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Using enzymatic amplification by aldolase for the optical detection of DNA by an artificial signal cascade

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ABSTRACT

A two-step reaction cascade is applied to the sequence-specific detection of single-stranded DNA, including analyte-triggered re-activation of apo-aldolase by its cofactor Zn^{2+} and catalytic conversion of a chromophore.

Sensing in living systems depends, on the cellular level, on signal cascades, that is, a chain of steps, which usually results in a small stimulus eliciting a large response. Such cascades combine several signal transduction and amplification steps. Application of nature-inspired, allosteric signal transduction strategies for the development of novel bio- and chemosensors is an emerging research field.[1] We have recently described the prototype of an autonomous two-step artificial signal cascade which includes allosteric transduction and catalytic amplification.[2] The concept was applied to the sequence-specific detection of DNA,[3] the 'primary signal', which triggers the release of a metal ion (Cu^{2+}), and the latter assembles as a cofactor with a precatalyst into the active, signal-amplifying chemical catalyst. A significant limitation is the low turnover frequency of the chemical catalyst, which results in poor amplification and long response time.

We were therefore exploring the re-activation of apoenzymes by their metal ion cofactors[4] as an alternative amplification strategy. Carbonic anhydrase (CA), a zinc-containing enzyme which catalyzes the hydration of CO_2, was selected due to its very high turnover rate. While response time of the CA-based system is short, its application is complicated by the use of gaseous CO_2, and its sensitivity is limited by spontaneous background hydration of CO_2.[5]

Here, we describe the extension of these studies to fructose-1,6-bisphosphate aldolase, which catalyzes the cleavage of fructose-1,6-bisphosphate into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (Scheme 1). Reagents are easily handled and uncatalyzed background conversion of the substrate is slow. The zinc(II)-dependent, dimeric Class II aldolase[6,7] was isolated from yeast. Substrate conversion is monitored indirectly by coupled enzymatic reactions, which result in the consumption of NADH (Scheme 1).

Enzyme isolation and apoenzyme synthesis. Isolation and purification of aldolase from baker's yeast was done according to the procedure reported by Belasco et al.[8] The yield was 30 mg with a purity of 67%. The apoenzyme was obtained by incubating aldolase with EDTA (pH 7.5) and by purifying by size exclusion chromatography. Concentration of the apoenzyme was determined by measuring absorbance at 280 nm ($\varepsilon_{280}^{0.1\%} = 1.02\ (mg/ml)^{-1}\ cm^{-1}$).[6] The resulting Zn content was determined by flame atom absorption spectroscopy as 2%. Further details are reported in the Supporting Material.

DNA detection. As described previously,[9] 20 mer DNA **1** modified by the tridentate chelator 2,2′:6′,2″-terpyridine (tpy) at both 3′ and 5′ termini, forms a stable circular 1:1-complex with Zn^{2+} ions, in which the metal ion is coordinated by both tpy moieties with a very high stability constant $\log K = 15$. Formation of the Zn complex, which is not decomposed by 10-fold excess EDTA ($\log K_{eff} = 13$ at pH 7), is followed by UV spectroscopy ($\lambda_{max} = 320$ nm). On addition of complementary DNA **2**, the bis-chelation of the metal ion is disrupted due to formation of the rigid double helix, and the metal complex is destabilized (Scheme 2).[5] Complexes between Zn^{2+} and one tpy-moiety of DNA **1**, and eventually formed intermolecular complexes of Zn^{2+} with two tpy-modified hybrids, are much less stable; 320 nm absorbance of the corresponding DNA **1**–Zn-mixtures is strongly reduced on addition of one equiv-

