Physical properties of oligonucleotides containing phosphoramidate-modified internucleoside linkages

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ABSTRACT

Because of their nuclease resistance and ability to form substrates for RNase H, antisense oligodeoxynucleotides (ODNs) possessing several methoxyethylphosphoramidate linkages at both termini have proven effective at targeting the degradation of specific mRNAs in Xenopus embryos. The efficacy of these compounds subsequently observed in tissue culture focused our attention on the issue of cellular uptake. To investigate the extent to which phosphate backbone modifications may increase the lipophilicity of ODNs, and thereby increase passive uptake by cells, the partitioning of a series of phosphoramidate-modified compounds between aqueous and organic phases was examined. The octanol:water partition coefficient of an unmodified, mixed-sequence 16-mer was 1.75×10^{-5}. The log of the partition coefficient increased in a sigmoidal manner with the number of methoxyethylphosphoramidate internucleoside linkages, indicating a nonlinear free energy relationship. The highest level of partitioning demonstrated was approximately 4×10^{-3} (a 230-fold increase), attained when 11 of the 15 phosphodiesters were modified. An increase in hydrophobicity was also attained with C_8 and C_{10} alkylamines acting as phase-transfer agents. The melting temperatures of heteroduplexes formed between a phosphoramidate-modified ODN and a complementary unmodified DNA strand decreased by approximately 1.5°C for every phosphate group modification. ODNs can thus be extensively derivatized without substantially compromising duplex formation under physiological conditions.

INTRODUCTION

Two important factors which determine the effectiveness of antisense oligodeoxynucleotides (ODNs) *in vivo* are susceptibility to degradation and intracellular uptake. The major route of degradation, both intracellularly and in biological fluids, is the rapid hydrolysis of phosphodiester bonds by exo- and endonucleases (1–4). Xenopus laevis embryos, for example, possess a very active 3′ exonuclease and a less active endonuclease which together reduce the half-life of a native ODN to about one minute (1). To counteract nucleolytic activities, it is advantageous to stabilize ODNs with phosphate linkages that are resistant to enzymatic cleavage. Replacement of one of the nonbridging oxygens of the phosphodiester bond with an alcohol, amine or an alkyl group to form a phosphotriester, phosphoramidate or alkylphosphonate linkage, respectively, generates a nonionic phosphate analog that is completely resistant to nucleolytic attack (1,5,6). When all of the internucleoside linkages in an ODN are modified in such a way, the resulting molecule is extremely stable. Unfortunately, the heteroduplexes formed between completely modified ODNs and their complementary RNAs are not substrates for ribonuclease H (RNase H) (7–9), and, consequently, the antisense activity of these derivatives is markedly reduced (7,9,10).

Recently we have described a new class of ODNs in which separate domains of the molecule were designed to serve specific purposes (1,9,11). Several 3′- and 5′-terminal internucleoside linkages were modified as phosphoramidates to prevent exonucleolytic and to retard endonucleolytic degradation of the ODNs. A stretch of six to eight consecutive unmodified diesters were included in the center of the molecule as an effector domain, to both interact with the targeted mRNA and form a substrate for RNase H. Because of their increased intracellular half-lives, ODNs of the general structure amidate-diester-amidate have been shown to be effective at directing the elimination of targeted mRNAs in Xenopus embryos (1,9).

Subsequently, we have shown that ODNs having this tripartite structure are also active in tissue culture. We have utilized such a derivative to inhibit the expression of a specific isoform of transforming growth factor β in embryonic chick heart explants to demonstrate its role in the induction of migration of cardiac endothelial cells (11). In this case, we also observed degradation of the targeted message, demonstrating the importance of RNase H. Unmodified ODNs were shown to be inactive, even at relatively high concentrations. Biological activity in this tissue explant system implies that the partially modified ODNs were able to penetrate the cell membrane. A number of other laboratories have also provided evidence for the intracellular uptake of ODNs (12, 13).

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The mechanisms by which ODNs are taken up into cells are not fully understood. Although a membrane protein has been identified that binds to oligo(dT) and has been suggested to act as an ODN transporter (14,15), the relative contribution of active versus passive transport has not yet been established. The ability of nonionic analogs, such as methylphosphonates, to enter cells suggests that passive diffusion of these compounds through cellular membranes occurs (6). Since phosphoramidate-modified ODNs possess a reduced negative charge density compared to their unmodified counterparts, their hydrophobicity, and hence rate of passive cellular uptake, should be enhanced. The partitioning of compounds between 1-octanol and a buffered aqueous phase has been used extensively to model the interactions of drugs and other organic molecules with cell membranes (16). Similar measurements, however, have not been reported previously for antisense ODNs.

