, tyrosine phosphoresces in the region 350–450 nm, and the sharp maxima of the tryptophan phosphorescence are in the region 400–580 nm.

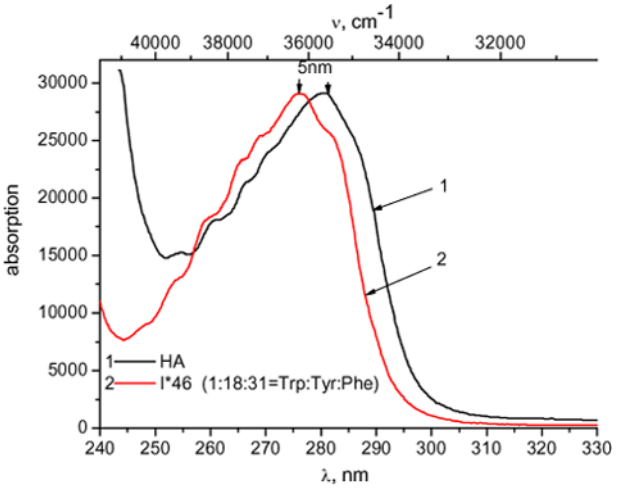

Figure 12. The optical absorption spectra of HA and the combination of π–electron-containing amino acids spectra. Aqueous solution, $T = 293$ K, $C_{HA} = 5 \cdot 10^{-2}$ mM.

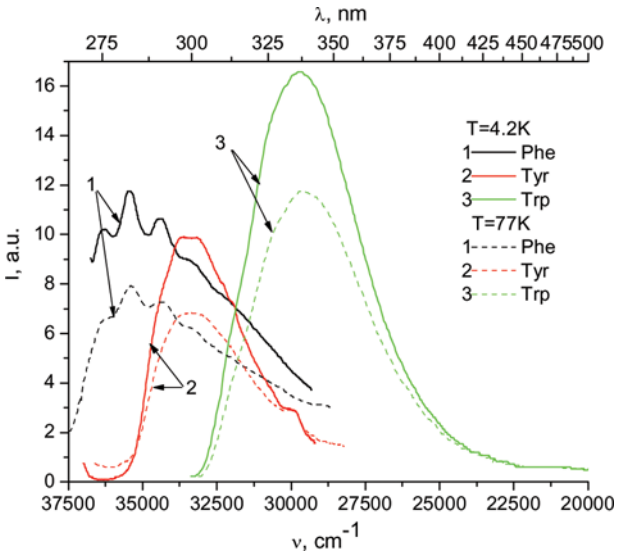

Figure 13. The fluorescence spectra of π-electron-containing amino acids at $T = 4.2$ K (1–3 solid) and 77 K (1′–3′ dash). Aqueous solution, excitation at 260 nm, $C_{Trp,Tyr,Phe} = 10^{-2}$ M.

To vary the excitation wavelength is a way to excite selectively only a tryptophan group, and then the fluorescence and the phosphorescence of HA are connected only with the tryptophan emission (Fig. 16). It is clear that the phosphorescence spectrum of a tryptophan group is more structured than its fluorescence spectrum. This last fact gives the ground to use namely the phosphorescence spectrum of a tryptophan group for the sensing of changes in the surrounding and the interactions of various types.

As an example of this fact, the phosphorescence spectrum of a tryptophan group in human albumin is shifted by 5 nm to the short wavelength region against to the phosphorescence spectrum of "free" tryptophan molecules in a solution. In the literature, such a phenomenon is explained by the great sensitivity of tryptophan to the surrounding including both the tryptophan-containing protein and the solvent. Moreover, not only the spectral position, but the spectral line shape depends on the type of protein [17].

Thereby, both singlet and triplet energy levels of π-electron-containing amino acids in HA are shifted in comparison with energy levels of "free" amino acids.

The next system of energy levels of the π-electron-containing groups in albumin can be obtained from spectral data (Fig. 17).

