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Amphiphilic Counterion Activators for DNA: Stimuli-Responsive Cation Transporters and Biosensors in Bulk and Lipid Bilayer Membranes

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We report that amphiphilic counterions can enable DNA to act as cation carrier, enzyme detector and biosensor. Calf thymus DNA is used as example throughout the study. Evaluation of a series of counterion activators suggests that strong amphiphilicity, alkyl or calix[4]arene tails and guanidinium cations give best results, whereas weak amphiphilicity, bola-amphiphilicity, planar aryl tails and ammonium cations are less satisfactory for various reasons. In the U-tube, DNA–counterion complexes can carry cations such as safranin O or *p*-xylene-bis-pyridinium bromide (DPX) across bulk chloroform membranes, whereas anions such as carboxyfluorescein (CF) and (8-Hydroxy-1,3,6-pyrenetrisulfonate (HPTS) are not transported. Uptake of DNA–counterion complexes into intact vesicles is demonstrated by DNA trapping experiments with internal polylysine. Comparison of results from different assays suggests that DNA–counterion complexes act as cation carriers under mild conditions, whereas pore formation and lysis dominate at higher concentrations. Applicability of DNA–counterion transporters for the detection of enzyme activity is demonstrated with phytate as an inactivating substrate and phytase as a reactivating enzyme. Compatibility with biosensing is exemplified with the fluorometric monitoring of phytate levels in almond extracts. The conceptual significance of these findings is briefly discussed, as are promising perspectives such as the application of DNA chemistry to multianalyte sensing in fluorogenic vesicles.

Introduction

In this report, we describe that calf-thymus DNA can be activated by amphiphilic cations to act as a cation transporter in bulk and lipid bilayer membranes and show that this unusual activity of DNA can be of interest for the development of biosensors.

These results are interesting in the context of recent progress made with cell-penetrating peptides (CPPs).[1] CPPs are guanidinium-rich oligomers and polymers that have attracted much attention because of their biological importance on the one hand and their complex behavior on the other hand.[1] We have suggested early on that the activities of weakly acidic polycations in general are determined by their need to strongly bind multiple counteranions to minimize intramolecular charge repulsion.[2,3] Building on this concept of counterion-mediated function, balanced combinations of amphiphilic and hydrophilic counteranions were found to enable oligo-/polyarginines to dissolve in chloroform,[2] partition from water into chloroform,[2] move across bulk and lipid bilayer membranes[2] and enter into live cells.[4] Other expressions of the same concept were shown to account for the voltage gating of biological potassium channels[5] and the conductance and the selectivity of synthetic ion channels.[6] Moreover, CPP–counteranion complexes were found to transport anions across bulk and bilayer membranes.[2] This anion transport activity was used to develop CPP–counteranion complexes into detectors of the activity of many different enzymes[7] and sensors for many different analytes.[8]

This rich multifunctionality obtained from counteranion activation of polycations such as CPPs naturally raised the question about charge inversion.[3] The ability of amphiphilic cations to mediate the uptake of DNA into cells has been studied and confirmed in many variations.[9,10] The cellular uptake of countercation-activated DNA and other weakly basic polyanions mirrors perfectly the cellular uptake of counteranion-activated CPPs and other weakly acidic polycations. The only difference is that the latter process can be spontaneous because anionic phospholipid activators are already present in biological membranes. To complete the picture and develop a multifunctionality as rich as for CPPs, we wondered whether or not countercations could activate DNA to function as cation transporters, enzyme detectors and biosensors.[3] To answer this question, we here first characterize counterion-activated DNA as ion carriers in the "U-tube"[2,11] and in vesicles, use then a collection of amphiphilic, bola-amphiphilic, aliphatic, aromatic and macrocyclic ammonium and guanidinium countercations to outline the nature of DNA activation, and explore sensing applications[12,13] with phytase and phytate as examples.

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Results and Discussion

DNA as cation carriers in bulk membranes

"U-tube" experiments were preferable to begin with because, contrary to the more complex lipid bilayer membranes, they provide straightforward and unambiguous evidence for the existence and selectivity of ion carriers.[2a,11] In a typical experiment, a hydrophobic chloroform phase or "bulk membrane" was placed at the bottom of the U-tube and covered at both the *cis* and the *trans* side with separate aqueous phases (Figure 1 D). Calf thymus DNA (ctDNA) as representative carrier

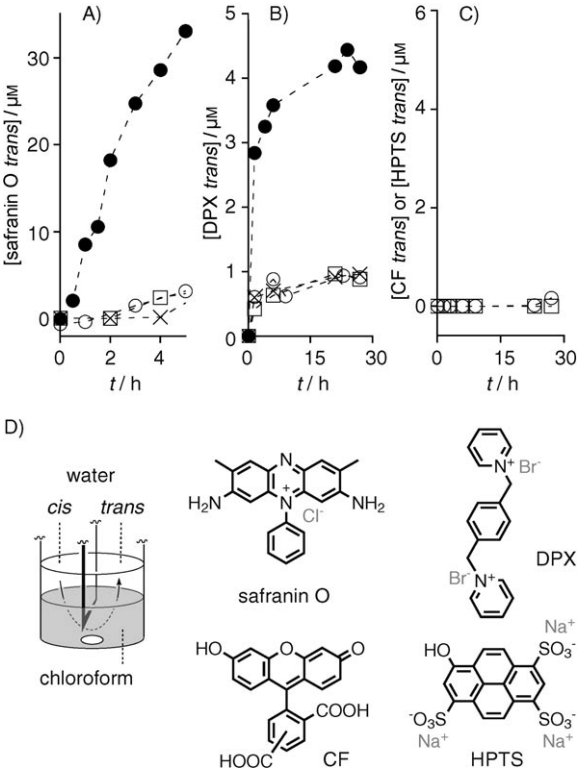

Figure 1. DNA–counterion complexes as cation carriers in the U-tube. A) Transport of safranin O (100 μM) from *cis* buffer (0.5 mL) across bulk chloroform membranes (3 mL) into *trans* buffer (0.5 mL) as a function of time (●) with calf thymus DNA (1.25 μg mL^{-1} *cis*) and DG (50 μM *cis*), (×) with ctDNA but without DG, (□) with DG but without ctDNA, and (○) without ctDNA and DG. Buffer: 10 mM Tris, 107 mM NaCl, pH 7.4). B) Same for DPX (100 μM *cis*). C) Same for CF (○, 100 μM *cis*) and HPTS (□, 100 μM *cis*) with ctDNA and DG. D) Experimental setup for U-tube experiments, and structure of cationic and anionic probes used. The term "U-tube experiment" is maintained although modern versions of U-tubes are often not U-shaped.

and/or dodecylguanidinium (DG) as representative amphiphilic counterion activator were added to the *cis* buffer together with a hydrophilic "reporter ion,"[2a] and the concentration of this reporter ion in the *trans* buffer was monitored as a function of time (Figure 1).

