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Modulation of binding properties of amphiphilic DNA containing multiple dodecyl phosphotriester linkages to lipid bilayer membrane

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ABSTRACT

DNA is a promising functional molecule to modify and design lipid membrane functions. In order to use DNA in a hydrophilic–hydrophobic interface including lipid membrane, we have developed an amphiphilic DNA having dodecyl phosphotriester linkages (dod-DNA). Herein, we report the binding of a series of amphiphilic dod-DNAs to the lipid bilayer membrane. Surface plasmon resonance (SPR) assay and fluorescent microscopy showed that dod-DNA having three dodecyl groups at each end strongly bound to lipid membrane due to the slow dissociation rate and the dod-DNA can be used as a linear template for molecular arrangement on the membrane surface.

Cell membrane provides hydrophilic–interface where a large number of proteins exert a variety of functions. Molecules that can modify the membrane properties and add novel functions are promising molecular tools for biotechnology. DNA has attracted much attention as a functional molecule due to its high specificity in hybridization and designable self-assemble property through Watson–Crick base pairing. In order to use the unique DNA properties on the membrane surface, amphiphilic (lipophilic) DNAs having hydrophobic functionalities has been developed.[1–3] The amphiphilic DNAs are anchored to the surface of lipid bilayer membrane and add new properties and functions to the membrane, such as specific labeling,[4–7] precise molecular arrangement,[8,9] vesicle aggregation[10–12] and fusion.[13–15] Recent progress on DNA nanotechnology combined with the amphiphilic DNAs has expanded the potential of DNA-based designer functions on the lipid bilayer membrane,[16–24] including light harvesting devices[23,24] and synthetic ion channels.[21,22] Control of DNA binding properties to the lipid membrane, including binding affinity and orientation with respect to the membrane surface, is important to make the best possible use of a variety of functional DNA structures on the lipid membrane.

We have recently developed amphiphilic DNAs containing hydrophobic dodecyl phosphotriester linkages instead of natural hydrophilic phosphate backbone (dod-DNA, Fig. 1a).[25,26] The dodecyl-modified hydrophobic backbone has no electric charge and the dodecyl groups positioned at the outermost surface of the DNA helix. Similar to amphipathic α-helix peptides having hydrophobic faces, dod-DNA having appropriate hydrophobic regions could be a suitable binder to lipid membrane surface. We herein report the binding of a series of amphiphilic dod-DNAs to the lipid membrane surface. The binding properties were investigated by surface plasmon resonance (SPR) assay and were optimized for the use of dod-DNA on the lipid membrane.

While introduction of hydrophobic functionalities at the end of one DNA strand has been widely used to prepare membrane-anchored DNAs (Fig. 1b),[1–3] we anticipated that binding affinity and orientation of amphiphilic DNAs to the lipid membrane could be tuned by the number and location of the hydrophobic modifications in the DNA sequence (Fig. 1). Single hydrophobic region at the terminal of DNA would anchor the DNA to membrane surface (Fig. 1b). Multiple hydrophobic regions at intervals of 1 turn (10.5 nucleotides) of B-DNA helix would strictly define orientation of the membrane-bound DNA with respect to the membrane plane, where the DNA lies down on the membrane surface (Fig. 1c). We first evaluated a binding of amphiphilic dod-DNAs having hydrophobic dodecyl phosphotriester linkages at the terminal, which can act as an anchor on the membrane surface.

We have synthesized a series of dod-DNAs having one to three dodecyl groups at the 5′-end (dod-DNA 1–3, Table 1) according to previously established synthetic procedure.[25] Bindings of the dod-DNAs to lipid membrane were evaluated by surface plasmon resonance (SPR) technique with a L1 sensor chip (Biacore) immobilized with

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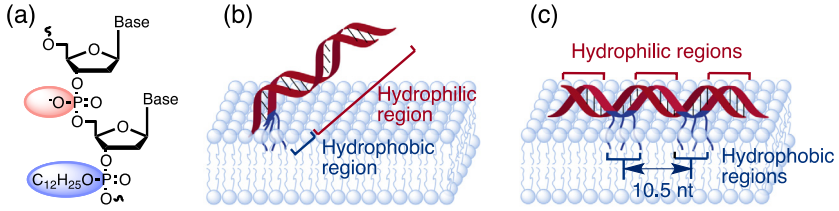

Figure 1. (a) Chemical structure of amphiphilic DNA (dod-DNA) used in this study. Schematic illustration of lipid membrane anchored dod-DNAs with (b) a single hydrophobic region at the terminal and (c) two hydrophobic regions in the same face of the DNA helix by introducing at intervals of 1 turn (10.5 nucleotides, nt) of the helix.

