Sensitized photomodification of oligonucleotide-binding proteins displayed on the eucaryotic cell surface

T. I. Gainutdinov, O. E. Shestova, L. A. Yakubov, M. I. Dobrikov, and V. V. Vlassov*

Novosibirsk Institute of Bioorganic Chemistry, Siberian Branch of the Russian Academy of Sciences, 8 prosp. Akad. Lavrent´eva, 630090 Novosibirsk, Russian Federation. Fax: +7 (383 2) 33 3677. E-mail: timurg@niboch.nsc.ru

Interactions of double-stranded nucleic acids with cell surface proteins, which are involved in binding and transport of extracellular nucleic acids, were studied by the photoaffinity modification with a binary system of oligonucleotide conjugates. The photoreactive double-stranded complex involved an oligonucleotide template and two complementary to adjacent sequences oligonucleotide conjugates. One conjugate contained a photoreagent, *viz.*, 4-azido-2,3,5,6-tetrafluorobenzaldehyde *N*-(3-aminopropionyl)hydrazone, at the terminus located in proximity to the terminus of another conjugate containing the sensitizer, *viz.*, 9-aminomethylanthracene. Binding of photoreagent and the sensitizer to a single-stranded template yields the photoreactive center. Upon irradiation with visible light (400—580 nm), this photoreactive double-stranded complex forms covalent cross-linkages with oligonucleotide-binding surface proteins of eucaryotic SPEV cells.

Key words: oligonucleotide-binding proteins, sensitized photomodification of proteins, 4-azido-2,3,5,6-tetrafluorobenzaldehyde *N*-(3-aminopropionyl)hydrazone, 9-aminomethylanthracene.

The development of antisense techniques and progress in the practical gene therapy are essentially hindered by low penetrability of oligonucleotides and polymeric nucleic acids through cell membranes.[1,2] It is believed that procedures for the efficient delivery of nucleic acids to cells can be developed based on the use of the mechanisms of nucleic acid transport in living organisms.

The results of the studies[3—11] provided evidence for the existence of proteins on the cell surface, which are responsible for binding and transport of extracellular nucleic acids. Data were obtained on the involvement of these proteins in the transport of single-stranded oligonucleotides from the extracellular space to intracellular compartments and then to the cell nucleus.[12—14] The mechanism of oligonucleotide transport into the cell nucleus performed by these proteins was studied by the affinity modification with alkylating and photoactivated oligonucleotide derivatives.[9,10,12—14] The same set of cell surface proteins was subjected to the modification independently of the type of the reactive group.[13] It was demonstrated that both the reactions of cell surface proteins with alkylating oligonucleotide derivatives and binding of oligonucleotides to cells are inhibited in the presence of double-stranded DNA (dsDNA). These data indicate that the same protein can be involved in binding and transport of both oligonucleotides and double-stranded nucleic acids. It is of interest to examine interactions of oligonucleotide-binding proteins with dsDNA because these proteins may also be responsible for the uptake of DNA by cells. The gene expression, which was observed after the introduction of DNA into the bloodstream or muscle tissues in the absence of any delivery agents,[15—19] also counts in favor of this assumption.

Previously,[20—29] we have proposed an approach aimed at improving the specificity and efficiency of photomodification of biopolymers. This approach is based on the use of a pair of oligonucleotide conjugates, which are complementary to the adjacent regions of a DNA template and bear residues of the photoactivated group (perfluoroarylazide) and the sensitizer. In the complementary complex, these groups are located in proximity to each other and form a photoreaction site, which can be activated by visible light through the radiationless energy transfer from the sensitizer to perfluoroarylazide. The structure of this photoactivated reactive duplex is virtually identical to that of the dsDNA fragment and can serve as a convenient tool for studying interactions of dsDNA with proteins possessing affinity for nucleic acids.[30]

Earlier, we have demonstrated[28,29] that under the conditions of irradiation with visible light (at a wavelength longer than 400 nm), the photoactivated oligonucleotide derivative containing the residue of 4-azido-2,3,5,6-tetrafluorobenzaldehyde *N*-(3-aminopropionyl)hydrazone exhibits low reactivity. The presence of an oligonucleotide bearing the residue of the sensitizer, *viz.*, 9-amino-

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 7, pp. 1108—1112, July, 2002.