Safranin O[14,15] was selected first as a convenient reporter cation (Figure 1). In the presence of ctDNA–DG complexes, safranin O moved efficiently from the *cis* water across the bulk membrane to the *trans* water (Figure 1 A, ●). In the absence of DG activators, ctDNA was incapable of transporting safranin O with comparable efficiency in the U-tube (Figure 1 A, □). The same was true for DG activators in the absence of ctDNA (Figure 1 A, x), and safranin O alone did not move across the bulk membrane either (Figure 1 A, ○).

The ability of DNA–counterion complexes to transport *p*-xylene-bis-pyridinium bromide (DPX)[14,15] across bulk chloroform membranes was studied next. Changes in the RP-HPLC profiles of the *trans* phase with time demonstrated that ctDNA–DG complexes act as carriers of DPX in bulk membranes (Figure 1 B, ●). Controls confirmed that DPX without complete DNA–counterion complexes does not show similar activity (Figure 1 B, ○, □, x). The transport of aromatic cations such as DPX by ctDNA-DG carriers was potentially interesting because binding to DNA duplexes could occur not only by competitive counterion exchange at the polyphosphate backbone, but possibly also by noncompetitive intercalation into the π-stack. However, DPX transport by other counterion-activated polyions such as RNA or polyphosphate suggested that possible contributions from intercalation are insignificant.[3]

The ability of DNA–counteranion complexes to carry anions across bulk chloroform membranes was evaluated with 5(6)-carboxyfluorescein (CF)[14,15] and 8-hydroxy-1,3,6-pyrenetrisulfonate (HPTS) as classical reporter anions. No anion transport activity was found under conditions in which ctDNA–DG complexes functioned as efficient cation carriers (Figure 1 C). Taken together, these U-tube experiments provided compelling experimental evidence that DNA–counteranion complexes can function as selective cation carriers.

DNA as transporters in lipid bilayer membranes

The ability of DG-activated ctDNA to act as a cation transporter in lipid bilayer membranes was determined in EYPC-LUVs⊃DPX/HPTS (i.e., egg yolk phosphatidylcholine large unilamellar vesicles loaded with the anionic fluorophore HPTS and the cationic quencher DPX).[2c] In this assay, the export of either the cationic quencher or the anionic fluorophore from the vesicle increases the relative distance between the two. As a result, cation export (DPX), anion export (HPTS), and vesicle lysis are all reported as an increase in emission (Figure 2). The HPTS/DPX assay thus is convenient because activity is observable for many different transport systems with different selectivities. However, the U-tube experiments described in the previous section demonstrate that counterion-activated DNA acts as a cation-selective carrier that clearly prefers to transport DPX rather than HPTS (Figure 1 B, ● vs. C, □).

According to the HPTS/DPX assay, ctDNA and DG alone were inactive. Together, however, they were active (Figure 2). The fluorescence recovery in response to the addition of ctDNA–DG complexes was calibrated against vesicle lysis and reported as fractional activity, *Y*. Dose–response curves for *Y* against the concentration of either the countercation activator or the DNA transporter gave the corresponding EC_{50} values, the effective concentration needed to reach $Y_{max}/2$ (Figures 2 B and S1).

