BioSensor 2017

1st European & 10th German BioSensor Symposium

20-23 March 2017 • Potsdam • Germany

Program and Book of Abstracts

www.ebs2017.com

Development of acoustic sensor for detection chymotrypsin activity at surfaces

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Casein protein makes up about 80% of bovine milk. There are four main types of casein: α-s1, α-s2, β and κ. In milk, casein forms micelles that are stabilized by calcium phosphate. They are of porous structure which allows water to flow into the core of the micelle. The surface of the β casein micelle is hydrophobic and terminated by κ casein. Degradation of casein micelles in milk are tied to the processes of milk getting sour or cheese making. Proteases like plasmin and trypsin are part of this process and therefore it is interesting to analyze protease activity in milk or during the process of casein cleaving. In order to study this process we used acoustic biosensor based on Quartz Crystal Microbalance (QCM). This method measures the change of the resonant frequency of the quartz crystal by deposition of mass on the crystal or change of the medium. Using the self assembled monolayer (SAM) of mercaptoundecanoic acid (MUA) we can activate the surface of the gold electrode on the quartz crystal. This monolayer is then able to bind casein - the substrate that can be cleaved by proteases like plasmin or chymotrypsin. This results in measurable change in resonant frequency which roughly translates to the mass of the casein bound to the surface and casein cleaved away by protease interaction. This measurement allows us to observe the cleaving of casein by different proteases. In addition the experimental set-up allowed us for change of the medium, therefore it is possible to measure cleaving of casein in different conditions, for example using different pH. We performed experiments using QCM on the cleaving of β casein by chymotrypsin. Chymotrypsin was detected in the range of concentrations 1 pM – 20 nM. Limit of detection of this acoustic transducer was 1 pM. At this concentration approx 6% of casein layer was cleaved (this corresponds to frequency increase by approx. 9 Hz). This method can be used also for study of the activity of other proteases, such as plasmin. Topography of casein layers and its changes following chymotrypsin cleavage was also studied by atomic force microscopy.

Acknowledgments:
This work was financially supported by European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 690898.