Tetrahedron Letters 56 (2015) 5804–5807

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Microwave radiation accelerates trypsin-catalyzed peptide hydrolysis at constant bulk temperature

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ARTICLE INFO

Article history:
Received 2 August 2015
Revised 22 August 2015
Accepted 2 September 2015
Available online 8 September 2015

Keywords:
Microwave heating
Conventional heating
Trypsin
Casein
Enzyme activity

ABSTRACT

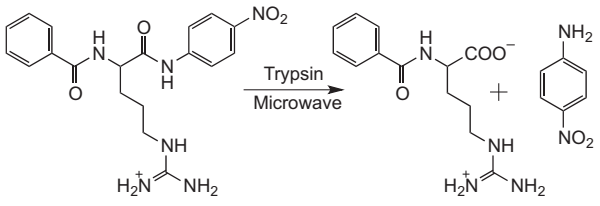
The influence of microwave radiation on trypsin activity was explored using a CEM CoolMate apparatus at a constant bulk temperature. Digestion of *N*α(±)-benzoyl-D/L-arginine-4-nitroanilide hydrochloride, azocasein and casein catalyzed by trypsin from the bovine pancreas was found to be accelerated when the reaction mixture was exposed to microwave radiation, while the bulk temperature was maintained constant through external cooling. Trypsin activity was found to be increased significantly when the reaction mixture was irradiated with microwave at a constant temperature. Cyclic dichroism measurement of trypsin exposed to microwave radiation suggests that there are changes in the secondary structure of trypsin exposed to microwave and conventional heating, however, these changes are presumably due to self-cleavages of trypsin.

Microwave heating has become an increasingly popular heating method in a wide range of applications over the past few decades. In organic synthesis, microwave heating has been shown to be very useful, often leading to faster reactions, higher yields and better selectivity.[1–6] One of the areas that has attracted heated debate relates to whether 'microwave specific effects' exist.[7–12] Some recent publications suggest that such effects are possible, which could presumably be attributed to 'selective heating' of reactants with high absorption cross sections.[13,14] We are interested in the effect of microwave irradiation on reactions that involve biomolecules such as enzymes, particularly when the reaction mixture is maintained at a constant bulk temperature while exposed to low power microwave, as these experiments could provide some insight into the influence of microwave exposure on biological systems, where the macroscopic temperature is mediated by bulk surroundings.

While some work has demonstrated that microwave radiation affects protein/enzyme structures,[15–18] bulk literature evidence has suggested that enzymatic activity can be affected when enzymes are exposed to microwave. The literature in this area prior to 2007 is summarized in a review.[19] This area of research was further demonstrated in more recent work.[20–32] It is worth noting that among the challenges in establishing reproducible results in these observations, monitoring and regulation of the reaction temperature accurately has been the most difficult one.[33] In this respect, Kappe and co-workers[34] showed that there was no difference in reactivity and enantioselectivity in the kinetic resolution of *rac*-1-phenylethanol catalyzed by immobilized lipases under conventional or microwave heating, when the temperatures were maintained the same. In this work, we further explored the influence of microwave exposure on trypsin activity while the bulk reaction temperature is kept constant through external cooling.

Towards this goal, trypsin from the bovine pancreas was chosen as the enzyme and a dipeptide, *N*α(±)-benzoyl-D/L-arginine 4-nitroanilide hydrochloride (BAPNA), as the substrate (Scheme 1). BAPNA is readily hydrolyzed by trypsin to give *N*-benzoyl arginine and *p*-nitroanaline. As the latter can be quantified readily by a colorimetric assay, this system provides an easy approach to study the effect of microwave irradiation on enzyme activity.

In order to maintain the reaction temperature while the reaction mixture is exposed to microwave, the CEM CoolMate



Scheme 1. Hydrolysis of *N*α(±)-benzoyl-D/L-arginine 4-nitroanilide hydrochloride by trypsin.

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http://dx.doi.org/10.1016/j.tetlet.2015.09.003
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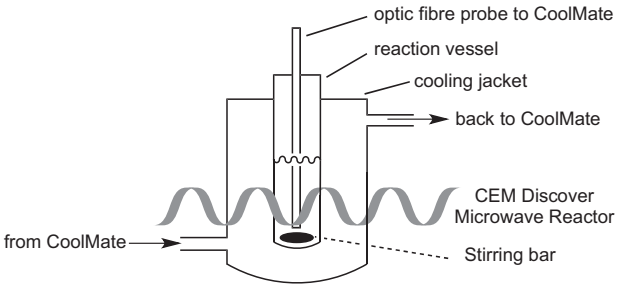

Figure 1. The CEM CoolMate Microwave System that is used in this study.

