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Hemin-utilizing G-quadruplex DNAzymes are strongly active in organic co-solvents

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ABSTRACT

The widespread use of organic solvents in industrial processes has focused in recent years on the utility of "green" solvents – those with less harmful environmental, health, and safety properties – such as methanol and formamide. However, protein enzymes, regarded as green catalysts, are often incompatible with organic solvents. Herein, we have explored the oxidative properties of a Fe(III)-heme, or hemin, utilizing catalytic DNA (heme·DNAzyme) in different green solvent-water mixtures. We find that the peroxidase and peroxygenase activities of the heme·DNAzyme are strongly enhanced in 20–30% v/v methanol or formamide, relative to water alone. Protic solvent content of >30% v/v gradually diminishes heme·DNAzyme catalytic activity; however, the heme·DNAzyme is still active in as high as 80% v/v methanol. In contrast to protic solvents, aqueous dimethylformamide solutions largely inhibit heme·DNAzyme activity. In view of the strong catalytic activity of heme·DNAzyme in aqueous methanol, we were able to determine that a 60% v/v methanol-water mixture gives the most optimal yield of the dibenzothiophene sulfoxide (DBTO) oxidation product of petroleum-derived dibenzothiophene (DBT). The high product yield reflects both DNAzyme catalysis and a high substrate availability. Overall, these results emphasize the excellent promise of G-quadruplex forming DNA catalysts in application to "greener" industrial chemistry.

1. Introduction

In industrial chemistry organic solvents find wide use, owing to the poor water solubility of many organic compounds and also the relative ease of product purification and isolation from organic solvents [1]. Given the common use of environmentally problematic solvents in industry, it is of little surprise that efforts are being made to promote the use of "green" solvents – those solvents with promising environmental, health, and safety properties [2,3]. Of note, methanol and methanol/water mixtures are commonly included among these green solvents [4]. However, the use of enzymes, ideal "green" catalysts, in green solvents is limited since many enzymes lose much of their activity in organic solvents [3]. For this reason, much investigation has focused on enzymatic catalysis under conditions of minimal water. This has included the use of pegylated (polyethylene glycol-modified) enzymes [5–7], biphasic solvent systems [8], lyoprotectants [9], organic salts [10], and immobilization [11].

The heme enzymes, including peroxidases and monooxygenases (such as the Cytochromes P450) have been the subject of significant interest as industrial reagents. However, as a rule, heme enzymes exhibit substantially poorer activity in water-organic solvent mixtures. Some cytochrome P450 enzymes become notably less active in as little as 5% of dimethyl sulfoxide (DMSO), methanol, ethanol, or acetonitrile (v/v) [12,13]. Similar effects have been observed with horseradish peroxidase (HRP), whose activity is negatively affected by the presence of ethanol, acetone, tetrahydrofuran, acetonitrile, and DMSO [14,15]. Such incapacity has stimulated research into pegylation of HRP (PEG-HRP modifications [16–18]) and in HRP immobilization [10,13]. The one heme enzyme that appears to be a relatively promising catalyst in aqueous organic solvent systems is chloroperoxidase. Van Deurzen et al. [19] found chloroperoxidase to retain 100% activity in up to 30% t-butanol, albeit with activity decrease at higher concentrations of the alcohol. However, even for chloroperoxidase the choice of organic co-solvent is limited, since solvents such as methanol and DMSO are bona fide substrates for this enzyme [20–22].

In light of these limitations, it is interesting to note that certain DNA folds (such as G-quadruplexes) maintain their native structure in water-organic solvent mixtures [23]; indeed, ethanol has been reported to actively stabilize G-quadruplexes [24]. Therefore, catalytic DNAs

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(DNAzymes) may prove to be valuable reagents in this area of research. Indeed, a series of earlier studies on ribozymes and DNAzymes that do not form G-quadruplexes report their persistently high activity in organic co-solvents [25–28].

A family of DNAzymes of particular interest to us, G-quadruplexes capable of binding and activating heme, were first reported in the 1990s [29]. We demonstrated that ferric heme [Fe(III)-protoporphyrin IX] binds tightly to guanine-rich single-stranded DNAs that fold to form G-quadruplexes [29,30]. The complexation is usually tight, with dissociation constant (K_d) values as low as ~10 nM [31]. These DNA-heme complexes display a number of remarkable properties, including UV–vis spectra closely resembling those of ferric hemoproteins such as metmyoglobin [29]. Most notably, these complexes show a strong, DNA-enhanced, oxidative activity — both 1-electron [29] and 2-electron oxidation [32]—with initial kinetics of substrate oxidation typically of the order of those observed with proteinaceous heme enzymes such as peroxidases and monooxygenases [29,32–34]. Such oxidative catalysis by heme-G-quadruplex complexes (henceforth referred to as heme·DNAzymes) requires the participation of an oxidant, such as hydrogen peroxide (or of dioxygen in the presence of reducing agents including NADH [34] or ascorbate [35]. Unlike hemoproteins, in which the heme prosthetic groups are buried more or less deeply the protein, the heme moiety in heme·DNAzymes remains stacked on the surface of the DNA G-quadruplex, free to interact with the environment. Travascio et al. [36] identified that with this particular exposure to the solvent, the iron moiety within heme·DNAzymes exists in a six-coordinate, high-spin state (this observation has been corroborated recently by an NMR study—Ref. [38]). Six-fold coordination implies the existence of two axial ligands to the heme iron, one of which is a water molecule on the outward face of the heme (distal to the DNA). It is inferred that this water ligand can exchange with hydrogen peroxide, leading to heme activation and oxidative catalysis [29,36,37].

