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Microwave-Induced Inactivation of DNA-Based Hybrid Catalyst in Asymmetric Catalysis

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Abstract

DNA-based hybrid catalysts have gained strong interests in asymmetric reactions. However, to maintain the high enantioselectivity, these reactions are usually conducted at relatively low temperatures (e.g. $< 5^{\circ}\text{C}$) for 2–3 days. Aiming to improve the reaction's turnover rate, we evaluated microwave irradiation with simultaneous cooling as potential energy source since this method has been widely used to accelerate various chemical and enzymatic reactions. However, our data indicated that microwave irradiation induced an inactivation of DNA-based hybrid catalyst even at low temperatures (such as 5°C). Circular dichroism (CD) spectra and gel electrophoresis of DNA suggest that microwave exposure degrades DNA molecules and disrupts DNA double-stranded structures, causing changes of DNA–metal ligand binding properties and thus poor DNA catalytic performance.

Keywords

DNA-based hybrid catalyst; microwave irradiation; simultaneous cooling; Michael addition; asymmetric catalysis

1. Introduction

Recently, DNA molecules have been explored as chiral scaffolds for metal complexes to design so called 'DNA-based hybrid catalysts'. These novel hybrid catalysts have shown high activities and enantioselectivities in asymmetric reactions [1-3], including the Michael addition [4-6], Diels-Alder reaction [5, 7-9], Friedel-Crafts alkylation [5, 10], intramolecular cyclopropanation [11], electrophilic fluorination of β -keto esters [12], and asymmetric hydration [8], etc. However, to maintain the high enantioselectivity/regioselectivity, most of these reactions were kept below 5°C for 2-3 days; thus the reaction efficiency awaits further improvement.

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To accelerate the reaction at low temperatures, microwave irradiation with simultaneous cooling has become an attractive tool. This unique combination can increase the kinetic energy of reactant molecules through microwaves while the cooling keeps the temperature low by removing the unnecessary thermal energy from intermolecular friction. As a result, this method allows more microwave energy to be introduced into the reaction system for a faster reaction. Although only a few studies dealt with this subject, most of them observed enhanced reaction rates through two methods: cooling with a stream of compressed air [13, 14] or with a microwave transparent cooling liquid (such as liquid N₂, solid CO₂ in hexane, or cryogenic fluid) [15, 16]. The latter cooling method enables the reaction to be conducted at very low temperatures, which is especially favorable for improving the stability of biomolecules and achieving high enantioselectivity. Currently, only very limited studies on the low-temperature enzymatic reaction irradiated by microwaves have been conducted in either organic solvents or ionic liquids [17].

The effect of microwaves on DNA structures depends on the strength and duration of the irradiation. Varma and Traboulay [18] irradiated Swiss male mice by 1.7 GHz microwave with a power density of 50 mW/cm² for 30 min, and then compared the nucleotide composition of normal and irradiated DNA isolated from mice. Since the decrease in optical density (i.e. hyperehmocicity) of DNA from Swiss mice testes indicates a decrease in hydrogen bonding, they suggested the possibility of DNA strand separation due to irradiation. Jebai et al. [19] studied the pUC18 plasmid DNA from *E. coli* irradiated by a low energy of 900 MHz microwave with a field intensity 6 V/m at room temperature for 10 days, but observed no change in the quantity of DNA strands and hyperehmocicity. Sagripanti and Swicord [20] irradiated the plasmid DNA by 2.55 GHz microwaves at 20 °C for 20 min, and found that the minimal temperature change had no impact on DNA structures, but the irradiation decreased the supercoiled DNA and increased the relaxed and linear DNA as confirmed by electrophoretic analysis. Lai and Singh [21] reported that microwave-exposure (continuous-wave 2.45 GHz microwaves, a power density of 1 mW/cm², an average whole-body specific absorption rate of 0.6 W/kg) led to notably higher levels of DNA single- and double-strand breaks in rat brain cells than in sham-exposed animals.

