Biochemical Pharmacology, Vol. 55, pp. 1221–1227, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S0006-2952(97)00595-9

Interaction of Human Plasma Membrane Proteins and Oligodeoxynucleotides

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ABSTRACT. Two oligodeoxynucleotide (oligodN) binding proteins of 100–110 kDa on plasma membranes of human cell lines were recently identified by us. These two proteins seemed to play a role in oligodN uptake. In this study, the impact of the chain length and the sequence of the oligodN on the interaction with those two proteins was investigated. Chain length of oligodN was an important determinant, but not the sole determinant for the interaction. Binding affinity of oligodNs was determined predominantly by base composition, where pyrimidine bases but not purine bases were required in the sequence to retain high affinity. The binding kinetics of the homopolymers of deoxycytidine (dC_{21}) and deoxythymidine (dT_{21}) suggests that the proteins may have different binding sites, with one site preferring thymine bases and the other cytosine bases. Moreover, some additional plasma membrane proteins were identified, with an apparent molecular mass ranging from 40 to 58 kDa, which could bind thymine bases but not cytosine bases. BIOCHEM PHARMACOL **55**;8:1221–1227, 1998.

KEY WORDS. oligodeoxynucleotide uptake; antisense; oligodeoxynucleotide binding proteins

The oligodNs‡ are potentially new chemical entities for the treatment of human diseases, which can act as antisense, antigene, or protein inhibitors [1]. To exert their intracellular action, oligodNs must enter the cell. The mechanism of cellular uptake of oligodNs could be affected by chemical modification of the phosphodiester linkage. The uptake of ionic molecules such as PO and PS oligodNs occurs via both fluid-phase pinocytosis and adsorptive endocytosis [1–3].

Currently, there is little information about which plasma membrane proteins are involved in the process of oligodN uptake. Several plasma membrane oligodN binding proteins ranging from 79 to 90 kDa were reported by different investigators [4, 5]. These proteins bind PO and PS oligodNs on the surface of HL-60 cells in a calcium-dependent manner [6]. Another oligodN binding protein of approximately 28–34 kDa, which binds oligodNs with much greater affinity under acidic conditions, has been found in lymphoid cells [7]. Subsequently, biotinylated PS oligodNs were found to bind to several proteins ranging from 137 to 147 and 79 to 85 kDa, as well as to species of lower molecular mass, on the cell membranes of K562 cells [8]. Rappaport *et al.* [9] have also identified two PS oligodN binding proteins of 46 and 97 kDa, isolated from purified renal tubular brush border membranes. Additionally, PO and PS oligodNs have been found to bind to both αM and β2 chains of the Mac-1 receptor on the surface of polymorphonuclear leukocytes [10].

More recently, using photolabeling technology, two oligodN binding proteins of 100–110 kDa were identified on the surface of several human cell lines [11]. These two proteins, which seem to be present in the outer aspect of the plasma membrane, appear to differ from the proteins identified by others, not only because of their size, but also because of different substrate specificities. Moreover, the amount of these two proteins correlates with the uptake of oligodNs.

In the present study, we further characterized these two proteins in terms of their binding affinity to oligodNs of different chain length and base composition. The binding kinetics of dC_{21} and dT_{21}, and the relationship between oligodN binding proteins of different molecular weights are also investigated.

MATERIALS AND METHODS

Chemicals

All chemicals were of reagent grade or better. RPMI 1640 medium and fetal bovine serum were purchased from JRH

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‡ *Abbreviations:* oligodN, oligodeoxynucleotide; PO oligodN, phosphodiester oligodeoxynucleotide; PS oligodN, phosphorothioate oligodeoxynucleotide; dC_n, homopolymer with *n* residues of deoxycytidine; SdC_n, phosphorothioate homopolymer with *n* residues of deoxycytidine; dT_n, homopolymer with *n* residues of deoxythymidine; dA_{21}, homopolymer with 21 residues of deoxyadenosine; dG_{21}, homopolymer with 21 residues of deoxyguanosine; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; and RNase, ribonuclease.

