

REVERSIBLE ATTACHMENT OF SUGAR BINDING PROTEIN (CONCAVALIN A) TO A GLUCOSE-COATED QUARTZ CRYSTAL SENSOR

Introduction

Concanavalin A (Con A) is a member of a group of proteins called lectins that are characterized by their specific affinity for sugar residues. Con A has broad applicability primarily because it binds strongly and selectively to α -D-glucose and α -D-mannose. Since a wide variety of serum and membrane glycoproteins have a "core oligosaccharide" structure, many glycoproteins can be examined or purified with Con A and its conjugates. Briefly, Con A has been utilized in hormone receptor studies, mitogenic assays, characterization of normal and malignant cells, glycoprotein purification, viral antigen isolation, dextran and mannan fractionation, cell agglutination studies, bacterial aggregation, membrane fluidity and lateral mobility investigations, turbidimetric assays for sugars, lymphokine production, as well as in many other applications.

This study illustrates the usefulness of Dissipative QCM (KSV QCM-Z500) for the investigation of sugar binding protein reactions. Here, the quartz crystal was coated with α -D-Glucose using special attachment chemistry. Apart from α -D-glucose, many other sugars coated quartz crystal sensors are available.

Results

Figures 1,2 and 3 show for the three measured overtones the frequency change (Δf_N), normalized frequency change ($\Delta f_N/N$) and the mass area density ($\Delta M/\Delta A$) calculated using the Sauerbrey equation. Solutions were introduced to the measurement cell in the following order: 600 s - 10 μ M Con A, 1500 s - regeneration liquid, 2100 and 2500 s - 2x PBS rinses, 2700 s - 10 μ M BSA. The final addition was performed to determine non-specific binding to the regenerated surface.

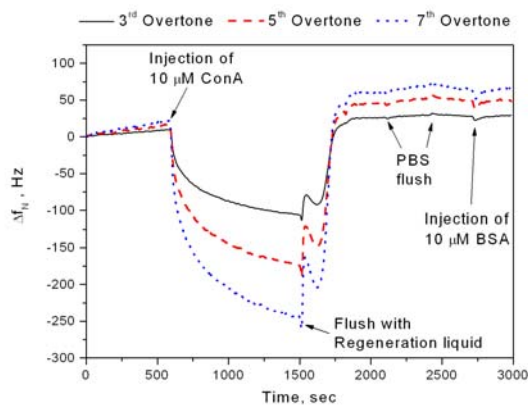


Figure 1

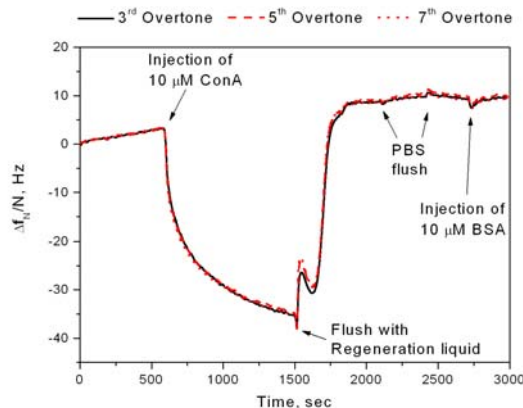


Figure 2

The frequency decreases because Con A binds to the sugar residues on the quartz crystal, and the binding kinetics are clearly visible. The regeneration step clearly shows that Con A binding is completely reversible. The slight and steady increase in the Δf profile before injecting Con A indicates a continuous and slow detachment of the sugar residues from the surface, which is supported by the fact that a straight line can be drawn between the Δf profile before Con A is introduced to that after regeneration. However, the normalized Δf is the same for all of the measured overtones (Figure 2), which means that the formed layer is rigid and the Sauerbrey equation can be used for calculating the adsorbed mass (Figure 3). The rather small change



Dissipative QCM Application Example

in dissipation (viscous energy loss, referred to as D) during the binding also indicates that the layer remains rigid (Figure 4).