1066-5285/02/5107-1198 $27.00

methylanthracene, in the duplex enables one to activate the photoreagent by visible light. Hence, the sensitized photomodification of nucleic acid-binding proteins with the use of a reactive duplex can be performed under the conditions such that the photomodification with the participation of a single-stranded conjugate bearing the photoreagent residue proceeds with a lower rate and efficiency, while the side effects of cell irradiation are reduced to a minimum.

The aim of the present study was to examine the possibility of the use of binary systems of oligonucleotide conjugates as a tool for investigating *in vivo* interactions between dsDNA and proteins. For this purpose, we dwelled on the sensitized photomodification of oligonucleotide-binding proteins, which are displayed on the surface of cultivated eucaryotic cells.

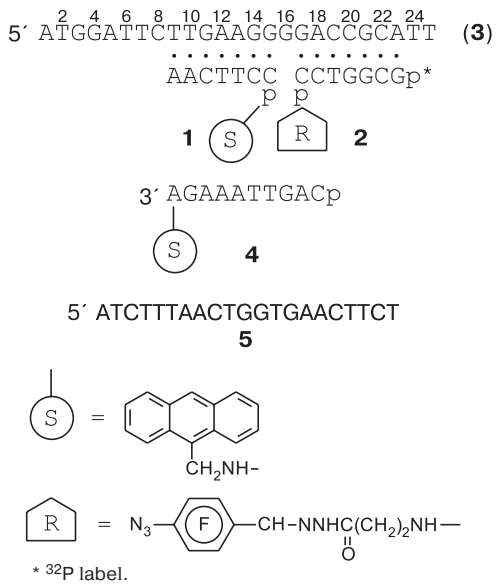
Results and Discussion

Interactions between oligonucleotide-binding proteins and dsDNA were studied with the use of a binary system of oligonucleotide derivatives* (Scheme 1). The oligonucleotide containing the residue of the sensitizer, *viz.*, 9-aminomethylanthracene (**1**), and the 5′-[^{32}P]-labeled oligonucleotide bearing the residue of the photoreagent, *viz.*, 4-azido-2,3,5,6-tetrafluorobenzaldehyde *N*-(3-aminopropionyl)hydrazone (**2**), are complementary to the adjacent regions of the single-stranded oligonucleotide template (**3**). In the test experiments, oligonucleotide **5** was used instead of oligonucleotide **3** and oligonucleotide conjugate **4** containing the 9-aminomethylanthracene residue was used instead of derivative **1**.

With the aim of limiting the possibility of the oligonucleotide transport into cells under the conditions of photomodification and ensuring the interaction between the oligonucleotide-binding protein and the reactive oligonucleotide derivative directly on the cell surface, incubation of the cells with the modified oligonucleotides and irradiation were carried out at 4 °C. The melting points of the complexes formed by oligonucleotide derivatives **1** and **2** with oligonucleotide **3** are 28 and 19 °C, respectively. Hence under the conditions used, oligonucleotide derivatives **1** and **2** are involved in a complementary complex with oligonucleotide **3**.

To reveal cell surface proteins capable of reacting *in vivo* with the photoreactive duplex, we carried out the photomodification of eucaryotic SPEV cells.** The photomodification was initiated by irradiation with filtered light (365—580 nm). The photomodification products of cell surface proteins were analyzed by electrophoresis according to Laemli's method.[31] It was found

Scheme 1



that the photomodification of a cell monolayer with derivative **2** (Fig. 1, lane *1*) and the double-stranded com-

