PERKIN 2

'Molecular-imprinting' of AMP utilising the polyion complex formation process as detected by a QCM system

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The formation of a polyion complex was applied for molecular imprinting of AMP on a QCM resonator surface. The gold electrode on the resonator surface was modified with anionic thiols. Subsequently, layers of polycations and boronic acid-containing polyanions were adsorbed onto the QCM anionic surface utilising an alternating adsorption process. When the polymer adsorption was conducted in the presence of AMP, the anionic charges of the phosphate group introduced into polyanion by a boronate–*cis*-diol interaction altered the film growth behaviour: *viz.*, excess polycationic units were adsorbed onto the polyanionic surface. After removal of the AMP from the surface polyion complex, a swollen gel layer with excess cationic charge is created. It was proven that this QCM system sensitively responds to AMP. The responsiveness is derived from the mass decrease in relation to the shrinking of the surface gel layer.

Introduction

'Foot-printing' is a convenient technique to protect a specific segment of DNA (or RNA) from scission by complexation with its complementary oligonucleotide, intercalators or proteins. It occurred to us that this technique would also be applicable to molecular imprinting (MI) in order to retain the memory of specific guest molecules. The conventional MI comprises a vinyl polymerisation process and consists of three steps: (i) synthesis of functional vinyl monomers that can interact with a target template, (ii) radical polymerisation of the vinyl monomers with cross-linking reagents in the presence of the template and (iii) removal of the template from the cross-linked copolymer resin.[1–10] Actually, this technique has already achieved success to some extent.[1,2] More recently, our group exploited a new MI method utilising the mechanism of the polyion complex formation: *viz.*, (i) the polyion complex formation between boronic acid-containing polyanion and polycation in the presence of an anionic template which is bound to the boronic acid group and (ii) removal of the anionic template by extensive extraction of the precipitate. The 'cleft' thus created in the polyion complex should show a 'memory' for the original anionic template molecule.

To test this working hypothesis we previously attempted 'molecular imprinting' of AMP utilising a polyion complex formation process.[11] The ribose moiety in AMP has a *cis*-diol function which can be bound to the phenylboronic acid group at basic pH, where the boronic acid groups exist as anionic boronate groups.[12–18] Hence, this boronate anionic site and the pendant phosphate anion site, in addition to the carboxylate anion site in polyanion **1**, are responsible for the formation of a polyion complex with polycation **2**. As expected, a 'cleft' created after removal of AMP showed high affinity for AMP.[11] In addition, we noticed that the removal and re-binding processes for AMP coincide with swelling and shrinking phenomena of this polyion complex.[11] The present paper reports an extension of this novel AMP imprinting method to an AMP sensing system using a QCM (Quartz Crystal Microbalance).[19–21] The basic concept for the present methodology is illustrated in Fig. 1. Since the re-binding of AMP induces the shrinking of the AMP-imprinted polyion complex, this change should be conveniently read out with a frequency change in the QCM system.[22] We have found that the multilayers deposited by an alternating adsorption method on a gold-coated QCM resonator only show satisfactory responsiveness to AMP when it is imprinted during the multi-layer adsorption process.

Table 1 Composition of the polyelectrolyte solutions for the non-imprinting system

Polycation solution	Polyanion solution
[cation unit]: 2 mmol dm^{-3}	[carboxylate unit] = [boronate unit]: 1 mmol dm^{-3}
Na_2CO_3: 5 mmol dm^{-3}	Na_2CO_3: 5 mmol dm^{-3}
$NaHCO_3$: 5 mmol dm^{-3}	$NaHCO_3$: 5 mmol dm^{-3}

Results and discussion

Preparation of a QCM resonator surface depositing multi-layered polyion complexes

In order to obtain an anionic QCM resonator surface, the resonator was exposed to 3-mercaptopropionic acid.[23,24] Then, the multi-layered polyion complex consisting of polyanion **1** and polycation **2** was deposited in a sequence of steps onto the anionic resonator surface. The film growth in the absence of AMP (see Table 1) is shown in Fig. 2 (plot a). A gradual decrease in QCM frequency was observed, which supports the view that the multi-layers were successfully stacked on the surface. When 14 layers had been deposited, a frequency decrease ($-\Delta F$) of 1189 Hz was observed. This change corresponds to film thickness (d) of 190 Å according to the calculation from equation (1).[25]

$$d(\text{Å}) = -0.16\,\Delta F(\text{Hz}) \qquad (1)$$

Addition of AMP into both the polycation and polyanion solutions (see Table 2) significantly changed the film growth