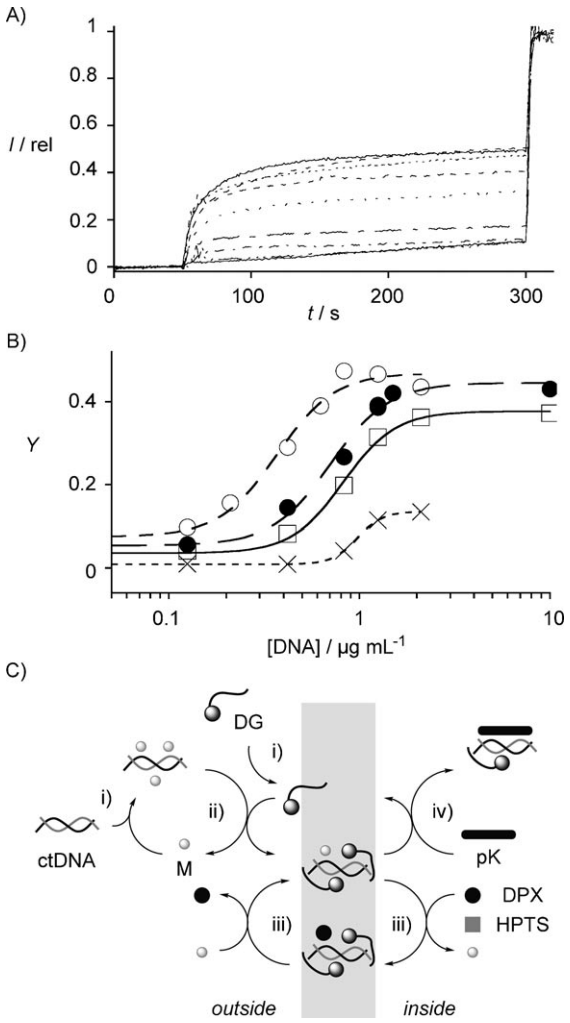

Figure 2. Dependence of the activity of DNA–counterion complexes on the concentration of DNA (A, B) and internal counterion inactivators (B). A) Fractional HPTS emission I (λ_{ex} 413 nm, λ_{em} 511 nm) as a function of time during the addition of calf thymus DNA ($t = 50$ s, i) 0, ii) 0.2, iii) 0.3, iv) 0.5, v) 0.7, vi) 1.0 and vii) 2.0 μg mL^{-1} final concentrations) to EYPC-LUVs⊃HPTS/DPX (~12.5 μM lipid) in the presence of external DG (50 μM). Conditions: 5 mM HPTS, 16.5 mM DPX, 10 mM Tris, 72 mM NaCl, pH 7.4 (inside); 10 mM Tris, 107 mM NaCl, pH 7.4 (outside), 25 °C, calibrated by final addition of triton X-100, $t = 300$ s). B) Dose–response curves for DNA at constant concentration of external activator DG and increasing concentration of internal inactivator pK (0 (○), 1 (●), 10 (□) and 100 μM (×) inside; with curve fits to Hill equation, see Table 1). C) Experimental setup for vesicle experiments: i) Addition of polyions (e.g., ctDNA) and counterion activators (e.g., DG) to vesicles with internal reporter ions (e.g., DPX, HPTS) and counterion inactivators (e.g., pK) possibly causes ii) the formation of membrane-active polyion-counterion complexes (e.g., ctDNA-DG), iii) DPX export, iv) the formation of internal polyion-counterion complexes (e.g., ctDNA-DG-pK), etc; M = Na.

The uptake of carriers into intact vesicles can be difficult to detect unambiguously.[2c,16] However, with DNA–counterion complexes that are known to enter cells[9,10] and shown above to move across bulk chloroform membranes (Figure 1), it seemed obvious that DNA uptake into vesicles would be no problem. To confirm that this is really the case, also under the present conditions, EYPC-LUVs⊃DPX/HPTS were loaded with increasing concentrations of poly-L-lysine (pK). The response of ctDNA–DG transporters was an increasing EC_{50} and a decreasing Y_{max} (Figure 2B and Table 1). This inactivation of extravesicularly added DNA by intravesicular pK could be interpreted with DNA uptake and inactivation by binding to the hydrophilic polycation in the vesicles. Decreasing Y_{max} with increasing EC_{50} in dose–response curves for DNA suggested that DG activators are inactivated together with DNA (that is, the formation of inactive pK-ctDNA–DG complexes within the vesicles).

Table 1. Inactivation of ctDNA-DG transporters by intravesicular polylysine (pK).[a]

	Poly-L-lysine [μM]	EC_{50} [μg mL^{-1}][b]	Y_{max} [%][c]
1	0	0.37 ± 0.04	47 ± 2
2	1	0.71 ± 0.06	45 ± 2
3	10	0.83 ± 0.04	38 ± 1
4	100	0.98 ± 0.01	14 ± 0.1

[a] From DPX/HPTS export from EYPC-LUVs⊃DPX/HPTS at constant concentration of DG activators (50 μM), 10 mM Tris, 107 mM NaCl, pH 7.4, 25 °C, see Figure 2. [b] Effective DNA concentration needed to reach 50% activity ($Y_{max}/2$), data ± SE. From Hill analysis of dose–response curves in Figure 2. [c] Maximal fractional fluorescence emission relative to lysis.

This interpretation, however, was not conclusive because the binding of DNA to intravesicular pK, extravesicular pK and freshly released pK should give exactly the same result. To detect uptake into intact vesicles more convincingly, sequential DNA addition to vesicles with internal pK inactivators and external DG activators was explored (Figure 3). The total DNA concentration was selected to produce nearly complete fluorescence recovery when added all at once (Figures 3A, solid, and 2B). Addition of the same amount of DNA in several small portions produced essentially no activity (Figure 3A, dashed). Clearly, stepwise DNA addition was not additive. This nonadditivity demonstrated that, at low concentrations, DNA–DG complexes move across bilayer membranes nondestructively and are trapped by pK within intact vesicles. pK release during counterion-mediated DNA uptake would make sequential DNA addition additive. Control experiments confirmed that sequential DNA addition to vesicles without intravesicular pK inactivators is additive (Figure 3B).

The same additivity was confirmed to occur with extravesicular rather than intravesicular pK inactivators (Figure 3C). The resulting uniqueness of nonadditive activity of DNA–counterion with intravesicular pK only confirmed that this phenomenon originates from nondestructive DNA uptake into and trapping within intact vesicles.

In contrast to the HPTS/DPX assay, the CF assay fails to detect cation export and reports on anion export and lysis only.[15] As representative examples from the collection of cations **1**–**17**, the activation of calf thymus DNA by DG **5**[3a] or calix[4]arene **15** was nearly identical in EYPC-LUVs⊃HPTS/DPX and EYPC-LUVs⊃CF (Figures 4A and 5). As a response to vesicle dilution, the HPTS/DPX assay revealed decreasing EC_{50} values, whereas constantly high EC_{50}s were found in the CF assay (Figure 4A–C). High EC_{50} values independent of vesicle concentration supported the fact that the CF assay detects