Table 3. Positions of the first excited singlet electronic energy levels of π-electron-containing amino acids evaluated at 77 K and 4.2 K

	S_1 (4.2 K), cm^{-1}	S_1 (77 K), cm^{-1}
Phe	37050	37150
Tyr	34850	34850
Trp	32900	32850

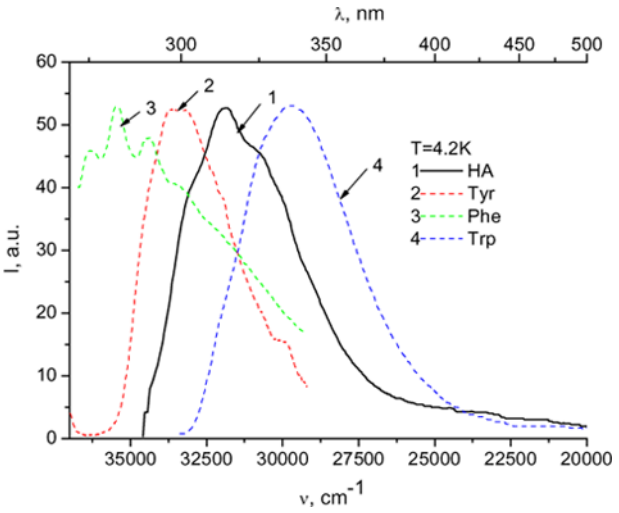

Figure 14. The fluorescence spectra of HA and normalized fluorescence spectra of π-electron-containing amino acids at $T = 4.2$ K. Aqueous solution, excitation at 260 nm, $C_{HA} = 0.6$ mM, $C_{Trp,Tyr,Phe} = 10^{-2}$ M.

The main contribution to the albumin phosphorescence at various excitation wavelengths gives tryptophan. We have to observe a significant phenylalanine contribution to the phosphorescence at the excitation wavelength $\lambda = 260$ nm, but it is not the case. Tyrosine gives some contribution to the albumin phosphorescence at the excitation wavelengths $\lambda = 260$ nm and $\lambda = 280$ nm. These data mean that the electronic excitation energy transfer is realized via the triplet levels by the scheme $T_{1phe} \rightarrow T_{1tyr} \rightarrow T_{1trp}$. This is in a good agreement with the results obtained in [17].

3.3. *The Spectral Manifestation of the RNA-Albumin and Oligonucleotides-Albumin Interaction*

The diagrams of the π-electron-containing amino acids of albumin and compounds (rAMP, rGMP, rCMP, rUMP) that model an elementary fragment of the RNA energy levels are presented in Figure 18.

This diagram was obtained from the spectral data presented above. As follows from this diagram, the binding of poly- and oligonucleotides to albumin can be manifested in the excitation electronic energy transfer from RNA to tryptophan. As a

Table 4. Positions of the first excited triplet electronic energy levels of π-electron-containing amino acids evaluated at T = 4.2 K and T = 77 K

	T_1 (4.2 K), cm^{-1}	T_1 (77 K), cm^{-1}
Phe	29400	29300
Tyr	28950	28750
Trp	25000	24050

