Nucleoside, nucleotide and oligonucleotide based amphiphiles: a successful marriage of nucleic acids with lipids

Arnaud Gissot,[a,b] Michel Camplo,[c] Mark W. Grinstaff[d] and Philippe Barthélémy*[a,b]

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Amphiphilic molecules based on nucleosides, nucleotides and oligonucleotides are finding more and more biotechnological applications. This Perspective highlights their synthesis, supramolecular organization as well as their applications in the field of biotechnology.

Introduction

Life requires the recognition and function of simple and complex molecules operating together. Among the numerous biomacromolecules and biomolecules, nucleic acids and lipids are of primary importance. While the former store and propagate genetic information, the latter, as the structural components of cell membranes, act as boundaries and allow for compartmentalization. The central dogma of molecular biology (one gene → one RNA → one protein) has viewed DNA as the carrier of genetic information and RNA (messenger, transfer or ribosomal) as the simple mediator between DNA and proteins. This theory is nowadays viewed as over simplistic. RNA is capable of i) storing genetic information (RNA viruses[1]), ii) catalyzing organic reactions (ribozymes) and iii) regulating gene expression (micro-, si-, s-RNAs...)[2] In other words, the three fundamental pre-requisites of life; i) information storage, ii) replication and iii) regulation, can be encoded solely in RNA molecules thus paving the way to the concept of a primary RNA world.[3] Beside their numerous biological functions, nucleic acids are possibly the most promising biomacromolecules for applications in biotechnology,[4–6] medicinal chemistry[7] and material sciences.[8–10]

One central feature of all autotrophic living systems is the use of a lipid boundary to separate the inside from the outside of the cell as well as the individual cellular compartments.[11] Besides their structural and compartmentalization function as a bilayer component, lipids are also known to play an important role in the regulation and control of cellular functions and diseases. Lipids are indispensable participants in many events of signal transduction.[12] Our understanding of biological membranes has evolved in the last decade and the current model of the membrane bilayer exhibits small heterogeneous nano-domains, called rafts, which differ in their composition from the surrounding membrane.[13] These membrane regions are highly dynamic sterol- and sphingolipid-enriched domains that compartmentalize cellular processes.[14]

Hybrid molecules and macromolecules combining nucleic acids and lipids have therefore attracted significant attention,[15] as for example in the design of artificial molecular devices,[10,16–19] and novel therapeutic strategies[20] The aim of this Perspective is to highlight recent advances in the area of amphiphilic structures derived from nucleosides, nucleotides and oligonucleotides with an emphasis on composition, structure, properties, and biotechnological applications. In the first part, we focus on nucleoside-based amphiphiles. This section includes several examples of natural structures and synthetic nucleoside based amphiphiles. In the next section, we present the use of nucleolipids as synthetic vectors for the transport of nucleic acids into cells. Finally, in the last section of this Perspective, we describe oligonucleotide-based amphiphiles (ONA).

[a]*Université Victor Segalen, Bordeaux, F-33076, France. E-mail: barthelemy@bordeaux.inserm.fr; Fax: +33 5 5757 1015; Tel: +33 5 5757 1014*
[b]*INSERM U869, Bordeaux, F-33076, France*
[c]*CINaM, UPR CNRS 3118, Faculté des Sciences de Luminy, case 913, 13288, Marseille, cedex 09, France*
[d]*Departments of Chemistry, Biomedical Engineering, Metcalf Center for Science and Engineering, Boston University, Boston, MA, USA*

Philippe Barthélémy (left), Michel Camplo (central) and Mark W. Grinstaff (right)

The authors' research interests are in molecular recognition and the use of bio-inspired amphiphiles to create novel supramolecular assemblies. These new nucleoside-based amphiphiles have applications ranging from bio-materials to drug delivery. Philippe Barthélémy (left) is Professor at the University of Victor Segalen Bordeaux 2 (INSERM U869) in Bordeaux, France. Michel Camplo (central) is an Associate Professor in Organic Chemistry at the University of Aix-Marseille II, France. Mark W. Grinstaff (right) is an Associate Professor of Biomedical Engineering and Chemistry at Boston University. Arnaud Gissot is an Associate Professor at the University of Victor Segalen Bordeaux 2 (arnaud.gissot@bordeaux.inserm.fr). The authors can be reached at barthelemy@bordeaux.inserm.fr, camplo@univmed.fr and mgrin@bu.edu, respectively.

1. Natural and synthetic nucleoside-based amphiphiles

Darwinian evolution has yielded a diversity of macromolecules and supramolecular assemblies from nucleic acids[21] and amphiphiles.[22,23] In recent years, nucleic acid features have been used in the development of numerous artificial structures, including polynucleotides[24–26] and nucleolipid analogues.[27–29] In these biomimetic approaches, two fundamental objectives have been pursued in combining nucleic acid and lipid families; i) the development of new therapeutic strategies and/or ii) the construction of new supramolecular assemblies.

