Communication

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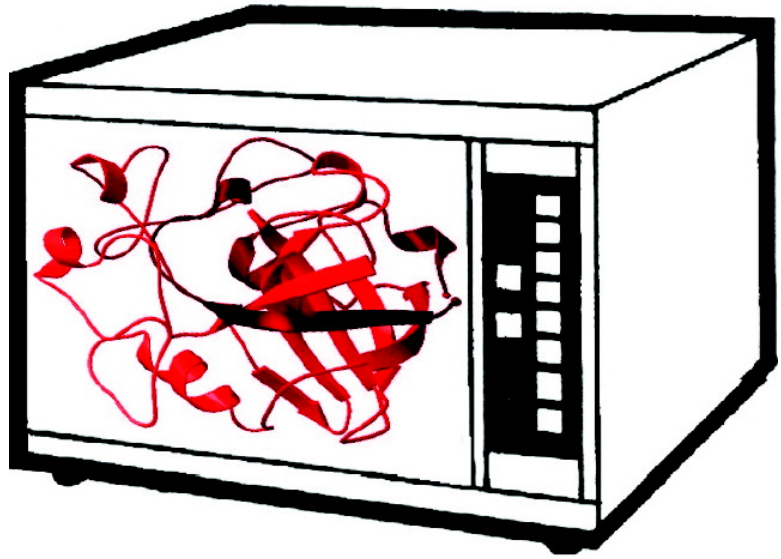

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Microwave Activation of Enzymatic Catalysis

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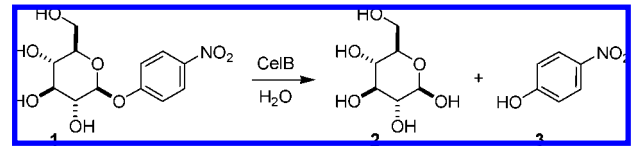
Microwave irradiation has become an effective tool in synthetic organic chemistry, dramatically increasing reaction rates and yields.[1–3] In microwave-assisted chemistry, the concept of "specific" microwave effects is controversial in large part because these effects are difficult to determine.[4,5] In general, specific effects triggered by microwave irradiation enable reactions, which cannot proceed by thermal heating alone. These effects likely arise from direct coupling of molecules with the microwave field, independent of the reaction temperature.[3] Generally, microwave irradiation induces molecular rotation arising from dipole alignment with the external, oscillating electric field.[5,6] As a result, microwaves significantly impact molecules with high dipole moments. Proteins and peptides have significant dipole moments, and thus may be especially susceptible to microwave irradiation.[7,8] Microwave induction of enzymatic reactions has been considered;[9] however, whether specific microwave effects are important in biocatalysis is not clear.[10] These effects are difficult to measure because rapid heating of aqueous solutions under high-power microwave irradiation can result in protein denaturation and inactivation.[11] Moreover, the intrinsic catalytic ability of enzymes at a relatively low temperature can obscure the potential rate enhancement through microwave irradiation. There have been attempts to conduct enzymatic reactions under microwave irradiation in nonaqueous solvents, but this has yielded inconclusive results because of greatly reduced enzymatic activity.[10,12]

Specific microwave effects in enzymatic catalysis could be observed if, at high levels of microwave irradiation, minimal catalytic activity arises from thermal heating of the bulk solvent. This can be achieved in an aqueous environment, if care is taken to (1) effectively cool the reaction mixture during irradiation using a jacketed reaction vessel and cryogenic cooling, while precisely measuring the temperature using a fiber optics probe (CEM Coolmate), and (2) use hyperthermophilic enzymes which have minimal catalytic activity at temperatures below 40 °C and denature at much higher temperatures than their mesophilic counterparts. Here, a β-glucosidase (CelB) from the hyperthermophilic archaeon, *Pyrococcus furiosus*, was examined for biocatalytic function under microwave irradiation; this enzyme is optimally active at ~110 °C. *P. furiosus* CelB cleaves exoglycosidic linkages in both natural (e.g., cellobiose) and synthetic substrates (e.g., **1**, Scheme 1).[13]

Enzymatic assays were conducted by cooling the nitrophenoate substrate **1** in reaction buffer (50 mM NaOAc, pH 5.5, 10% DMSO) to −20 °C, followed by the addition of heat-pretreated *Escherichia coli* cell extract containing recombinant CelB. Microwave irradiation (300 W) was applied until the reaction temperature reached 40 °C (all temperatures were measured using a fiber optics temperature sensor); coolant at −60 °C was simultaneously circulated through the jacketed reaction vessel. The reaction was then quenched with

Scheme 1. Colorimetric CelB Assay: The Enzymatic Hydrolysis of **1** Produces Glucose (**2**) and Nitrophenol (**3**)



a 1 M Na_2CO_3 solution, and the absorbance of the alkoxide of **3** was measured at 405 nm (Figure 1, note the logarithmic activity scale). No significant substrate hydrolysis was observed under microwave irradiation without Pfu CelB addition. Furthermore, under identical thermal conditions (−20 to 40 °C) (see Figure 2), no significant enzymatic activity ($<10^{-11}$ mol min^{-1} μg^{-1}) was detected in the absence of microwave irradiation (0 W). However, a greater than 4 orders of magnitude increase in enzymatic activity of Pfu CelB was achieved with 300 W of microwave irradiation (2.3×10^{-6} mol min^{-1} μg^{-1}). Thus, a substantial microwave effect on enzymatic catalysis was observed.[1,5] This illustrates for the first time that microwaves can trigger high biocatalytic rates of a hyperthermophilic enzyme at bulk solution temperatures far below (ΔT = 70 °C or more) its thermal optimum.