Fig. 1 shows, schematically, both the make-up of a heme·DNAzyme, and a classic 2-electron oxidation reaction (of a thioether to a sulfoxide) known to be catalyzed by these DNAzymes [32,39]. A recent study on the potential compatibility of heme·DNAzymes with pure organic solvents used the pegylation approach that has been used with proteinaceous heme enzymes [16–18]. It was found that robustly pegylated DNAzymes do indeed show catalytic activity in methanol [40]. Nevertheless, the compatibility (solubility as well as persistence of catalytic properties) of natural, non-PEG modified heme·DNAzymes in organic solvents and solvent/water mixtures has not been seriously investigated to date. We have therefore tested the activity of a typical heme·DNAzyme ("G4"), with proven peroxidase and oxygenase activity, in a range of organic solvent-water mixtures. We have backed up our observations on oxidative catalysis with, wherever possible, UV–vis and CD spectroscopy, to determine the status of the heme and the DNA components of the heme·DNAzyme. Finally, we have used our novel findings to determine the optimal conditions for the use of green solvents in oxidizing poorly water-soluble, industrially relevant compounds such as dibenzothiophene. Findings from these tests exemplify the high potential for the use of organic solvents in heme·DNAzyme catalysis.

2. Materials and methods

2.1. Materials

Oligonucleotides – All oligonucleotides used in this study were purchased from Integrated DNA Technologies and the University of Calgary CORE DNA Services. Sequences used in this study were: G4 (CatG4) (5′-TGGGT AGGGC GGGTT GGGAA A-3′), SS (5′-AATAC GACTC ACTAT AGGAA GAGAT G-3′), and $T_4G_5T_4$ (5′-TTTTG GGGGT TTT-3′). $T_4G_5T_4$ was used to generate a tetramolecular G-quadruplex, $(d\text{-}T_4G_5T_4)_4$. Hemin was purchased from Frontier Scientific, Inc. Fresh 1 mM heme stocks were made as required by dissolving hemin in dimethylformamide. *Chromogenic substrates* – Amplex Red (Resorufin), was purchased from Sigma Aldrich, and dissolved in dimethylformamide to create a 10 mM stock solution. ABTS [2.2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] was purchased from Sigma Aldrich. Fresh ABTS stock solutions were made for each separate experiment, by dissolving ABTS in ddH_2O to 100 mM.

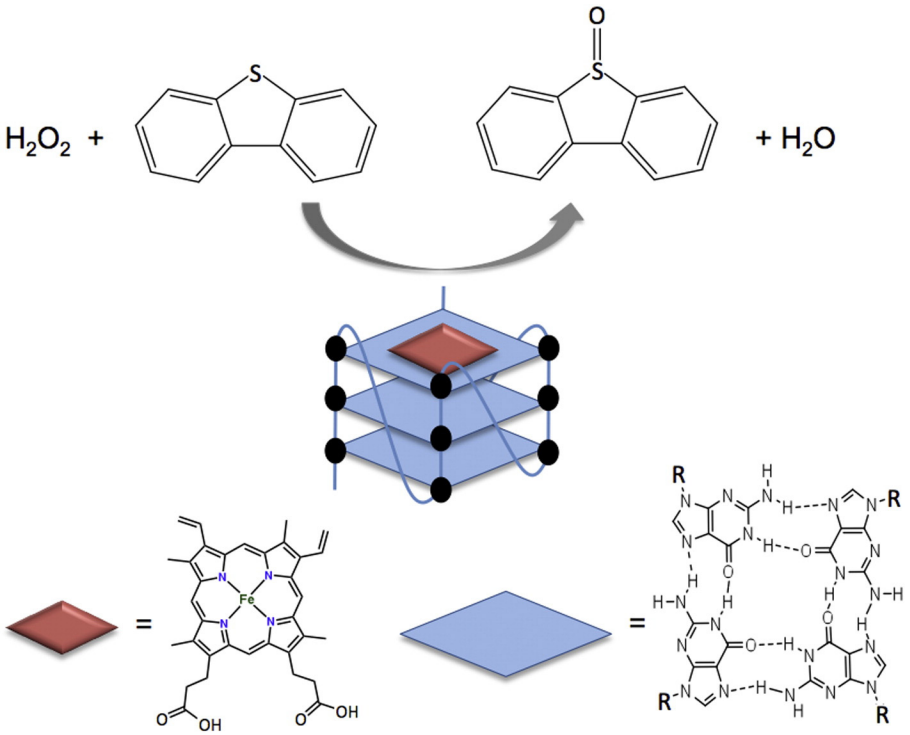

Fig. 1. A schematic drawing of a heme·DNAzyme.

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