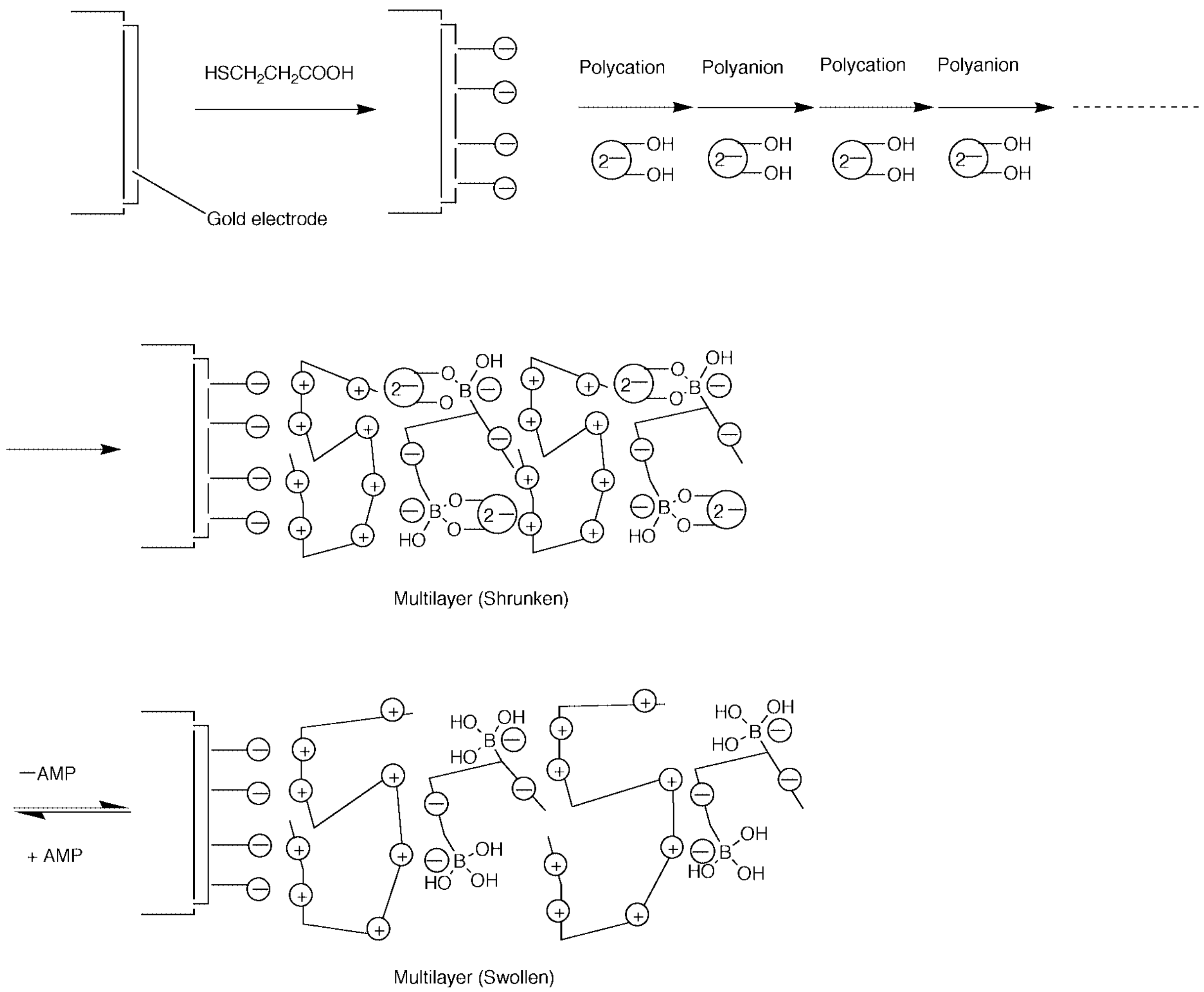
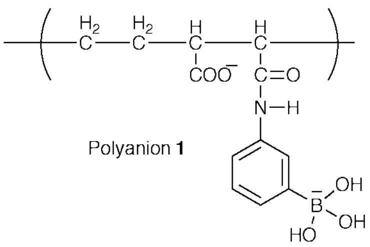
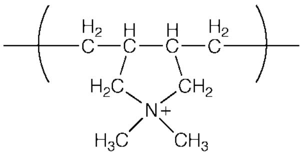
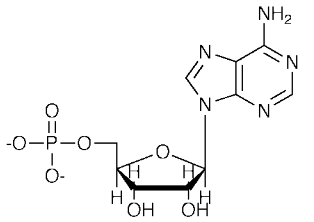
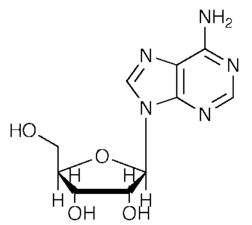