This study aims to evaluate the effect of microwave-irradiation with simultaneous cooling on the DNA structural changes, as well as on the rate and enantioselectivity of DNA-based asymmetric Michael addition.

2. Materials and methods

2.1 Materials and Instruments

Deoxyribonucleic acid (DNA) sodium salt from salmon testes (catalog # D1626), *N*-methylimidazole, *n*-butyllithium, *trans*-cinnamic acid, 4,4'-dimethyl-2,2'-dipyridyl (dmbipy), 3-(*N*-morpholino) propanesulfonic acid (MOPS), and MOPS sodium salt were acquired from Sigma-Aldrich and used as received. Ionic liquids (ILs) were purchased or prepared as shown in our earlier paper [22]. Deep eutectic solvent, choline chloride/glycerol (1:2, molar ratio), was prepared following our earlier study [23]. The synthesis of (*E*)-(1-methyl-1*H*-imidazole-2-yl)-3-phenylprop-2-en-1-one (**1**) was a modification of literature

methods [24-26] and is described in details in our recent paper [6]. The preparation of copper complex, $\text{Cu}(\text{dmbipy})(\text{NO}_3)_2$, was based on a literature method [7].

A CEM Discover Lab-Mate microwave reactor from CEM Corporation (Matthews, NC) was connected to a CEM Cool-Mate for simultaneous cooling for all microwave-irradiated reactions. The microwave reactor was operated at 2.45 GHz. The system was controlled by a computer using the Synergy software. Solvay Solexis Galden HT-55 heat-transfer fluid (HTF) was used, which was cooled by dry ice or liquid N_2 (see Figure S1. in *Electronic Supplementary Material*).

2.2 DNA-based catalytic enantioselective Michael reaction

All aqueous solutions including buffer were prepared with nuclease-free water. A solution of salmon testes DNA (2.5 mL of a 2.0 mg/mL solution in 20 mM pH 6.5 MOPS buffer) was sonicated in ice bath for 5 min. In a 20 mL microwave tube, the DNA solution was mixed with a solution of 2.5 mL 0.45 mM $[\text{Cu}(\text{dmbipy})(\text{NO}_3)_2]$ in 20 mM pH 6.5 MOPS to prepare a buffered solution of DNA bound catalyst. To this mixture, 7.5 μmol of substrate (**1**, see Scheme 1) in 25 μL acetonitrile/2-propanol (3/2, v/v) was added and cooled to below 5 °C. Additional co-solvent/additive in MOPS was added to make a total volume of 7.5 mL. The reaction was initiated by adding 100 eq. dimethylmalonate (86 μL). A fiber optic probe in a plastic tube was inserted into the reaction mixture. The mixture was microwaved with simultaneous cooling at 5 °C for a predetermined length of time. The product was isolated by extraction with diethyl ether followed by drying with Na_2SO_4 and removal of the solvent. The crude product (**2**), was dissolved in 1.0 mL CD_3OD for analysis by ^1H -NMR and HPLC (Chiralpak AD 2.1 $\times$ 150 mm, 10 μm , *n*-heptane/*i*-PrOH 90/10, 0.2 mL/min, 210 nm) [4].

2.3 Circular dichroism (CD) spectra of DNA in aqueous solutions

The stock solution of DNA from salmon testes dissolved in 20 mM MOPS buffer (pH 6.5) was at a nominal concentration of 2/3 mg/mL. Co-solvents/additives were weighted to meet the desired final concentrations. An aliquot of the mixture was scanned in the range of 220-320 nm by a JASCO J-825 CD Spectrometer. The instrument parameters were set as data pitch 0.1 nm, scanning speed 100 nm/min, band width 1.00 nm, slit width 100 μm , DIT 16 sec, standard sensitivity, 10 accumulations, and the cell temperature of 5 °C.

