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Investigation of Ferritin Desorption from Gold Initiated by *In Situ* pH-Change

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One of the main questions regarding protein adsorption is about the reversibility of the adsorption process. To get a deeper understanding of this, adsorption of ferritin on Au was studied by quartz crystal microbalance and the pH of the buffer was changed *in situ* between two values that favour adsorption by different amounts. We found that although some ferritin desorbs from Au, the desorption is incomplete. When the desorption reached a constant value, we returned to the original conditions and investigated the readsorption. Our experiments show that the adsorption of ferritin onto Au is a partly reversible process. We found that for different initial ferritin coverages the proportion of ferritin that had been subsequently desorbed was approximately constant.

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1. Introduction

Protein adsorption on surfaces is a well-studied phenomenon, as protein–surface interactions are present in life and are useful for technological applications [1–7]. At the same time they are sometimes undesirable, as they can lead to formation of biofilms or cause thrombosis [5].

Therefore the reversibility of protein adsorption onto a wide range of surfaces is a broadly studied subject. Various theories were developed to analyse these processes and there are several experimental reports [6–9]. However, there exists no entirely satisfactory, comprehensive theory.

To help the development of further theories and to gain deeper understanding of the protein adsorption onto surfaces, we decided to monitor *in situ* the adsorption and desorption processes of a selected protein induced by the change of pH. The protein needs to be stable in the investigated pH-range, and be easy to describe and model.

We have chosen native horse spleen ferritin as a useful tool for our investigation of protein adsorption on Au surfaces. Ferritin is a quasispherical protein, whose function is to store iron in living organisms. The inorganic core of ferritin has a diameter of about 8 nm and can contain up to 4500 Fe(III) ions [10], while the external diameter of the protein is 12 nm. Ferritin is a good choice for gravimetric investigation by quartz crystal microbalance (QCM) [1–3] as the inorganic iron-containing core has a much higher density than a typical protein without inorganic component, which means that the signal-to-noise ratio on the QCM is enhanced, giving more accurate measurements of the adsorbed amount. Even though not every ferritin core has the same mass, when averaging over a large surface the differences are negligible.

In their previous work, Caruso et al. [1] showed that the adsorption of ferritin is dependent on concentration in the range of 1–20 μg ml^{-1}, while Hemmersam et al. [2] showed that the adsorption of ferritin is also dependent on the surface properties such as the isoelectric point and the pH.

In this paper we show for the first time that it is possible to alternate between adsorption and desorption of ferritin on Au by changing the pH of the solution *in situ*. We also show that although there is some degree of reversibility in the adsorption of ferritin, it is far from being a completely reversible process. We further present results of how ferritin desorption depends on the amount previously adsorbed.

2. Materials and methods

The horse spleen ferritin (Sigma–Aldrich, in 0.150 M NaCl) was used as received. (Tests carried out with ferritin that we have purified further did not show qualitative differences to the as-received material.) The buffer solution contained 5 mM HEPES (Fisher Scientific, 99%+), 5 mM acetic acid (Aldrich, 99.7%+) and 100 mM KCl (Sigma-Aldrich, 99–100.5%), dissolved in MilliQ water (resistivity 18.2 MΩ cm). The pH of the buffer was changed by addition of KOH and HCl.

The QCM measures the frequency shift caused by the adsorbed mass on an oscillating piezoelectric crystal. In the case of small mass changes the Sauerbrey equation [11] applies

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$$\Delta f = C_f \Delta m, \quad (1)$$

where $f(5\ \mathrm{MHz})$ is the frequency of the crystal, C_f is a constant for a given quartz crystal and Δm is the adsorbed mass. The Sauerbrey equation is deduced for oscillation in a gas environment, therefore in our case viscoelastic effects from the solvent and the adsorbed layer complicate the situation. Based on the results of Caruso et al. [1] the theoretical value of C_f for in-air measurements has to be multiplied by ≈ 2.3 when applied to ferritin adsorbed from liquid. Due to the method used by Caruso et al. [1], this estimate should probably be considered as a maximum value.