1.1 Natural nucleolipids

Nucleolipids are hybrid molecules composed of a lipid covalently linked to a nucleobase, or a nucleoside, or a nucleotide or an oligonucleotide. Hybrid lipid–nucleoside structures occur in eukaryotic and prokaryotic cells. Tunicamicyns for instance possess antimicrobial, antifungal, antiviral and antitumor activities.[30,31]

Cytidinediphosphate diacylglycerol coenzyme (CDP-DAG, Fig. 1), a diphosphorylated nucleolipid (or liponucleotide), is present in mammalian cells and plays a central role in the metabolism of phosphoinosides and cardiolipins.[32] CDP-DAG and its analogue 2′-deoxy-CDP-DAG are also present in prokaryotic cells as key intermediates in the synthesis of all glycerophospholipids. In addition to their biochemical function, nucleolipids are unique in terms of molecular structure (lipid, phosphorus, sugar, heterocyclic moieties) and biophysical properties. Consequently, nucleolipid analogs have been investigated as anticancer drugs and pro-drugs (see next section). Other examples and biological activities of natural nucleolipids can be found in a comprehensive review published by Rosemeyer.[15]

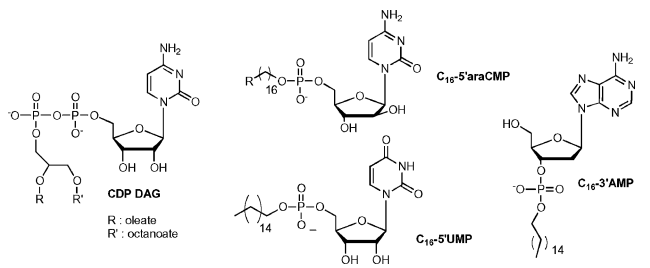

Fig. 1 Chemical structures of CDP-DAG and synthetic amphipatic nucleotides. C_{16}-5′UMP (see reference 62) and C_{16}-3′AMP are examples of nucleoside-monophosphate amphiphiles. C_{16}-5′araCMP is a lipidic derivative of ara-C.

1.2 Artificial nucleolipids

Nucleotides are natural substrates of transcriptase or reverse transcriptase. Accordingly, many analogs have been designed to block the enzymatic elongation of RNA chains and are utilized as anti-tumor or antiviral drugs. Given their inherent toxicity, many prodrugs of these chemotherapeutic agents have been evaluated over the years. For instance, amphiphilic nucleoside molecules derived from arabinofuranosylcytosine (ara-C)[33–36] (Fig. 1) and arabinofuranosyladenosine (ara-A)[37,38] were synthesized to enhance the anti-tumor activity of the drugs. The phosphoric acid ester function is slowly hydrolyzed in the cytoplasm thus liberating the active molecule, the aliphatic chain was also expected to enhance cellular uptake. In addition, analogs of cytidine diphosphate diacylglycerol (CDP diacylglycerol) prolonged the life spans of L5178Y and P388 tumor-bearing mice by 93% and 357% respectively when administered intraperitoneally at identical single doses of 50 mg kg^{-1} per day.[36,39]

In the mid 80's, several research groups combined the pharmacologically active nucleolipids with the aggregation properties of vesicle-forming lipids. The resulting drug carrier systems were capable of incorporating large amounts of amphipatic drugs. By loading the nucleolipid pro-drug into liposomes, the following properties were expected. The liposome would maintain and ensure a minimal leakage of the lipidic prodrug from the delivery system for an efficient transport, and protect the drug from enzymatic degradation. Hence, the *in vivo* anti-tumor activities of the liposome preparations of amphiphilic ara-C and 5-fluorouracil (5-FU) against L1210 lymphoid leukemia were clearly enhanced (2–8 times).[40,41] Yet, therapeutic effects of acyclovir derivatives on the replication of Herpes simplex virus using this strategy were only modest.[42,43]

1.3 Supramolecular assemblies of nucleolipids

In contrast to classical detergents, nucleolipids possess a highly informative polar head (A, T, C, G, U or analogs) with additional H-bonding and π-stacking capabilities that can specifically base-pair with other nucleobases. The interplay between these specific base pairing interactions,[24–26] and the unique aggregation properties of lipids is very attractive for the design of supramolecular assemblies with new and interesting properties. These concepts of molecular recognition with nucleolipids were initially applied to a supramolecular film at the air–water interface.[27,44,45] More recently, base-pairing between complementary nucleolipids at mesoscopic interfaces such as those found in vesicular and micellar systems have been investigated.[28,29,46–51] The data collected from several techniques (Langmuir–Blodgett, UV-vis, FTIR, ATR, *etc.*), provided direct evidence for the formation of multiple hydrogen bonds between the base pairs. For example, it was recently reported that a nucleotide bolaamphiphile (a nucleolipid with two thymine headgroups connected *via* an aliphatic spacer) self-assembled with polyA of various lengths to form nanofibers.[52] Additionally, nucleolipid–nucleolipid interactions have been studied in different types of supramolecular systems including micelles,[53] vesicles,[54] and monolayers.[55,56] For these purposes a variety of synthetic strategies have been used to prepare nucleolipids,[33,57,58] including coupling reactions of monoalkyl phosphate with a nucleoside and enzymatic catalysis.[59–62] Nucleolipids with fatty esters on the 5′ and 4′ positions were also prepared and their molecular recognition capabilities were studied.[55,62] Since 2002, our groups have been designing novel amphiphilic structures derived from nucleobases. We have investigated how changes in the molecular structure of the nucleolipid (hydrophobization of the 2′ and/or 3′ position in contrast to the classical 5′-modification) affect their physiochemical and self-assembling properties. Thus we have synthesized 1) nonionic (compounds $\mathbf{I}_{1-3}$),[63,64] 2) zwitterionic (phosphocholine derivatives, $\mathbf{I}_{4-6}$),[65,66] 3) anionic[67] (single chain nucleotides $\mathbf{I}_7$) and 4) cationic compounds ($\mathbf{I}_8$ and $\mathbf{I}_9$).[68,69] Examples of nucleolipid structures synthesized are shown in Fig. 2.