2.4 Binding constants between DNA and $[\text{Cu}(\text{dmpiby})(\text{NO}_3)_2]$

DNA from salmon testes was dissolved in distilled water to make a nominal concentration of 2/3 mg/mL. The concentration of base pairs was calculated from spectrophotometrical absorbance at 260 nm using $\epsilon_{260} = 12,800 \text{ M}^{-1} \text{ cm}^{-1}$. An aliquot of stock DNA solution (0, 90, 180 and 270 μL) was added into 100 μL of 0.45 mM $\text{Cu}(\text{dmbipy})(\text{NO}_3)_2$ in MOPS (20 mM, pH 6.5), followed by the addition of MOPS buffer to make the total volume of 1.5 mL. The copper complex was maintained at 30 μM . The mixture was then analyzed by a Thermo Scientific NanoDrop 2000 for its absorbance at 260 nm. The binding constant (K_b) can be determined by the following equation [5, 27],

$$\frac{D}{\Delta\epsilon_{ap}} = \frac{1}{\Delta\epsilon}D + \frac{1}{\Delta\epsilon K_b}$$

where $\epsilon_{ap} = |\epsilon_a - \epsilon_f|$, $\epsilon = |\epsilon_b - \epsilon_f|$, ϵ_a , ϵ_f and ϵ_b the apparent, free and bound extinction coefficients for the complex respectively, and D is the DNA concentration in base pairs. D/ϵ_{ap} was plotted against D , and the K_b value was calculated from the ratio of the slope to the y-intercept.

2.5 Gel electrophoresis of DNA

The compositions of DNA samples were characterized using agarose gel electrophoresis. Briefly, 10 μ L Sybr safe DNA stain (10,000 $\times$) was added into 100 mL 0.5% agarose gel during gel casting. DNA samples were prepared by mixing 10 μ L DNA solution (2/3 mg/mL) with 2 μ L Bio-Rad nucleic acid sample loading buffer. For each well, 6 μ L of DNA sample or DNA standard (EZ Load 1 kb Molecular Ruler, Bio-Rad) was loaded. Gel electrophoresis was conducted at 110 V for 65 min. Gel images were acquired on a Bio-Rad Gel Doc EZ system. The concentrations of DNA samples were quantitated by measuring their UV absorption at 260 nm on a Thermo Scientific Nanodrop 2000 system.

3. Results and discussion

The DNA-based hybrid catalyst was prepared by mixing salmon testes DNA in MOPS buffer (20 mM, pH 6.5) with Cu(dmbipy)(NO₃)₂ in MOPS (dmbipy = 4,4'-dimethyl-2,2'-dipyridyl), as shown in Scheme 1 [4, 5]. To evaluate the catalytic activity of hybrid catalysts, a Michael addition reaction was selected as our primary model system: the conversion of (*E*)-(1-methyl-1*H*-imidazole-2-yl)-3-phenylprop-2-en-1-one (**1**) to (*R*)-dimethyl 2-(3-(1-methyl-1*H*-imidazol-2-yl)-3-oxo-1-phenylpropyl)malonate (**2**) (see Scheme 1).

3.1 Effect of microwave irradiation on asymmetric catalysis

As illustrated by the reactor system in Figure S1 (see *Electronic Supplementary Material*), the reaction tube was cooled by circulating heat-transfer fluid (HTF), which was cooled by dry ice (or liquid N₂) in the Cool-Mate (CEM Corporation, Matthews, NC). Based on our observations, when dry ice was used, the temperature of HTF could reach -20 to -30 °C; when liquid N₂ was used, the HTF temperature could reach below -40 °C. To maintain the reaction temperature at 5 - 20 °C with this simultaneous cooling, microwave irradiation was initiated at an adjustable but relatively steady power mode. As shown in Figures S2 and S3, when HTF was cooled by dry ice, a relatively constant microwave power input of about 50 - 70 Watts was applied to the reaction mixture (60 - 100 Watts for liquid N₂ cooling).