Received 9 June 1997; accepted 25 September 1997.

Biosciences. Kanamycin was obtained from the Sigma Chemical Co.

Synthesis and Labeling of Oligodeoxynucleotides

PO and PS oligodNs were synthesized and purified as described previously [12, 13]. The SdN_{21} sequence was 5′-GCCGAGGTCCATGTCGTACGC-3′, while the dN_{21} sequence was 5′-AGATTTTTGGGATTGAAT-GAC-3′. The oligodNs were 5′ end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase according to an established procedure [14].

Cells

The hepatoma cell line HepG2 was grown in Minimal Essential Medium supplemented with 10% fetal bovine serum and 100 μg/mL of kanamycin at 37° in a humidified atmosphere containing 5% CO_2.

Preparation of Plasma Membranes

Plasma membranes from HepG2 cells were extracted as previously described [15]. Briefly, cells in the exponential phase of growth were washed with PBS and disrupted in lysis buffer (10 mM of Tris-HCl, pH 7.4, 10 mM of NaCl, 1.5 mM of $MgCl_2$, 1 mM of DTT, 0.5 mM of PMSF, 0.5 μg/mL of pepstatin A, and 0.5 μg/mL of leupeptin) using a ground glass homogenizer at 4°. The homogenate was then centrifuged at 1000 *g* for 10 min. The supernatant was overlaid on a 35% sucrose solution and centrifuged for 60 min at 18,000 *g*. Then the interfaces containing plasma membranes were centrifuged at 100,000 *g* for 60 min. The pellets were resuspended in a solution containing 250 mM of sucrose, 10 mM of Tris-HCl, pH 7.4, 0.5 mM of PMSF, 0.5 μg/mL of pepstatin A and 0.5 μg/mL of leupeptin, and stored at −80° until used. Protein concentration was determined by the Bio-Rad protein assay kit.

Photolabeling of Plasma Membranes and Cells

The photolabeling of plasma membranes was performed by a modification of the method of Sobol *et al.* [16]. Briefly, a fixed amount of membrane protein was mixed with a 100-nM concentration of radiolabeled oligodN in 50 μL of buffer A (20 mM of Tris-HCl, pH 7.5, 150 mM of NaCl, 6 mM of $MgCl_2$, 1 mM of EDTA, 10 mM of DTT, 2% glycerol, 0.02% NP-40, 0.2 mM of PMSF) for 2 min at 22°. Then the membranes were irradiated at 254 nm by using the Stratalinker UV Crosslinker 1800 for 1 min at 4°. Photolabeled membrane proteins were resolved by using 10% SDS–PAGE [17]. When HepG2 cells were used instead of a plasma membrane protein preparation, 5×10^5 cells were seeded overnight and then incubated with 100 nM of radiolabeled oligodN for 2 min at 22°. Then the cells were irradiated, lysed with 1% SDS, and analyzed by 10% SDS–PAGE.

Determination of IC_{50} and Inhibition Constants (K_i) of Various OligodNs

The inhibition constants (K_i) of various oligodNs were determined by the Cheng–Prusoff equation: $IC_{50} = K_i\ (1 + [S]/K_d)$ [18]. The IC_{50} (inhibitor concentration that causes 50% reduction of binding) was determined by incubating 2 μg of protein for 5 min at 22° with a fixed amount of radioactively labeled oligodNs and an increasing amount of unlabeled oligodNs in a final volume of 50 μL of buffer A (described previously). The membranes were then photolabeled as above. Free and bound oligodNs were separated by filtration on Whatman GF/C glass microfiber membranes that were washed with 15 mL of PBS and dried. Membrane-associated radioactivity was quantified by liquid scintillation spectrometry.