In the present study, we show an unexpectedly high and easily measurable partition coefficient for ODNs in an aqueous:octanol system. A native mixed-sequence 16-mer has a partition coefficient of 1.75×10^{-5}. A large increase in lipophilicity was attained by partial neutralization of the negative charges on the ODN. The conversion of eleven phosphodiester linkages to methoxyethylphosphoramidates increased the partition coefficient 230-fold, to 4×10^{-3}. By altering the degree of modification, and the nature of the substituent, predictable levels of hydrophobicity can be obtained. High levels of phosphate modification can be accommodated without compromising hybridization under physiological conditions. Each methoxyethylphosphoramidate group introduced decreased the thermal stability of a heteroduplex formed with an unmodified DNA strand by only about 1.5°C.

EXPERIMENTAL PROCEDURES

Oligonucleotide synthesis

ODNs were synthesized on an ABI Model 381A DNA synthesizer using hydrogen phosphonate chemistry and purified by reversed-phase HPLC as described previously (1). The compounds were then resuspended in sterile H_2O and quantitated by measuring absorbance at 260 nm (1 O.D. unit equals 40 μg ODN/ml).

Determination of thermal stability

Thermal denaturation of ODN duplexes was followed by observing the hyperchromicity at 260 nm using a Gilford Model Response II spectrophotometer. 3.0 nmol of modified ODN and 3.2 nmol of unmodified complement were dissolved in 0.4 ml of 150 mM NaCl, 10 mM sodium phosphate buffer, pH 7.3. The solutions were heated to 80°C for 10 min to destroy any secondary structure and then slowly cooled to ambient temperature. Absorbances of ODN solutions were measured every 0.5°C from 25−75°C. Melting temperatures were obtained from the maximum value of the first derivative plots of absorbance vs. temperature. The Van't Hoff enthalpy was derived from the slope of a plot of ln K vs. 1/T.

Electrophoretic Analysis

To determine the effect of phosphate modification on gel mobility, approximately 0.5 nmol of ODNs A-F and H-K (Table 1) were loaded onto a 20% polyacrylamide-7 M urea gel and electrophoresed for 1.5 hr. The gel was incubated for 1 hr at room temperature in formamide:water (1:1) containing 0.005% Stains-all (Aldrich). The ODNs were visualized by destaining the gel in several changes of distilled water. The migration of each ODN was then determined relative to that of bromophenol blue. The migration of the 5′-phosphorylated forms of ODNs A-G was also determined. The ODNs were 5′-end-labeled and gel-purified as previously described (1). Equal amounts of the labeled compounds were loaded onto a denaturing 20% polyacrylamide gel and electrophoresed for approximately 1.5 hr. Following visualization by autoradiography, the migration of each ODN was determined relative to that of bromophenol blue.

Chromatographic Analysis

A mixture of ODNs A-F and H-K were analyzed by reversed-phase HPLC. A solution containing approximately equal amounts of each ODN was loaded onto a Hamilton PRP-1 column equilibrated with 5% acetonitrile, 10 mM ethylenediamine acetate, pH 8.0. A linear acetonitrile gradient of 0.45%/min was employed at a flow rate of 1.0 ml/min.

