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Supramolecular Cocaine−Aptamer Complexes Activate Biocatalytic Cascades

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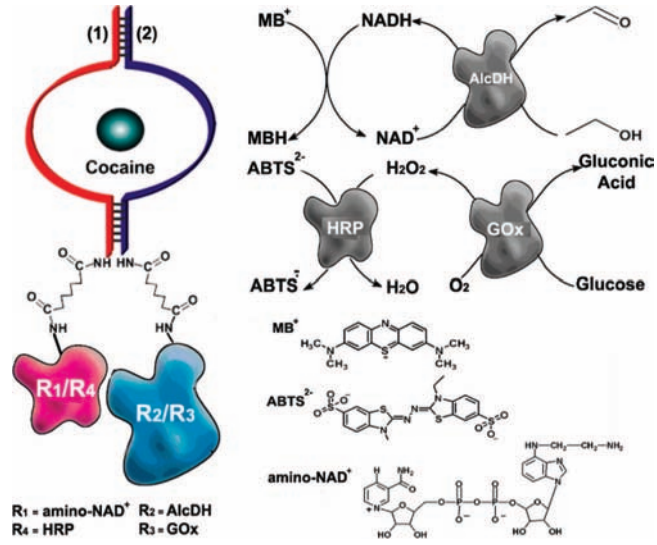
The molecule- or ion-induced self-assembly of complex supramolecular structures such as wires,[1] grids,[2] or capsules[3] attracts recent research efforts aimed to use molecular (or ionic) building blocks to construct nanoscale molecular electronic components, nanocontainers or nanotransporters, or new catalysts. Similarly, supramolecular macromolecular[4] or macromolecule−nanoparticle[5] composites were tailored as functional structures for programmed catalysis,[6] information storage,[7] controlled wettability,[8] the assembly of solar cells,[9] and optoelectronic[10] or nanoscale devices.[11] The self-assembly of biomolecular supramolecular structures combines the unique recognition and catalytic properties of biomolecules to yield systems of predesigned functionalities.[12] For example, supramolecular aptamer−protein interactions led to self-assembly of protein nanowires,[13] and DNA−DNA hybridization led to the programmed organization of linear polycatenated nanowires[14] or 2D and 3D nanostructures.[15] The base sequences of nucleic acids control their binding and catalytic properties (aptamers and DNAzymes, respectively), and supramolecular structures of aptamers and DNAzymes have been implemented for sensing, nanobiotechnology, and logic gate operations.[16] For example, supramolecular complexes of aptamer fragments were used to develop a series of logic gates.[17] In the present study we report on the use of two different enzymes tethered to the anti-cocaine aptamer fragments, or nicotinamide adenine dinucleotide (NAD^+)/enzyme tethered to the anti-cocaine aptamer fragments,[18] as functional components that self-assemble, in the presence of cocaine, to supramolecular structures that activate biocatalytic cascades. No analogous biocatalytic transformations occur in a diffusion-controlled homogeneous mixture of the biomolecular components. Furthermore, as the mutual orientation of the two enzymes, or the cofactor−enzyme units, on the aptamer fragments is regulated by the formation of the aptamer−substrate supramolecular structure, control over the functional reactivity of the biocatalytic systems is dictated by the concentration of the substrate.

