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Supramolecular micelle-based nucleopzymes for the catalytic oxidation of dopamine to aminochrome†

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Lipidated DNazymes or a lipidated Cu(II)-complex and lipidated aptamer sequences form supramolecular assemblies of micellar nucleopzymes for the enhanced oxidation of dopamine to aminochrome. The catalytic functions of the micellar nucleopzymes are attributed to the concentration of the substrate, using the aptamer units, in close proximity to the active sites.

Catalytically active nucleic acids (DNazymes) represent a novel class of bio-inspired catalysts that have attracted substantial research interest in recent years.¹ Different applications of DNazymes were reported, and these included their use as amplifying labels for sensing platforms,^{2,3} functional units for triggering DNA devices,⁴ triggers for programmed synthesis,⁵ functional components for logic gates and computing circuits,⁶ catalysts for driving chemical transformations,⁷ and catalytic units for the controlled release of loads (*e.g.* drugs from nano-carriers⁸). One of the most studied DNazymes is the hemin/G-quadruplex (hGQ), a horseradish peroxidase (HRP)-mimicking DNzyme.⁹ Similar to HRP, the hGQ DNzyme catalyzes the H₂O₂-mediated oxidation of organic substrates and the formation of chromophoric¹⁰ or fluorescent¹¹ products, or the generation of chemiluminescence in the presence of luminol and H₂O₂.¹² Also, the hGQ DNzyme induces a variety of oxidation processes, such as the oxidation of phenols,¹³ thiols,¹⁴ NADH,¹⁵ or aniline.¹⁶ Furthermore, the hGQ catalyzed the growth of nanoparticles¹⁷ and was found to control the aggregation of Au nanoparticles.¹⁸ The catalytic functions of the hemin/G-quadruplex were extensively applied to develop amplified optical and electrochemical sensing platforms,¹⁹ to synthesize conducting polymers²⁰ and assemble

supramolecular DNA switches and machines.²¹ Furthermore, continuous efforts are directed to improve the catalytic functions of peroxidase-mimicking DNazymes. For example, hemin/isoguanine pentaplexes were reported²² as new peroxidase-mimicking DNazymes. These DNazymes show, however, lower activities than native peroxidase and lack substrate selectivity. Recently, we reported that the functions of the hGQ DNzyme can be significantly enhanced by the conjugation of the hGQ to an aptamer that binds the substrate being oxidized by the hGQ catalytic site.²³ The DNzyme-aptamer conjugate was termed by us “nucleopzyme”, and its improved catalytic properties were attributed to the concentration of the substrate, by means of the aptamer, in spatial proximity to the catalytic site. That is, the nucleopzyme conjugates act as enzyme-mimicking structures. Specifically, we demonstrated the effective oxidation of dopamine and *N*-hydroxy-L-arginine by H₂O₂ to aminochrome and L-citrulline, respectively, using hGQ/anti-dopamine or hGQ/anti-L-arginine aptamer nucleopzyme structures. Furthermore, we demonstrated that by a rational design of hGQ-aptamer conjugates, structural rigidification could be realized, resulting in nucleopzymes with improved catalytic functions.²⁴ We realized, however, that other methods to assemble functional nucleopzyme structures may be envisaged. Specifically, we were intrigued by the possibility of using micelles formed by lipidated oligonucleotide sequences as functional nucleopzyme catalytic nanostructures.²⁵ That is, the integration of lipidated catalytic oligonucleotides and lipidated aptamers into micellar structures is anticipated to yield functional structures, where the substrate is concentrated in spatial proximity to the active sites, thus leading to enhanced catalytic functions of the supramolecular aggregate. Furthermore, dissociation of the micellar aggregate by means of surfactants allows us to inhibit the catalytic functions of the system. In the present study, we demonstrate the assembly of micellar nanostructures consisting of the lipidated hemin/G-quadruplex (hGQ) units and the lipidated dopamine binding aptamer (DBA) units. We reveal enhanced catalytic functions of the micellar aggregate toward the H₂O₂-mediated oxidation of dopamine to aminochrome, as compared to the separated lipidated hGQ and DBA units. Also, we demonstrate