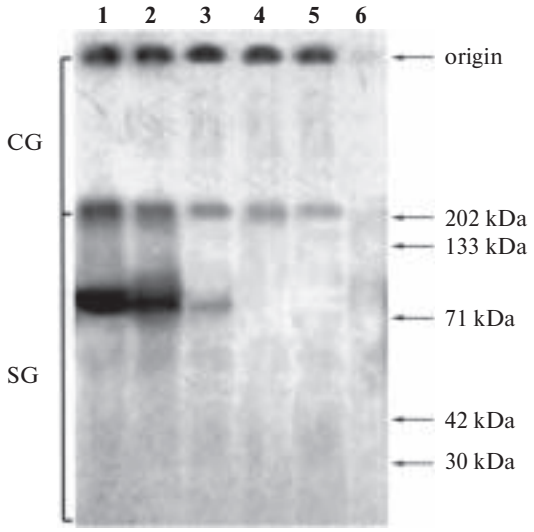


Fig. 1. Electrophoretic separation of the modification products of SPEV cell surface proteins photoactivated by the oligonucleotide derivative. A cell monolayer was incubated in a DMEM medium at 4 °C for 5 min in the presence of the 0.5 μM photoreagent [^{32}P]-**2** (*1*, *4*), the 0.5 μM photoreagent and 0.5 μM complementary oligonucleotide template **3** (*2*), or the 0.5 μM photoreagent, 0.5 μM oligonucleotide **1**, and 0.5 μM complementary oligonucleotide template **3** (*3*, *5*). Then the cells were incubated in the dark at 4 °C for 30 min (*4*, *5*) or irradiated with filtered light (λ = 365—580 nm) under the same conditions (*1*, *2*, *3*, *6*). An aliquot of the medium in which the cells were subjected to photomodification was applied onto lane *6*. The photomodification products were analyzed by electrophoresis in 12% PAAG—SDS; CG is the concentrating gel; SG is the separating gel.

* The prefix d in the notations of the deoxyribooligonucleotides is omitted.

** SPEV is the line of porcine embryonic kidney cultivated cells.

plex (Fig. 1, lanes *2* and *3*) afforded the same reaction product. The molecular weight of this product is ~79 kDa as estimated by comparing its mobility in polyacrylamide gel (PAAG) electrophoresis with those of molecular weight standard markers. This value corresponds to the molecular weight of the oligonucleotide-binding protein involved in binding and transport of oligonucleotides to the cell nucleus, which has been described earlier.[13,14] In the test experiments, modification products of cell proteins were not found upon dark incubation of the cells in the presence of the single-stranded photoreagent (Fig. 1, lane *4*) or the double-stranded complex involving oligonucleotides **1**, **2**, and **3** (Fig. 1, lane *5*). It can be assumed that an oligonucleotide-binding protein (or a group of proteins with the same mobility), which is responsible for binding and transport of single-stranded DNA (ssDNA), is involved also in dsDNA binding.

The kinetic data on accumulation of modification products of cell surface proteins in the course of direct and sensitized photomodifications under irradiation with filtered light in the range of 365—580 nm are presented in Fig. 2. It can be seen that the efficiency of the sensitized photomodification (Fig. 2, curve *3*) is lower than that of the direct (non-sensitized) photomodification with the double-stranded complex of photoreagent **2** with complementary oligonucleotide **3** (Fig. 2, curve *2*), which, in turn, is lower than the efficiency of the photomodification observed under the action of only photoreactive oligonucleotide derivative **2** without the addition of the oligonucleotide template and sensitizer **1** (Fig. 2, curve *1*).

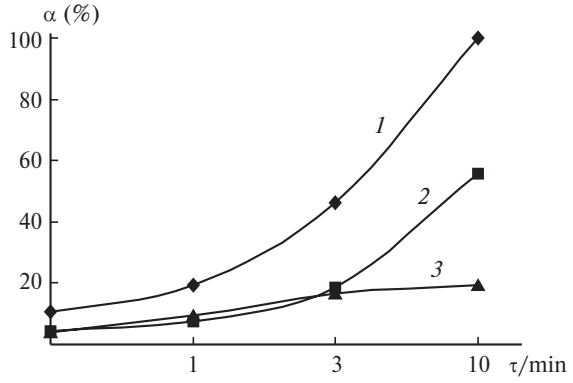


Fig. 2. The relative degree of modification (α) for the direct and sensitized photomodifications of SPEV cell surface proteins *vs*. the time of irradiation (τ). A cell monolayer was incubated at 4 °C for 5 min in the presence of the photoreagent 0.5 μM [^{32}P]-**2** (*1*) (the degree of modification achieved in this series during the maximum time of irradiation was taken as 100%); the 0.5 μM photoreagent, and 0.5 μM complementary oligonucleotide template **3** (*2*); the 0.5 μM photoreagent, 0.5 μM oligonucleotide **1**, and 0.5 μM complementary oligonucleotide template **3** (*3*). Then the cells were irradiated with filtered light (λ = 365—580 nm). The photomodification products of the cell proteins were analyzed by electrophoresis in 12% PAAG—SDS. The degree of modification was determined by densitometry of the autoradiograph.

