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of the 9th International Conference on Fundamental and Applied Aspects of Physical Chemistry

Volume I

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EFFECTS OF MICROWAVE TREATED SUBSTRATE ON PEPSIN REACTION KINETICS

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Abstract

Microwave (MW) irradiated bovine serum albumin (BSA) and bromphenol blue (BPB) complex was used as substrate for the assay of pepsin by kinetic method. Decreased reaction velocity under absorbed MW energy and constant temperature was observed.

Introduction

Identifying and evaluating the biological effects of microwave (MW) have been complex and controversial. Because of the paucity of information on the mechanism of interaction between MW and biological systems, there has been a persistent view that MW fields are incapable of inducing bioeffects other than by heating. Using microwaves to promote reaction rates of chemical reactions become routine [1, 2]. Their application in enzyme-catalyzed reactions is relatively limited [3]. In general, it is believed that the reactions are accelerated since the molecules absorb energy by two modes: dipole rotation and ionic movement. There is evidence that microwaves cause different biological effects depending upon field strength, frequencies, waveforms, modulation and duration of exposure [4].

Experimental

Bovine serum albumin (BSA), Brom-phenol blue sodium salt (BPB) and Porcine pepsin A were purchased from Sigma Aldrich and used without further purification. MW irradiation was performed in a single mode focused CEM reactor (Model Discover, CEM Co., Matthew, NC) working at 2.45 GHz. External cooling the reaction mixture provided the constant temperature and irradiation power of 0.45 W/g. Enzyme assay was performed in Gly-HCl buffer solution at pH 2 and 37^{o} C using BSA-BPB as a substrate [6]. Lineweaver-Burk double reciprocal plots were used to analyze the effects of MW treated substrate on reaction kinetics.

Results and Discussion

The water solution of BSA (10 g/ 100 ml) was used for further substrate development. The appropriate amount of dye (BPB) was dissolved in the minimum amount of ethanol, diluted with glycine buffer pH 2.0 to the concentration of 1 mM. 1.80 ml of BSA solution was added to 3.0 ml of BPB solution, mixed well

and make up to 20 ml with glycine buffer pH 2.0. The final concentration of substrate solution was 150 μM [6]. Albumin combines with BPB at pH 2.0 to give a product having a different color from the original dye (Figure 1).

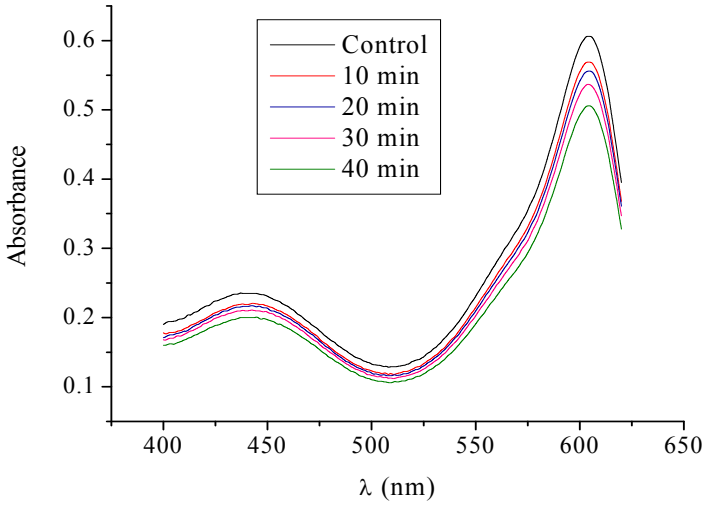

Fig. 1. Changes in absorption spectra of BSA-BPB complex under different MW exposure time at 37°C and pH 2.

The composition of BSA-BPB complex was determined by application of the low of mass action to the assumed equilibrium:

$$mBSA + nBPB \Leftrightarrow BSA_m - BPB_n$$

where *m* and *n* represents of molecule reacting. We also determined that albumin and bromphenol blue react in one-to-one molar ratio. There are two absorption peaks, at 445 nm and 605 nm. Induced MW irradiation did not degrade formed complex, but influence on its protein part, probably causing conformational changes in BSA, which manifests in decreased absorbance at characteristic maximum. Pepsin acting on this complex appears to break it up and regenerate the free BPB (the characteristic absorption maximum at 445 nm increased with release of free dye, while maximum at 605 nm decrease implying degradation of BSA-BPB complex). The change in absorbance during enzymatic reaction was followed at 605 nm.

To evaluate kinetic parameters of induced inhibition effects by microwave irradiated substrate, Lineweaver – Burk linearization of Michaelis equation was used. The Michaelis Menten constant (K_m) and maximum reaction rate (V_{max}) as well as apparent values for K_{mapp} and V_{maxapp} in a presence of inhibition effects of microwave irradiated substrate were derive from double reciprocal Lineweavre-Burk plot (Table 1). Catalytic constant k_{cat} and catalytic effectiveness $k_{cat}\ K_m^{-1}$ in a presence of MW treated substrate were also calculated.

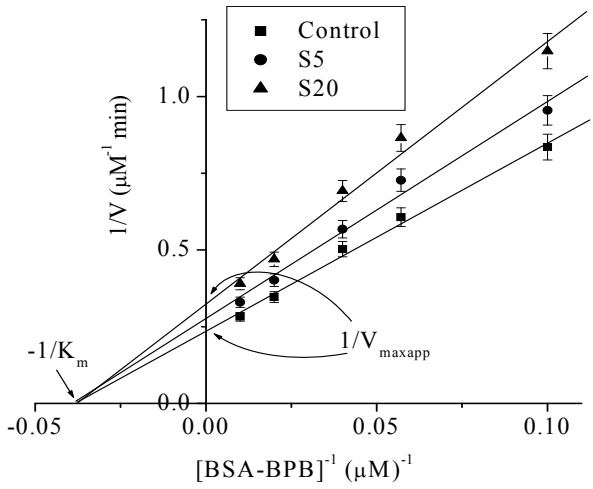

Fig. 2. Lineweaver-Burk plot of pepsin inhibition caused by MW treated substrate. ! - control reaction under conventional heating in water bath at 37°C; , - reaction with irradiated BSA-BPB solution for 5 minutes; 7 - reaction with irradiated BSA-BPB solution for 20 minutes.

Tabe 1. Kinetic parameters evaluated from double reciprocal Lineweaver-Burk plots

	V_{maxapp} ($\mu M\ min^{-1}$)	K_{mapp} (μM)	k_{cat} (min^{-1})	$k_{cat}\ K_m^{-1}$ ($min^{-1}\ \mu M^{-1}$)
Control	3.51	19.28	2.34	0.121
S5	3.03	18.02	2.02	0.112
S20	2.56	18.23	1.71	0.094

Conclusion

The MW irradiation causes conformational changes in protein part of substrate complex and consequently a decrease of enzymatic activity as a function of the absorbed MW irradiation dose of substrate.

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