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Fig. 1 Schematic depictions of: (A) the lipidated G-quadruplexes (1) and (2), and (B) the tetra-lipidated dopamine binding aptamer (DBA) sequence (3). The five residues that form the dopamine binding pocket are highlighted by the colored circles; these are replaced with thymine residues in the mutated sequence, lipodBA_m (4). (C) Schematic depiction of the DNAzyme/DBA–aptamer micelle structures composed of hemin/²lipoGQ (1) and lipodBA (3). (D) Schematic depiction of the hemin/⁴lipoGQ (2) and lipodBA (3). The H₂O₂-mediated micellar nucleopzyme-catalyzed oxidation of dopamine (1) to aminochrome (2) and the structure of hemin are also depicted in panel C.

that the hGQ DNAzyme catalytic units in the micellar structures can be substituted with an artificial catechol oxidase-mimicking lipidated dinuclear Cu(II)-complex that leads to the catalyzed H₂O₂-mediated oxidation of dopamine.

To assemble the desired catalytic nucleopzyme micellar nanostructures, we prepared various lipidated DNAzyme sequences. Specifically, we synthesized two versions of lipidated G-quadruplexes, *i.e.* di- and tetra-lipidated G-quadruplexes (²lipoGQ, 1, and ⁴lipoGQ, 2, respectively, Fig. 1A). We also prepared a lipidated dopamine binding aptamer, DBA, sequence that contained four lipidated 2'-deoxyuridines in the linker-region of the aptamer (lipoDBA, 3, Fig. 1B). In order to assess the effect of the five residues that were previously determined to form the dopamine binding site,²⁶ we prepared a mutated version of lipoDBA (3) in which these bases were substituted with thymines, resulting in the formation of lipodBA_m (4). All lipidated DNAs were prepared by previously described procedures,²⁷ purified by HPLC, and characterized by MALDI-TOF mass spectrometry (Fig. S1–S3, ESI†).

The critical micelle concentrations, CMCs, for the different lipidated G-quadruplex-functionalized DNAzymes and lipidated DBA aptamer were evaluated using 1,6-diphenyl-1,3,5-hexatriene (DPH) as a fluorescent probe.²⁸ The CMC values for the isolated components 1, 2, or 3, as well as for various ratios of the two components, *i.e.* 1 and 3, and 2 and 3 (see Fig. 1C and D for a schematic depiction) were evaluated. We find that the CMC values correspond to 8–9 μM for the isolated components (²lipoGQ, 1, ⁴lipoGQ, 2, and lipodBA, 3), as well as for various

Fig. 2 (A and B) Saturation curves for the various ratios of ²lipoGQ (1) (A) or ⁴lipoGQ (2) (B) with lipodBA (3). Ratios of hemin/lipoGQ (1, for A) or (2, for B): lipodBA present in the different systems: (a) 20% : 80%, (b) 40% : 60%, (c) 60% : 40%, and (d) 80% : 20%.

combinations of 1 and 3, or 2 and 3 (see Fig. S4, ESI†). We note that the CMC values were not significantly affected by the number of lipids attached to the G-quadruplex sequences: both the di- and tetra-lipidated G-quadruplex structures, and their mixture with the tetra-lipidated DBA sequence, revealed similar CMC-values of 8–9 μM. Accordingly, we applied a concentration that corresponded to 10 μM of the various micelle constituents in our subsequent dopamine oxidation studies so that the lipidated components were retained in the micellar structures.

Fig. 2(A) depicts the rates of oxidation of dopamine (5) to aminochrome (6) at different concentrations of dopamine by micelles composed of hemin/²lipoGQ (1) and lipodBA (3) at variable ratios of components (1) and (3). One may realize that the maximum efficiency is observed at a (1):(3) ratio corresponding to 40% : 60%. The maximum saturation rate of this system corresponds to $V_{\text{max}} = 62 \pm 7 \text{ nM s}^{-1}$. Fig. 2(B) shows the rates of oxidation at different concentrations of dopamine, using variable ratios of hemin/⁴lipoGQ (2) and lipodBA (3) as constituents of the micellar structures. We also observe, in this case, a maximum rate for the oxidation of (5) to (6) at a (2):(3) ratio that corresponds to 40% : 60%, yet with a lower $V_{\text{max}} = 38 \pm 5 \text{ nM s}^{-1}$ value. Here we note that the concentration of hemin introduced into different systems was equimolar to the concentration of the lipidated G-quadruplexes. This ensured that the catalytic oxidation rates originate from the hemin/G-quadruplex catalytic units in the respective structures. Evidently, as the relative concentrations of (1) to (3) or (2) to (3) increase up to 40% to 60%, the rates of oxidation of (5) to (6) are enhanced. A further increase of the content of (1) or (2) beyond this ratio results in a decrease in the rates of oxidation of (5) to (6) by the DNAzyme/aptamer micelles. The detailed catalytic parameters corresponding to the kinetics curves of the two micellar systems shown in Fig. 2(A) and (B) are