Kinetics of Binding and Determination of K_d Values of dT_n to Plasma Membrane Proteins

Plasma membrane protein (2 μg) from HepG2 cells was incubated with increasing concentrations of ^{32}P-labeled dT_n for 2 min at 22°. The samples were irradiated and electrophoresed on 10% SDS–PAGE. The results were analyzed by laser scanning densitometry, and the concentrations of ^{32}P-labeled dT_n were plotted as a function of gel band intensity. The dissociation constants (K_d) were then calculated from the saturation curve.

Renaturation Study of Binding Proteins

Plasma membrane proteins were separated by 10% SDS–PAGE. The gels were washed with 200 mL of renaturing buffer (10 mM of Tris-HCl, pH 7.5, 100 mM of NaCl, 4 M of urea, 0.5 PMSF) for 30 min at room temperature, with one change of buffer after 30 min. Then the proteins were transferred to nitrocellulose filters by capillary action in the renaturing buffer at room temperature for 48 hr. The filters were washed with binding buffer A (described above) followed by quenching with 3% BSA in binding buffer. The filters were incubated with 7.5 nM of ^{32}P-labeled SdN_{21} (2 Ci/mmol) or ^{32}P-labeled dT_{21} (16 Ci/mmol) in binding buffer for 30 min, and then washed with the same buffer two times, 30 min each time, at room temperature.

RESULTS

Effect of Different Chain Lengths of OligodN on Protein Binding

It was demonstrated previously that dC_{21} was able to bind with high affinity to the 100–110-kDa plasma membrane proteins with an apparent dissociation constant (K_d) of 60 ± 24 nM [11]. In the present study, the protein binding affinity of oligodNs with different chain lengths was investigated in competitive binding experiments, and the inhibition constants (K_i) of the various oligodNs were determined (Table 1). Among the PO oligodNs, the K_i values

TABLE 1. Values of K_i for competitors of dC_{21} binding to plasma membrane proteins

OligodNs	K_i* (nM)
dC_{28}	5.1 ± 1.0
dC_{18}	278.0 ± 98.0
dC_{14}	> 700.0
SdC_{28}	0.2 ± 0.03
SdC_{21}	0.5 ± 0.14
SdN_{21}	2.9 ± 1.12

*K_i (inhibition constant) values, calculated from the Cheng–Prusoff equation [18], represent the mean ± SD from at least three experiments with duplicate samples in each experiment.

for dC_{28}, dC_{18}, and dC_{14} were 5.1, 278 and >700 nM, respectively. With PS oligodNs, the K_i values for SdC_{28}, SdC_{21}, and SdN_{21} were 0.2, 0.5, and 2.9 nM, respectively. These results indicate that these membrane proteins bind longer oligodNs more efficiently. There is a 25-fold difference between the K_i values determined for SdC_{28} and for dC_{28}. This reflects, as reported previously [11], a stronger binding affinity for PS oligodNs than for PO oligodNs to the two proteins. Although homopolymers of deoxycytidine and sequence-specific oligodNs were able to bind to these proteins, the K_i value for SdC_{21} was 6-fold lower than the value for SdN_{21}, suggesting that the sequence or composition of the oligodN could affect the affinity of binding.

Effect of the Base Composition of the OligodN Sequence on Protein Binding

To further investigate the impact of the base composition of the oligodN, we photolabeled homopolymers of deoxycytidine, deoxythymidine, and deoxyadenosine of 21 nucleotide length, dC_{21}, dT_{21}, and dA_{21}, respectively, to plasma membranes from HepG2 cells. As shown in Fig. 1A, both dC_{21} and dT_{21} were able to cross-link the 100–110-kDa proteins, whereas dA_{21} showed no cross-linking efficiency. It should be noted that dT_{21} bound preferentially to the 100-kDa protein; binding to the 110-kDa protein could be shown only after longer autoradiography exposure (data not presented). Moreover, dT_{21} was able to bind four proteins with apparent molecular masses ranging from 40 to 58 kDa. Because the results obtained by photolabeling dG_{21} to plasma membrane proteins were inconsistent (data not shown), the ability of dG_{21} to bind to plasma membrane proteins could not be evaluated. In competition binding experiments (Fig. 1B, right panel), a 10-fold excess of dC_{21} was able to inhibit efficiently the cross-linking of dT_{21} to the 100-kDa protein, whereas the cross-linking to the smaller proteins was unchanged. dN_{21} was less efficient in inhibiting the cross-linking of dT_{21} to the 100-kDa protein, and totally ineffective in inhibiting the binding to the smaller proteins. The cross-linking ability of an oligodN, with a sequence containing a stretch of 5 thymine bases (see Materials and Methods), to plasma membrane proteins, and its ability to be competitively displaced by