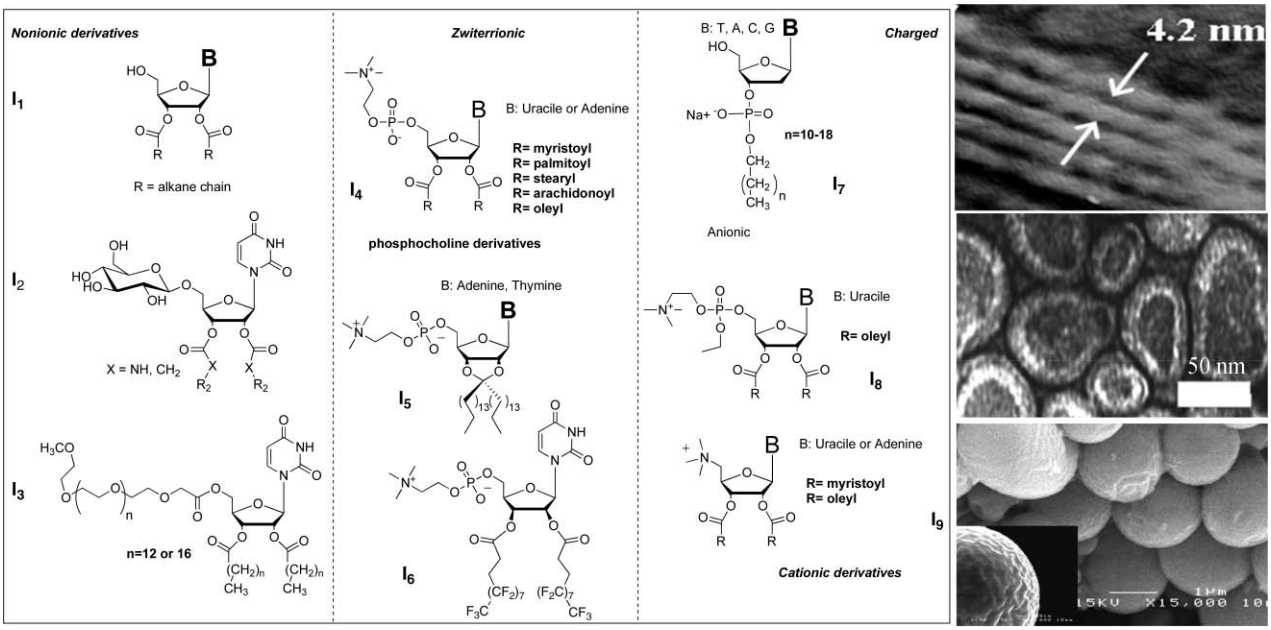

Fig. 2 Left, Examples of synthetic nucleoamphiphiles (**I**). Right, TEM and SEM images showing examples of nucleolipid self-assemblies: nano-fibers, vesicles, and microspheres.

We discovered that these molecules possess unique properties such as the formation of aggregates including fibers, vesicles and micro-spheres. Additionally, these nucleolipids form hydrogels and organogels. The supramolecular systems obtained are promising in many aspects and could lead to new types of materials for transport of biomacromolecules such as DNA and RNA. Non-toxic transfection reagents derived from nucleolipids with high transfection efficiencies have been developed.[70,71]

Interestingly, beside cationic nucleolipids, we recently reported alternative approaches to form stable nucleic acid supramolecular assemblies. For this purpose, neutral amphiphiles derived from uridine featuring two hydrophobic chains and either a glucose (compound $\mathbf{I}_2$, Fig. 2)[63] or polyethylene glycol (compound $\mathbf{I}_3$, Fig. 2)[64] hydrophilic moieties were prepared using simple synthetic routes. The data collected from physico-chemical studies demonstrated that amphiphile–nucleic acid complex formation was a consequence of the amphiphilic character of the molecule, phosphate–sugar, and nucleobase–nucleobase interactions in the case of glycosyl amphiphiles. Likewise, AFM studies show that the three distinct structural components of the poly(ethylene glycol) amphiphile (*i.e.*, nucleobase, alkyl chains, and poly(ethylene glycol), compound $\mathbf{I}_3$, Fig. 2) are required for the formation of DNA–amphiphile supramolecular assemblies on a mica surface.

2. Nucleolipids for transfection

The delivery of nucleic acids to cells and the resulting ability to correct a defective gene, introduce a new gene, or knock-down a gene provides a method to study biological processes, to manufacture proteins, and to treat many diseases. The delivery of nucleic acids to cells requires a synthetic vector to complex the DNA, formation of a supramolecular DNA:vector assembly, and uptake of this assembly in the cell. Cationic-based synthetic nonviral systems are routinely used *in vitro* as convenient biological tools for transporting nucleic acids to cells. Several cationic lipids have been synthesized and evaluated for plasmid DNA delivery[72] since the discovery of *N*-[1-(2,3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride (DOTMA) as a cationic transfecting agent.[73,74] Numerous structural features have been altered on the basic cationic lipid structure, leading to compositions such as DOTAP (*N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride) which are now commercially available and under clinical evaluation (Fig. 3).[75]

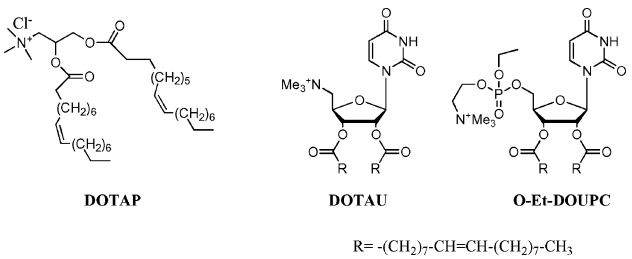

Fig. 3 Structures of different nucleolipids used for transfection.