Partition Analysis

ODNs A-G were 5′-end-labeled and gel-purified (1). Approximately 4×10^6 cpm of each ODN were dried under vacuum in a 1.5-ml screw-top centrifuge tube. The ODN residue was resuspended in 200 μl 1.0 M potassium phosphate, pH 7.2 (previously equilibrated with 1-octanol). This solution was shaken vigorously and briefly centrifuged. To this mixture were added 200 μl 1-octanol (previously equilibrated with 1.0 M potassium phosphate, pH 7.2). The sample was shaken vigorously at 24.5 ± 1°C for at least 2 hr to ensure that ODN transfer between the two phases was at equilibrium, and subsequently centrifuged for a few seconds to separate the phases. 120 μl of the octanol layer were carefully removed from the tube and added to a liquid scintillation vial containing 15 μl of fresh potassium phosphate solution. Next, 15 μl of the aqueous layer was removed from the sample and added to a liquid scintillation vial containing 120 μl of fresh 1-octanol. In this way, any possible differences in quenching were eliminated. 5.0 ml of Budget-Solv (RPI) were added to the scintillation vials, and the radioactivity present in each phase was quantitated with a Beckman Model LS-1801 liquid scintillation counter. Several vials containing 15 μl potassium phosphate solution, 120 μl octanol and 5 ml scintillation cocktail were counted to serve as background controls. The determination of partition coefficients in the presence of exogenous amines was performed in the same manner as described above, with one exception. Prior to adding 200 μl of buffer-saturated 1-octanol, 2 μl of the desired amine were added to the 200 μl of the phosphate buffer containing the radiolabeled ODN. The partition coefficient is defined as the ratio of the amount of ODN present in the organic phase to the amount present in the aqueous phase.

RESULTS

Characterization of Modified ODNs

Several ODNs of identical sequence but differing in the number of methoxyethylphosphoramidate linkages were synthesized and are shown in Table 1. Figure 1A demonstrates the relative migration of ODNs A-F (open circles) and the 5′-phosphorylated forms of ODNs A-G (filled circles) on a 20% polyacrylamide-7 M urea gel. In both series of ODNs, there was a steady decrease in mobility as internucleoside phosphate groups were modified. As expected, the addition of a 5′-phosphate group increased the

Table 1. Phosphoramidate-modified ODNs

Designation	Sequence Modifications	# modified[a]
A	C T G A C A A C A T G A C T G C	0 (0)
B	C T G A C A A C A T G A C*T*G*C	3 (20)
C	C T G A C A A C A T G*A*C*T*G*C	5 (33)
D	C T G A C A A C A*T*G*A*C*T*G*C	7 (47)
E	C T G A C A A*C*A*T*G*A*C*T*G*C	9 (60)
F	C T G A C*A*A*C*A*T*G*A*C*T*G*C	11 (73)
G	C T G*A*C*A*A*C*A*T*G*A*C*T*G*C	13 (87)
H	C*T*G*A C A A C A T G A*C*T*G*C	7 (47)
I	C*T*G*A*C A A C A T G*A*C*T*G*C	9 (60)
J	C*T*G*A*C*A A C A T G*A*C*T*G*C	10 (67)
K	C*T*G*A*C*A A C A T*G*A*C*T*G*C	11 (73)

* represents a 2-methoxyethylphosphoramidate internucleoside linkage.
[a]Number of modified linkages (% modified).

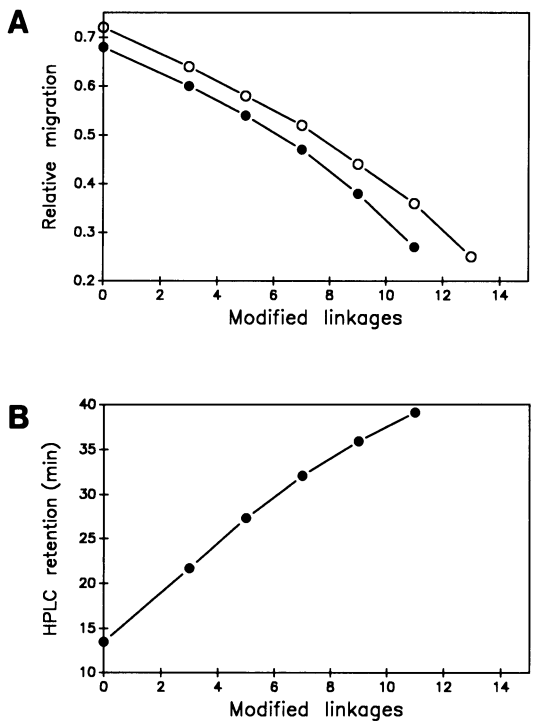

Figure 1. Effect of phosphate modification on the electrophoretic and chromatographic behavior of ODNs. (A) ODNs, either unlabeled (filled circles) or 5'-end-labeled (open circles) were electrophoresed for 90 min on a denaturing 20% polyacrylamide gel. Migration was determined relative to bromophenol blue. (B) ODNs were loaded onto a PRP-1 column equilibrated with 5% CH_3CN and eluted with a linear CH_3CN gradient of 0.45%/min.

rate of migration. This effect became more prominent as the compounds were more extensively modified and the 5' phosphate accounted for an increasing fraction of the total charge.