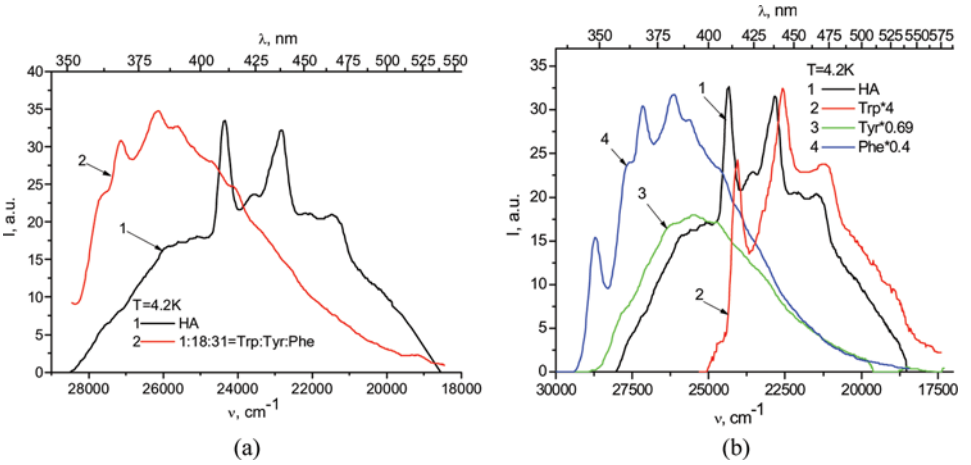

Figure 15. (a) The phosphorescence spectrum of HA (1) and the combination of π–electron-containing amino acids spectra (2) at $T = 4.2$ K. Aqueous solution, excitation at 260 nm, $C_{HA} = 0.6$ mM; (b) The phosphorescence spectrum of HA (1) and π-electron-containing amino acids (2–4) at $T = 4.2$ K. Aqueous solution, excitation at 260 nm, $C_{HA} = 0.6$ mM, $C_{Trp,Tyr,Phe} = 10^{-2}$ M.

result, the tryptophan fluorescence and the phosphorescence emission should dominate in binary solutions of albumin-RNA and albumin-oligonucleotide. Nevertheless, at low temperatures (77–4.2 K) at the 260-nm excitation wavelength, both components are present in the fluorescence and phosphorescence spectra. Only at room temperature, the tryptophan fluorescence dominates. This is related to a very low quantum yield of fluorescence (10^{-5}) of nucleotides at room temperature [4].

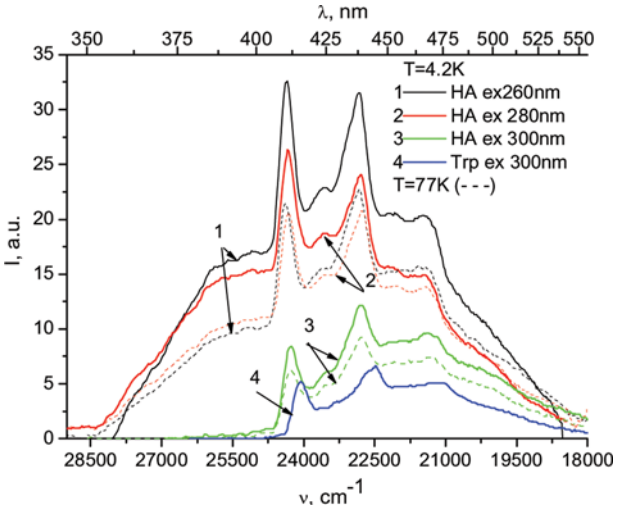

Figure 16. The phosphorescence spectra of the HA at various excitation wavelengths. Aqueous solution, $C_{HA} = 0.6$ mM.

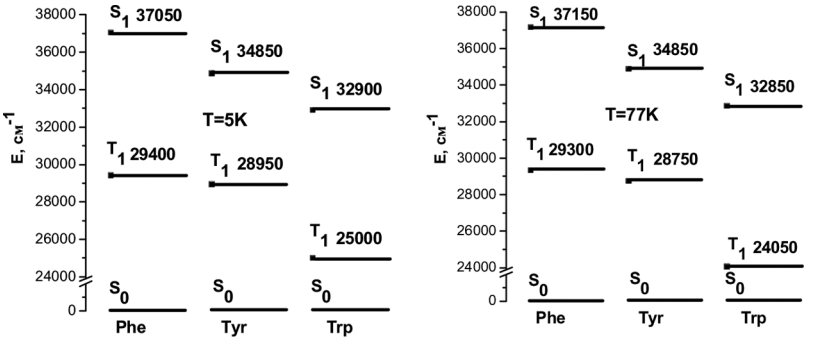

Figure 17. The position of the first excited singlet and triplet electronic energy levels of π-electron-containing amino acids in albumin evaluated at $T = 4.2\,\mathrm{K}$ and $T = 77\,\mathrm{K}$.

That's why the fluorescence and the phosphorescence of RNA and $2'5'A_3$ are not observed yet under this condition.

The absence of the triplet excitation capturing from nucleotides by a tryptophan group in albumin proves that if the binding of the studied compounds exists or its mutual penetration takes place, the nucleotides are at the distance $R_{A\text{-}Trp} > 20\,\text{Å}$ from tryptophan groups in albumin.