N^6-(2-Aminoethyl)-nicotinamide adenine dinucleotide (amino-NAD^+) was covalently tethered to the nucleic acid (**1**) by the bis(sulfosuccinimidyl) suberate, BS^3, cross-linker. Nucleic acid (**1**) includes the base sequence that corresponds to one segment of the anti-cocaine aptamer. Nucleic acid (**2**) was covalently linked to the NAD^+-dependent enzyme, alcohol dehydrogenase, AlcDH, by the BS^3 linker. The average loading of AlcDH corresponded to ca. 3 nucleic acids per protein. The activity of the (**2**)-modified AlcDH corresponded to ca. 90% of the native enzyme activity. The nucleic acid (**2**) includes the base sequence of the second segment of the anti-cocaine aptamer. Addition of cocaine to the system resulted in the formation of the supramolecular complex between cocaine and the two aptamer segments, Scheme 1. The close proximity between the cofactor unit and the enzyme, AlcDH, activated the electron transfer communication between the components, and this stimulated the biocatalyzed oxidation of ethanol. The AlcDH-mediated oxidation of ethanol yields the reduced NADH cofactor, and this reduces Methylene Blue, MB^+, to the colorless product that enables us to probe the kinetics of the biocatalytic reaction. Figure 1 curve (a) depicts the time-dependent absorbance changes of MB^+ in the presence of the NAD^+-modified (**1**) and the (**2**)-functionalized AlcDH, in the presence of ethanol, 10 mM, without adding cocaine to the system. Minute depletion of the absorbance of the dye is observed, implying that under the experimental conditions no electron transfer between the enzyme and the cofactor occurs, and thus, the biocatalyzed oxidation of ethanol is prohibited. Figure 1, curves (b) to (f), shows the time-dependent spectral changes of the system upon adding different concentrations of cocaine. As the concentration of cocaine increases, the biocatalyzed oxidation of ethanol is enhanced. These results are consistent with the formation of the supramolecular complex between the two aptamer fragments and cocaine. The localization of the NAD^+ cofactor and AlcDH in the supramolecular structure facilitates the biocatalyzed reduction of

Scheme 1. Cocaine-Induced Self-Assembly of Cofactor/Enzyme or Enzyme/Enzyme-Tethered Aptamer Fragments into Coupled Biocatalytic Systems



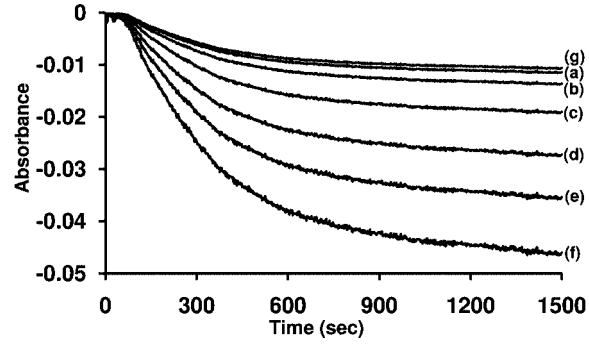


Figure 1. Time-dependent absorbance changes corresponding to the reduction of MB^+ by the NAD^+/AlcDH supramolecular complex generated upon the addition of variable concentrations of cocaine: (a) 0 M, (b) 5×10^{-7} M, (c) 1×10^{-6} M, (d) 1×10^{-5} M, (e) 1×10^{-4} M, (f) 1×10^{-3} M, (g) negative control where sequence (**1**) was substituted by the perturbed aptamer (**3**) in the presence of 1×10^{-3} M cocaine (see Supporting Information). All experiments were performed in Tris buffer (10 mM, pH 7.4, 100 mM NaCl).

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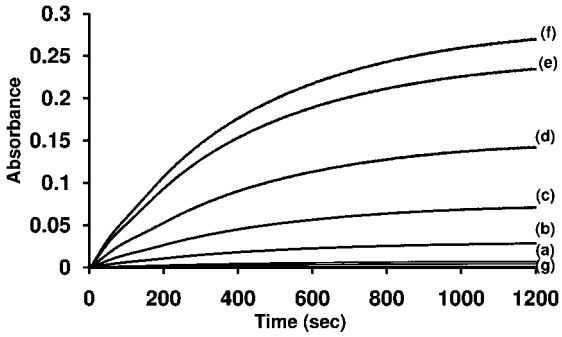

Figure 2. Time-dependent absorbance changes corresponding to the oxidation of $ABTS^{2-}$ by the GOx/HRP enzyme cascade activated by the supramolecular aptamer complex generated by variable concentrations of cocaine: (a) 0 M, (b) 5×10^{-7} M, (c) 1×10^{-6} M, (d) 1×10^{-5} M, (e) 1×10^{-4} M, (f) 1×10^{-3} M, (g) negative control where sequence (**1**) was substituted by the perturbed aptamer (**3**) in the presence of 1×10^{-3} M cocaine (see Supporting Information). All experiments were performed in phosphate buffer (10 mM, pH 7.4, 100 mM NaCl).