As confirmed in our earlier study [6], at 5 °C to room temperature without microwave irradiation, salmon testes DNA-based hybrid catalyst exhibited high activities and enantioselectivities in MOPS solutions of 0.4 M glycerol (trial 1 in Table 1), 0.2 M [BMIM] [CF₃COO] (trial 11), 0.2 M choline chloride (ChOCl)/glycerol (1:2, molar ratio) (trial 15), and 0.5 M dipropylene glycol dimethyl ether (trial 18). As a baseline for comparison, the

same reaction in 0.4 M glycerol at 5 °C without microwave irradiation gave >99% enantiomeric excess (ee) and 19.8% yield in 4 h (trial 2). However, when microwave irradiation was applied with simultaneous cooling, the reaction yield in 0.4 M glycerol at 5 °C was only 7.8% at 1 h, 8.8% at 2 h and then decreased to 4.9% at 4 h (trials 5, 6, 7 and 8), and the yield at 20 °C was only 1.7% (trial 9), although high ee values (>99%) were always maintained. When DNA was not sonicated but the reaction was microwaved, even lower yields were observed in 0.4 M glycerol (trials 3 and 4). A similar DNA-based catalyst deactivation (in term of low yields and/or low ees) under microwave irradiation was observed in other co-solvents systems in Table 1, 0.2 M [BMIM][CF₃COO] (trial 12, 13 and 14), 0.2 M ChOCl/glycerol (1:2) (trials 16 and 17), and 0.5 M dipropylene glycol dimethyl ether (trial 19). It is noteworthy to point out that when the HTF was cooled by liquid N₂ (trial 17), a low yield of 4.5% was also obtained under microwaves for 4 h in 0.2 M ChOCl/glycerol (1:2).

3.2 Effect of microwave irradiation on DNA structures and binding properties

As shown in Figure 1, without microwave treatment, the CD spectrum of salmon testes DNA showed characteristics of B-form DNA with a long wave positive band at 278 nm corresponding to p-p base packing and a shorter wave negative band at 243 nm corresponding to helicity [28]. In the absence of [Cu(dmbipy)(NO₃)₂], DNA microwaved for 20 min in MOPS showed the best match to this characteristic pattern; other DNA samples microwaved in 0.4 M glycerol showed a slight red shift of negative bands although the overall B-form pattern was maintained. In the presence of 0.15 mM [Cu(dmbipy)(NO₃)₂], DNA samples microwaved in 0.4 M glycerol (vs not microwaved, either with or without pre-sonication) exhibited a slight red shift of negative bands and a slight blue shift of positive bands, and a lower intensity of negative bands but a higher intensity of positive bands (Figure 2). These results suggest that microwave irradiation could induce some DNA structural changes especially in the presence of copper (II) complexes. It is known that some copper (II) complexes (in particular, with mixed ligands) could cleave the supercoiled plasmid DNA [29, 30]. Gel electrophoresis in Figure 3 suggests that after microwave exposure for 2–4 h, salmon testes DNA (in MOPS without 0.15 mM [Cu(dmbipy)(NO₃)₂]) was substantially degraded in solutions as shown by the much lighter color of lanes 6, 7, and 8. Therefore, a long time microwave irradiation could cause the destruction of double-strand structures of some DNA molecules. In the presence of 0.15 mM [Cu(dmbipy)(NO₃)₂], gel electrophoresis in Figure 4 (Lane 3 vs 2, Lane 5 vs 4) indicates a similar degree of DNA degradation (comparing with Lanes 7 and 8 in Figure 3). Therefore, microwave irradiation itself seems the main cause of DNA degradation with or without the copper complex. For this reason, under microwave exposure for a period of time, only a small fraction of DNA molecules survived and could act as chiral scaffolds, resulting in a low reaction yield; however, this small fraction of DNA still maintained the typical B-form (as shown by CD spectra in Figures 1 and 2) leading to effective catalysis, thus the product ee was not affected considerably.