As follows from Figure 19, no manifestations of the interaction of these biomacromolecules are observed in the fluorescence spectra of RNA-HA solutions, because there are no additional bands or shifts of the main bands. These solutions demonstrate obviously the structured and clear phosphorescence spectra with the 387-nm narrow band that is typical of adenine groups present in the RNA macromolecule.

Thus, if RNA (it consists of 25 links, as well as oligoRNA) binds HA, this binding is "outer" when the RNA macromolecule is not "plunging" into the protein surrounding.

Contrary to the system mentioned above, the dramatical effects are observed in the binary solutions of HA and a short adenine-containing compound (trimer

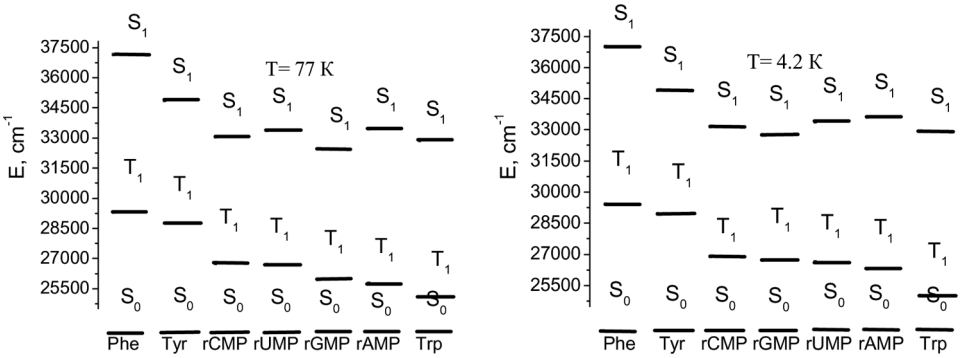

Figure 18. The position of the first excited singlet and triplet electronic energy levels of π-electron-containing amino acids in albumin compounds (rAMP, rGMP, rCMP, and rUMP) that model an elementary fragment of RNA.

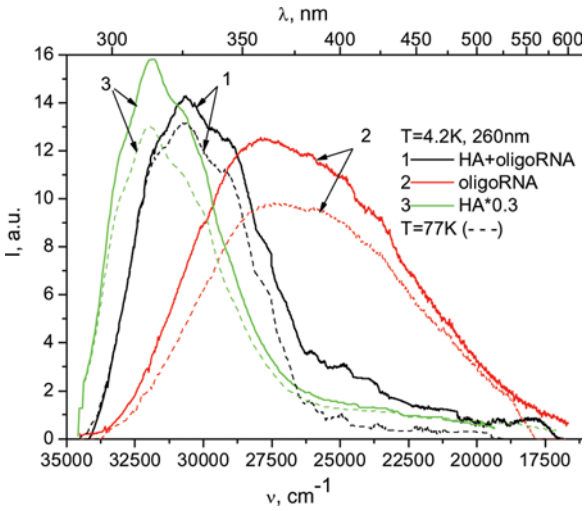

Figure 19. The fluorescence spectra of oligoRNA, HA, and a binary solution of RNA-HA at 4.2 and 77 K. Aqueous solution, excitation at 260 nm, $C_{oligoRNA} = 10$ mM, $C_{HA} = 0.6$ mM.

that contains three rAMP). In the fluorescence spectra at low temperatures, the significant wavelength shifts of the phosphorescence maximum are observed under the transition from solutions of $2'5'A_3$ to binary solutions of $2'5'A_3$-HA (Fig. 21).

The shifts (5 nm) are observed especially neatly for adenine maxima 387 nm at 4.2 K in the phosphorescence spectra of binary $2'5'A_3$-HA that is not overlapped with the trypthophan phosphorescence bands. The 5-nm shift is appeared to be