Microwave System (Fig. 1) was used. This microwave reactor operates at a frequency of 2.45 GHz, and provides the ability to keep constant bulk reaction temperatures while the reaction mixture is exposed to microwave irradiation. This system features a reaction vessel circumvented by a jacket where a microwave-transparent fluid, such as perfluoropolyether Galden HT 110, circulates. The coolant is pre-cooled in a reservoir by either dry ice or liquid nitrogen. An important feature of this setup is that the reaction temperature is monitored in situ with an optic fiber temperature probe, which provides feedbacks to the system so that the microwave output can be adjusted in a real-time fashion to maintain a pre-determined temperature in the reaction mixture. Note that in all the experiments described in this work, stirring was set at 'high' to ensure homogeneity of reaction mixtures.

When BAPNA was subjected to trypsin digestion, it became clear that hydrolysis was accelerated by microwave radiation, while the bulk reaction temperature was kept constant by simultaneous cooling. Figure 2 shows the progress of two reactions carried out at 25 °C, as measured in the absorbance of product formed over time, one in the absence of microwave (control) and another exposed to up to 20 W microwave radiation while the temperature

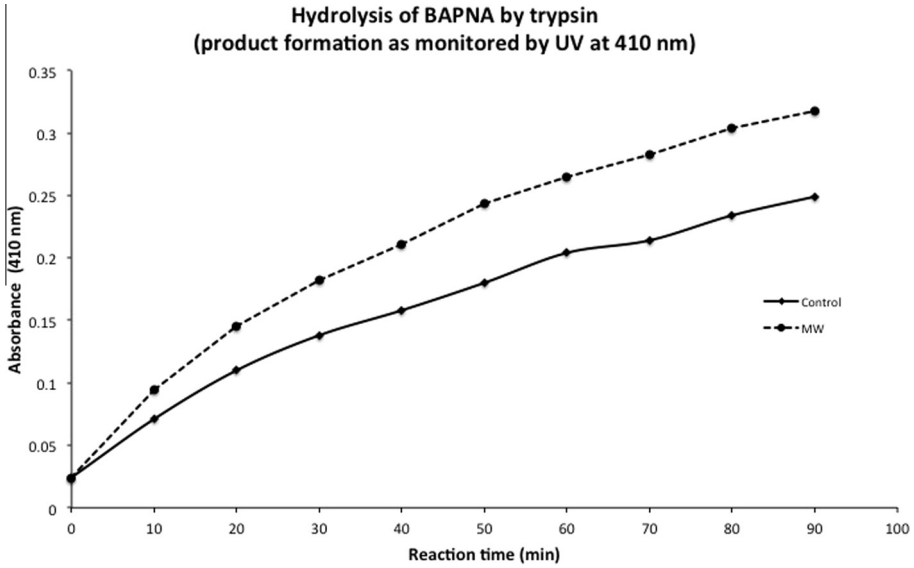

Figure 2. Digestion of BAPNA (1.0 mM) by trypsin (5.0 μM). Solid line: reaction at 25 °C in the absence of microwave; dashed line: reaction mixture was exposed to microwave radiation of varying power of up to 20 W, while the bulk temperature was kept at 25 °C through external cooling by a CoolMate system. The flow rate of the CoolMate system was kept constant.

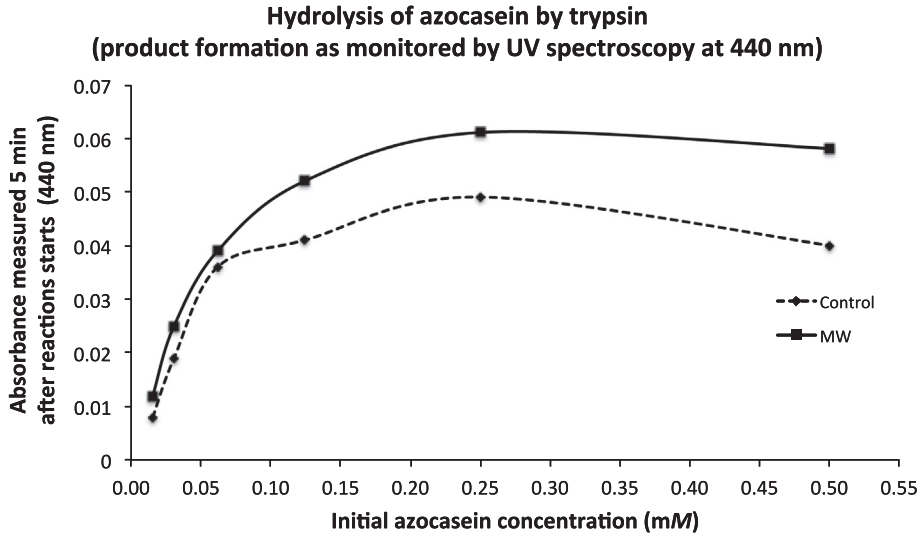

Figure 3. Plot of changes in absorbance at 440 nm of samples taken 5 min after reactions initiate, versus initial azocasein concentrations. Both control (reactions carried out in the absence of microwave) and microwave (exposed to microwave radiation of varying power of up to 20 W) reactions were carried out at 22 °C. The flow rate of the CoolMate system was kept constant.