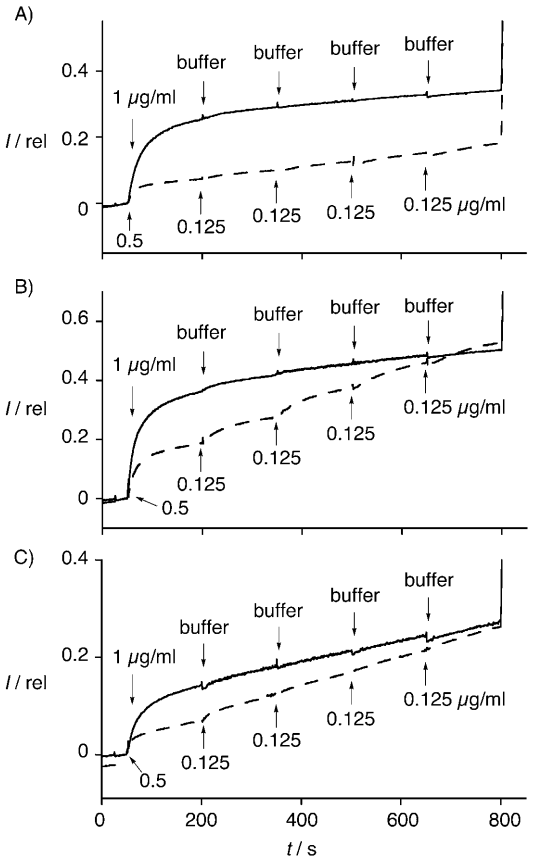

Figure 3. Fractional HPTS emission *I* during the addition of ctDNA at $t = 50$ s (1.0 μg mL^{-1} final; ——) or at $t = 50$ s (0.5 μg mL^{-1}), $t = 200$ s (0.125 μg mL^{-1}), $t = 350$ s (0.125 μg mL^{-1}), $t = 500$ s (0.125 μg mL^{-1}) and $t = 650$ s (0.125 μg mL^{-1}; -----) to EYPC-LUVs⊃HPTS/DPX (~12.5 μM lipid) with external DG (50 μM) and A) with internal pK (10 μM), B) without pK, or C) with external pK (10 nM). Conditions as in Figure 2.

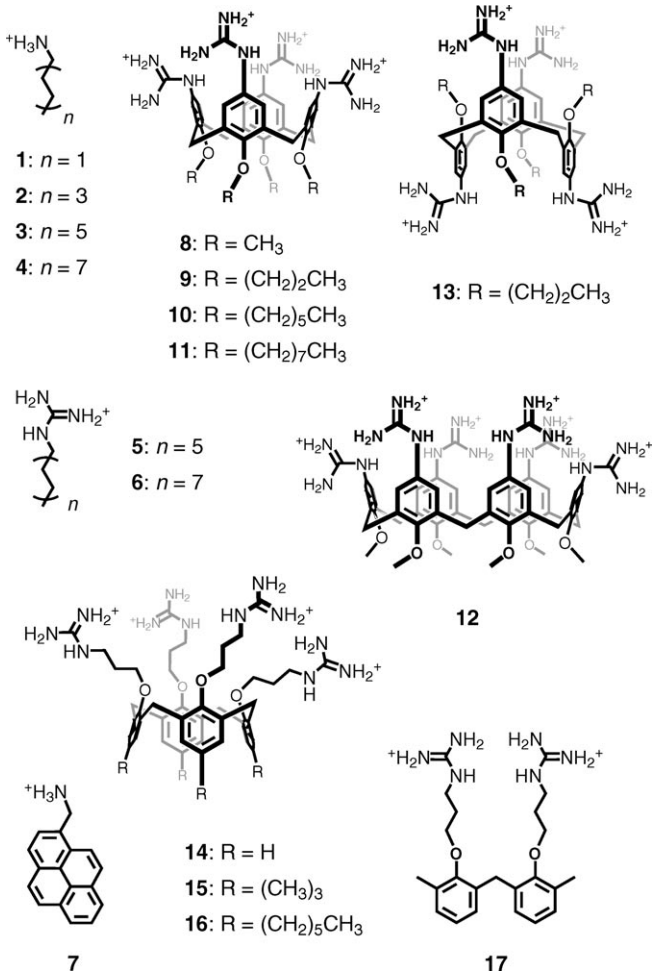

Figure 5. Countercation activators for DNA carriers, detectors and sensors.

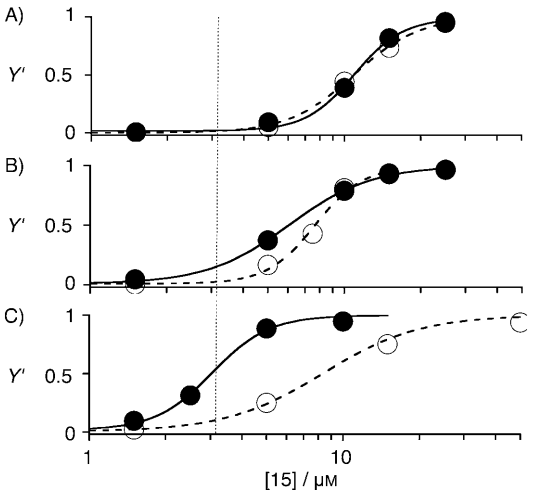

Figure 4. Dose–response curves for the activation of calf thymus DNA (12.5 μg mL^{-1}) by calix[4]arene **15** (increasing concentrations) in EYPC-LUV⊃HPTS/DPX (●) and EYPC-LUV⊃CF (○) at A) ~75 μM, B) ~50 μM and C) ~25 μM EYPC. Fractional activities *Y* from conventionally calibrated curves were normalized to maximal ($Y' = 1 = Y_{max}$) and minimal ($Y' = 0$) before lysis.

larger pores or more dramatic events that depend only on the critical micelle concentration (cmc) of higher-order DNA–counterion assemblies (Figure 4 A–C, ○). Low EC_{50} values with dependence on vesicle concentration demonstrated that the HPTS/DPX assay indeed reports on cation transporters (Figure 4 A–C, ●). The differences found between HPTS/DPX and CF assay thus suggested that under mild conditions, DNA–counterion complexes can act as cation transporters in intact vesicles (Figure 4 C, ● vs. ○). In the present example, this is the case around 3 μM **15**, 12.5 μg mL^{-1} ctDNA, and ~25 μM EYPC (Figure 4, dotted line). The validity of this interpretation was confirmed by U-tube and intravesicular trapping experiments (Figures 1 and 3). The apparent transition from nondestructive cation transport toward the formation of larger pores and more dramatic events at higher concentrations of DNA–counterion complexes was not surprising. The concentration dependence of transport mechanisms, usually shifting from carriers over channels and pores toward detergents with increasing concentration, has been suggested to occur in many cases, including prominent examples such valinomycin, melittin or Triton X-100.[17]

