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Lipophilic nucleic acids — A flexible construction kit for organization and functionalization of surfaces

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ABSTRACT

Lipophilic nucleic acids have become a versatile tool for structuring and functionalization of lipid bilayers and biological membranes as well as cargo vehicles to transport and deliver bioactive compounds, like interference RNA, into cells by taking advantage of reversible hybridization with complementary strands. This contribution reviews the different types of conjugates of lipophilic nucleic acids, and their physicochemical and self-assembly properties. Strategies for choosing a nucleic acid, lipophilic modification, and linker are discussed. Interaction with lipid membranes and its stability, dynamic structure and assembly of lipophilic nucleic acids upon embedding into biological membranes are specific points of the review. A large diversity of conjugates including lipophilic peptide nucleic acid and siRNA provides tailored solutions for specific applications in bio- and nanotechnology as well as in cell biology and medicine, as illustrated through some selected examples.

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1. Introduction

1.1. Dynamical assembly due to weak interactions

Life is based on dynamical supramolecular assembly ruled by the interplay of many weak intermolecular interactions. Constant reformation of the weak interactions allows control and adjustment for the appropriate structure, whereas strong interactions would produce amorphous static structures [1,2]. While the covalent bonds between atoms have interaction energies of 100–400 kJ/mol, those of weak forces are in the order of or slightly higher than a few k_BT (2.5 kJ/mol at room temperature, with k_B the Boltzmann constant and T the absolute temperature). Weak forces include hydrogen bonding (4–120 kJ/mol), van der Waals interactions (<5 kJ/mol), π stacking (<1–50 kJ/mol), forces that arise due to the hydrophobic effect, and to some degree electrostatic forces.

These interactions are responsible for numerous structures found in nature, but also for the recognition of chemical signals and their translation into a biological response. One important example demonstrating the structural power of weak forces is the hydrophobically driven self-assembly of lipids and detergents into membranes, micelles or other supramolecular assemblies [3]. Weak forces are not only sufficient for formation of these assemblies but are also responsible to provide them with appropriate lateral fluidity, they allow for shape changes in the process of spontaneous curvature formation, endo- or exocytosis, caveolae formation, and virus entry into cells [4–7]. They also allow for binding and dissociation of molecules, e.g. to lipid membranes, without very large energy barriers separating the two states. The balance of a wealth of single weak interactions leads in some other cases to bistability phenomena, with coexistence of different assemblies (i.e. phase-separated lipid domains in fluid bilayers, raft-like domains [8]).

In general, most biological functions are accomplished by a molecular switch mechanism, based on the interplay between multiple weak forces, e.g. protein complex formation, oligomerization, change of phosphorylation state, or reversible lipid modification. Also, nucleic acid strands are held together by multiple hydrogen bonds and π-stacking giving rise to a large variety of DNA and RNA structures, while simultaneously allowing for the easy separation of DNA in the process of transcription or cell division. A chemical modification of nucleic acids permits further degree of organization, e.g. an attachment of a hydrophobic moiety allows assembly in micelles or presentation on lipid membrane surfaces.

1.2. Lipophilic nucleic acids

A lipophilic conjugate is a chemical structure of one or many hydrophobic (lipid-like) moieties with a polar molecule, e.g. nucleoside, nucleotide, peptide, protein, nucleic acid, or synthetic polymer. Some lipophilic conjugates exist in nature, e.g. cytidine diphosphate diacylglycerol is a precursor of phosphatidylglycerol and phosphatidylinositol synthesis [9] and lipid modified proteins constitute an essential part of cell-signaling system [10–12]. Other conjugates are synthesized de novo on demand as described in, e.g. Rosemeyer, 2005; Kaczmarek, 2008; Gissot, Barthélémy, 2008; Berti, 2011, Soft Matter; and Allain, Couvreur, 2012 [9,13–16]. Another example of a functional head group conjugated to lipids is nitrilotriacetic acid (NTA), a well characterized chelator that allows isolation of His-tagged proteins. Starting from the first lipophilic modification of NTA introduced by Tampe and coworkers [17], multivalent conjugates with high-affinity for His-tagged protein were developed [18] and further tested for analytical and drug delivery applications [19–21]. Recently protein targeting into cell rafts by a multivalent NTA lipophilic conjugate was reported [22].