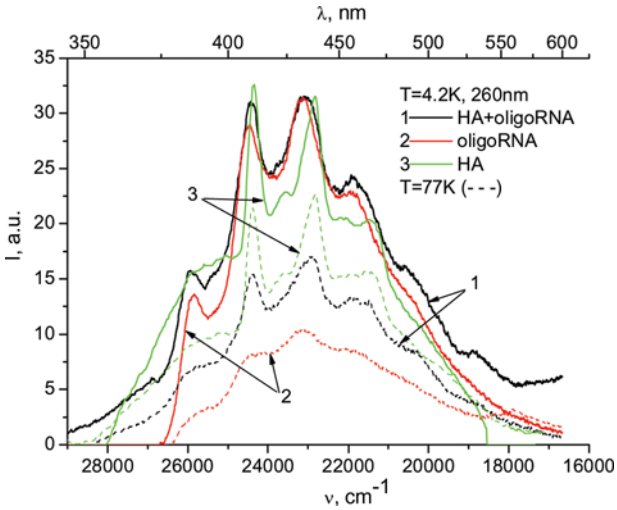

Figure 20. The phosphorescence spectra of oligoRNA, HA, and a binary solution of RNA-HA at 4.2 and 77 K. Aqueous solution, excitation at 260 nm, $C_{oligoRNA} = 10$ mM, $C_{HA} = 0.6$ mM.

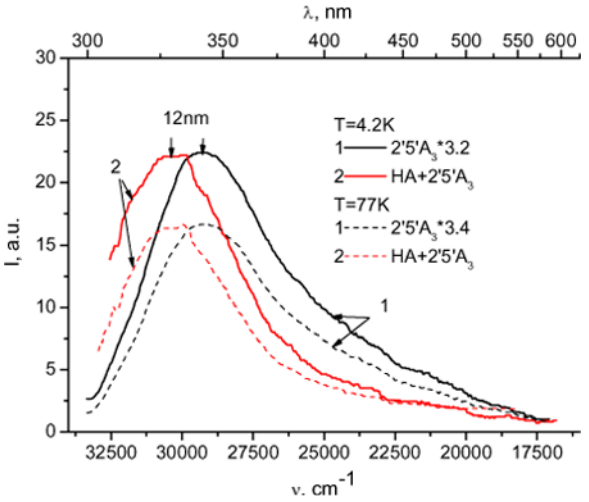

Figure 21. The fluorescence spectra of a $2'5'A_3$ solution and a binary solution of $2'5'A_3$-HA. Aqueous solution, excitation at 260 nm, $C_{2'5'A3} = 1$ mM.

typical. Really, as was shown above, the trypthophan phosphorescence maxima at 4.2 K are shifted just by 5 nm under the condition of the "plunging" of trypthophan groups into the protein surrounding (the transition from water solutions of $2'5'A_3$ to binary solutions of $2'5'A_3$-HA).

However, the main evidence of the binding of $2'5'A_3$ and HA is the appearance of a sharp structure of the adenine phosphorescence of $2'5'A_3$ molecules at 77 K (when the phosphorescence spectrum of $2'5'A_3$ is low-structured) at the addition of $2'5'A_3$ molecules in a HA solution (Figs. 22 and 23).

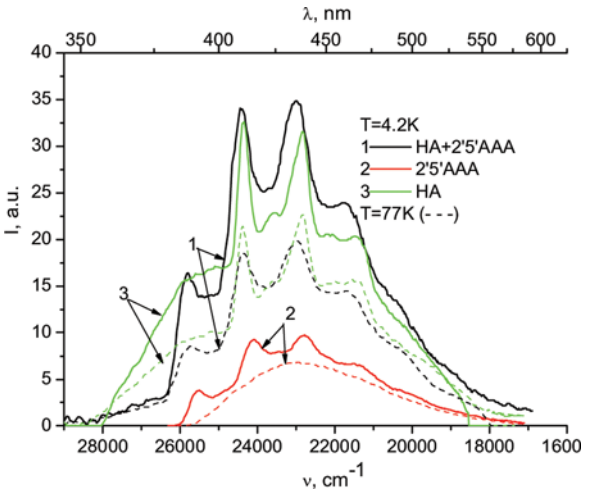

Figure 22. The phosphorescence spectra of a $2'5'A_3$ solution and a binary solution of $2'5'A_3$-HA. Aqueous solution, excitation at 260 nm, $C_{2'5'A3} = 1$ mM.