Based on the data obtained, it can be suggested that a decrease in the degree of modification of surface cell proteins with the photoreactive duplex (as compared to the degree of modification of surface proteins with the single-stranded photoreactive conjugate) is caused by at least two factors: a) competitive inhibition of binding of the photoreagent to the nucleic acid receptor and b) the reaction of activated arylazide with oligonucleotide **3** involved in the duplex. To test this assumption, we studied the influence of the concentrations of competitor oligonucleotide **5**, which is noncomplementary to conjugates **1** and **2**, and oligonucleotide template **3** on the yields of the photomodification products of the oligonucleotide-binding protein (Fig. 3).

It appeared that the use of oligonucleotide **3** complementary to the photoreagent (Fig. 3, curve *2*) led to a sharp decrease in the efficiency of modification of the protein under study. When oligonucleotides **2** and **3** were taken in an equimolar ratio (under the conditions of formation of the double-stranded complex), the degree

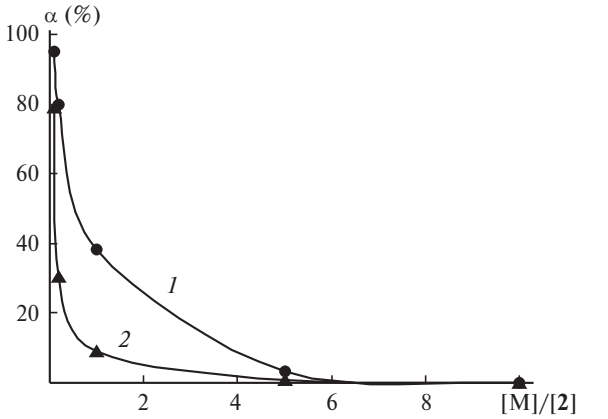


Fig. 3. The relative degree of affinity modification (α) of the SPEV cell oligonucleotide-binding protein by photoreactive oligonucleotide derivative [^{32}P]-**2** *vs*. the concentration of complementary (**3**) or competitor (**5**) oligonucleotides (expressed as the ratio of the concentration ([M]) of **3** (or **5**) to the concentration of photoreagent **2**): *1*, competitor oligonucleotide **5** noncomplementary to photoreagent [^{32}P]-**2**; *2*, complementary oligonucleotide template (**3**). A cell monolayer was incubated in the presence of 0.5 μM photoreagent 5′-[^{32}P]-**2** and at different concentrations of the competitor oligonucleotide at 4 °C for 5 min and then irradiated with light in the range of 365—580 nm at 4 °C for 30 min. The photomodification products of the cell proteins were analyzed by electrophoresis in 12% PAAG—SDS, the degree of modification was determined by densitometry of the autoradiograph (the corresponding autoradiograph is not shown). The degree of modification achieved upon irradiation of the cells under the same conditions in the absence of the oligonucleotide template (competitor) was taken as 100%.

of modification of the oligonucleotide-binding protein was only ~10% of the degree of direct photomodification observed in the absence of oligonucleotide **3** under the same conditions of incubation and irradiation. Such a sharp decrease in the efficiency of modification of the protein molecules is attributable to the fact that a large portion of photoreagent **2** was consumed in the photomodification reaction of oligonucleotide **3** involved in the complementary complex.

In the presence of oligonucleotide **5** noncomplementary to the photoreagent (Fig. 3, curve *1*), the efficiency of modification of the protein is decreased to a smaller degree. Thus when conjugates **2** and **5** were taken in an equimolar ratio, the degree of modification of the protein was about 40% of the degree of direct photomodification observed in the absence of oligonucleotide **3** under the same conditions of incubation and irradiation. Most likely, this decrease in efficiency of photomodification of the oligonucleotide-binding protein is attributed to competitive inhibition of binding of oligonucleotide derivative **2** to cell surface proteins in the presence of oligonucleotide **5**.

In order to more rigorously prove the binding of the oligonucleotide-binding protein to the duplex, we performed irradiation under milder conditions (400—580 nm) and carried out the test experiment with the use of conjugate **4**, which is noncomplementary to the oligonucleotide template (see Scheme 1) and also bears the 9-aminomethylanthracene residue, instead of modified oligonucleotide **2**.