Table 1 Kinetic parameters of the various dopamine oxidizing micelles having different ratios of ²lipoGQ (1) or ⁴lipoGQ (2) and lipoDBA (3)

Entry	Ratio lipoGQ:lipoDBA	$V_{\max}$ (nM s ⁻¹)	k_{cat} (10 ⁻³ s ⁻¹)	K_M (μM)	
1	² lipoGQ (1)	2:8	19.8 ± 0.1	13.4	5.9 ± 0.4
2		4:6	61.9 ± 6.8	20.9	24.2 ± 12.3
3		6:4	56.7 ± 8.3	12.8	64.2 ± 30.5
4		8:2	52.8 ± 10.5	8.9	35.0 ± 10.5
5	⁴ lipoGQ (2)	2:8	17.9 ± 1.6	12.1	22.1 ± 9.4
6		4:6	38.4 ± 5.1	13.0	47.6 ± 22.7
7		6:4	30.7 ± 5.3	6.9	64.1 ± 35.9
8		8:2	32.5 ± 5.3	5.5	78.7 ± 38.6

Conditions: 20, 40, 80, 150, 250, and 500 μM dopamine (5), 1 mM H₂O₂. The micelles were composed of 10 μM lipoDNA, which contain 2, 4, 6, or 8 μM lipoGQ, either ²lipoGQ (1) or ⁴lipoGQ (2), which formed 1.48, 2.96, 4.44, or 5.92 μM catalytically active hGQ units (equals [catalyst]). Buffer: 5 mM MES, pH = 5.5, 200 mM KCl, 2 mM MgCl₂. Note: $k_{\text{cat}} = V_{\max}/[\text{catalyst}]$.

summarized in Table 1. Evidently, the $V_{\max}$ values in the two systems increase up to a ratio of 4:6 of (1):(3) or (2):(3), and then at higher contents of (1) or (2) the rates decrease. That is, even though the content of the catalytic sites increases, the overall catalytic oxidation rate of (5) to (6) is retarded. This is reflected by a substantial drop in the k_{cat} value of the systems that contain increased contents of (1) or (2) beyond the ratio of 4:6. We attribute the maximum catalytic performance of the micellar structures at this ratio to the optimal concentration of the dopamine substrate, using the aptamer units, close to the catalytic sites present in the micelles. Furthermore, the fact that the maximum rates for the oxidation of (5) to (6) are observed at a ratio, where the aptamer concentration in the micelles slightly exceeds the concentration of the hemin/G-quadruplex units implies that binding of the substrate and release of the product from the DBA sites are the rate-limiting steps in the oxidation process. Also, we note that the oxidation of (5) to (6) by the hemin/²lipoGQ (1) is *ca.* 2-fold more efficient than in the hemin/⁴lipoGQ (2) containing system (particularly visible at a 4:6 ratio). Presumably, the hemin/²lipoGQ unit is more flexible in the micellar structure, allowing the positioning of the catalytic center in a favored spatial organization with respect to the dopamine binding site of the aptamer, leading to enhanced catalytic functions of these micelles.

In further experiments, we evaluated the catalytic functions of the lipidated hemin/G-quadruplex and lipidated DBA micelles at a high concentration of dopamine, *i.e.* 500 μM at which saturation of the dopamine binding sites occurred (see Fig. 2), and compared the catalytic efficiencies to the separated non-micellar constituents. Fig. 3, curve a, shows the V_{corr} values of the micelles containing hemin/²lipoGQ (1) and lipoDBA (3) at different ratios (V_{corr} corresponds to $V_{\max}$ values that were corrected for the increase in activity caused by the increasing percentage of hemin/lipoGQ in the micelles, see Fig. S5, ESI[†]). Clearly, the rate of oxidation of (5) to (6) reaches an optimal value at a ratio of 4:6. Similar results were observed for the hemin/⁴lipoGQ (2) and lipoDBA (3) micelles (see Fig. S5, ESI[†]). Treatment of all micellar compositions with 0.1% triton X-100 separated the components and led to similar inefficient rates for the oxidation of (5) to (6),

Fig. 3 Corrected rate of oxidation of 500 μM dopamine (5) to aminochrome (6) in the presence of nucleosynzyme micelle systems composed of different ratios ²lipoGQ (1) and lipoDBA (3) (open circles, curve a). When 0.1% of triton X-100 was added, the corrected rates dropped significantly, up to 3.8-fold (closed circles, curve b).