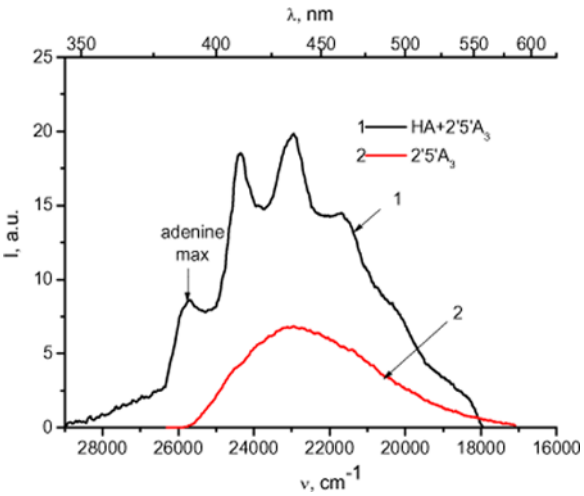

Figure 23. The phosphorescence spectra of 2′5′-AAA-Albumin system (1) and free 2′5′-AAA (2) at $T = 77$ K. Aqueous solution, excitation at 260 nm, $C_{2'5'A3} = 1$ mM.

The same effect is observed in the phosphorescence of a $2'5'A_3$ solution under the passage from 77 to 4.2 K (Fig. 24). This fact is an additional evidence of the penetration possibility of $2'5'A_3$ molecules in the protein surrounding that is more rigid in contrast to a water solution. It appears that increasing the water solution ''rigidity'' under the passage from 77 to 4.2 K leads to the same effect as that under the transition from the water surrounding of $2'5'A_3$ molecules to the protein surrounding (Figs. 23 and 24).

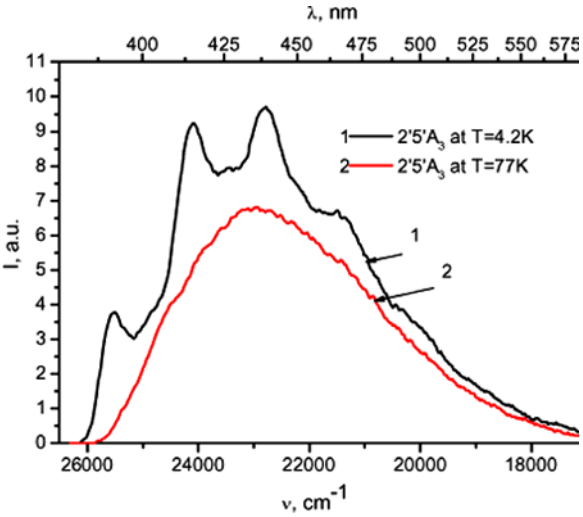

Figure 24. The phosphorescence spectra of 2′5′-AAA at $T = 4.2$ K (1) and 77 K (2). Aqueous solution, excitation at 260 nm, $C_{2'5'A3} = 1$ Mm.

4. Conclusions

Thus, the conclusion about the possibility of the RNA/oligoRNA binding to HA can be made:

1. The positions of the RNA singlet and triplet energy levels, as well as the nature of the traps for mobile excitons, are defined more exactly. The relative disposal of electronic levels is:

$$1.\ S_A > S_U > S_C > S_G \qquad 2.\ T_C > T_G > T_U > T_A$$

2. Adenine bases are traps for mobile triplet excitons in RNA (contrary to DNA, in which AT complexes are the triplet traps). This is confirmed by phosphorescence spectra.
3. The phosphorescence spectra of the compounds investigated due to their sharp structure at 4.2 K are the most suitable for study the RNA-albumin interaction. All types of RNA investigated in a biological surrounding (proteins, etc.) can be identified using the 387-nm sharp adenine band.
4. The addition of human albumin macromolecules to $2'5'A_3$ solutions leads to the same effect as a decrease in the temperature and, as a result, to an increase in the rigidity of the surrounding solvent. In our opinion, these two facts prove that $2'5'A_3$-molecules penetrate into macromolecules of albumin. The penetration of $2'5'A_3$ into albumin is not deep enough for the energy exchange between optical centers (A-group of $2'5'A_3$, Tyr and Trp centers of albumin; the distance is at least >20 Å) or the corresponding complex creation.
5. We do not observe any spectral changes neither for short RNA (25 links), nor for native RNA. This means that these macromolecules do not penetrate into albumin. At least, only the surface weak binding of short and native RNAs with albumin can be realized.

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