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Volume I

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MICROWAWE IRRADIATION INFLUENCE ON ENZYME KINETICS

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Abstract

Kinetic study of microwave (MW) irradiated enzyme pepsin was performed. Decreased enzyme activity was observed, under constant temperature and absorbed MW energy per time unit. In accordance with experimental conditions, V_{max} and K_m were calculated for irradiated pepsin solutions and compared with control reaction with non-irradiated pepsin.

Introduction

Microwaves (MWs), now in wide use (i.e. mobile phone, cooking), cause different biological effects depending on applied field strength, frequencies and duration of exposure. Two types of effects are ascribed to MW, i.e. thermal and non-thermal. The thermal effects are related to the fast increase of temperature due to the efficient absorption of MW energy by the irradiated medium and specific thermal effects due to the nonstandard evolution of heat in the reaction media [1]. Knowledge about molecular mechanisms involved in non-thermal effects that could involve energy transfer from the electromagnetic field to the vibration modes of macromolecules, altering their conformation is still obscure [2]. Kinetic properties of the enzyme under MW irradiation are essential for understanding of MW effects on enzyme catalysis. Here we present results of kinetics study of microwave-irradiated pepsin.

Experimental

Porcine pepsin A, Bovine serum albumin (BSA), Brom-phenol blue sodium salt (BPB) were 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. All experiments were done with a same working conditions, *i.e.* the emitted power by the instrument was 30 W, the absorbed MW power, $P_{abs} = m \cdot C_p \cdot (dT/dt)_i$, calculated by calorimetric method measuring temperature increase during the initial heating period was (2.8 ± 0.3) W, and specific absorbed rate (SAR) of (0.47 ± 0.05) W/g. Temperature in the sample was measured by fiber optic temperature sensor preventing interaction with MW and influence on temperature reading. External cooling reaction mixture provided the constant temperature and irradiation power. Enzyme assay was performed in buffer solution at pH 2 and 37^o C using BSA-BPB as a substrate [3]. The K_m and V_{max} were determined using a linear regression on Lineweaver-Burk double reciprocal plots.

Results and Discussion

Depending on experimental conditions, the degrees of inhibition caused by absorbed MW radiation vary from 39.11% to 45.91% for 5 and 20 min of pepsin MW irradiation respectively (Figure 1).

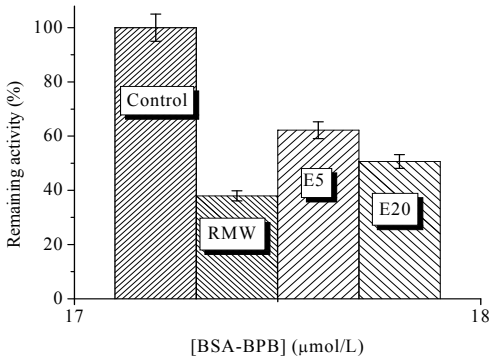

Fig. 1. Percentage of remaining activity: Control - without microwave irradiation; RMW- reaction in microwave field; E5 - reaction with irradiated enzyme solution for 5 min; E20- reaction with irradiated enzyme solution for 20 min. Each point represents the mean of three determinations.

The initial velocity (V_0) of the reactions were calculated and plotted versus BSA-BPB concentrations, generating saturation plots for each monitored reaction. The plots follow Michaelis-Menten kinetics (Fig. 2. - inset) with MW induced decrease of the reaction rates in all cases. A double reciprocal plot of velocity *vs*. substrate concentration at increasing exposure time (5 and 20 min, due to the discernible changes in enzyme activity) resulted in a linear plots intersecting at different points at 1/[S] axis. From Lineweaver-Burk plots the obtained values of V_{max} for the control and reactions for the irradiated pepsin were (3.521±0.176) μM/min and (2.198±0.109) μM/min respectively. The decrease of reaction velocity in a presence of MW irradiated enzyme is not proportional to increased time of irradiation. It is noticeable that for the irradiation times of 5 and 20 min the apparent velocity of enzyme reaction, i.e. the value of V_{max} is not significantly changed (Figure 2). Compared to the control reaction, V_{max} of irradiated samples was decreased. However, the apparent Michaelis-Menten constants varied significantly. At exposure time of 5 min of MW irradiation calculated Michaelis-Menten constant was K_{mapp} = 17.391 μM, while at exposure time of 20 min of MW irradiation K_{mapp} = 24.938 μM, (Figure 2). As the temperature of samples were kept constant in all experiments (37°C) the observed inactivation can be ascribed to the non-thermal effects of MW irradiation. The observed inactivation of pepsin can not be explain by numerous models available for mechanism-based inactivation systems. Based on Schnell and Hanson model [4] for quantitative prediction of non-mechanism-based enzyme inactivation, we consider independent proceeding of enzyme inactivation and product formation.

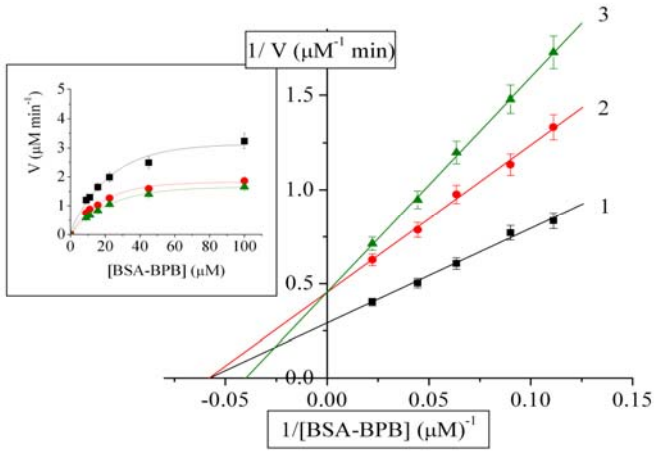

Fig. 2. Lineweaver-Burk plot of pepsin inhibition caused by MW radiation. **1**- control reaction under conventional heating in water bath at 37°C; **2**- with 5 min irradiated enzyme; **3**- with 20 min irradiated enzyme. **Inset** - initial velocity versus BSA-BPB concentration with non-irradiated and MW irradiated pepsin at pH 2 and 37°C.

We approximate reaction model which show that the rate of enzyme transformation in the MW field may be described by the reaction of order 0.85 and rate constant $k_{MW} = (0.38 \pm 0.02)$ $(\mu M/dm^3)^{0.15}$ min^{-1}. The same approach give possibility to kinetically characterize MW-modified enzyme with $V_{max} = (2.194 \pm 0.110)$ μM min^{-1} $K_m = (25.018 \pm 1.251)$ μM which is in good agreement with the experimental results.

Conclusion

The MW irradiation, in controlled experimental conditions (constant absorbed MW energy and temperature) causes a decrease of enzymatic activity, as well as V_{max}. Although MW quanta have no energy to break peptide bonds, decreased enzyme activity can be ascribed to relaxing protein structure, assuming excessive breaking and reforming hydrogen bonds. Accordingly, kinetic behavior of pepsin was described with approximate, "non-mechanism based inactivation", reaction model that is in good agreement with obtained results.

Acknowelagement

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