The elution time of ODNs A-F from a reversed-phase HPLC column is shown in Figure 1B. With increased modification the ODN became more hydrophobic resulting in longer retention time. As the ODN was more extensively derivatized the effect of each added substituent became somewhat attenuated. Table 2 shows that the position of modifications within a given ODN has little effect on either their electrophoretic or chromatographic behavior. ODNs H, I and K, having an amidate-diester-amidate

Table 2. Comparison of equally modified ODNs

% modified (structure)	ODN	Rm[a]	HPLC Retention (min)
47 (DA)[b]	D	0.47	32.1
(ADA)[c]	H	0.46	32.1
60 (DA)	E	0.38	35.9
(ADA)	I	0.37	35.8
73 (DA)	F	0.27	39.1
(ADA)	K	0.26	38.7

[a]Electrophoretic migration relative to bromophenol blue.
[b]5'phosphodiester-phosphoramidate.
[c]5'phosphoramidate-phosphodiester-phosphoramidate.

Table 3. Partition coefficients of modified ODNs

ODN	K_{oct}[a]
A	$1.75 \pm 0.58 \times 10^{-5}$
B	$3.93 \pm 1.33 \times 10^{-5}$
C	$5.48 \pm 0.88 \times 10^{-5}$
D	$1.74 \pm 0.62 \times 10^{-4}$
E	$1.07 \pm 0.26 \times 10^{-3}$
F	$4.01 \pm 1.60 \times 10^{-3}$
G	$4.46 \pm 1.36 \times 10^{-3}$

[a]Ratio of the amount of ODN present in the octanol phase to that present in the aqueous phase. The values represent data from at least 6 experiments.

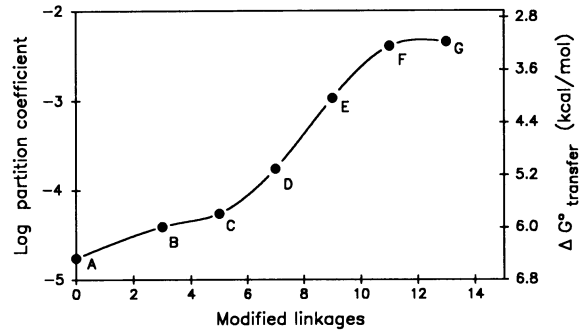

Figure 2. Effect of phosphoramidate modification on the partition coefficients of ODNs. 5'-end-labeled ODNs were shaken vigorously for several hr in the presence of equal volumes of 1-octanol and 1 M potassium phosphate, pH 7.2. The radioactivity present in each phase was determined and the partition coefficient calculated. The letters next to the data points refer to ODN designations made in Table 1.

structure, have properties virtually identical to ODNs D, E, and F, respectively, which are modified solely at the 3' terminus.

Effect of Modification on ODN Partitioning

HPLC analysis demonstrated qualitatively that ODNs become more hydrophobic with increasing phosphate modification. To obtain a more quantitative measurement, the partition coefficients of ODNs A-G between 1-octanol and a 1 M potassium phosphate buffer were determined. ODNs A-G were chosen for these studies because they could be labeled at their 5' termini with ^{32}P to a high specific activity. Even with this sensitvity, it might be expected that the partition coefficient of an ODN into an organic solvent would be too low to determine. This is most likely the reason that ODN partitioning has not been previously investigated. However, the octanol:water partition coefficient for

Table 4. Effect of exogenous primary amines on the partitioning of an unmodified ODN

Amine	Partition coefficient	Relative increase[a]
none	$1.75 \pm 0.58 \times 10^{-5}$	1.0
1% 2-methoxyethylamine	$1.60 \pm 1.20 \times 10^{-5}$	0.9
1% 1-butylamine	$3.70 \pm 2.20 \times 10^{-5}$	2.1
1% ethylenediamine	$2.78 \pm 1.10 \times 10^{-5}$	1.6
1% 1-decylamine	$1.02 \pm 0.03 \times 10^{-3}$	58
1% 1-octylamine	$1.17 \pm 0.34 \times 10^{-3}$	67
0.1% 1-octyamine	$2.40 \pm 0.30 \times 10^{-4}$	14
0.01% 1-octylamine	$1.55 \pm 0.61 \times 10^{-5}$	0.9