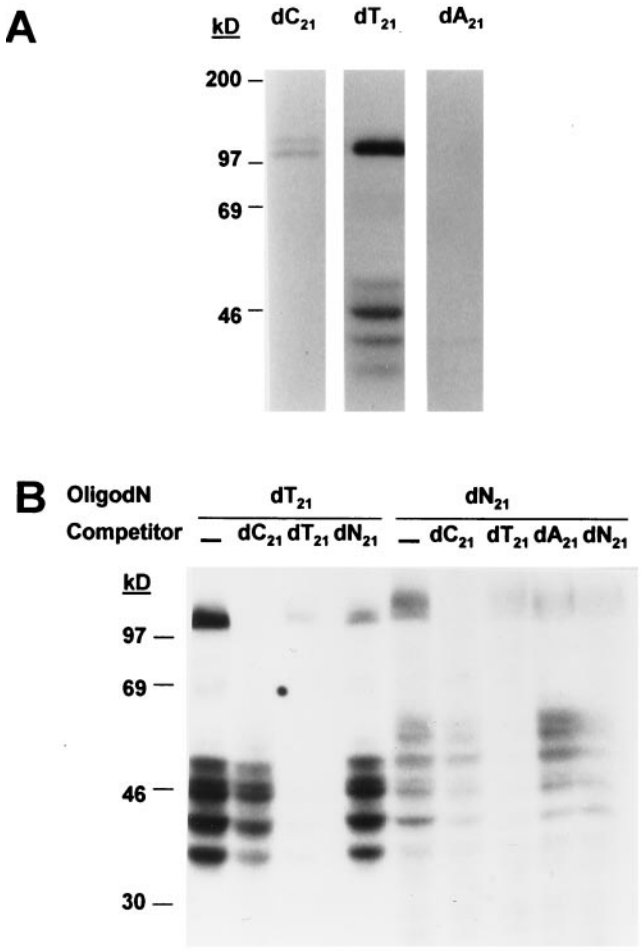

FIG. 1. (A) Photolabeling of homopolymers to plasma membranes of HepG2 cells. HepG2 cells (5×10^5) were incubated with 100 nM of [^{32}P]dC_{21}, [^{32}P]dT_{21}, or [^{32}P]dA_{21} for 2 min at 22°. The cells were photolabeled for 1 min, lysed with 1% SDS, and analyzed by 10% SDS–PAGE. (B) Competition of dT_{21} and dN_{21} binding by different oligodNs. HepG2 cells (5×10^5) were incubated with 100 nM of [^{32}P]dT_{21} or [^{32}P]dN_{21} for 2 min at 22° in the presence or absence of a 1-μM concentration of competitor. The cells were photolabeled for 1 min, lysed with 1% SDS, and analyzed by 10% SDS–PAGE.

homopolymers of different base compositions, were studied. dN_{21} photolabeled not only the 100–110-kDa proteins but also smaller proteins around 50 kDa (Fig. 1B, right panel). Those proteins have a molecular weight similar to those photolabeled by dT_{21}. In competition experiments (Fig. 1B, right panel), a 10-fold excess of dC_{21} was able to inhibit dN_{21} binding to the 100–110-kDa proteins, whereas dT_{21} and dA_{21} showed a lower inhibitory ability. Moreover, dN_{21} binding to proteins around 50 kDa was inhibited strongly only by excess of dT_{21}. These results suggest that for the 100–110- kDa proteins the presence of pyrimidine bases, but not purine bases, seem to be a major determining factor for the binding, whereas for the proteins around 50 kDa, the major determining factor seems to be the presence of thymine bases only.