The adsorption data were measured using a Stanford Research Systems QCM 200, and quartz crystals (2.54 cm diameter, AT-cut Stanford Research Systems) coated with sputtered Au.

The Au-coated crystals were cleaned using Piranha solution (1 part 30% H_2O_2 and 3 parts 96% H_2SO_4) for 20 min, then rinsed thoroughly with MilliQ water [1]. The cleanliness of the crystal was ensured by checking the frequency in air.

The solution was stirred (300 rpm) and the pH was monitored *in situ*, using a PHM210 standard pH meter (MeterLab, Radioanalytical, Copenhagen). The measurements were conducted at room temperature.

3. Results

We made various measurements to find out more about the adsorption and desorption of ferritin onto/from Au. The reproducibility of our results was around 20%, which is comparable to the reproducibility reported by Feraponova et al. [4].

The charge on the ferritin surface can be varied by a change of pH. The isoelectric point (pH(I)) of the ferritin being around 4.6 [12] implies that the ferritin is negatively charged above this pH. If the pH of the buffer is 5, there is a weak electrostatic repulsion between the ferritin molecules themselves — which increases considerably if the pH is changed to higher values making the surface charge of ferritin more negative [2]. An IP cannot be stated for Au, but previous research suggests that the adsorption of ferritin on Au reaches its maximum at pH 5 and then diminishes as the pH of the solution is increased [2]. In our case we changed the pH to 7.5 *in situ*, to get a deeper insight into the reversibility of ferritin adsorption on Au.

Figure 1 shows how the system reacts to a pH change *in situ*. The ferritin was adsorbed at pH 5.0 ± 0.1 for 10 min, after which the pH was changed to 7.5 ± 0.1 by the injection of 5 M KOH solution, to initiate desorption. After 15 min at pH 7.5±0.1 the pH of the solution was reset to 5.0±0.1 by the injection of the appropriate amount of 20% HCl solution to favour adsorption again. As the solution was stirred during all of our experiments, homogeneity was reached after seconds. This experiment demonstrates the feasibility of alternating between adsorption and desorption of ferritin on Au by changing the pH of the solution *in situ*.

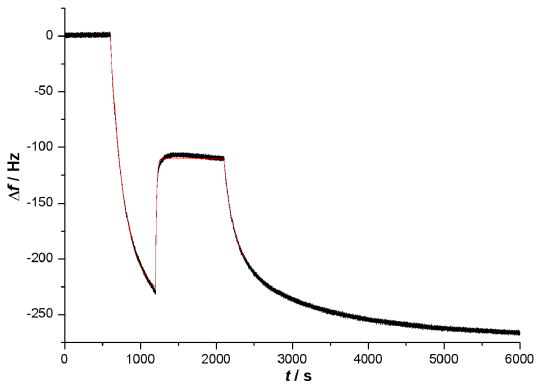

Fig. 1. pH-dependent adsorption and desorption of ferritin. Ferritin (final concentration 100 μg ml^{-1}) was added at $t = 600$ s. The adsorption started at the moment of addition. At $t = 1200$ s the pH of the solution was changed to 7.5±0.1 from the initial value of 5.0±0.1, and this induced immediate desorption. At $t = 2100$ s the pH of the buffer was returned to 5.0±0.1, when readsorption occurs. Exponential curves (for further details see in the text) were fitted to the adsorption and desorption curves. In our raw data there is a small linear term which we attribute to drift, and which is constant and independent of pH. We subtracted this term prior to plotting.

To see whether the frequency change reached at the higher pH is an absolute value, we measured the desorption after various adsorption times (2, 5 and 10 min). Different adsorption times correspond to different amounts of ferritin initially adsorbed. Figure 2 shows that the steady-state value reached at pH 7.5 ± 0.1 is indeed dependent on the initial adsorption time or the adsorbed amount. The data show that under our experimental conditions the proportion of initially adsorbed ferritin that desorbs is approximately fixed at around $50 \pm 5\%$.