[a]Fold increase in partitioning relative to no added amine.

even an unmodified ODN was easily measurable. For ODN A, an unmodified 16-mer, the value was 1.75×10^{-5} (Table 3). ATP in the same system had a partition coefficient of 5.9×10^{-6}. When the log of the partition coefficient is plotted against the number of modified residues present in each ODN, the result is a sigmoidal curve (Fig. 2), i.e., a non linear free energy relationship is obtained. The steepest rise in the partition coefficient is observed as the ODN proceeds from being 33% to 67% modified. The partition coefficient then reaches a plateau at about 4×10^{-3}, when eleven of the fifteen phosphodiesters are substituted as phosphoramidates.

Increased Hydrophobicity with Exogenous Amines

Removal of the negative charge of an ODN through the conversion of phosphodiesters to phosphoramidates is an effective way to increase partitioning of the compound into an organic environment. To investigate whether the same degree of hydrophobicity could be attained without covalent modification, we determined the partition coefficient of ODN A in the presence of exogenous aliphatic amines. The use of alkylamines to increase the organic partitioning of ODNs is reminiscent of early work by Plaut et al. investigating strategies to separate various phosphoric acid esters (17). The results of the present study are shown in Table 4. Short-chain aliphatic amines, such as methoxyethylamine, were ineffective at substantially altering the partition coefficient of the unmodified ODN. This result suggests that the increased hydrophobicity of the methoxyethylphosphoramidate-modified ODNs is due to charge neutralization alone. Longer-chain alkylamines, on the other hand, were able to greatly increase the extent of ODN transfer from an aqueous environment into octanol. The addition of 1% 1-octylamine or 1-decylamine increased the equilibrium constant of ODN partitioning into octanol to 1.17×10^{-3} and 1.02×10^{-3}, respectively, almost two orders of magnitude. Reducing the concentration of octylamine from 1% to 0.1% decreased the partition coefficient fourfold, to 2.40×10^{-4}. At 0.01%, octylamine was ineffective at altering the partition coefficient of ODN A.

Thermal Stability

To examine the effect of individual phosphate modifications on the hybridization properties of an ODN, we determined the melting temperatures of heteroduplexes formed between the ODNs in Table 1 and an unmodified complementary 16-mer. The thermal denaturation profiles of the 3′-modified ODNs are shown in Figure 3A. Figure 3B shows the profiles of ODNs possessing both 3′ and 5′ modifications. All of the melting curves appear monophasic and the transitions are sharp. Plots of the first

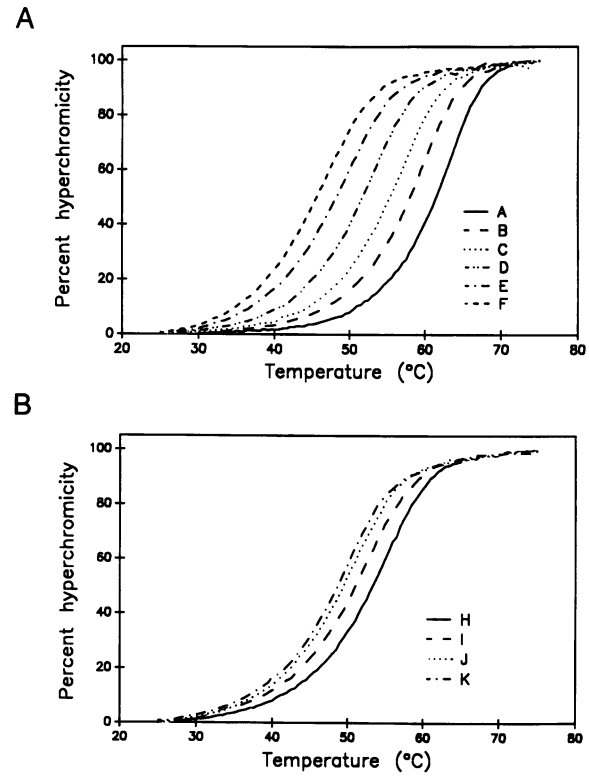

Figure 3. Melting curves of phosphoramidate-modified ODNs with an unmodified complementary 16-mer. Absorbance values at 260 nm were normalized and converted to percent hyperchromicity. Measurements were carried out in 150 mM NaCl, 10 mM sodium phosphate, pH 7.3. Curves were generated for both 3′-modified ODNs (A) and those derivatized at both termini (B).