OligodN	Sequence (5′->3′)	Binding (%)
1	AGA TTT TTG GGA TTG AAT GAC	100
2	AGA TTT TCG GGA TTG AAT GAC	39
3	AGA TTT CTG GGA TTG AAT GAC	29
4	AGA TTC TCG GGA TTG AAT GAC	14
5	AGA TCT CTG GGA TTG AAT GAC	8

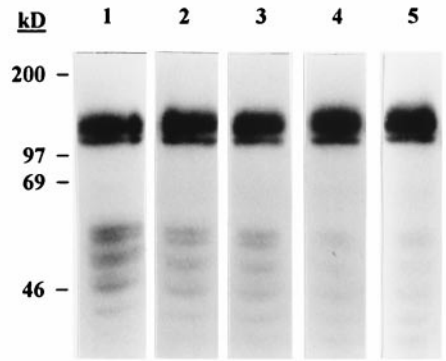

FIG. 2. Effect of oligodN sequence on binding to plasma membrane protein around 50 kDa. Base changes to the oligodN-1 were made as shown (underlined bold). A 100-nM concentration of [^{32}P]oligodN-1, -2, -3, -4, and -5 was photolabeled to 5 × 10^5 HepG2 cells and analyzed by 10% SDS–PAGE. The gel bands were quantitated by laser scanning densitometry, and the total sum of the intensity of the four bands near 50 kDa was reported as percent of sequence 1. The results from a representative experiment are shown.

Effect of OligodN Sequence on Binding to Proteins around 50 kDa

It was shown that dN_{21} was able to bind the 100–110-kDa proteins and the smaller proteins around 50 kDa, whereas some other oligodNs with different sequences did not show binding to the proteins around 50 kDa (data not shown). To elucidate the binding requirements of those proteins, a sequence and binding relationship study was performed, and the results are depicted in Fig. 2. Replacing one or two T residues with C was able to decrease the cross-linking efficiency of oligodN to the 50-kDa proteins, while the cross-linking to the 100–110-kDa proteins was unchanged. Moreover, even if the differences in percentage of binding were minor, the cross-linking efficiency to the small proteins seemed to be proportional to the total number of sequential Ts in the sequence, suggesting that stretches of Ts in the oligodN sequence seemed to be responsible for cross-linking to the 50-kDa proteins. However, additional studies are required to better investigate this issue.

TABLE 2. Values of the apparent dissociation constant (K_d) of dT_{21} and dT_{28} on binding to the 100-kDa plasma membrane protein*

	K_d (nM)
dT_{21}	190 ± 100
dT_{28}	80 ± 34

*Plasma membrane proteins (2 μg) from HepG2 cells were photolabeled with increasing concentrations of ^{32}P-labeled dT_n, and the results were quantitated by laser scanning densitometry. The saturation curve was obtained by plotting the concentration of dT_n as a function of gel band intensity. The K_d values represent the means ± SD from three different experiments.