All the synthetic vectors currently in use rely either on electrostatic forces or electrostatic and hydrophobic interactions for DNA binding and gene delivery. Our groups have pioneered the use of nucleolipids with additional hydrogen bonding and π stacking capabilities to further modulate the interactions between DNA and the synthetic vector.[70,71] Accordingly, several promising nucleic acid vectors based on a cationic amphiphilic uridine have been developed like DOTAU and O-Et-DOUPC (Fig. 3).

Their synthesis is straightforward starting from the protected uridine acetonide, and the *in vitro* transfection assays are very encouraging. For example, O-Et-DOUPC at high nucleolipid : DNA (w : w) ratios (18 : 1–36 : 1) is an efficient transfection agent in CHO cells using a reporter β-gal gene assay. These results

compare favorably with DOTAP and Transfast both used under optimal conditions (Fig. 4).

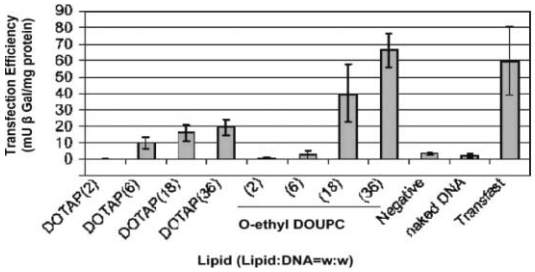

Fig. 4 Transfection efficacies of O-Et-DOUPC compared to 2 conventional transfection reagents (DOTAP and Transfast).

Last but not least and in sharp contrast to conventional transfecting agents, the cytotoxicity of DOTAU was shown to be virtually null (Fig. 5).[70,71] DOTAU can therefore be used in a wider range of concentration compared to Lipofectamine.

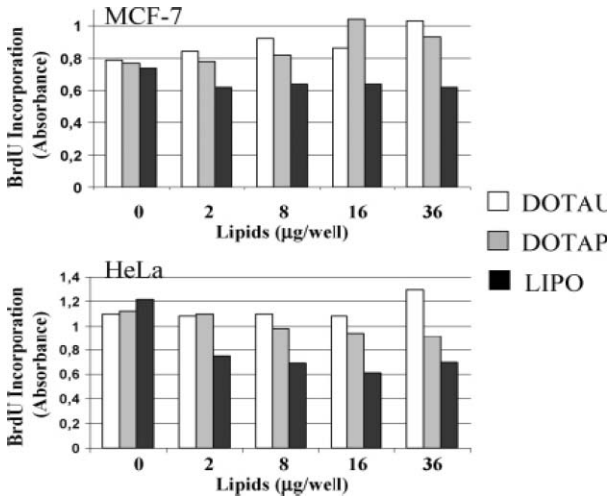

Fig. 5 Effects of DOTAU, DOTAP and Lipofectamine (LIPO) on human breast adenocarcinoma cell line (MCF-7) (top) and HeLa cells (bottom) proliferation. The cells were incubated with various quantities of lipids and 5-bromo-2-deoxyuridine (BrdU) to quantify cell proliferation.

The supramolecular assemblies made of calf thymus DNA (CT-DNA) and DOTAU or DOTAP have been investigated. Data collected from several SAXS experiments at room temperature show that CT-DNA–lipid complexes arrange into L_{α}^{C} lamellar phases. Interestingly, the DOTAU–polyA lipoplexes exhibit more compact systems than DOTAP–polyA lipoplexes due to tighter association between the uracil base of DOTAU and adenine of the polyA single strand. The infrared spectrum of the DOTAU–polyA lipoplex in KBr shows a carbonyl stretch at 1712 cm^{-1} for the C=O uracil as well as C=N and C=C ring vibrations reminiscent of a U:A base pair in RNA.[76] If most of the nucleic acid–amphiphile assemblies rely on electrostatic compensation between the polyanionic nucleic acids and cationic amphiphiles, DNA supramolecular assemblies were also observed in the presence of neutral nucleoside based amphiphiles. In particular, the nucleic acid binding capabilities of the glycosyl nucleoside derivatives (compound $\mathbf{I_2}$, Fig. 2) were investigated by a variety of techniques including UV-vis, quasi-elastic light scattering (QELS), transmission electronic microscopy (TEM), gel electrophoresis, ^{31}P NMR, IR, and circular dichroism (CD).[63] The results obtained strongly support amphiphile–nucleic acid complex formation thanks to the amphiphilic character of the molecule and phosphate–sugar interactions. Importantly, the FTIR data and the lower ellipticity values measured by CD indicate that the uridine moiety of the amphiphile is also involved in the stabilization of the amphiphile–polyA-polyU complex. Finally, Berti *et al.* recently reported a promising strategy for the base-specific recognition between micelles composed of dioctanoylphosphatidyluridine (a negatively charged amphiphile) and short oligoadenylic acids.[77] Very interestingly, the recognition takes place in the absence of divalent cations and the selectivity of polyU for this amphiphile resembles that observed between complementary bases in duplex DNA. Accordingly, no interaction is observed between polyU and dioctanoylphosphatidyluridine. Cationic polymers in use for the delivery of nucleic acids into cells are usually associated with aspecific coulombic interactions and cytotoxicity. Therefore, the system developed by Berti *et al.* clearly indicates the trends that should be pursued for the development of future innocuous and specific synthetic transfecting agents.