Table 5. Melting temperatures of phosphoramidate-modified ODNs

ODN	Tm[a] (°C)	Enthalpy[b] (kcal/mol)
A	63.3	−88
B	59.6	−81
C	57.0	−78
D	52.7	−74
E	50.0	−68
F	46.2	−68
H	55.0	−68
I	52.2	−67
J	50.7	−68
K	49.3	−70

[a]Average of at least 3 experiments. Standard deviations are less than ± 0.3°C.
[b]Van't Hoff enthalpy for duplex formation.

derivative of absorbance with respect to temperature for each ODN produced a single peak from which the melting temperature was obtained. The T_m values are compiled in Table 5. A plot of melting temperature versus number of unmodified linkages for ODNs A-F and H-K produced two straight lines ($r = 0.996$ and 0.999, respectively) shown in Figure 4. The slope of the lower line indicates that, in the case of ODNs derivatized at the 3′ terminus, each modification decreased the melting temperature of the duplex with an unmodified ODN by 1.6°C. Similarly, the thermal stability of duplexes containing ODNs that are derivatized on both ends was reduced by about 1.4°C for each modification.

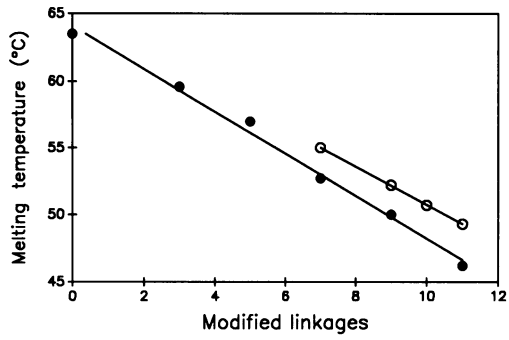

Figure 4. Effect of ODN modification on duplex stability. The melting temperatures from Table 3 were plotted against the number of modified linkages in each ODN. Closed circles represent ODNs A-F, while open circles represent ODNs H-K.

The thermal stability data was used to generate a Van't Hoff plot, from which the enthalpy of duplex formation was determined. These values are shown in Table 5.

DISCUSSION

The degradation and cellular uptake of ODNs are major determinants of the efficacy of antisense molecules. We have previously examined the degradation of ODNs possessing the general structure amidate-diester-amidate by various nucleolytic activities present in Xenopus embryos (1,9). ODN stability was increased 10 to 20-fold by phosphoramidate modification of several terminal internucleoside linkages, and, as a result, the concentration of ODN required for effective elimination of targeted mRNAs was decreased to subtoxic levels. Consequently, altered developmental phenotypes which resulted from the depletion of specific messages could be observed and evaluated. In the present study, we have determined the cumulative effect of individual phosphate modifications on the hydrophobicity, as well as the hybridization properties, of phosphoramidate-modified ODNs.

The introduction of methoxyethylphosphoramidate groups into an ODN was found to decrease progressively the thermal stability of heteroduplexes formed with a complementary unmodified DNA strand. In the case of ODNs modified only at the 3′ terminus, each modified internucleoside linkage reduced the melting temperature by 1.6°C. When both termini of the ODN were modified, leaving a central unmodified region, the thermal stability decreased 1.4°C for each phosphate modification. The T_m of the duplex with ODN F, where 11 of 15 linkages were modified, was reduced about 17°C. This value is similar to that observed previously, following modification of 12 of 14 linkages in a mixed-sequence 15-mer with methoxyethylphosphoramidates (18). The modest reductions in thermal stabilities observed following phosphate modification indicate that ODNs may be extensively derivatized without significantly impairing hybridization under physiological conditions. The higher T_m values observed for ODNs modified at both ends compared to those derivatized to the same extent but modified only at the 3′ terminus probably reflects the lower contribution of flanking residues to duplex stability. The sharp transitions of the melting curves in Figure 1 suggest that, following modification, there is little heterogeneity in the thermal stability of the resulting ODN diastereomers. Alternatively, significant destabilizing effects may only be observed for minor subpopulations of diastereomers (e.g., all R or all S).