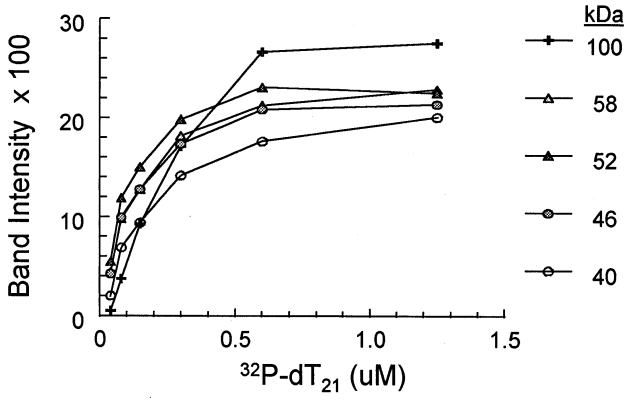

FIG. 3. Kinetics of binding of dT_{21} to plasma membrane proteins. Plasma membrane proteins (2 μg) from HepG2 cells were incubated with 0.04, 0.08, 0.15, 0.3, 0.6, and 1.2 μM of ^{32}P-labeled dT_{21} for 5 min at 22°. The samples were photolabeled and subjected to 10% SDS–PAGE. The result was analyzed by laser scanning densitometry, and the concentrations of ^{32}P-labeled dT_{21} were plotted as a function of gel band intensity. The results from a representative experiment are shown.

Kinetics of Binding of dT_n to Plasma Membrane Proteins

The association of the 100-kDa protein with dT_{21} and dT_{28} exhibited saturation binding with apparent K_d values of 190 ± 100 and 80 ± 34 nM, respectively (Table 2). This indicates that, as was already observed for dC_n, longer oligodNs bind more efficiently to this protein. The binding of dT_{21} to the four proteins around 50 kDa was also examined (Fig. 3). The four proteins not only exhibited similar binding kinetics with K_d values ranging from 100 to 200 nM, but also their saturation binding curves almost overlapped the saturation curve of the 100-kDa protein. It should be noted that the relative amount of cross-linking to the four proteins varied from experiment to experiment, while the saturation curves and K_d values were always similar.

Displacement of dC_{21} Binding to Plasma Membrane Proteins by Different OligodNs

Based on the apparent K_d values, the affinities of binding of dC_{21} and dT_{21} to the 100–110-kDa proteins are similar. Surprisingly, when dT_{21} was used to displace the binding of ^{32}P-labeled dC_{21} in competitive binding experiments, no inhibition was found up to 50 μM (Table 3). One inter-

TABLE 3. Displacement of dC_{21} binding to plasma membrane proteins by different oligodNs

Competing oligodN	IC_{50}* (nM)
dT_{21}	> 50,000
dC_{21}	300 ± 0.1
dA_{21}	> 50,000
dN_{21}	> 20,000
SdC_{21}	23 ± 6.0
SdN_{21}	136 ± 4.0

*The IC_{50} (concentration of inhibitor that causes 50% reduction of binding) values represent the mean ± SD from three experiments with duplicates in each experiment.

pretation of this result is that those two oligodNs may bind to different sites on the same protein. Moreover, dN_{21} and dA_{21} at 100-fold or greater molar excess were not able to displace dC_{21} binding, indicating, as had been suggested previously by photolabeling experiments (Fig. 1), that the two oligodN binding proteins have a preference to bind oligodNs containing pyrimidine bases.

Renaturation Study of Binding Proteins

To investigate the apparent molecular weight of the oligodN binding proteins and to understand more about their different affinities for oligodNs of different base composition, we carried out renaturation studies of plasma membrane proteins after SDS–PAGE electrophoresis. As indicated in Fig. 4, the binding pattern of proteins prephotolabeled with SdN_{21} was indeed different from that of dT_{21} (lanes 1 and 3). Similar results were obtained when filters containing renatured proteins were incubated with ^{32}P-labeled SdN_{21} or with ^{32}P-labeled dT_{21} (lanes 2 and 4). After protein renaturation, SdN_{21} bound preferentially to a 100-kDa protein and, with a relatively lower affinity, to a ~50-kDa protein (lane 2), whereas dT_{21} bound with high affinity to three or four proteins around 50 kDa and with lower affinity to the 100-kDa protein (lane 4). It should be noted that the relative amount of dT_{21} binding to proteins of different