Table 1
Sequences of DNAs used in this study[a]

dod-DNA1	5′-**T_R**TTTTCACCGACCACGC-3′
dod-DNA2	5′-**T_RT_R**TTTCACCGACCACGC-3′
dod-DNA3	5′-**$T_RT_RT_R$**TTCACCGACCACGC-3′
dod-DNA4	5′-TAGCTACT**$T_RT_RT_R$**TCATCGT-3′
dod-DNA5	5′-**$C_RT_RC_R$**CAGTTCGTGAGTGATGTATAGCGATGTC**$G_RT_RC_R$**T-3′
DNA6	5′-GCGTGGTCGGTGAAAAA-3′
DNA7	5′-CTCCAGTTCGTGAGTGATGTATAGCGATGTCGTCT-3′
DNA8	5′-AGACGACATCGCTATACATCACTCACGAACTGGAG-3′
Cy3-DNA9	5′-**Cy3**-ATCACTCACGAACTGGAG-3′
Cy5-DNA10	5′-AGACGACATCGCTATAC-**Cy5**-3′

[a] Modified nucleotides and fluorescent dyes are shown in bold face and hydrophobic triester linkage is indicated by a subscript 'R'.

Table 2
Melting temperatures of DNA duplexes (2 μM) with and without POPC liposome[a]

	T_m (°C) without liposome	T_m (°C) with liposome	ΔT_m (°C)
dod-DNA3/DNA6	67.4	66.2	−1.2
dod-DNA5/DNA8	68.5	73.5	5.0
DNA7/DNA8	77.9	78.4	0.5

[a] ΔT_m values were calculated from the equation: $\Delta T_m = T_m$ (with liposome) $- T_m$ (without liposome).

1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC) liposome.[27,28] For each SPR experiment, DNA hybridization with the surface-bound dod-DNA was monitored by a subsequent injection of the complementary DNA. dod-DNA1 having a single dodecyl group showed no apparent increase in SPR response, indicating a very weak interaction to the lipid membrane under the condition (Fig. 2a, injection of the dod-DNA was depicted by solid arrow). In contrast, apparent rises in SPR response were observed upon injection of dod-DNA2 and dod-DNA3. The curve shapes indicate the different kinetics of the dod-DNAs binding to the lipid membrane; dod-DNA2 having two dodecyl groups showed rapid association and dissociation (Fig. 2b), while dod-DNA3 having three dodecyl groups showed slow association and dissociation resulting in retention of the dod-DNA3 on the membrane (Fig. 2c). With increasing number of dodecyl groups, the binding affinity to the lipid membrane increased because of the slow dissociation rate. Although the K_d value of dod-DNA3 with the lipid membrane could not be precisely determined because of the slow dissociation, dod-DNA3

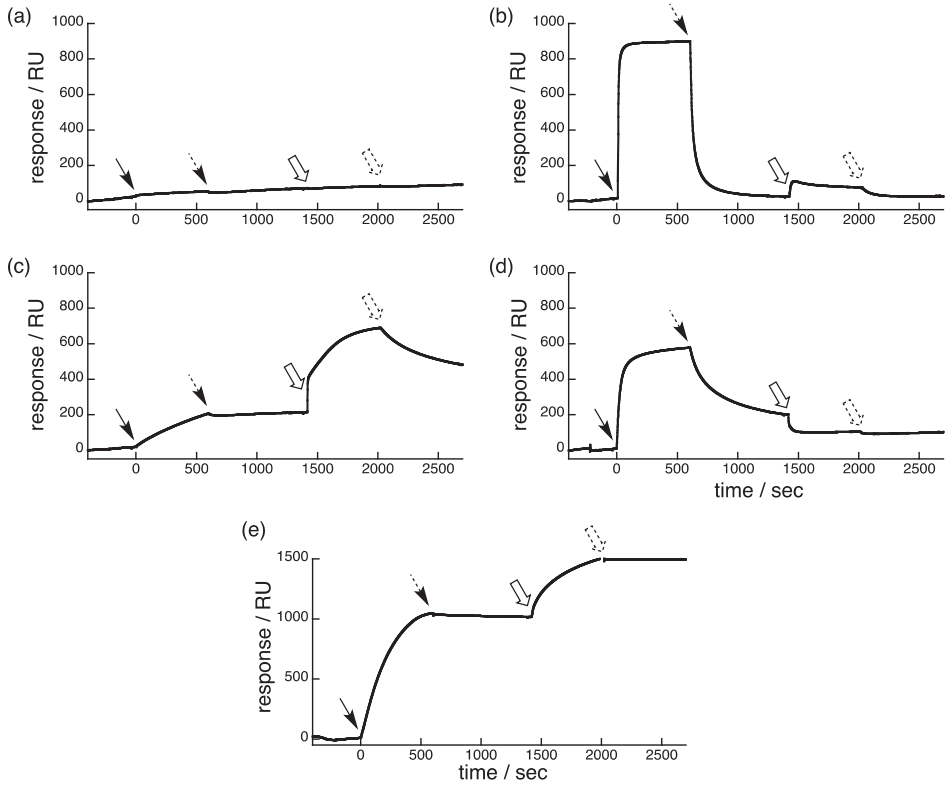

Figure 2. Sensorgrams of dod-DNA bindings to the lipid bilayer and their hybridizations. POPC liposomes are deposited on L1 sensor chip, followed by injections of dod-DNAs (t = 0, black solid arrows) and their complementary DNAs (t = 1400, white arrows). (a) dod-DNA1. (b) dod-DNA2. (c) dod-DNA3. (d) dod-DNA4. (e) dod-DNA5. Dashed arrows indicate the beginning of running buffer injection (washing steps, t = 600 and 2000).

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