Under these conditions of irradiation, the efficiency of sensitized modification was higher than that of direct photomodification (Fig. 4, *cf.* curves *1* and *2*). Upon irradiation of a cell monolayer in the presence of conjugate **1**, the initial rate of accumulation of photomodification products was 3—4 times higher than that in the case of the direct modification.

This situation is in good agreement with the data obtained previously for the sensitized photomodification of DNA.[28,29] Under these conditions of irradiation, the photomodification of single-stranded DNA sensitized by the oligonucleotide conjugate with 9-aminomethylanthracene proceeded rapidly and with high efficiency.

In the test experiment, which was performed with the use of oligonucleotide **4** bearing the 9-aminomethylanthracene residue instead of oligonucleotide derivative **2** noncomplementary to template **3** (Fig. 4, curve *3*), the initial rate of accumulation of modification products of surface proteins was equal to that observed in the case of the direct photomodification (Fig. 4, curve *1*), whereas the relative degree of photomodification was somewhat lower.

Earlier, it has been demonstrated[22] that the photomodification can be sensitized only in the presence of the photoreaction site, which is completely assembled

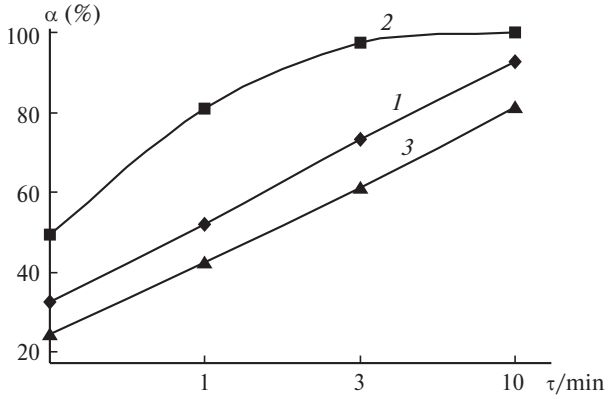


Fig. 4. The relative degree of modification (α) and the initial rates of direct and sensitized photomodifications of SPEV cell surface proteins *vs*. the time of irradiation (τ). A cell monolayer was incubated at 4 °C for 5 min in the presence of 0.5 μM photoreagent [^{32}P]-**2** and 0.5 μM complementary oligonucleotide template **3** (*1*); the 0.5 μM photoreagent, 0.5 μM oligonucleotide **1**, and 0.5 μM complementary oligonucleotide template **3** (*2*); the 0.5 μM photoreagent, 0.5 μM complementary oligonucleotide template **3**, and 0.5 μM oligonucleotide **4** (*3*). The cells were irradiated with visible light (λ 400—580 nm). The photomodification products of the cell proteins were analyzed by electrophoresis in 12% PAAG—SDS, the degree of modification was determined by densitometry of the autoradiograph (the corresponding autoradiograph is not shown).

on an oligonucleotide template, because it is necessary that the distance between the photosensitizing and photoactivated groups should be no larger than the Förster radius (which is generally equal to 2—7 nm) at which the efficiency of the energy transfer is halved. In the case of oligonucleotide derivative **4**, sensitization does not take place, which indicates that the groups of the sensitizer and photoreagent are located at a distance, which is too large for the energy transfer to occur and photomodification to be initiated. The data obtained suggest that the sensitized photomodification of the oligonucleotide-binding protein proceeds only after binding of the protein to the completely assembled double-stranded photoreactive complex **1** + **2** + **3**.

To summarize, we demonstrated that binary systems of oligonucleotide conjugates can be used as a tool for studying *in vivo* interactions between double-stranded nucleic acids and proteins. It was shown that an oligonucleotide-binding protein (or proteins), which is displayed on the eucaryotic cell surface, is capable of interacting not only with single-stranded but also with double-stranded nucleic acids.