The binding study in Table 2 illustrates that upon microwave irradiation, binding constants (K_b) of DNA with [Cu(dmpiby)(NO₃)₂] increased for trials 4–7 (unsonicated DNA), particularly for trial 4 ($38.9 \times 10^4 \text{ M}^{-1}$). A reasonable K_b value of $7\text{--}8 \times 10^4 \text{ M}^{-1}$ (trials 2

and 3 in Table 2) afforded high yields and ees (trials 1 and 2 in Table 1). Excessively strong binding between DNA and the ligand could negatively affect the accessibility of Cu^{2+} as the catalytic center. On the other hand, a very low binding constant ($0.80 \times 10^4 \text{ M}^{-1}$) for trial 8 (sonicated DNA) means a weak interaction between DNA and the ligand, which also decreases the catalyst's performance (note that $[\text{Cu}(\text{dmpib})](\text{NO}_3)_2$ is a poor catalyst without DNA [6]).

4. Conclusions

Microwave irradiation with simultaneous cooling induced an inactivation of DNA-based hybrid catalyst in asymmetric Michael addition. Although some double-stranded DNA molecules were preserved, a substantial amount of DNA molecules degraded and lost their characteristic structures. As a result, the binding constants of DNA with metal ligand were drastically changed. Therefore, microwave irradiation may not be a suitable tool for accelerating the DNA-based catalytic reactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Highlights

- Microwave irradiation with simultaneous cooling inactivates the DNA-based hybrid catalyst.
- Microwave treatment disrupts DNA double-stranded structures.
- Microwave irradiation leads to changes in binding properties between DNA and metal ligand.

Figure 1.

CD spectra of DNA from salmon testes with or without microwave (MW) irradiation (2/3 mg/mL DNA in 20 mM pH 6.5 MOPS, no $[\text{Cu}(\text{dmbipy})(\text{NO}_3)_2]$; DNA was not pre-sonicated except the last sample; if MW, the reaction was maintained at 10 °C and the heat-transfer fluid was cooled by dry ice).

Figure 2.

CD spectra of DNA from salmon testes with or without microwave (MW) irradiation (2/3 mg/mL DNA, 0.4 M glycerol, and 0.15 mM [Cu(dmbipy)(NO₃)₂] in 20 mM pH 6.5 MOPS; if MW, the reaction was maintained at 10 °C and the heat-transfer fluid was cooled by dry ice).

Figure 3.

Effect of microwave irradiation on DNA degradation as illustrated by gel electrophoresis of salmon testes DNA (2/3 mg/mL DNA in 20 mM pH 6.5 MOPS) (0.5% Agarose gel containing 10 μ L Sybr[®] safe DNA gel stain, TAE buffer, 6 μ L sample for each lane, 110 V, 65 min).

Lane legend

1. 0.08 mg/ml Bio-Rad EZ Load 1 kb Molecular Ruler
2. DNA in MOPS (20 mM, pH 6.5)
3. DNA in MOPS sonicated in ice bath for 5 min
4. DNA in MOPS microwaved at 10 °C for 20 min
5. DNA in MOPS containing 0.4 M glycerol microwaved at 10 °C for 20 min
6. DNA in MOPS containing 0.4 M glycerol microwaved at 10 °C for 2 h
7. DNA in MOPS containing 0.4 M glycerol microwaved at 10 °C for 4 h
8. DNA in MOPS sonicated for 5 min, adding 0.4 M glycerol microwaved at 10 °C for 4 h

Figure 4.

Effect of copper complex on DNA degradation under microwave as illustrated by gel electrophoresis of salmon testes DNA (2/3 mg/mL DNA, 0.4 M glycerol, and 0.15 mM [Cu(dmbipy)(NO₃)₂] in 20 mM pH 6.5 MOPS) (0.5% Agarose gel containing 10 µL Sybr® safe DNA gel stain, TAE buffer, 6 µL sample for each lane, 110 V, 65 min).