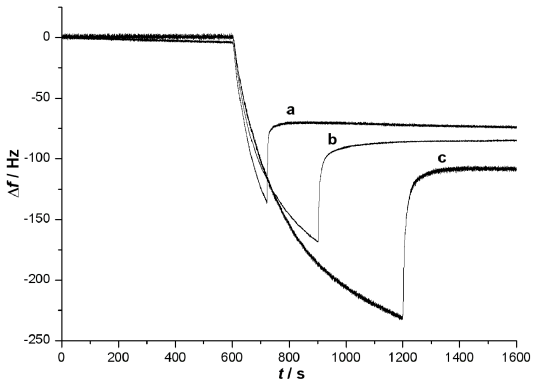

Fig. 2. Time dependence of ferritin desorption. Ferritin (final concentration 100 μg ml^{-1}) was added at $t = 600$ s. After letting ferritin adsorb for: (a) 120 s, (b) 300 s, and (c) 600 s, desorption was initiated by changing the pH from 5.0 ± 0.1 to 7.5 ± 0.1.

4. Discussion

4.1. The kinetics of adsorption

Providing the fitting interval is not too long, the time-dependence of the frequency change can be well described by a simple exponential approach to saturation

$$\Delta f = l\,[1 - \exp(-\Delta t/t_{\mathrm{ads}})]\,, \tag{2}$$

where Δf [Hz] is the frequency change of the oscillating quartz crystal, Δt [s] is the adsorption time, l is the saturation frequency change, which is a measure of the maximum amount of ferritin that may be adsorbed, and t_{ads} is a time constant for adsorption. t_{ads} will likely depend on the protein concentration close to the crystal surface.

For longer intervals (2) breaks down, which is perhaps not surprising as it is based on a very simple model of irreversible adsorption. The effective time constant for adsorption t_{ads} is smaller for readsorption (143 ± 14 s) than for the initial adsorption (186 ± 18 s), perhaps because in the former case the local concentration is higher due to some ferritin that was desorbed remaining in the vicinity of the substrate.

4.2. Desorption induced by pH change

To see whether the adsorption of ferritin on Au is a reversible process, the pH and hence the charge on the protein was changed *in situ*. The fact that changing the pH initiates desorption already implies that there is some reversibility in the system. The desorption is not complete, which could be caused by a change in state of some of the adsorbed protein. Our result that $50 \pm 5\%$ of the adsorbed protein is desorbed independently of the initial adsorption time/adsorbed amount suggests that the proportion of protein that adsorbs reversibly is approximately the same at all stages of the process. Further experiments are needed to determine whether protein that is initially adsorbed reversibly can transform to an irreversibly adsorbed form on longer timescales.

4.3. The kinetics of desorption

The kinetics of desorption can also be described by an exponential curve, where the limit for large t is dependent on the preceding adsorption time/previously adsorbed amount. The equation of the fitted curve is

$$\Delta f = r\,[\exp(-\Delta t/t_{\mathrm{des}})] + i\,, \tag{3}$$

where t_{des} is a time constant for desorption. In this equation r is proportional to the reversibly adsorbed protein amount and i to the irreversibly adsorbed amount. In the case of desorption after 10 minutes adsorption time, r equals -101 ± 10 Hz, $t_{\mathrm{des}} = 22 \pm 10$ s and $i = -110 \pm 11$ Hz. In the case of initial adsorption for 2 min i equals -72 ± 7 Hz; in the case of 5 min $i = -86 \pm 9$ Hz.

If we compare t_{des} and t_{ads}, we see that t_{des} is an order of magnitude smaller than t_{ads}, which means that the kinetics of desorption is faster than those of the adsorption. This result is consistent with the adsorption being limited by the near-surface concentration while in the case of desorption this is not a major factor.

5. Conclusions

We have shown that the desorption of ferritin from Au can be induced by changing the pH of the buffer *in situ*. By comparing rates of adsorption and desorption we find evidence that the adsorption of ferritin is limited by the near-surface concentration of protein in our system. We do not observe significant dependence of the proportion of ferritin that desorbs on the initially adsorbed amount. Although at the atomic and molecular level, protein adsorption is a highly complex process, our results show that a simple model appears sufficient to describe the observed kinetics. Similarly simple pictures have been used to interpret the results of previous studies [1, 2].

Acknowledgments

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