The dependence of the activity of ctDNA–DG complexes on other variables was not surprising. For instance, EC_{50} values in-

creased and Y_{max} decreased with increasing ionic strength (Figure S3). This finding confirmed the central importance of ion pairing and ion exchange for the activity of ctDNA–DG complexes. Low membrane fluidity gave poor efficiencies (Figure S4). This trend was consistent with but not exclusive for an ion carrier mechanism.[2,15,18] The presence of anionic lipids in the bilayer did not influence efficiencies much (Figure S5). This insensitivity of polyanionic DNA to anionic phospholipids was in meaningful contrast to the strong dependence of polycationic CPPs and K^+ channels, in which anionic lipids in the membrane can act as intrinsic counterion activators.[2,5]

Counterion activators

The activity of countercation-activated polyanions beyond DNA decreased with increasing basicity of the involved anions (DNA, RNA, polyphosphate ≫ hyaluronan, polyglutamate).[3] This series complemented the decreasing activity of counteranion-activated polycations with increasing acidity of the involved cation and thus confirmed intramolecular charge repulsion as general origin of counterion-mediated function.[2,3]

To quantify the activation of calf thymus DNA by amphiphiles **1**–**17**, their EC_{50} values, maximal activities Y_{max} and efficiencies η[2b] were determined (Figure S6 and Table 2). The results revealed that high activator hydrophobicity is essential for high η. Ammonium cations (**1**–**4**) were less efficient than guanidinium cations (**5**, **6**). This trend was not surprising considering the preference of phosphate anions to pair with guanidinium rather than ammonium cations.[2,19]

Countercations with alkyl tails (**1**–**6**) were better than aryl tails (**7**, **17**). This trend was contrary to the charge-reversed situation with CPPs, in which the high efficiency of aromatic counteranion activators was explained by their favourable partition into and translocation across bilayer membranes.[2,20] The reversed preference for alkyl over aryl activators with DNA carriers could suggest that activators **7** and **17** preferably intercalate into the DNA duplex and thus prevent their aromatic tails to mediate partitioning into the bilayer membrane. However, poor activity found with other counterion-activated polyions such as RNA or polyphosphate did not support this interpretation.[3]

Intercalation into DNA can be excluded with nonplanar aromatic macrocycles such as calixarenes.[10] However, tetraguanidinium calix[4]arenes with insufficient amphiphilicity such as **8** nevertheless failed to activate DNA transporters. Expansion to calix[6]arenes (**12**) did not solve the problem. Increasing the amphiphilicity of calix[4]arenes resulted in increasing efficiency to activate DNA transporters. Considering the presence of four guanidinium cations per activator, the efficiency of calixarenes **9**–**11** and **14**–**16** was comparable to or slightly better than that of the best alkylguanidinium activator **6**. However, comparisons at high activator efficiency should be carried out with caution because the onset of stoichiometric binding can obscure significant differences.[21]

The bola-amphiphilic 1,3-*alt* isomer **13** was clearly less active and caused only 34% as compared to 52% fluorescence recovery with the amphiphilic isomer **9** (Figure 6, ● vs ○). This important difference confirmed amphiphilicity as an essential characteristic of counterion activators. The acyclic analogue **17** was more than 100-fold less efficient than the calix[4]arene **14**. Multivalency, macrocyclic preorganization and competing intercalation into DNA are likely to contribute to this quite important calixarene effect.

Table 2. Countercation activators of DNA transporters.[a]

Activator	Charge[b]	EC_{50} [μM][c]	Y_{max} [%][d]	η[e]
1	+1	–	–	–
2	+1	–	–	–
3	+1	108 ± 5	100 ± 3	34
4	+1	7.7 ± 1.4	73 ± 5	57
5	+1	33 ± 2	81 ± 3	44
6	+1	4.6 ± 0.2	83 ± 3	72
7	+1	–	–	–
8	+4	–	–	–
9	+4	0.86 ± 0.02	52 ± 2	59
10	+4	0.78 ± 0.09	70 ± 4	81
11	+4	1.0 ± 0.01	90 ± 4	100
12	+6	–	–	–
13	+4	6.1 ± 0.7	34 ± 3	28
14	+4	1.5 ± 0.1	57 ± 3	60
15	+4	1.3 ± 0.1	90 ± 5	96
16	+4	1.4 ± 0.1	85 ± 5	90
17	+2	190 ± 12	81 ± 3	22

[a] From DPX/HPTS export from EYPC-LUVs⊃DPX/HPTS at constant concentration of calf thymus DNA (1.25 μg mL^{-1}, ~4 μM phosphate), 10 mM Tris, 107 mM NaCl, pH 7.4, 25 °C, see Figure 6 and S6; **1**, **2**, **7**, **8** and **12** were inactive (Y_{max} < 15%). [b] Maximal charge corresponding to the number of guanidinium/ammonium cations, ignoring possible charge reduction by proximity effects. [c] Effective activator concentration needed to reach 50% activity (Y_{max}/2), data ± SE. From Hill analysis of dose–response curves (e.g., Figure 6). [d] Maximal fractional fluorescence emission at saturation with activator relative to **3**, calculated after calibration to constant values after lysis. [e] Relative activator efficiency $\eta = f \times Y_{max} \times pEC_{50}$ [mM], $f = 0.37$.

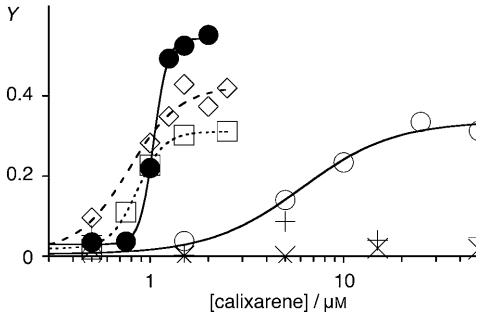

Figure 6. Dose–response curves for the activation of calf thymus DNA (1.25 μg mL^{-1}) by calixarenes **8** (x), **9** (□), **10** (◇), **11** (●), **12** (+) and **13** (○) in EYPC-LUV⊃HPTS/DPX.

The discovery that CPP–counteranion complexes function as anion carriers in bulk and bilayer membranes was useful for the development of new drug delivery[4] and sensing systems.[8] The herein reported discovery of the charge-inverted DNA–countercation complexes as cation carriers could thus be

expected to have a similarly diverse and important impact. Whereas cellular uptake of DNA–countercation complexes is extensively studied and routinely used in many variations,[9,10] their potential to function as multienzyme detectors and multianalyte sensors[12,13] remained to be clarified.

