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Catalytically active nucleic acids (DNAzymes) represent a novel class of bio-inspired catalysts that have attracted substantial research interest in recent years. Different applications of DNAzymes were reported, and these included their use as amplifying labels for sensing platforms, functional units for triggering DNA devices, triggers for programmed synthesis, functional components for logic gates and computing circuits, catalysts for driving chemical transformations, and catalytic units for the controlled release of loads (e.g. drugs from nano-carriers). One of the most studied DNAzymes is the hemin/G-quadruplex (hGQ), a horseradish peroxidase (HRP)-mimicking DNAzyme. The catalytic functions of the micellar nucleoazymes are attributed to the concentration of the substrate, using the aptamer units, in close proximity to the active sites.

Supramolecular micelle-based nucleoazymes
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 nucleoazymes for the enhanced oxidation of dopamine to aminochrome. The catalytic functions of the micellar nucleoazymes are attributed to the concentration of the substrate, using the aptamer units, in close proximity to the active sites.

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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 H$_2$O$_2$-mediated oxidation of dopamine to aminochrome.

To assemble the desired catalytic nucleoazyme 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 (lipoGQ, 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, we prepared a mutated version of lipoDBA (3) in which these bases were substituted with thymines, resulting in the formation of lipoDBAm (4). All lipidated DNAs were prepared by previously described procedures, 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. 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 (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 (lipoGQ, 1, 4lipoGQ, 2, and lipoDBA, 3), as well as for various 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 of 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/4lipoGQ (1) or 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
summarized in Table 1. Evidently, the \( V_{\text{max}} \) 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 \( k_{\text{cat}} \) 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/\( \text{lipoGQ} \) (1) is ca. 2-fold more efficient than in the hemin/\( \text{lipoGQ} \) (2) containing system (particularly visible at a ratio of 4:6). Presumably, the hemin/\( \text{lipoGQ} \) 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 \( \mu \text{M} \) 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 \( V_{\text{corr}} \) values of the micelles containing hemin/\( \text{lipoGQ} \) (1) and lipoDBA (3) at different ratios (\( V_{\text{corr}} \) corresponds to \( V_{\text{max}} \) values that were corrected for the increase in activity caused by the increasing percentage of hemin/\( \text{lipoGQ} \) in the micelles, see Fig. S5, ESI\(^f\)). 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/\( \text{lipoGQ} \) (2) and lipoDBA (3) micelles (see Fig. S5, ESI\(^f\)). 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), compared to the catalytic efficiencies to the separated non-micellar constituents. Fig. 3, curve a, shows the time-dependent absorbance changes at \( \lambda = 480 \text{ nm} \) observed upon the \( \text{H}_2\text{O}_2 \)-mediated oxidation of 500 \( \mu \text{M} \) dopamine (5) to (6)
by the catalytic micelles. The rate of oxidation for this system corresponds to 15.4 ± 0.4 nM s⁻¹. Fig. 4(B), curve (b), depicts the rate of oxidation of (5) to (6) by H₂O₂ 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 ± 0.3 nM s⁻¹. Furthermore, micelles composed of only lipo-BPMPCu₂ (3) in the presence of 10 μM non-complexed Cu(II) displayed a rate of 4.1 ± 0.8 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 hemin²lipoGQ (1)/(3) micelles (15.4 ± 0.4 nM s⁻¹ vs. 57 ± 6 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 nucleo-enzyme nanostructures consisting of micelles composed of lipidated hemin/GQ or lipidated dinuclear Cu²⁺-complexes as catalytic units and the approach to construct organized nucleoapzyme nanostructures.