Experimental

The following materials were used in this study: acrylamide, *N*,*N*-methylenebis(acrylamide), tris(hydroxymethyl)aminomethane, sodium dodecyl sulfate (SDS), glycine, a DMEM

medium for cell cultivation (Sigma), protein molecular weight markers for SDS-PAAG electrophoresis (Sigma), Triton X-100 detergent (LKB), ammonium persulfate (Fluka), an Agfa CP-BU X-ray film, [γ-^{32}P]ATP (Biosan, Novosibirsk) with specific activity of 5000 Ci mmol^{-1}, triphenylphsophine (Fluka), dipyridyl disulfide (Aldrich), and T4 polynucleotide kinase (BioLabs). The remaining reagents of special purity grade or reagent grade were domestically produced.

The oligonucleotide derivatives were isolated by HPLC on a Milikhrom liquid chromatograph (Russia) equipped with a 60×2-mm column; Nucleosil C-18 as the sorbent (5—20 μm; Merck, Germany); stepwise concentration gradient of acetonitrile in 0.05 *M* $LiClO_4$ (400-μL steps: 0—20—40—60%); the rate of elution was 100 μL min^{-1}.

The incident light intensity was determined on a Yu-117 luxmeter.[29]

The molar absorption coefficients (ε_{260}) of the oligonucleotides were calculated with the use of the Oligos program employing the dinucleotide method.[32]

9-Aminomethylanthracene hydrochloride was prepared as described previously.[28]

Oligonucleotides 3 and 5 and the oligonucleotide addresses containing 5′-(CCTTCAA, CAGTTAAAGA) and 3′-terminal phosphate (GCGGTCC) were synthesized by the phosphoramidite method on an automated Bioset synthesizer (Russia)[33] at the Novosibirsk Institute of Bioorganic Chemistry of the Siberian Division of the Russian Academy of Sciences. The calculated molar absorption coefficients of the oligonucleotides (ε_{260}/L mol^{-1} cm^{-1}) were 244400 (**3**) and 219400 (**5**).

Oligonucleotide conjugate 2 was prepared and isolated as described previously.[28]

The radioactive ^{32}P label was introduced into the resulting derivative by the transfer of the terminal phosphate group from [γ-^{32}P]ATP with the use of T4[34] polynucleotide kinase. The labeled oligonucleotide derivative was separated from the non-incorporated label by gel filtration on Micro-Spin G-25 columns (Pharmacia-Biotech).

Oligonucleotide derivatives 1 and 4 were prepared and isolated as described previously.[29]

Cell cultivation. The experiments were carried out with the use of cultivated cells of porcine embryonic kidney (SPEV) fibroblasts. The cells were cultivated in a DMEM medium containing 10% of bovine embryonic serum and gentamycin (40 mg mL^{-1}) at 37 °C under 5% CO_2. The cell viability was determined by staining with Trypan Blue. In the experiments, the cell viability was no lower than 90%.

Modification of cell proteins with photoactivated derivatives of oligonucleotide 2. The specific photomodification of oligonucleotide-binding proteins was carried out with the use of cells, which were grown in 48-well plates. Prior to the experiment, a cell monolayer was washed with a DMEM medium and then incubated on ice in a DMEM medium containing a mixture of oligonucleotides for 5 min. Then the cell monolayer (in plates placed in ice) was irradiated with condensed light using a DRK-120 mercury lamp of an OI-18A illuminator (LOMO, St. Petersburg) equipped with the following sets of glass light filters (λ/nm; W/mW cm^{-2}): BS-7, SZS-23 (365—580; 10); ZhS-10, SZS-23 (400—580; 8). Then the cell monolayer was rinsed three times with a DMEM medium to wash it from the oligonucleotide remained unbound, mechanically removed, placed in the DMEM medium (0.2 mL), and precipitated by centrifugation (1 min, 4000 g). To perform electrophoretic analysis of the modified proteins, the precipitate of the cell pellet was dissolved in a buffer (0.03 *M* Tris-HCl, pH 6.8, 1% SDS, 5% β-mercaptoethanol, 20% glycerol) and heated at 100 °C for 3 min. The modified proteins were separated by SDS electrophoresis in 10% PAAG and visualized by autoradiography.

Electrophoretic analysis of proteins. Electrophoresis was carried out according to Laemli's method.[31] The concentration gel contained 5% acrylamide, 0.08% bis-acrylamide, 0.125 *M* Tris-HCl (pH 6.8), and 0.1% SDS. The separation gel contained 12% acrylamide, 0.375 *M* Tris-HCl (pH 8.8), and 0.1% SDS. The electrode buffer was composed of 0.025 *M* Tris-HCl, 0.192 *M* glycine, and 0.1% SDS (pH 8.3). Electrophoresis was carried out at the current of 5 mA and then (when the dye reached the interface between the concentration and separation gels) at 10—15 mA.

