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In-situ activity of α-amylase in the presence of controlled-frequency moderate electric fields

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ABSTRACT

Studies on the effects of electric fields on enzymatic activity have typically focused on post-treatment residual activity. However, information on their effects on *in-situ* activity is scant. The objective of this study was to investigate the effect of controlled-frequency Moderate Electric Fields (MEF) on α-amylase activity. Relative α-amylase activities were measured *in-situ* in the presence of an electric field (1 V/cm, 1 Hz-1MHz) at 60 °C, with a zero electric field at the same temperature serving as a control treatment. Results show a significant nonthermal effect of electric field frequency on α-amylase activity, with up to 41% enhancement of the activity in the 1–60 Hz frequency window. At higher frequencies, activity was either unaffected, or slightly inhibited by the presence of the electric field. Molecular dynamics simulation results show that in the 1–60 Hz frequency window, the translational electrophoretic enzyme displacement approaches the intermolecular spacing of water. Interestingly, enzyme rotational motion transitions from oscillatory behavior to full rotation below 60 Hz. Application of higher frequencies restricts the electrophoretic displacement of the enzyme due to rapid reversal of the direction of the electric field.

1. Introduction

For many decades, α-amylase has been used in a wide range of applications in industries such as foods, brewing, textiles, adhesives, pharmaceuticals, biofuel, and sewage treatments (Liu, Chen, & Chou, 2003; Sundarram & Murthy, 2014). In the food industry, starch hydrolysis products are widely used for their functional properties (BeMiller & Whistler, 1996), and the industry has shifted from acid to enzymatic hydrolysis due to the high efficiency and specificity of biocatalysts (Sundarram & Murthy, 2014; van der Maarel, van der Veen, Uitdehaag, Leemhuis, & Dijkhuizen, 2002).

α-amylase (E.C.3.2.1.1) is the most widely studied endo-enzyme that catalyzes the hydrolysis of α-1, 4-glycosidic bonds resulting in α-oligosaccharides of varied chain lengths (Janecek, Svensson, & MacGregor, 2014; van der Maarel et al., 2002). Its action on the amylose fraction (contains α-1, 4-glycosidic bonds) of starch is known to proceed in two steps: initially, a rapid and complete degradation of amylose causing a sudden loss of viscosity (i.e. liquefaction) due to random attack on the substrate by the enzyme, and, subsequently, a slow hydrolysis of oligosaccharides forming glucose and maltose as final products (Kulp, 1975). Similarly, amylopectin also undergoes random degradation by α-amylase yielding glucose, maltose, as well as a series of dextrins and oligosaccharides containing α-1, 6-glycosidic bonds (Kulp, 1975), which are further hydrolyzed by a combination of enzymes such as pullulanase and glucoamylase in the process known as saccharification (Chopra et al., 2010). While enzymatic hydrolysis can be continued to produce sweeteners, the intermediate hydrolytic products are clearly important to achieve desirable functional properties of food products. Consequently, it is evident that controlling the rate of enzyme activities is advantageous in manipulating the level of conversion during the hydrolysis of starch.

The interaction of electricity with biological materials has been of interest for several decades (Astrakas, Gousias, & Tzaphlidou, 2012; Hill, 1958; Neumann, 1986; Schwan, 1959). Potato tissues treated by pulsed electric fields have shown electric field-induced tissue damage and enhanced release of intracellular molecules (Jalte, Lanoiselle, Lebovka, & Vorobiev, 2009; Janositz, Noack, & Knorr, 2011). A number of studies relate the effects of electric fields with structural alterations in biological macromolecules and, thereby, their biological functions. Influence of high-voltage pulsed electric field treatments on several food enzymes has been studied by Ohshima, Tamura, and Sato (2007). Their results show up to 20% increase of catalytic activity after subjecting the enzymes to a pulsed electric field intensity of 12 kV/cm. Tian, Fang, Du, and Zhang (2016) have observed up to 22% increase of α-amylase activity when the pulsed electric field intensity is maintained below 15 kV/cm. Furthermore, structural analysis by circular dichroism

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and fluorescence spectra reveals that the electric field increases the α-helices (by 34.76%) and reduces the random coils (by 12.04%) on the protein structure of α-amylase. Moderate Electric Fields (MEF) applied during ohmic heating (typically at less than 100 V/cm, 50–60 Hz) have been shown to exhibit nonthermal effects on enzyme activities (Castro, Macedo, Teixeira, & Vicente, 2004; Jakob et al., 2010). Durham (2015) investigated the activity of cellulase subjected to MEF treatments at various treatment variables. The results show that cellulose activity can be enhanced or inhibited by manipulating the electric field strength, frequency, and temperature. Samaranayake and Sastry (2016a & 2016b) have shown similar results for pectin methylesterase and polygalacturonase in tomato homogenate subjected to MEF treatments.