The discovery of natural RNAzymes and synthesis of DNAzymes, DNA origami, and aptamers showed clearly the versatility of nucleic acid structures [23–26]. The double helix size, its bending rigidity, the high fidelity and cooperativity of base pairing, provide a material with high (subnanometer) spatial resolution and addressability. Moreover the paired structures can be (dis)-assembled via reversible thermal cycling in accessible temperature ranges (20–80 °C). Nucleic acid nanostructures can be functionalized with single or multiple chemical units, whose positioning and distance within the nano-constructs are programmed by design.

Conjugation of lipophilic moieties with a nucleic acid results in lipophilic nucleic acid (LiNA) combining self-assembly properties of the anchors and specific recognition of the nucleic acid strand. Some LiNA self-assemble into defined structures capable of enhanced affinity binding due to multivalency, e.g. aptamer-presenting micelles [27], and others allow easy and controllable functionalization of surfaces, including cell membranes [15,28–34]. LiNA were used to enhance gene delivery and gene silencing by siRNA and antisense PNA [35–39]. LiNA were found to inhibit viruses, e.g. Hepatitis C virus translation [37] and HIV fusion with cells [40]. They were used for development of drug delivery systems [16,27,41], as a detection tool for microRNA in living cells [42], and for DNA detection [43,44]. For recent reviews describing applications of LiNA see [45,46].

In this review, we discuss biophysical aspects of lipophilic nucleic acid self-organization, interaction with lipid membranes, and formation of structures on the surfaces. A LiNA conjugate consists of an anchor, linker, and a molecular recognition unit (nucleic acid). Fig. 1 presents several examples of lipophilic conjugates used in recent studies, which will be discussed below. Different types of backbone structures were used as shown in Fig. 1 (B1, B2). The respective aim of a study (and availability) determines the choice of the nucleic acids. DNA and RNA can be easily synthesized, and their charged phosphate groups make them well water soluble. Solubility in water is important if working in aqueous solutions, especially for living systems. DNA is chemically more stable than RNA and lipophilic DNA is often used in biophysical studies, assembly studies, and for building of nucleic acid detection devices [47]. RNA is more vulnerable to enzymatic degradation by RNases that are both ubiquitous and highly resistant against denaturation, but has a higher thermal stability than DNA [48]. RNA is a regulating molecule in cells and, therefore, lipid modifications of antisense, micro, and small interfering RNA were studied [45]. Stability against nucleases can be significantly enhanced while increasing target affinity and specificity by attaching or substituting nucleobases with artificial nucleobases, such as locked nucleic acid (LNA), which is stable even in cellular environment [49,50].

One of the most stable forms of nucleic acid is peptide nucleic acid (PNA). It consists of a peptide backbone conjugated to the canonical nucleobases [51] (Fig. 1). PNA is not only chemically highly stable [52], there are also no enzymes known capable of degrading PNA [38]. Also, DNA/PNA hybrids are highly insensitive to changes in ionic strength [53]. Lacking phosphate groups, PNAs are uncharged and natively hardly water soluble. Solubility of PNA can be significantly enhanced by extending the PNA strand with charged amino acids [54]. To prevent self-aggregation of neutral PNA, a negatively charged linker is reasonable to use. Addition of a total of four glutamic acids to the sequence was used recently for improving solubility of dipalmitoylated PNA [30,55]. Further increase in solubility can be achieved by pre-hybridizing a lipophilic PNA with the charged complementary DNA.

Choosing an anchor, a linker, and a nucleic acid adequate for the aim of a study is always a compromise between the probability of a conjugate to aggregate, kinetics and stability of incorporation into membranes, and accessibility of the nucleic acid for hybridization with complementary strands. Linkage of a lipophilic anchor is now possible to any position of a DNA or RNA sequence, e.g. using phosphoramidites of the lipophilic nucleotides or “click chemistry”. Often just 5′end or 3′ end are modified [28,56]. Commercially available 5′end or 3′end modifications with tri-ethylene-glycol cholesteryl (TEG-cholesteryl) are used often for membrane targeting and cell uptake studies as synthesis of