NAD^+ to NADH, resulting in enhanced reduction of MB^+. As the concentration of cocaine is higher, the formation of the supramolecular complex is favored, resulting in faster depletion of MB^+.

The cocaine−aptamer complex was further used to activate an enzyme cascade. Glucose oxidase, GOx, was functionalized with the nucleic acid (**2**) by BS[3]. Similarly, horseradish peroxidase, HRP, was modified with the nucleic acid (**1**). The average loading of the (**2**)-functionalized GOx and of the (**1**)-modified HRP corresponded to 3.5 and 6 nucleic acids per protein, respectively, and their activities corresponded to ca. 90% of the native enzymes activities. Scheme 1 depicts the schematic activation of the enzyme cascade by the cocaine−aptamer complex. The GOx-mediated oxidation of glucose yields gluconic acid and H_2O_2 as products. The H_2O_2 formed acts as the substrate for HRP that catalyzes the oxidation of 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonate), $ABTS^{2-}$ to the colored product, $ABTS^{\bullet-}$ ($\lambda = 414$ nm). Figure 2 curve (a) shows the time-dependent absorbance of the system at $\lambda = 414$ nm consisting of the (**2**)-functionalized GOx and (**1**)-modified HRP, in the presence of glucose, 25 mM, with no added cocaine. A minute increase in the absorbance is detected, indicating that the two enzymes do not communicate one with another, under the experimental conditions. Figure 2, curves (b) to (f), shows the time-dependent absorbance changes of the systems that include variable concentrations of cocaine. Addition of cocaine activates the biocatalytic cascade, and as the concentrations of cocaine increase, the rate of the two-enzyme cascade is enhanced. These results are consistent with the fact that higher cocaine concentrations favor the formation of the supramolecular cocaine−aptamer complex that brings to proximity the GOx-HRP enzymes. The H_2O_2 generated by the GOx-mediated oxidation of glucose yields a high local concentration of H_2O_2 close to the HRP active site, leading to the effective oxidation of $ABTS^{2-}$. The initial rates of $ABTS^{2-}$ oxidation increase as the concentration of glucose is elevated, and the rate levels off to a saturation level, consistent with the complete assembly of the two aptamer fragments in the form of the cocaine−aptamer complex (see Supporting Information). Assuming that the rate of $ABTS^{2-}$ oxidation relates directly to the concentration of the cocaine−aptamer complex, the derived dissociation constant of the cocaine−aptamer complex corresponds to ca. $K_d = 200\ \mu M^{-1}$, consistent with the previously reported value.[18] The rate of the enzyme cascade is controlled by the biocatalytic oxidation of glucose that follows the Michaelis−Menten kinetic model (see Supporting Information, Figure S1).

It should be noted that the activation of the biocatalytic transformations by the cocaine−aptamer supramolecular complex might also be viewed as a method for the amplified detection of cocaine. Indeed, the two methods enabled the analysis of cocaine with a detection limit of 5×10^{-7} M (for the selectivity of the system, see Supporting Information). It should be noted that the absence of Mg^{2+}, the low salt concentration, and the low concentration of the nucleic acid fragments prohibited the activation of the biocatalytic cascade by sole base-pairing hybridization. Furthermore, a control experiment that substituted a segment in the anticocaine aptamer by a foreign sequence did not yield catalytically active constructs in the presence of cocaine (see Supporting Information, and Figure 1 curve (g), Figure 2 curve (g)).

To conclude, the present study has introduced the use of supramolecular aptamer−substrate complexes as organized structures that controls biocatalytic transformations that do not proceed (at similar experimental conditions) in a random, nonorganized system. This paradigm can be extended to numerous biocatalytic scaffolds of further complexity. Such biocatalytic structures may be used to amplify aptamer−substrate recognition events. Furthermore, the present system demonstrated the activation of biocatalytic reactions by aptamer structures and, thus, may be considered as a biomimetic prototype for systems biology.

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Supporting Information Available: Sequences and synthesis of the functionalized aptamer fragments, a set of further control experiments, the selectivity of the resulting complex, the Michaelis−Menten analysis of the enzyme cascade, and the model of the cocaine−aptamer supramolecular complex are provided. This information is available free of charge via the Internet at http://pubs.acs.org.

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