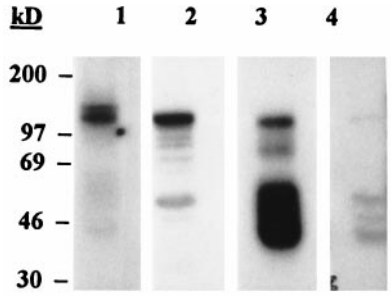

FIG. 4. Renaturation study of oligodN binding proteins. After SDS–PAGE, plasma membrane proteins (5 μg) from HepG2 cells were renatured and transferred to nitrocellulose filters. The profile of proteins prephotolabeled with 100 nM of [^{32}P]SdN_{21} or with [^{32}P]dT_{21} is shown in lanes 1 and 3, respectively. Binding of renatured proteins with 7.5 nM of [^{32}P]SdN_{21} or [^{32}P]dT_{21} is shown in lanes 2 and 4, respectively.

molecular weights could vary slightly with different membrane protein preparations. Moreover, unlike the prephotolabeling results, the 110-kDa protein was never detected after protein renaturation.

DISCUSSION

The antisense approach involves the binding of a synthetic oligodN to a complementary sequence in the target nucleic acid through a highly selective process governed by the Watson–Crick base pair hybridization. Because PO oligodNs have turned out to be very sensitive to nucleases, chemically modified oligodNs such as PS oligodNs have been developed to avoid degradation [1]. PS oligodNs, although quite effective as antisense agents, have shown several nonsequence-specific effects. The nonsequence-specific effects are mainly caused by the interaction of PS oligodNs with DNA binding proteins such as human DNA polymerases and RNase H [19] or with proteins such as the heparin binding proteins, basic fibroblast growth factor (bFGF), and laminin, through a charge interaction [20, 21]. Recently, it was shown that the nonsequence-dependent binding may still be influenced by the sequence. For instance, the ability of PS oligodN to bind bFGF or laminin is heavily dependent upon the presence of four contiguous guanine residues (the G-quartet) in the oligodN sequence, but the presence of the G-quartet may not account for all of the sequence-specificity observed with the PS oligodNs [22].

Another example of sequence-selective non-antisense action of oligodNs was offered by PS oligodNs containing the CpG motif, which was found to be profoundly immunomodulating. The CpG motif-containing oligodNs was reported to induce cell proliferation [23] and antibody response when administered to mice [24] or to produce interferon and activate natural killer cells [25, 26].

In this study, we describe the binding of oligodNs to the 100–110-kDa plasma membrane proteins, which is dependent upon the base composition of the oligodN. Stretches of pyrimidine bases seemed to be required in the oligodN sequence for effective binding, while the presence of contiguous purine bases decreased the affinity of binding to the two proteins. Because most of the experiments have been performed by cross-linking of oligodNs to plasma membrane proteins, and binding is only one of several prerequisites for cross-linking, we also performed binding competition experiments and protein renaturation studies, and the results correlated well with the photolabeling results. Moreover, by using gel-mobility shift analysis, it was found that dC_{21}, dT_{21}, and dN_{21} were able to bind efficiently to the highly purified 100-kDa protein, whereas dA_{21} showed much lower binding affinity (data not shown), which is in agreement with results shown in this paper. However, the reason for the selectivity shown by the 100-kDa protein on binding homopolypyrimidine is not clear. Because the structures of homopolymers like poly(dA), poly(dC), and poly(dT) may be very different in

solution, this may contribute to the different affinities of binding observed. Nevertheless, this issue needs to be investigated further.

The apparent dissociation constants of dC_{21} and dT_{21} for the 100–110-kDa proteins were found to be similar, while dT_{21} at 100-fold molar excess was not able to competitively displace dC_{21} binding. Because the kinetics of binding of those two homopolymers to highly purified 100-kDa protein (data not shown) were found to be the same as that of protein from crude membrane preparations, we suggest that the lack of competition of dT_{21} toward dC_{21} is not related to interaction of dT_{21} with other proteins present in crude extract. Instead, this result indicates that the 100–110-kDa protein could have at least two different binding sites, with one site preferring thymine bases and the other cytosine bases. Attempts to evaluate the binding affinity of homopolymers of dG to those two proteins were inconclusive, due to the formation of secondary structures of oligodG. Recently, oligodNs rich in guanosine have been shown by different investigators to have an antiviral activity that could be ascribed to their ability to form intra- and intermolecular superstructures [27–29].