3. Oligonucleotide-based amphiphiles (ONA)

The development of oligonucleotide-based amphiphiles (ONA) dates back to the late 80's, early 90's and is closely associated with the development of the antisense strategy for gene therapy.[78] In fact and as seen in the previous section, cellular membranes usually constitute a physical barrier for the internalization of polyanionic, hydrophilic antisense oligonucleotide therapeutics. The basic idea underlying the synthesis of ONA was therefore obvious: the appended hydrophobic segment of the ONA was hypothesized to provide an anchor for the antisense oligonucleotide into the membrane thus facilitating internalization of the ONA.[79,80] This idea was correct and many ONAs were shown to be efficiently taken-up by different cell lines.[81–83]

The synthesis of ONAs is anything but trivial requiring extensive expertise in both organic chemistry and solid phase synthesis (SPS) of oligonucleotides. Following the successes of ONA-based antisense strategy, many hydrophobic phosphoramidite building blocks are now commercially available.

As shown in Fig. 6, these phosphoramidites allow for the incorporation of simple hydrophobic modification(s) anywhere in the sequence of the oligonucleotide (3′- and/or 5′-termini and/or between two consecutive nucleotides) and simple ONA structures are nowadays readily custom-made by DNA synthesis companies.

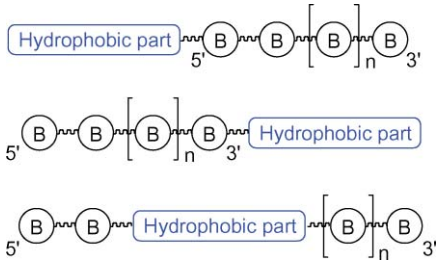

Fig. 6 Examples of ONA structures. Hydrophobic parts (blue) can be incorporated either at the termini (3′- and/or 5′) or between two consecutive nucleotides (B represents a nucleotide).

Yet, the hydrophobic modification of the oligonucleotide at the level of the nucleotide itself still requires extensive know-how in organic synthesis. These modifications have been accomplished at the sugar,[84,85] at the inter-nucleoside phosphate group,[86] or at the heterocyclic base.[87] It is beyond the scope of this article to review all these syntheses in detail,[15] the more prominent results only will be highlighted in the remainder of this paragraph.

Basically, the hydrophobic modification can be incorporated either during the synthesis of the oligonucleotide on the solid support or after the synthesis of the oligonucleotide part has been accomplished. The synthesis and properties of DNA block copolymers have been reviewed recently and the reader is referred to this article.[88]

3.1 Hydrophobic modification during the oligonucleotide solid-phase synthesis

The strategy of modifying the oligonucleotide during solid-phase synthesis (SPS) is by far the most widely used approach to add hydrophobic moieties to oligonucleotides. Due to the elongation of the oligonucleotide chain in the 3′-5′ direction during oligonucleotide SPS, the modification is more conveniently incorporated at the 5′-end compared to the 3′-end which requires substantial additional efforts in chemical synthesis.[85,89] An elegant strategy to functionalize the 3′-terminal internucleoside phosphorus was for instance developed by Letsinger from the corresponding H-phosphonate manually attached beforehand to the solid support (Fig. 7).[86]

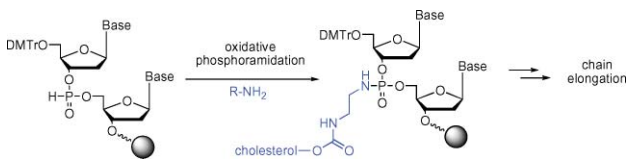

Fig. 7 Example of a strategy developed by Letsinger to functionalize the 3′-terminal internucleoside.

The wealth of chemical functions compatible with oligonucleotide SPS is still restricted. For instance, esters do not usually withstand the final treatment with ammonia required for the deprotection of the nucleobases and cleavage from the control pore glass (CPG) solid support. As a result, only polydT—which does not require base protection—was fully synthesized on CPG with an ester linkage.[90,91] The ester function is indeed very attractive for tethering a hydrophobic residue to oligonucleotides. The resulting ONA behaves as a prodrug in that case and is slowly hydrolyzed by esterases present in the cytoplasm thus liberating the active antisense oligonucleotide after internalization. This in turn eliminates the risk for the antisense ONA to remain adsorbed into the membrane away from its targeted mRNA. In that context, Guzaev developed the phosphoramidite (shown in Fig. 8) whose aliphatic ester moieties are only slowly cleaved off by ammonia under the conditions routinely used for the deprotection of oligonucleotides thus allowing the classical synthesis of ONA prodrugs on solid support.[92]

Given the importance of prodrugs in the realm of pharmaceutical sciences, an alternative strategy for the deprotection and cleavage from the solid support has been devised that maintains the integrity of the ester bond.[91]

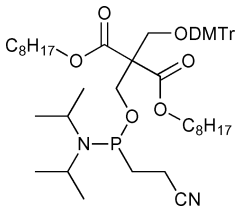

Fig. 8 A phosphoramidite building block suitable for the incorporation of ester moieties in ONAs.