DNA as sensors in lipid bilayer membranes

Among several possibilities to develop counterion-activated DNA transporters into sensing systems, inactivation by hydrophilic and anionic analytes was explored first. Phytate (or IP_6) was selected as important representative of this family.[22] Competitive pairing of phytate with calixarene activators **15** cleanly inactivated DNA transporters (Figure 7A, ●, $IC_{50} = 450 \pm 30$ nM).

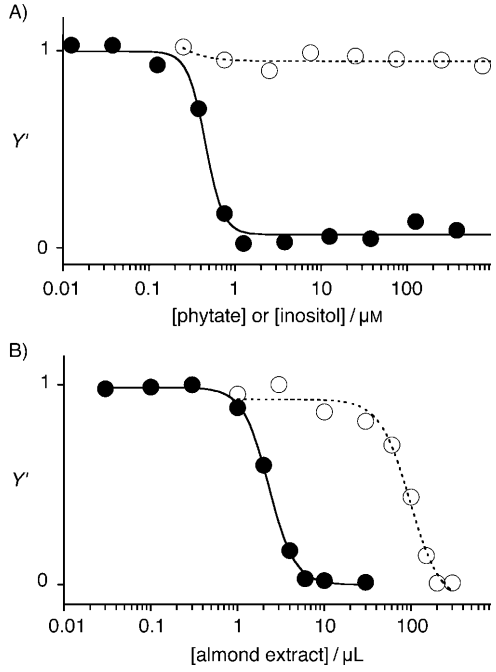

Figure 7. Dose–response curves for the inactivation of calf thymus DNA (1.25 μg mL^{-1}) and calixarene **15** (2.5 μM) in EYPC-LUV⊃HPTS/DPX by A) phytate (●) and inositol (○) and B) almond extract before (●) and after (○) phytase treatment. Fractional activities *Y'* are as in Figure 4.

Similar inactivation did not occur with the completely dephosphorylated inositol (Figure 7A, ○). Enzymatic phytate hydrolysis by incubation with phytase was detectable as fluorescence recovery caused by the activation of DNA–calixarene complexes (Figure S7). This successful example for the detectability of enzyme activity with DNA–calixarene complexes implied that other approaches developed for synthetic pores[23] and CPP–counterion complexes[7,8] will be applicable to DNA–counterion complexes as well.

The applicability of DNA–calixarene complexes to sensing in complex matrices was explored next. Almond extracts were prepared following routine procedures.[22] DNA–counterion complexes were efficiently inactivated by these almond extracts (Figure 7B, ●). Incubation of almond extracts with phytase practically removed their ability to inactivate DNA–counterion complexes (Figure 7B, ○). This demonstrated that the inhibitory activity of almond extract originated mainly from phytate. Comparison of the IC_{50} values before and after incubation with phytase with calibration curves afforded the expected phytate content (21 mg g^{-1} expected,[22] 25 ± 1 mg g^{-1} found).

The sensing of phytate and the related IP_7 has been explored previously with synthetic pores and CPP–counteranion complexes.[22] In CPP–counteranion complexes, phytate competes with the counteranion activator for binding to the polycation. In the complementary DNA–countercation complexes, phytate competes with the polyanion for binding to the countercation activator. Interestingly, phytate sensing turns out to be more sensitive with DNA ($IC_{50} = 450 \pm 30$ nM) than with CPP transporters ($IC_{50} = 5.4 \pm 1.1$ μM). However, counterion exchange in both sensing systems is less efficient than phytate binding within synthetic pores ($IC_{50} = 45 \pm 5$ nM). Successful phytate sensing implied that related sensing approaches will be applicable to DNA–counterion complexes as well,[23] including the recent method to sense hydrophobic analytes with CPP-counterion complexes.[8] However, the most important sensing applications of DNA–counterion transporters will take advantage from DNA chemistry in the broadest sense, including the use of aptamers,[13] enzymes, intercalators or more complex topologies.[24]

Conclusions

In this report, we provide experimental evidence that amphiphilic counterions can enable DNA to act as cation carrier, show that the active complexes can be used as detectors of enzyme activity and as biosensors in complex matrices, and we underscore that these results are important from a conceptual point of view.[2,3] Counteranions have been shown previously to activate polycationic CPPs[1] not only for cellular uptake[4] but also as anion carriers,[2] enzyme detectors[7] and biosensors,[8] and the theoretical basis of counteranion-mediated multifunctionality has been clarified.[2,3] Complementary to CPPs as weakly acidic polycations, DNA molecules are weakly basic polyanions. Countercations are very well known to activate DNA for cellular uptake.[9,10] The herein provided evidence that DNA–counterion complexes can function as cation carriers, enzyme detectors and biosensors is important because it demonstrates that the multifunctionality of polyion-counterion complexes is general and occurs with low-basicity polyanions exactly as with low-acidity polycations.

DNA transporters, detectors and sensors are of general and of practical interest. Application of the lessons learned with CPP sensors concerning hydrophobic analytes, signal amplification, and so on, will be straightforward and useful.[8,23] The perspective to use DNA chemistry for biosensing in fluorogenic,[15] chromogenic[25] or chirogenic[26] vesicles is particularly attractive. Current efforts focus on the use of DNA aptamers[13] as analyte-responsive transporters in membrane-based sensing systems. Preliminary results are promising and will be reported in due course.[24]

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Keywords: biosensors · calixarenes · DNA · ion pairs · membranes