It was shown that the nonsequence-specific effect of PO and PS oligodNs on target proteins is highly dependent on the length of the oligodN [30]. The longer the chain length of an oligodN, the more potent the interaction with the oligodN binding proteins. This correlation was observed in the case of oligodN binding proteins such as rsCD4 [31], rgp120 [32], RNase H1 [19], and p80 on the surface of HL-60 cells [5, 6]. Therefore, it is not surprising to observe that increasing chain length of the oligodN can have a more pronounced effect on inhibiting the binding of dC_{21} to the 100–110-kDa plasma membrane oligodN binding proteins.

The uptake of oligodNs represents a critical issue in antisense technology because in order to be useful agents, oligodNs must be able to enter cells and achieve an effective intracellular concentration. Recently, to improve their cellular uptake, oligodNs have been modified in several ways including modifications with a cholesteryl moiety [33], by coupling with cationic lipids [34], or by poly-L-lysine conjugation [35]. Unfortunately, even if effective in increasing oligodN internalization, these interesting strategies have shown some limitations due to cellular toxicity. The 100–110-kDa proteins were correlated with oligodN uptake [11], and, as we have demonstrated in this study, the binding affinity of oligodNs to those proteins is dependent predominantly on the base composition of the oligodN. Therefore, if those two proteins do play an important role in oligodN uptake, the design of antisense oligodNs should take into consideration not only the ability of oligodNs to efficiently hybridize to the target but also their capability to bind these two proteins and gain entry into the cells.

The 100- and the 110-kDa oligodN binding proteins were hypothesized previously to be the same protein with different post-translational modifications. Both proteins were detected by photolabeling oligodNs to plasma membrane proteins, whereas after protein renaturation only the 100-kDa protein could be detected. It is possible that post-translational modifications could interfere with appropriate protein renaturation after SDS–PAGE, and consequently the 110-kDa protein could lose the ability to bind oligodNs. However, it is also possible that the 110-kDa protein could result from different amounts of oligodN cross-linking to the same 100-kDa protein. Moreover, another possibility could be that the 110-kDa protein is a totally different protein that may be tightly associated with the 100-kDa protein on plasma membrane and, therefore, accidentally cross-linked to oligodNs. This is still under investigation.

It is unclear if the four oligodN binding proteins with apparent molecular masses ranging from 40 to 58 kDa are related to the 100-kDa protein. These four proteins are able to be renatured after SDS gel electrophoresis, and stretches of thymine bases are required for the binding to oligodNs. Their kinetics of binding were evaluated, and they were found to be similar to the 100-kDa proteins. Moreover, because we have demonstrated previously that serine proteases were able to degrade the 100–110-kDa proteins to a 50-kDa protein [11], it is possible that those 50-kDa proteins could be the proteolytic products of the 100-kDa protein. Additional studies are in progress to address this issue.

In conclusion, this study demonstrated that chain length and base composition are important determinants for the binding affinity of oligodNs to the 100–110-kDa proteins. Pyrimidine bases but not purine bases are required in the oligodN sequence to retain good binding. The two proteins seem to have different binding sites, one site preferring thymine bases and the other cytosine bases. Considering that these two proteins are the major oligodN binding proteins on the cellular plasma membrane and that they are correlated with oligodN uptake, their characteristics of binding need to be considered carefully by investigators in order to develop oligodNs that are going to be efficiently delivered to the intracellular target site.

The authors wish to thank Christopher L. Gaj for editorial assistance. This work was supported by the National Cancer Institute, Grant CA 44358. S.C. was the recipient of a fellowship from the Istituto Superiore di Sanità, Project AIDS.

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