Enzymatic Hydrolysis of Potato Amylopectin Immobilized on a Quartz Crystal Microbalance by Attraction between Opposite Charges

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Introduction
Enzymatic hydrolysis of starch is an important digestive process, whose rate and extent affect metabolic response. Restraining the rate and extent of starch digestion by enzymes induces the increase of slowly digestible or resistant starch contents in food materials. The rate of starch hydrolysis is measured in vitro procedures developed in attempt to mimicking human starch digestion. The quartz crystal microbalance with dissipation monitoring (QCMD) is known to provide a very sensitive mass measuring device and be a tool to study real-time procedures developed in attempt to mimicking human starch digestion. The objective of this study was to investigate the method to immobilized starch on the QCM electrode and evaluate the enzmatic hydrolysis of starch by α-amylase, β-amylase, and amyloglucosidase in real-time using QCMD.

Materials and Methods
1) Quartz crystal microbalance with dissipation monitoring (QCMD)

![QCMD Diagram](Q-Sense, Sweden)

- Am = ∆f/∆m (Sauerbrey equation)
- n: mass sensitivity constant
- m: frequency shift
- D = E_{stored}/2f
- D: dissipation which is associated with the viscoelasticity of deposited layers

2) Method to immobilize starch on the QCM electrode

- Immobilizing potato amylopectin by attraction between opposite charges
- Potato amylopectin (AP) → slightly anionic with phosphate groups
- Poly(L-lysine) (PLL) → multiply charged polycation

3) Polyelectrolyte and enzyme solutions

- 10mM sodium acetate buffer (pH7.0) containing 100mM NaCl to stabilize the QCMD signal
- 6.6mg/ml PLL (Sigma P6516) in 10mM sodium acetate buffer (pH7.0, 100mM NaCl)
- Flash out the redundant PLL solution with buffer
- Equilibrated in pH4.5 10mM acetate buffer containing 100mM NaCl for β-amylase and amyloglucosidase
- α-amylase (porcine pancreas, Sigma A6255) solution in pH7.0 10mM sodium acetate buffer
- Amyloglucosidase (Aspergillus niger, Sigma A1602) solution in pH4.5 10mM acetate buffer

Results

- The extent of hydrolysis 96.6±1.9%
- The extent of hydrolysis 37.5-49.5%
- The extent of hydrolysis 75.4-88.6%

Conclusions
- PLL was proved to be an appropriate linker for immobilizing potato amylopectin on the silica surface of the QCMD plate by opposite charge attraction.
- The extent of hydrolysis by α-amylase was over 95% at any tested concentration. In the case of adding β-amylase and amyloglucosidase, the limit dextrin was detected by QCMD and the extent of degradation depended on the concentration of enzyme solutions.
- The initial hydrolysis rate increased linearly with the concentration of enzyme solutions in double logarithmic approximation for α-amylase, β-amylase, and amyloglucosidase.

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