[1] a) K. Inomata, A. Ohno, H. Tochio, S. Isogai, T. Tenno, I. Nakase, T. Takeuchi, S. Futaki, Y. Ito, H. Hiroaki, M. Shirakawa, *Nature* **2009**, *458*, 106–109; b) A. E. Jablonski, W. H. Humphries, C. K. Payne, *J. Phys. Chem. B* **2009**, *113*, 405–408; c) I. Nakase, T. Takeuchi, G. Tanaka, S. Futaki, *Adv. Drug Delivery Rev.* **2008**, *60*, 598–607; d) P. A. Wender, W. C. Galliher, E. A. Goun, L. R. Jones, T. H. Pillow, *Adv. Drug Delivery Rev.* **2008**, *60*, 452–472; e) A. Ziegler, *Adv. Drug Delivery Rev.* **2008**, *60*, 580–597; f) K. M. Stewart, K. L. Horton, S. O. Kelley, *Org. Biomol. Chem.* **2008**, *6*, 2242–2255; g) E. K. Esbjörner, P. Lincoln, B. Nordén, *Biochim. Biophys. Acta Biomembr.* **2007**, *1768*, 1550–1558; h) T. Shimanouchi, P. Walde, J. Gardiner, Y. R. Mahajan, D. Seebach, A. Thomae, S. D. Krämer, M. Voser, R. Kuboi, *Biochim. Biophys. Acta Biomembr.* **2007**, *1768*, 2726–2736; i) M. Tang, A. J. Waring, M. Hong, *J. Am. Chem. Soc.* **2007**, *129*, 11438–11446; j) J. M. Gump, S. F. Dowdy, *Trends Mol. Med.* **2007**, *13*, 443–448; k) E. P. Holowka, V. Z. Sun, D. T. Kamei, T. J. Deming, *Nat. Mater.* **2007**, *6*, 52–57; l) E. A. Goun, T. H. Pillow, L. R. Jones, J. B. Rothbard, P. A. Wender, *ChemBioChem* **2006**, *7*, 1497–1515; m) M. Martinell, X. Salvatella, J. Fernández-Carneado, S. Gordo, M. Feliz, M. Menéndez, E. Giralt, *ChemBioChem* **2006**, *7*, 1105–1113; n) I. Tsogas, D. Tsiourvas, G. Nounesis, C. M. Paleos, *Langmuir* **2006**, *22*, 11322–11328; o) Y. Wolf, S. Pritz, S. Abes, M. Bienert, B. Lebleu, J. Oehlke, *Biochemistry* **2006**, *45*, 14944–14954; p) S. Afonin, A. Frey, S. Bayerl, D. Fischer, P. Wadhwani, S. Weinkauf, A. S. Ulrich, *ChemPhysChem* **2006**, *7*, 2134–2142; q) R. Fischer, M. Fotin-Mleczek, H. Hufnagel, R. Brock, *ChemBioChem* **2005**, *6*, 2126–2142.

[2] a) N. Sakai, S. Matile, *J. Am. Chem. Soc.* **2003**, *125*, 14348–14356; b) M. Nishihara, F. Perret, T. Takeuchi, S. Futaki, A. N. Lazar, A. W. Coleman, N. Sakai, S. Matile, *Org. Biomol. Chem.* **2005**, *3*, 1659–1669; c) N. Sakai, T. Takeuchi, S. Futaki, S. Matile, *ChemBioChem* **2005**, *6*, 114–122.

[3] a) T. Takeuchi, N. Sakai, S. Matile, *Faraday Trans.* **2009**, *143*, 187–203; b) N. Sakai, S. Futaki, S. Matile, *Soft Matter* **2006**, *2*, 636–641.

[4] T. Takeuchi, M. Kosuge, A. Tadokoro, Y. Sugiura, M. Nishi, M. Kawata, N. Sakai, S. Matile, S. Futaki, *ACS Chem. Biol.* **2006**, *1*, 299–303.

[5] D. Schmidt, Q. X. Jiang, R. MacKinnon, *Nature* **2006**, *444*, 775–779.

[6] N. Sakai, N. Sordé, G. Das, P. Perrottet, D. Gerard, S. Matile, *Org. Biomol. Chem.* **2003**, *1*, 1226–1231.

[7] T. Miyatake, M. Nishihara, S. Matile, *J. Am. Chem. Soc.* **2006**, *128*, 12420–12421.

[8] S. M. Butterfield, T. Miyatake, S. Matile, *Angew. Chem.* **2009**, *121*, 331–334; *Angew. Chem. Int. Ed.* **2009**, *48*, 325–328.

[9] a) S. Bhattacharya, A. Bajaj, *Chem. Commun.* **2009**, 4632–4656; b) S. Fletcher, A. Ahmad, W. S. Price, M. R. Jorgensen, A. D. Miller, *ChemBioChem* **2008**, *9*, 455–463; c) L.-A. Tziveleka, A.-M. G. Psarra, D. Tsiourvas, C. M. Paleos, *J. Controlled Release* **2007**, *117*, 137–146; d) S. Horiuchi, Y. Aoyama, *J. Controlled Release* **2006**, *116*, 107–114; e) K. Kostarelos, A. D. Miller *Chem. Soc. Rev.* **2005**, *34*, 970–994; f) M. Sainlos, M. Hauchecorne, N. Oudrhiri, S. Zertal-Zidani, A. Aissaoui, J.-P. Vigneron, J.-M. Lehn, P. Lehn, *ChemBioChem* **2005**, *6*, 1023–1033; g) C. R. Dass, *J. Mol. Med.* **2004**, *82*, 579–591; h) S. Zhanga, Y. Xu, B. Wang, W. Qiao, D. Liu, Z. Li, *J. Controlled Release* **2004**, *100*, 165–180; i) R. Haag, *Angew. Chem.* **2004**, *116*, 280–284; *Angew. Chem. Int. Ed.* **2004**, *43*, 278–282; j) A. J. Kirby, P. Camilleri, J. B. F. N. Engberts, M. C. Feiters, R. J. M. Nolte, O. Söderman, M. Bergsma, P. C. Bell, M. L. Fielden, C. L. García Rodríguez, P. Guédat, A. Kremer, C. McGregor, C. Perrin, G. Ronsin, M. C. P. van Eijk, *Angew. Chem.* **2003**, *115*, 1486–1496; *Angew. Chem. Int. Ed.* **2003**, *42*, 1448–1457; k) P. L. Felgner, T. R. Gadek, M. Holm, R. Roman, H. W. Chan, M. Wenz, J. P. Northrop, G. M. Ringold, M. Danielsen, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 7413–7417.