The ability of an ODN to act as an effective antisense agent will depend upon its intracellular concentration and, therefore, upon its cellular uptake. In theory, ODNs can either passively diffuse through the cell membrane or enter cells via protein-mediated transport. The rate of passive diffusion across a lipid bilayer depends upon two factors. The first is the hydrophobicity of the compound, which can be quantitatively determined by measuring the partition coefficient. The second determinant is the ability of the compound to pass through the membrane and is expressed as the diffusion coefficient. The parameters contributing to the diffusion coefficient, with the exception of the volume of the diffusant, are related only to membrane properties (19). Since the volume of an ODN should not be significantly altered by phosphate modification, the membrane permeability of an ODN should depend primarily upon its partition coefficient. Assuming that the hydrophobicity of 1-octanol adequately mimics that of a biological membrane, the transfer of an ODN from an aqueous environment into octanol can be considered a valid system with which to examine factors affecting passive cellular uptake. Despite the value of this information and the ease with which it can be obtained, there have been no prior reports of the determination of partition coefficients of oligonucleotides more than a few residues in length into organic solvents (20).

The native 16-mer, ODN A, had a partition coefficient of 1.75×10^{-5}, two to threefold higher than that of ATP. This may initially seem surprising since ODN A contains many more negative charges than ATP. If, however, one considers the charge-to-base ratio, the situation becomes clearer. Because of its increased number of charges per residue, ATP partitoning into octanol is less favorable than the partitioning of an ODN. Interestingly, a sigmoidal curve resulted from plotting the log of the partition coefficient of ODNs A-G versus the number of modified linkages, indicating a nonlinear free energy relationship (Figure 2). The conversion of one-third of the 3′-terminal phosphodiester linkages to methoxyethylamidates increased the partition coefficient to 5×10^{-5}, only about threefold. The next few groups added were critical. In this transition zone, in which the percent of modification increases from 33 to 73%, the hydrophobic properties of the ODN were dramatically increased. At 73% modification (ODN F), the maximal degree of octanol partitioning ($\approx$0.4%) was observed. We have not yet determined the origin of the cooperativity in this system, but it may relate to interactions involving the hydration spheres around each phosphate group present in an ODN.

By altering the counterion associated with an unmodified ODN, a strategy comparable to the use of phase-transfer catalysts to facilitate certain chemical reactions, a relatively high level of ODN transfer into octanol was attained. The extent of transfer was highly dependent upon the hydrophobicity of the amine. The addition of 1% 1-butylamine increased the partition coefficient by only a fewfold, while the same concentration of 1-octylamine and 1-decylamine enhanced partitioning by almost two orders of magnitude. The phase-transfer ability of 1-octylamine was concentration-dependent and dilution of the amine to a final concentration of 0.01% completely abolished its activity. Interestingly, 2-methoxyethylamine, which was utilized to generate ODNs B-K had no significant effect on ODN partitioning when used as a counterion.

The conversion of an individual phosphodiester bond to a phosphoramidate, phosphotriester, or alkylphosphonate can potentially increase the partition coefficient of an ODN in two ways: neutralization of the negative charge of the phosphate backbone and addition of a lipophilic moiety. The lack of an effect of 2-methoxyethylamine on the partitioning of DNA suggests that the primary consequence of phosphoramidate modification with this amine is neutralization of charge. This is not surprising considering the structure of the amine, a short alkyl group possessing a terminal ether moiety capable of hydrogen bonding with water. Decylphosphoramidate linkages, in contrast, not only possess uncharged phosphate groups, but would also carry a long alkyl chain. ODNs synthesized with this substituent would be predicted to possess an extraordinarily high degree of hydrophobicity.

By altering both the number of modifications and the hydrophobicity of each substituent, the lipophilicity of an ODN can be adjusted over a broad range. The extent of hydrophobicity can be readily quantitated by measuring the octanol:water partition coefficient. Although phosphate modifications destabilize the duplexes in which they occur, the data presented here indicate that the level of perturbation should not compromise the hybridization of even extensively modified ODNs under physiological conditions. Quantitative comparison between the degree of lipophilicity and the intracellular transport of modified ODNs should provide further insights into the role of passive uptake.

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