Gel exposure on an Agfa CP-BU X-ray film with an intensifying screen was carried out at −20 °C for 8—24 h. The autoradiographs of the gels were scanned on a 2222 Ultroscan-XL laser densitometer (LKB, Sweden).

References

1. D. G. Knorre, V. V. Vlassov, and V. F. Zarytova, in *Oligonucleotides: Antisense Inhibitors of Gene Expression*, Ed. J. S. Cohen, CRC Press, 1989, 173.
2. E. Wickstrom, *TIBS*, 1992, **10**, 281.
3. L. A. Yakubov, E. A. Deeva, V. F. Zarytova, E. M. Ivanova, A. S. Ryte, L. V. Yurchenko, and V. V. Vlassov, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 6454.
4. S. L. Loke, S. A. Stein, X. H. Zhang, K. Mori, M. Nakanishi, C. Subasinghe, J. S. Cohen, and L. M. Neckers, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 3474.
5. V. V. Vlassov, E. A. Deeva, and L. A. Yakubov, *Dokl. Akad. Nauk SSSR,* 1989, **308**, 998 [*Dokl. Chem.*, 1989 (Engl. Transl.)].
6. P. Hawley and I. Gibson, *Antisense Res. Dev.*, 1992, **2**, 119.
7. C. Beltinger, H. U. Saragovi, R. M. Smith, L. LeSauteur, N. Shah, L. DeDionisio, L. Christensen, A. Raible, L. Jarett, and A. M. Gewirtz, *Clin. Invest.*, 1995, **95**, 1814.
8. J. Rappaport, B. Hanss, J. B. Kopp, T. D. Copeland, L. A. Bruggeman, T. M. Coffman, and P. E. Klotman, *Kidney International*, 1995, **47**, 1462.
9. P. Laktionov, J. E. Dazard, J. Piette, E. Vives, E. Rykova, V. Vlassov, and B. Lebleu, *Nucleosides Nucleotides*, 1999, **18**, 1697.
10. P. P. Laktionov, J. E. Dazard, E. Vives, E. Y. Rykova, J. Piette, V. V. Vlassov, and B. Lebleu, *Nucleic Acids Res.*, 1999, **27**, 2315.
11. G.-Q. Yao, S. Corrias, and C. Yung-Chi, *Biochem. Pharm.*, 1996, **51**, 431.
12. V. V. Vlasov, M. V. Nechaeva, S. I. Baiborodin, O. E. Shestova, I. V. Safronov, A. A. Koshkin, and L. A. Yakubov, *Dokl. Akad. Nauk,* 1995, **345**, 123 [*Dokl. Chem.*, 1995 (Engl. Transl.)].
13. L. A. Yakubov, O. E. Shestova, A. Yu. Andreeva, and V. V. Vlassov, *Dokl. Akad. Nauk,* 1998, **361**, 550 [*Dokl. Chem.*, 1998 (Engl. Transl.)].