3.2 Post-synthetic hydrophobic modification of oligonucleotides

Modification of oligonucleotides with hydrophobic moieties after they have been cleaved from the solid support and fully deprotected is quite challenging. Kabanov *et al.* described an interesting protocol for the derivatization of 5′-phosphate oligonucleotides in reverse micelles (Fig. 9).[93]

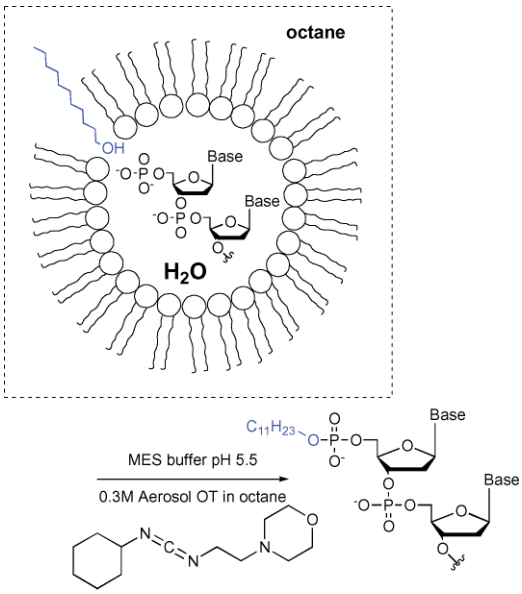

Fig. 9 A reverse-micelle strategy for the hydrophobization of oligonucleotides.

The efficient co-localization of the lipophilic alcohol with the hydrophilic nucleic acid and the carbodiimide at the lipid–water interphase is brought about by the micellar system and ensures the efficacy of the reaction. Very interestingly, this strategy can theoretically be applied to any natural oligonucleotides provided a phosphate group is present in the nucleic acid structure.

Abell and coworkers developed a more classical approach for the derivatization of oligonucleotides with activated acid or aliphatic thiols in an aqueous buffered medium (Fig. 10).[84,94]

The two derivatization chemistries are orthogonal in the sense that the underivatized oligonucleotide can form amides and disulfides linkages in a regio-controlled manner when both modifications are present simultaneously in the same strand.

3.3 ONAs as antisense

Antisense nucleic acid molecules have been used experimentally to bind to mRNA and block the expression of specific genes at the translational level. The development of a reliable antisense-based

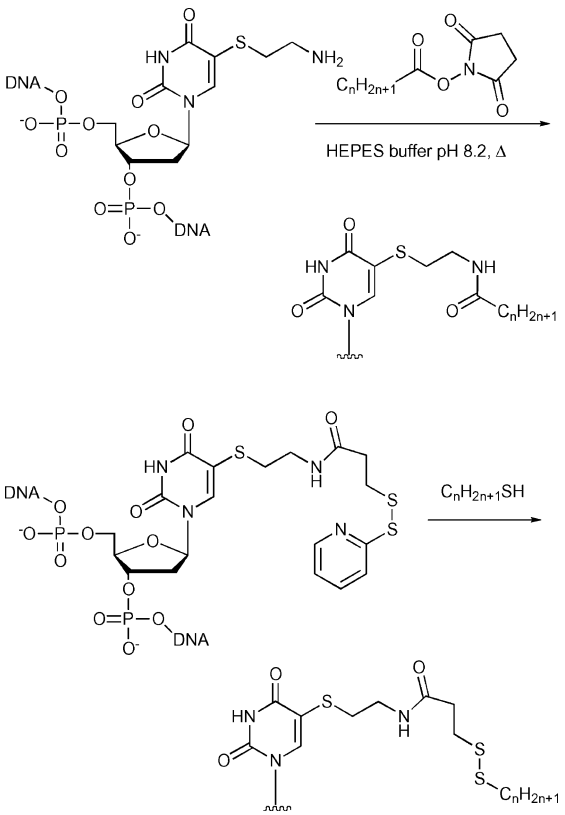

Fig. 10 Post-synthetic strategy for the direct hydrophobization of oligonucleotides.

therapy for *in vivo* implementation is still hampered by the poor cellular uptake of highly negatively charged and high molecular weight antisense molecules. Therefore, new antisense strategies are being developed today. One of the first strategies developed to improve cellular uptake consisted of the covalent tethering of a lipophilic group to the antisense oligonucleotide with the aim of adsorbing the antisense to the membrane thus facilitating membrane crossover. The uptake, and antisense activity of these ONAs are indeed greatly improved compared to underivatized analogs.[86,95–97] Additionally and not surprisingly, such lipophilic conjugates proved to be more resistant toward nucleases,[94,95,98] and the resistance increases with the size of the aliphatic chain.[99] This strategy proved also successful with si-RNAs.[100] In this example, the hydrophobic part of ONAs serve to anchor the oligonucleotide into the cellular membrane and thus facilitate adsorptive endocytosis of the antisense ONA.[95]

For example, Anada and co-workers developed a thermo-responsive antisense based on a polymer-bound oligonucleotide.[101] In spite of the numerous lipophilic residues available, most DNA-based antisense oligonucleotides feature a cholesteryl motif. A number of observations can be made regarding the hydrophobic effect including: 1) antisense efficacy does not correlate with high lipophilicity: very lipophilic conjugates were not capable of inhibiting intercellular adhesion molecule-1 (ICAM-1) production in murine liver in contrast to the cholesteryl analog.[95] 2) Cholesterol is recognized by low-density lipoprotein receptors and subsequently actively endocytosized.[102] Accordingly, upregulation of the LDL receptor on the cell surface promotes the uptake of cholesterol-based ONAs.[96] 3) Many viruses-like HIV-feature cholesterol units at their surface capable of cholesterol recognition. Consequently, several cholesterol-based ONAs were shown to inhibit viral replication more effectively than underivatized analogs.[86,103,104] The antiviral activity does not yet necessarily correlate with sequence-specificity suggesting alternative mechanisms of inhibition.[105] Although this lack of sequence-specificity is equally recurrent with unconjugated antisense oligonucleotides, ONAs with a pendant cholesteryl residue were shown to inhibit in a non specific manner both HIV reverse transcriptase and the interaction between HIV gp120 and host CD4.[97] The same behavior was also observed with phospholipid-based ONAs.[96]