Fig. 3, curve b. Furthermore, integration of the lipidated mutated DBA sequence, lipoDBAm (4), which has reduced affinity for the dopamine substrate, into the micellar structure that includes any of the lipidated hemin/G-quadruplexes, (1) or (2), yielded catalytic rates similar to those observed for the surfactant-induced separated components (Fig. S5, ESI[†]). These results highlight the significance of the DBA units in concentrating the substrate in spatial proximity to the active site within the micellar structure to yield the most active oxidation catalyst.

Furthermore, we explored the possibility of substituting the lipidated hemin/GQ catalytic sites in the micelles with a fully synthetic lipidated catechol oxidase-mimicking dinuclear Cu(II)-BPMP complex (BPMP = 2,6-bis[bis(2-pyridylmethyl)amino]-methyl-4-carboxymethylphenol).²⁹ The dinuclear Cu²⁺-BPMP complex was covalently anchored to 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine to yield the lipidated lipo-BPMP (Cu)₂ complex, (7), Fig. 4(A). In order to compare the activity of this complex with that of the hGQ DNAzyme, we applied a ratio of (7):(3) that corresponds to 40%:60%. Fig. 4(B), curve (a), depicts the time-dependent absorbance changes at λ = 480 nm observed upon the H₂O₂-mediated oxidation of 500 μM dopamine (5) to (6)

Fig. 4 (A) Structural formula of the lipidated catechol oxidase-mimicking dinuclear Cu(II)-BPMP complex (7). (B) Schematic depiction of the catalytic oxidation of dopamine to aminochrome by means of micelles composed of 60% lipoDBA (3) and 40% lipo-BPMP(Cu)₂ (7). The curves show the time-dependent formation of aminochrome by various systems (50 mM HEPES, pH = 7.0, 200 mM KCl, 2 mM MgCl₂, see the text for further details).

by the catalytic micelles. The rate of oxidation for this system corresponds to $15.4 \pm 0.4 \text{ nM s}^{-1}$. Fig. 4(B), curve (b), depicts the rate of oxidation of (5) to (6) by H_2O_2 in the presence of micelles of the same constituents but now treated with 0.1% triton X-100, leading to the separation of the micellar components and a drop in the rate to $6.2 \pm 0.3 \text{ nM s}^{-1}$. Furthermore, micelles composed of only lipo-BPMPCu₂, i.e. which lack the dopamine binding site, displayed a rate of $7.8 \pm 0.5 \text{ nM s}^{-1}$, Fig. 4(B), curve c, whereas micelles composed of only lipoDBA (3) in the presence of $10 \mu\text{M}$ non-complexed Cu(II) displayed a rate of $4.1 \pm 0.8 \text{ nM s}^{-1}$, Fig. 4(B), curve d. We note, however, that the rate of oxidation of dopamine (5) to aminochrome (6) by the (7)/(3) micelles is substantially lower compared to that of the hemin/²lipoGQ (1)/(3) micelles ($15.4 \pm 0.4 \text{ nM s}^{-1}$ vs. $57 \pm 6 \text{ nM s}^{-1}$, respectively). Nevertheless, the results demonstrate the successful catalytic oxidation of dopamine by micelles composed of the catechol oxidase-mimicking catalyst (7) and the dopamine binding aptamer, DBA.

In conclusion, the present study has introduced a novel approach to construct organized nucleopzyme nanostructures consisting of micelles composed of lipidated hemin/GQ or lipidated dinuclear Cu²⁺-complexes as catalytic units and the lipidated dopamine binding aptamer. The association between the substrate and aptamer with the catalytic micelles led to the concentration of the substrate at a close spatial position with respect to the catalytic sites, resulting in enhanced oxidation of the substrate. We observe, however, only moderate catalytic enhancement for the oxidation processes. This may originate from non-optimal positioning of the catalytic site with respect to the substrate–ligand site and/or due to the flexibilities of the micellar structures. By further optimization of the lengths of the lipidated chains, and eventually by rigidification of the micelles by crosslinking, the catalytic performance of the micellar nucleopzymes could be improved.

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