Lane legend

1. 0.08 mg/ml Bio-Rad EZ Load 1 kb Molecular Ruler
2. DNA without pre-sonication and without microwave irradiation
3. DNA without pre-sonication, and under microwave irradiation at 10 °C for 4 h
4. DNA with 5 min pre-sonication in ice bath and without microwave irradiation
5. DNA with 5 min pre-sonication in ice bath, and under microwave irradiation at 10 °C for 4 h

Scheme 1.

Microwave-assisted DNA-based asymmetric Michael addition with simultaneous cooling (dmbipy = 4,4'-dimethyl-2,2'-bipyridine).

Table 1

Effect of microwave irradiation on DNA-based asymmetric Michael addition

Trial	Co-solvent	Temperature, irradiation	Time (h)	ee (%)	HPLC yield (%)	Optical rotation
1	<i>0.4 M glycerol</i>	<i>5 °C, no microwave</i>	72	>99	96	–
2	<i>0.4 M glycerol</i>	<i>5 °C, no microwave</i>	4	>99	19.8	–
3	0.4 M glycerol (DNA not sonicated)	5 °C, microwave	0.5	>99	2.3	–
4	0.4 M glycerol (DNA not sonicated)	5 °C, microwave	1	>99	1.9	–
5	0.4 M glycerol	5 °C, microwave	1	>99	7.8	–
6	0.4 M glycerol	5 °C, microwave	2	>99	8.8	–
7	0.4 M glycerol	5 °C, microwave	3	>99	6.0	–
8	0.4 M glycerol	5 °C, microwave	4	>99	4.9	–
9	0.4 M glycerol	20 °C, microwave	4	>99	1.7	–
10	none	20 °C, microwave	4	>99	0.18	–
11	<i>0.2 M [BMIM][CF₃COO]</i>	<i>5 °C, no microwave</i>	72	95	93	–
12	0.2 M [BMIM][CF ₃ COO]	5 °C, microwave	4	90	4.3	–
13	0.2 M [BMIM][CF ₃ COO]	10 °C, microwave	4	92	17	–
14	0.2 M [BMIM][CF ₃ COO]	20 °C, microwave	4	46	19	–
15	<i>0.2 M ChOCl/glycerol (1:2)</i>	<i>r.t., no microwave</i>	24	94	88	–
16	0.2 M ChOCl/glycerol (1:2)	20 °C, microwave	4	>99	12	–
17	0.2 M ChOCl/glycerol (1:2)	10 °C, microwave (liquid N ₂)	4	>99	4.5	–
18	<i>0.5 M dipropylene glycol dimethyl ether</i>	<i>r.t., no microwave</i>	24	>99	87	–
19	0.5 M dipropylene glycol dimethyl ether	20 °C, microwave	4	79	2.5	–

Note: under microwave irradiation, the heat-transfer fluid (HTF) was cooled by dry ice except trial 17. ChOCl = choline chloride. Trials with italic fonts are for those reactions without microwave irradiation.

Table 2Binding constants (K_b) of DNA with [Cu(dmpiby)(NO₃)₂]

Trial	Co-solvent	Sonication (min)	Microwave condition	$K_b/10^4$ (M ⁻¹)
1	None	0	No microwave	2.02
2	None	5	No microwave	7.15
3	0.4 M glycerol	5	No microwave	8.15
4	None	0	Microwave at 10 °C for 20 min	38.9
5	0.4 M glycerol	0	Microwave at 10 °C for 20 min	10.6
6	0.4 M glycerol	0	Microwave at 10 °C for 2 h	8.12
7	0.4 M glycerol	0	Microwave at 10 °C for 4 h	9.61
8	0.4 M glycerol	5	Microwave at 10 °C for 4 h	0.80