Despite a few successes,[95] the targeting of a specific mRNA with conventional antisense nucleic acids or ONAs has remained elusive. The ONA-based antisense strategy may yet be revolutionized by the discovery that cholesterol-based ONAs—the so-called antagomirs—are specific and potent inhibitors of miRNAs *in vivo* in mice.[20] miRNA are an abundant class of short (*ca.* 20 nucleotides in length), non-coding RNAs involved in the regulation of gene expression.[106] Quite surprisingly, the targeted miRNAs were not only specifically blocked but also degraded by an unknown mechanism and the cholesterol was found essential in this degradation process. Noteworthy, the miRNA targeted by the antagomir is involved in the metabolism of cholesterol.[107] Even if many questions remain unanswered as to the exact role of the cholesterol in that process and how the targeted miRNA is degraded (is it delivered to hepatocytes and/or a result of changes in subcellular localization of the antagomir-miRNA complex?),[108] these observations highlight the exciting perspectives for the development of ONAs as antisense or siRNA therapeutics.[109]

3.4 ONAs in supramolecular assemblies

A prerequisite for ONAs to function as viable antisense oligonucleotides is their ability to form a supramolecular duplex—*via* canonical Watson–Crick base pairing—with the complementary, targeted, nucleic acid (mRNA, miRNA, *etc.*). Consequently, the influence of the hydrophobic substituent on the duplex stability is expected to be as minimal as possible. A detailed investigation on the biophysical aspects of the interaction and behavior of ONAs—with α-tocopherol as the lipophilic substituent—in lipid membranes has recently been reported.[110] This study suggests the preferential localization of ONAs into liquid-ordered domains ("rafts"). Moreover, the double helix formation between the oligonucleotidic part of the ONA and the complementary ss-DNA was found essentially unaffected when the ONA is present into the membrane,[110,111] even when the latter is negatively charged, provided a PEG spacer is inserted in-between the cholesteryl residue and the oligonucleotide segment of the ONA.[112] The strength of the duplex formed between the antisense ONAs and the "natural" targets can be estimated by UV-monitoring of the melting temperature (T_m) of the duplex.[113] No general trend is observed in the literature when one or more hydrophobic groups are tethered to the nucleic acid as for instance observed by Gryaznov and co-workers who thoroughly studied the influence of cholesterol-based ONAs in the formation of duplexes and triplexes.[94] Accordingly, destabilization,[86,104,114] stabilization[92] and no effect on the duplex stability,[86,89,115,116] have all been reported.

Although not relevant to the antisense strategy, as both strands bear a lipophilic substituent, Letsinger reported an impressive

23–24 °C increase in duplex stability when two complementary ONAs with hydrophobic groups favorably positioned to overlap and aggregate are allowed to hybridize.[116]

Even when the two hydrophobic groups are present on each strand at both ends of the duplex (each strand is modified at its 5′-end) only a modest increase in T_m is observed.[104] Finally and quite amazingly, the hydrophobic forces proved strong enough to drive polyA and polyT strands in a—normally unfavorable—parallel orientation (Fig. 11).[116]

Fig. 11 The presence of the two cholesteryl residues in DNA strands forces the formation of a duplex in an otherwise unfavourable parallel orientation.

Also worth mentioning is the propensity of ONAs to form supramolecular assemblies on their own. ONAs are in fact true surfactants with their oligonucleotide headgroups and their hydrophobic tails. As a result, ONAs can self-organize into micelles,[117] and vesicles[118,119] in the concentration range utilized for thermal denaturation experiments.[120] Gosse *et al.* observed the coexistence of micelles of ONAs in the micromolar concentration range with vesicles made of phosphatidylcholine at a concentration below 20 mmol L^{-1}.[117] The authors hypothesized that an entropic penalty exists for the anchoring of a single-stranded DNA ONA in a half-space at a bilayer surface. The ONA developed in this work was used to probe the polarity of the ONA environment.

3.5 Other supramolecular ONA-based assemblies and their applications

The unique recognition properties of oligonucleotidic headgroup associated with these amphiphiles make ONAs appealing building blocks for supramolecular applications. For instance, 2D arrays of mobile liposomes were created starting from liposomes formulated with ONAs of different oligonucleotidic sequences at their surface (A′ and B′, Fig. 12).[121,122]

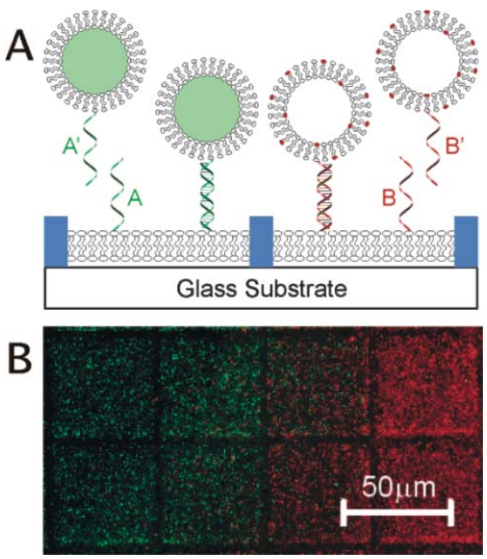

Fig. 12 Arrays of mobile tethered liposomes on supported lipid bilayers.