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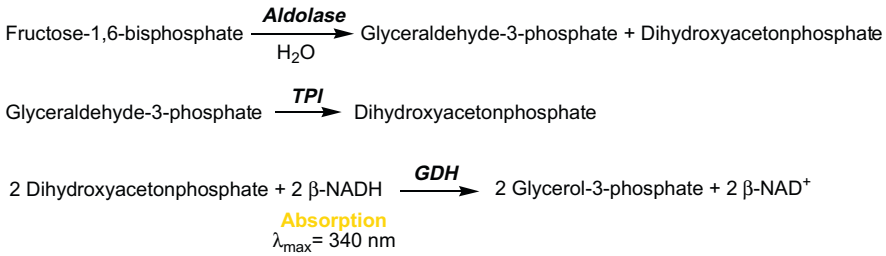
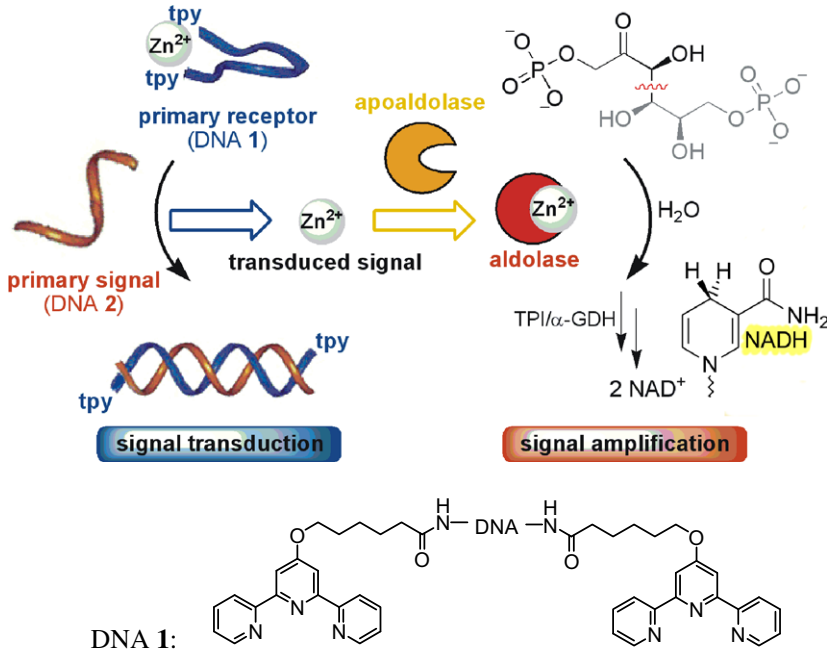


Scheme 1. Aldolase-catalyzed cleavage of fructose-1,6-bisphosphate, and coupled enzymatic reactions (TPI = Triose-phosphate isomerase, GDH = Glycerol-3-phosphate dehydrogenase), which allow indirect monitoring of substrate conversion by photometry of NADH concentration.



Scheme 2. Top: the two-step reaction cascade includes allosteric signal transduction (DNA **2** to Zn^{2+}) and enzymatic signal amplification. DNA **1**: 5′-tpyd(ATCGTTAC CAAAGCATCGTA)tpy, complementary DNA **2**: 5′-d(TACGATGCTTTGGTAACGAT). Bottom: structure of DNA **1**.