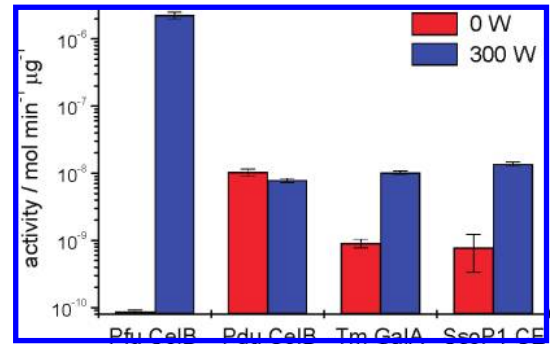


Figure 1. Effect on microwave irradiation on Pfu CelB, Pdu CelB, Tm GalA, and SsoP1 CE activity. All experiments were conducted in triplicate.

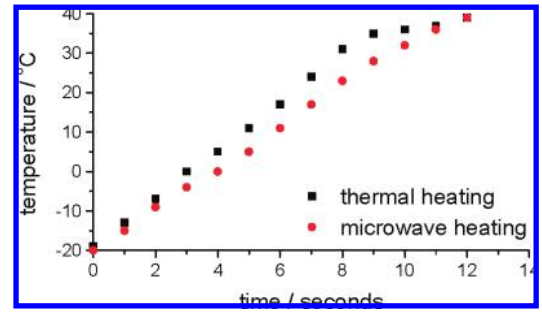


Figure 2. Temperature profiles (measured with a fiber optics probe) of the microwave mediated (300 W) and the thermal reactions.

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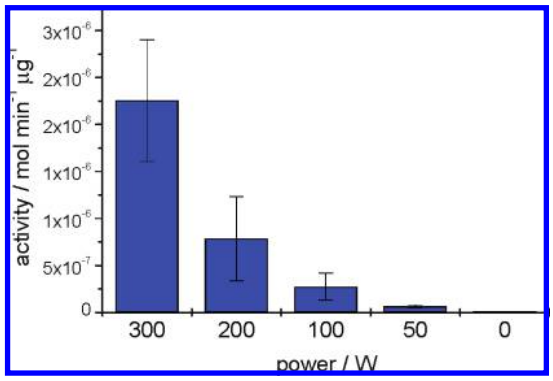

Figure 3. Pfu CelB enzymatic activity dependence on microwave power. All experiments were conducted in triplicate, 5 mM substrate concentration. In all cases, the temperature increased from −20 to 40 °C.

Note that no microwave biocatalytic activation was observed in the case of a mesophilic homologue of CelB from *Prunus dulcis* (Pdu CelB) (Figure 1); the enzyme activity that was observed was likely related to thermal stimulation during heating between −20 to 40 °C.

To further probe the efficacy of microwave activation, two other hyperthermophilic enzymes, an α-galactosidase from *Thermotoga maritima* (Tm GalA)[14] and a carboxylesterase from *Sulfolobus solfataricus* P1 (SsoP1 CE),[15] were investigated (Figure 1). A similar colorimetric assay was used, based on α-galactopyranoside or hexanoic acid analogues of **1** (Scheme 1).

In both cases, for the same thermal heating profile (Figure 2), microwave irradiation significantly stimulated enzyme activity. Note that both TmGalA and SsoP1 CE have lower T_{opt} than Pfu CelB (∼90−95 °C[14,15] vs 110 °C for CelB), such that some thermally induced activity was observed in the absence of microwave irradiation.

Hyperthermophilic enzymes have highly compact structures and limited conformational flexibility at temperatures far below their normal functional range. The observed specific microwave effect (enzyme activity at unusually low temperatures) most likely derives from molecular motion induced by a rapid dipole alignment of the peptide bonds with the oscillating electric field.[8,10] In fact, Pfu CelB activity at biocatalyically suboptimal temperatures is a function of input microwave power (Figure 3). The strong induction of molecular motion in the enzyme through microwave irradiation is corroborated by the observation that high microwave power (300 W) at moderate temperatures (75 °C) led to a denaturation of Pfu CelB. This was confirmed by a loss of activity and a loss of tertiary structure as measured by circular dichroism (see Supporting Information). Note that, without microwave irradiation, denaturation of the hyperthermophilic Pfu CelB occurs at approximately 115 °C.[13a] No denaturation of the enzyme was observed by CD under either the low temperature (−20 to 40 °C, 300 W) microwave conditions or under thermal heating (75 °C, 0 W). At such low temperatures, the mesophilic analogue Pdu CelB also did not denature, as determined by CD (Supporting Information).

The results reported here illustrate for the first time the intrinsic effect of microwave irradiation on biocatalysis. Furthermore, they indicate that hyperthermophilic enzymes can be activated at temperatures far below their optimum, presumably by microwave-induced conformational flexibility. This finding offers the prospect of using hyperthermophilic enzymes at ambient temperatures to catalyze reactions with thermally labile substrates and products. Furthermore, microwaves could be used to regulate biocatalytic rates at very low temperatures for enzymes from less thermophilic sources. Both of these possibilities are being considered.

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Supporting Information Available: Experimental protocols for enzymatic assays, and circular dichroism measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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