14. O. E. Shestova, A. Yu. Andreeva, V. V. Vlassov, and L. A. Yakubov, *Dokl. Akad. Nauk,* 1999, **368**, 264 [*Dokl. Chem.*, 1999 (Engl. Transl.)].
15. J. A. Wolff, R. W. Malone, P. Williams, W. Chong, G. Acsadi, A. Jani, and P. L. Felgner, *Science*, 1990, **247**, 1465.
16. J. Hartikka, M. Sawdey, F. Cornefert-Jensen, M. Margalith, K. Barnhart, M. Nolasco, H. L. Vahlsing, J. Meek, M. Marquet, P. Hobart, J. Norman, and M. Manthorpe, *Hum. Gene Ther.*, 1996, **20**, 1205.
17. V. Budker, G. Zhang, I. Danko, P. Williams, and J. A. Wolff, *Gene Ther.*, 1996, **5**, 272.
18. V. Budker, G. Zhang, S. Knechtle, and J. A. Wolff, *Gene Ther.*, 1996, **3**, 593.
19. G. Zhang, D. Vargo, V. Budker, N. Armstrong, S. Knechtle, and J. A. Wolff, *Hum Gene Ther.*, 1997, **8**, 1763.
20. M. I. Dobrikov, S. A. Gaidamakov, A. A. Koshkin, N. P. Luk´yanchuk, G. V. Shishkin, and V. V. Vlassov, *Dokl. Akad. Nauk,* 1995, **344**, 122 [*Dokl. Chem.*, 1995 (Engl. Transl.)].
21. V. V. Vlassov, M. I. Dobrikov, S. A. Gaidamakov, E. K. Gaidamakova, T. I. Gainutdinov, and A. A. Koshkin, in *DNA and RNA Cleavers and Chemotherapy of Cancer and Viral Diseases*, Ed. B. Meunier: NATO ASI Series; Dordrecht: Kluwer Acad. Publ., 1996, **479**, 195.
22. M. I. Dobrikov, S. A. Gaidamakov, E. K. Gaidamakova, T. I. Gainutdinov, A. A. Koshkin, and V. V. Vlassov, *Antisense Nucl. Acid Drug Develop.*, 1997, **7**, 309.
23. M. I. Dobrikov, S. A. Gaidamakov, A. A. Koshkin, T. I. Gainutdinov, N. P. Luk´yanchuk, G. V. Shishkin, and V. V. Vlassov, *Bioorg. Khim.*, 1997, **23**, 191 [*Russ. J. Bioorg. Chem.*, 1997, **23** (Engl. Transl.)].
24. M. I. Dobrikov, S. A. Gaidamakov, A. A. Koshkin, N. P. Luk´yanchuk, G. V. Shishkin, and V. V. Vlassov, *Bioorg. Khim.*, 1997, **23**, 553 [*Russ. J. Bioorg. Chem.*, 1997, **23** (Engl. Transl.)].
25. M. I. Dobrikov, S. A. Gaidamakov, T. I. Gainutdinov, E. D. Tenetova, G. V. Shishkin, and V. V. Vlassov, *Dokl. Akad. Nauk,* 1998, **358**, 403 [*Dokl. Chem.*, 1998 (Engl. Transl.)].
26. M. I. Dobrikov, S. A. Gaidamakov, T. I. Gainutdinov, E. D. Tenetova, G. V. Shishkin, and V. V. Vlassov, *Bioorg. Khim.*, 1999, **25**, 31 [*Russ. J. Bioorg. Chem.*, 1999, **25** (Engl. Transl.)].
27. M. I. Dobrikov, T. I. Gainutdinov, and V. V. Vlassov, *Nucleosides Nucleotides*, 1999, **18**, 1517.
28. M. I. Dobrikov, S. A. Gaidamakov, T. I. Gainutdinov, T. I. Ivanova, and V. V. Vlassov, *Bioorg. Khim.*, 1999, **25**, 137 [*Russ. J. Bioorg. Chem.*, 1999, **25** (Engl. Transl.)].
29. M. I. Dobrikov, T. I. Gainutdinov, T. I. Ivanova, and V. V. Vlassov, *Bioorg. Khim.*, 2000, **26**, 617 [*Russ. J. Bioorg. Chem.*, 2000, **26** (Engl. Transl.)].
30. M. V. Sokolov, *Prikladnaya biofotometriya* [*Applied Biophotometry*], Nauka, Moscow, 1982, 79 pp. (in Russian).
31. U. K. Laemli, *Nature*, 1970, **227**, 680.
32. C. R. Cantor and J. Tinoko, *J. Mol. Biol.*, 1965, **13**, 65.
33. S. M. Gryaznov, V. V. Gorn, V. F. Zarytova, V. P. Kumarev, A. S. Levina, A. S. Polishchuk, V. K. Potapov, G. A. Potemkin, Yu. G. Sredin, and Z. A. Shabarova, *Izv. SO Akad. Nauk SSSR, Ser. Khim.*, 1987, **1**, 119 [*Bull. Sib. Branch Russ. Acad. Sci., Div. Chem. Sci.*, 1987, **1** (Engl. Transl.)].
34. T. Maniatis, E. F. Fritsch, and J. Sambrook, in *Molecular Cloning*, Cold Spring Harbor, New York, 1982, 125.

Received November 20, 2001