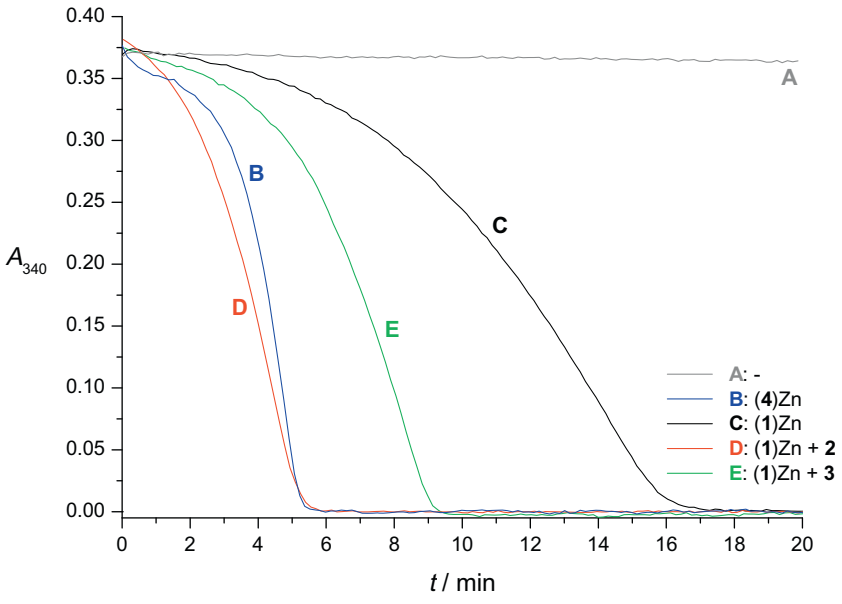


Figure 1. Detection of 20 mer DNA **2** by activation of fructose-1,6-bisphosphate aldolase, indirectly monitored by decrease of NADH absorbance at 340 nm with time. All reaction solutions contain 20 nM apoaldolase, 1.9 mM fructose-1,6-bisphosphate, 0.13 mM β-NADH, 1.7 U GDH/TPI (relating to GDH), 0.1 M KOAc, 0.1 M NaCl, 10 mM $MgCl_2$ and (except A) 50 nM $ZnSO_4$, respectively, at pH 7.3 (90 mM Hepes) and 25 °C. In addition, B–E contain 100 nM of the indicated DNA oligomer (**1**, **2**, **3**, or **4**, see legend). DNA 1: 5′-tpyd(ATCGTTACCAAAGCATCGTA)tpy, complementary DNA 2: 5′-d(TACGATGCTTTGGTAACGAT), mismatch DNA 3: 5′-d(TACTATGCTTTGGTAACGAT), mono tpy substituted DNA 4: 5′-tpyd(ATCGTTACCAAAGCATCGTA).

alent of the relatively weak Zn binder nitrilotriacetate ($\log K_{eff} = 8$ at pH 7). When apoaldolase is present in the reaction mixture in subμM concentration, it binds the released Zn^{2+}. Activity of the holoenzyme was indirectly monitored by a literature reported assay, observing the decrease of the absorbance of NADH ($\lambda_{max} = 340$ nm) with time (Scheme 2). Absorbance of the tpy-Zn moiety is close to zero at 340 nm and does not interfere with the photometric monitoring of NADH (see Fig. 1).

The decrease of NADH concentration in the presence and absence of complementary DNA is shown in Figure 1. In the absence of any Zn^{2+}-containing compounds, the background reaction of the apoenzyme is negligible (gray line A). Background reaction rate in the presence of the probe (DNA **1**)Zn, but in absence of complementary DNA (black line C) is initially slow, too, but increases with time, possibly due to slow spontaneous transfer of Zn^{2+} from the probe to the enzyme. Complementary DNA **2** at 100 nM concentration strongly accelerates NADH conversion (red line D), while single mismatch DNA **3** (green line E) is less effective. A 5′-mono tpy substituted DNA **4** (blue line B) does not strongly bind Zn^{2+} so that the aldolase is activated even in the absence of complementary DNA. The detection limit for DNA **2** is in the order of 20 nM at 20 nM probe concentration.

In conclusion, the zinc-dependent enzyme aldolase has been applied as a signal-amplifying catalytic module for the optical detection of DNA by an autonomous artificial signal cascade. A major complication previously observed with carbonic anhydrase, the significant uncatalyzed background reaction, is overcome by aldolase. In addition, compatibility of the novel DNA detection principle with a coupled enzymatic assay including three different enzymes was demonstrated.

Optimization of the catalytic module of the artificial signal cascade is another step toward the development of a fast, robust, and simple assay for the detection of nucleic acid sequences. Lowering the detection limit to the subnanomolar concentration range remains a challenge.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.102.

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