In addition to the possible alterations in protein structure by MEF (Pereira, Teixeira, & Vicente, 2011), it has been hypothesized that the electrophoretic motion resulting from MEF exerts either an activation or inactivation effect on enzyme molecules (Samaranayake & Sastry, 2016b), depending on the temperature. Since electrophoretic motion, when in an aqueous environment, depends on net charge and dipole moment of a molecule, the degree of activation (or inactivation) by MEF varies for different enzymes. We note that with the exception of Durham (2015), most studies have focused on the residual post-process enzyme activity. It is of interest to investigate the *in-situ* behavior of the enzyme while under the influence of an alternating electric field. Accordingly, the objective of this present study was to investigate the effect of MEF frequency on the *in-situ* activity of α-amylase. An additional objective was to simulate the molecular motion of α-amylase in response to the electric field, to gain further insight into frequency-dependent behavior.

2. Materials and methods

2.1. Materials

The α-amylase (liquid preparation) used in this study was received as a gift from an enzyme manufacturer (Novozymes North America Inc., NC, USA). The optimum activity conditions for this enzyme were pH 5.0 and 60 °C, as reported by the manufacturer. All other chemicals used were of analytical grade.

2.2. MEF treatments

The MEF treatments were conducted in a cylindrical glass treatment cell (internal diameter: 2.54 cm) equipped with platinized-titanium electrodes (Fig. 1). The selection of platinized-titanium is based on its high resistance to adverse electrochemical reactions, enabling smooth operation at low frequency electric fields (Samaranayake & Sastry, 2005). An external circulating water jacket provided temperature control for the test sample (Fig. 2). The electrodes were set 2 cm apart and gentle agitation was provided by magnetic stirring to ensure mixing and uniform temperature throughout. A dual channel function generator (Tektronix AFG 3252, Tektronix Inc., Richardson, TX) and Power Amplifier (Powertran Model 500A RF, Industrial Test Equipment, Port Washington, NY) supplied the electric field. MEF treatments were performed at various frequencies from 1 Hz to 1 MHz (sine wave) by applying a relatively small electric field (1 V/cm) to minimize the ohmic heating effect. The influence of electric field was identified by performing a control experiment without electric field (0 V/cm) at the same temperature. Three independent replicates were run at each MEF frequency; and relative α-amylase activity was expressed with respect to the α-amylase activity of the control.

2.3. In-situ evaluation of α-amylase activity

The measurement of dextrinizing power using the iodometric principle is one of the oldest and best-known methods of determining of α-amylase activity (Huggins & Russell, 1948; Xiao, Storms, & Tsang, 2006). In the present study, an *in-situ* method was developed, based on the iodometric principle, to determine the relative activity of α-amylase. This *in-situ* method is essentially a method modified from that initially proposed by Fuwa (1954), utilizing dilute starch solutions (below 0.1 g/100 mL), for the measurement of dextrinizing power. The test sample (i.e. the substrate) for the enzymatic hydrolysis reaction was therefore a dilute starch solution (0.03 g/100 mL) prepared as follows.

A stock solution was initially prepared by dissolving 0.3 g of soluble potato starch in 100 mL of pH 5.0 sodium acetate buffer (50 mmol/L) containing calcium chloride (1 mmol/L). The solution was then filtered for clarity since it aids the detection of the endpoint. This stock solution was prepared daily, and both MEF (at a given frequency) and control treatments were run using the same stock solution. To prepare the test solution, 1.0 mL of the stock solution was mixed with 100 μL of iodine reagent (5 mmol/L iodine + 5 mmol/L potassium iodide) and then diluted to 10.0 mL using pH 5.0 sodium acetate buffer. The test solution (dark blue color) was freshly prepared prior to fill into the MEF treatment cell (Fig. 1).

In each experimental run, a 8.5 mL aliquot of the test solution was pipetted into the MEF treatment cell (Fig. 1), which was preheated to 60 °C by means of external cooling jackets. When the test solution reached the target temperature (60 °C), the enzymatic reaction was initiated (t = 0) by injecting 500 μL of α-amylase solution, which was

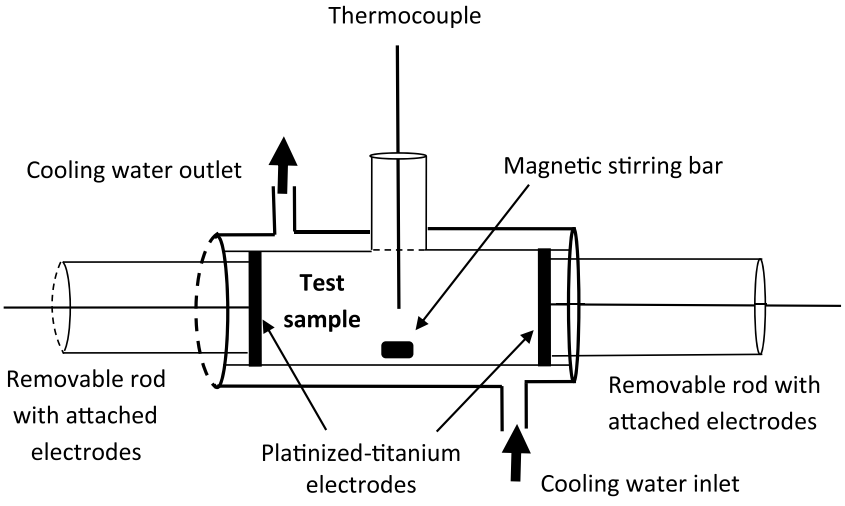

Fig. 1. Schematic diagram of the MEF treatment cell.