[10] a) V. Bagnacani, F. Sansone, G. Donofrio, L. Baldini, A. Casnati, R. Ungaro, *Org. Lett.* **2008**, *10*, 3953–3956; b) F. Sansone, M. Dudi, G. Donofrio, C. Rivetti, L. Baldini, A. Casnati, S. Cellai, R. Ungaro, *J. Am. Chem. Soc.* **2006**, *128*, 14528–14536.

[11] a) J. Rebek Jr. , B. Askew, D. Nemeth, K. Parris, *J. Am. Chem. Soc.* **1987**, *109*, 2432–2434; b) P. Breccia, M. Van Gool, R. Pérez-Fernández, S. Martín-Santamaría, F. Gago, P. Prados, J. de Mendoza, *J. Am. Chem. Soc.* **2003**, *125*, 8270–8284; c) R. Pérez-Fernández, M. Pittelkow, A. M. Belenguer, J. K. M. Sanders, *Chem. Commun.* **2008**, 1738–1740.

[12] a) J.-L. Reymond, V. S. Fluxa, N. Maillard, *Chem. Commun.* **2009**, 34–46; b) A. Wada, S. Tamaru, M. Ikeda, I. Hamachi, *J. Am. Chem. Soc.* **2009**, *131*, 5321–5330; c) L. Vial, P. Dumy, *New J. Chem.* **2009**, *33*, 939–946; d) *Creative Chemical Sensing Systems. Topics in Current Chemistry* (Ed.: T. Schrader), Springer, Heidelberg, **2007**; e) A. Hennig, H. Bakirci, W. M. Nau, *Nat. Methods* **2007**, *4*, 629–632; f) A. Buryak, A. Pozdnoukhov, K. Severin, *Chem. Commun.* **2007**, 2366–2368; g) A. T. Wright, E. V. Anslyn, *Chem. Soc. Rev.* **2006**, *35*, 14–28; h) C. Guarise, L. Pasquato, V. De Filippis, P. Scrimin, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 3978–3982; i) M. Sanz Alaejos, F. J. García Montelongo, *Chem. Rev.* **2004**, *104*, 3239–3265; j) J. J. Lavigne, E. V. Anslyn, *Angew. Chem.* **2001**, *113*, 3212–3225; *Angew. Chem. Int. Ed.* **2001**, *40*, 3118–3130; k) N. A. Rakow, K. S. Suslick, *Nature* **2000**, *406*, 710–713.

[13] a) M. Li, N. Lin, Z. Huang, L. Du, C. Altier, H. Fang, B. Wang, *J. Am. Chem. Soc.* **2008**, *130*, 12636–12638; b) I. Willner, M. Zayats, *Angew. Chem.* **2007**, *119*, 6528–6538; *Angew. Chem. Int. Ed.* **2007**, *46*, 6408–6418; c) J. Liu, Y. Lu, *Angew. Chem.* **2006**, *118*, 96–100; *Angew. Chem. Int. Ed.* **2006**, *45*, 90–94.

[14] R. P. Haugland, *The Handbook. A Guide to Fluorescent Probes and Labeling Techniques*, 10th ed., Invitrogen, **2005**.

[15] S. Matile, N. Sakai, *The Characterization of Synthetic Ion Channels and Pores. In Analytical Methods in Supramolecular Chemistry* (Ed.: C. A. Schalley), Wiley, Weinheim, **2007**, pp. 391–418.

[16] B. A. McNally, A. V. Koulov, T. N. Lambert, B. D. Smith, J. B. Joos, A. L. Sisson, J. P. Clare, V. Sgarlata, L. W. Judd, G. Magro, A. P. Davis, *Chem. Eur. J.* **2008**, *14*, 9599–9606.

[17] S. Matile, A. Som, N. Sordé, *Tetrahedron* **2004**, *60*, 6405–6435.

[18] A. V. Koulov, T. N. Lambert, R. Shukla, M. Jain, J. M. Boon, B. D. Smith, H. Li, D. N. Sheppard, J. B. Joos, J. P. Clare, A. P. Davis, *Angew. Chem.* **2003**, *115*, 5081–5083; *Angew. Chem. Int. Ed.* **2003**, *42*, 4931–4933.

[19] a) P. Blondeau, M. Segura, R. Pérez-Fernández, J. de Mendoza, *Chem. Soc. Rev.* **2007**, *36*, 198–210; b) K. A. Schug, W. Lindner, *Chem. Rev.* **2005**, *105*, 67–113.

[20] a) J. M. Sanderson, E. J. Whelan *Phys. Chem. Chem. Phys.* **2004**, *6*, 1012–1017; b) A. N. J. A. Ridder, S. Morein, J. G. Stam, A. Kuhn, B. de Kruijff, J. A. Killian, *Biochemistry* **2000**, *39*, 6521–6528; c) W. M. Yau, W. C. Wimley, K. Gawrisch, S. H. White, *Biochemistry* **1998**, *37*, 14713–14718.

[21] a) F. Mora, D.-H. Tran, N. Oudry, G. Hopfgartner, D. Jeannerat, N. Sakai, S. Matile, *Chem. Eur. J.* **2008**, *14*, 1947–1953; b) B. K. Shoichet, *J. Med. Chem.* **2006**, *49*, 7274–7277; c) O. H. Straus, A. Goldstein, *J. Gen. Physiol.* **1943**, *26*, 559–585.

[22] S. M. Butterfield, D.-H. Tran, H. Zhang, G. D. Prestwich, S. Matile, *J. Am. Chem. Soc.* **2008**, *130*, 3270–3271.

[23] a) S. Litvinchuk, H. Tanaka, T. Miyatake, D. Pasini, T. Tanaka, G. Bollot, J. Mareda, S. Matile, *Nat. Mater.* **2007**, *6*, 576–580; b) G. Das, P. Talukdar, S. Matile, *Science* **2002**, *298*, 1600–1602.

[24] T. Takeuchi, S. Matile, unpublished results.

[25] S. M. Butterfield, A. Hennig, S. Matile, *Org. Biomol. Chem.* **2009**, *7*, 1784–1792.

[26] A. Hennig, S. Matile, *Chirality* **2008**, *20*, 932–937.

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