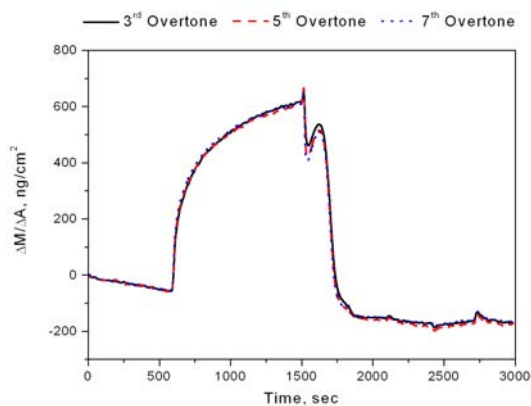


Figure 3

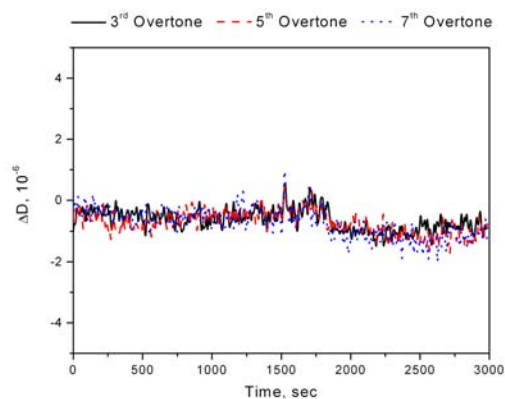


Figure 4

A closer examination of the dissipation change reveals a slight drop at about 1700 s, which coincides with the time at which the regeneration solution was introduced. If the resistance change (ΔR) is plotted instead of the dissipation change (ΔD), a more pronounced effect can be observed (Figure 5). R is coupled to D and hence dissipative process according to:

$$D = (R / L \times 2\pi f) ,$$

R = Resistance

L = Inductance

f = Resonance frequency

The analysis software of the QCM-Z500 instrument not only calculates the adsorbed/desorbed amount of material at the surface but also the adsorbed layer thickness. The amount of material adsorbed and the thickness are shown in Figure 6. The thickness of the Con A layer obtained from the modeling i.e. ~ 8 nm corresponds very well with the dimensions of Con A found in literature 7.88 nm x 7.93 nm x 13.3 nm (J Struct Biol. 1996 117(1):16-23).

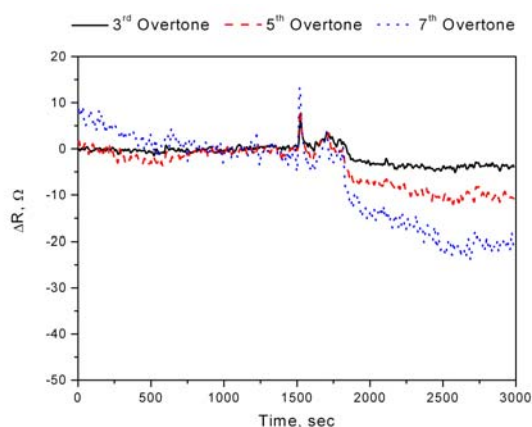


Figure 5

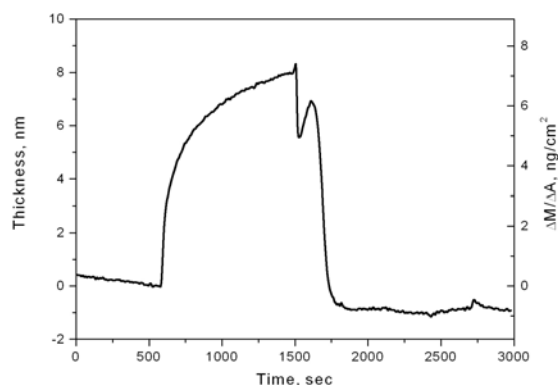


Figure 6



Dissipative QCM Application Example

Conclusion

This study clearly shows the usefulness and ease of use of QCM for the study of affinity interactions. The ability to extract information on dissipative processes at the surface increases the scope of the measurement compared with optical techniques. What's more, the availability of many different sugar residues facilitates the possibility of doing 'affinity fingerprinting' between protein samples and a matrix of sensor surfaces coated with different sugar residues.