The information encoded in the oligonucleotide sequence results in the specific binding of the vesicles to complementary strands displayed in a spatially-controlled way on a fluid-supported lipid bilayer. Interestingly, the assembly kept the ability to move freely in the plane of the lipid bilayer.

A similar strategy was used by Paunov and co-workers for the fabrication of DNA arrays based on microcontact printing of DNA surfactants on solid lipophilic substrates.[123,124] These ONA-based 2D-arrays may find many applications for sensing and biotechnological applications. For example, Goto and co-workers recently developed a reverse micelle-based liquid–liquid extraction method for the specific extraction of a targeted ss-DNA (Fig. 13).[125]

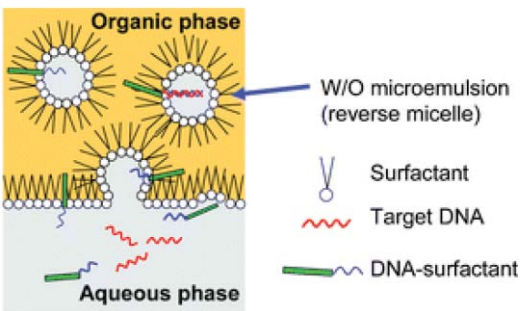

Fig. 13 Sequence-specific extraction of ss-DNA using reverse micelles in which hybridization between a DNA-surfactant and a target DNA having a complementary sequence allows selective transport of the target DNA to an organic phase from a mixture of DNA oligonucleotides.

A supramolecular network of liposomes was specifically assembled through Watson–Crick base pairing between two complementary ONAs present on two different vesicles (Fig. 14).[126]

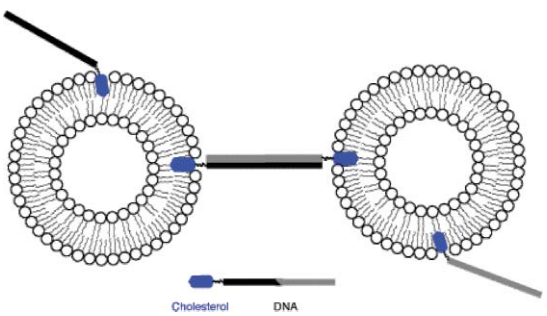

Fig. 14 Schematic drawing of a supramolecular assembly formed from vesicles functionalized with complementary DNA strands.

Interestingly, the aggregate proved reversible upon heating above the melting temperature of the duplex and then cooling.

An interesting ONA-based sensing platform was build by Dentinger.[119] The ONA spontaneously forms vesicles in phosphate buffer solutions at pH 7.4. Orange OT and pyrene dyes are entrapped into the lipophilic bilayer and are released upon exposure to the strand complementary to the ONA (Fig. 15).

Interestingly, 40% of the dyes are released in the presence of 10% complementary strand thus amplifying the detection signal.

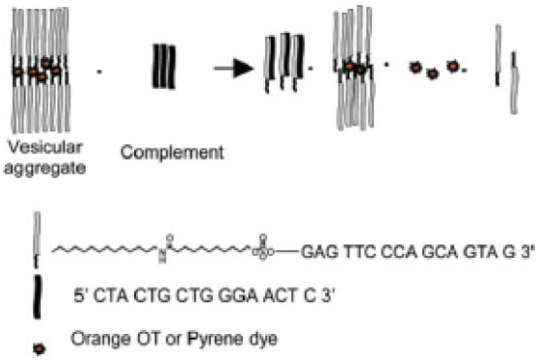

Fig. 15 Dye release from an ONA-based supramolecular assembly upon binding of the complementary DNA.

Conclusion

The large number of recent and relevant publications in the field of nucleolipids and oligonucleotide-based amphiphiles clearly demonstrates that the design and fabrication of nucleic acid-based amphiphilic molecules is more than a simple scientific curiosity. The emerging systems developed so far clearly open up interesting avenues for the design and development of supramolecular devices, biotechnological tools and therapeutic strategies. The above examples illustrate how efficient prodrug molecules and potent transfection agents with no associated cytotoxicity have for instance been devised from nucleolipids. Given the importance of delivering genetic materials and targeting specific cells in gene therapy, nucleolipids are very attractive candidates. In fact, the additional pyrimidine or purine bases present in the structure of nucleolipids bring about new favorable H-bonding and π-stacking interactions. The nucleolipoplex consisting of a nucleoside-based lipid base-paired with a single-stranded polyU developed by Baglioni and Berti clearly illustrates this point.[127] One challenge is therefore to design robust neutral supramolecular complexes less prone to aspecific binding to cell surfaces compared to conventional cationic delivery systems that could be more easily targeted to specific cells and deliver other biologically active compounds. On the other hand, ONA-based supramolecular assemblies are just starting to receive attention and biotechnological applications are burgeoning. Chemical synthesis of ONAs is yet still an issue. For instance, new synthetic strategies will be required to synthesize analogs of the antagomirs to unravel their mechanism of action and to develop even more potent inhibitors.

The purpose of this Perspective was to highlight some recent examples of nucleoside, nucleotide and oligonucleotide based amphiphiles, to show the varied applications being explored with these biomolecules and biomacromolecules, and to stimulate further discussion and research in this exciting area.

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