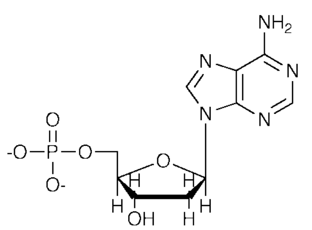
Fig. 1 Alternating adsorption of polyanion **1** and polycation **2** on a gold-coated QCM resonator.


Polyanion **1**


Polycation **2**


Adenosine 5'-monophosphate (AMP)


Adenosine (Ad)


2'-Deoxyadenosine 5'-monophosphate (DAMP)

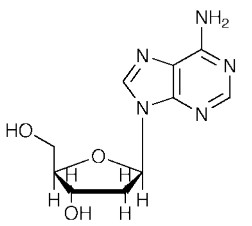

2'-Deoxyadenosine (DAd)

Table 2 Composition of the polyelectrolyte solutions for the AMP-imprinting system

Polycation solution	Polyanion solution
[cation unit]: 2 mmol dm^{-3}	[carboxylate unit] = [boronate unit]: 1 mmol dm^{-3}
AMP: 1 mmol dm^{-3}	AMP: 1 mmol dm^{-3}
Na_2CO_3: 5 mmol dm^{-3}	Na_2CO_3: 5 mmol dm^{-3}
$NaHCO_3$: 5 mmol dm^{-3}	$NaHCO_3$: 5 mmol dm^{-3}

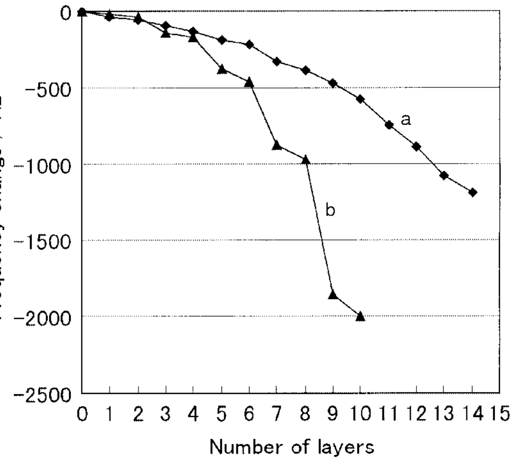


Fig. 2 *In air* frequency decrease in QCM induced by alternating adsorption of polycation **2** (odd layers) and polyanion **1** (even layers). Plot a: in the absence of AMP, plot b: in the presence of AMP.

behaviour (Fig. 2, plot b). The magnitude of frequency decrease for the polyanion adsorption steps was comparable with those for plot a (adsorption in the absence of AMP), but a much larger frequency decrease was observed for the polycation adsorption steps. When AMP was added only into the polyanion solution, the film growth curve was basically the same as that in plot a. These results suggest the following film growth mechanism: i) the complexation between the boronate group and AMP does not occur in the polyanion adsorption step owing to the electrostatic repulsion between the phosphate group in AMP and the carboxylate and boronate groups in the polyanion and ii) in the polycation adsorption step, AMP is adsorbed into the film layer by both the diol–boronate complexation and the electrostatic interaction between the polycation and the phosphate anionic charge. The difference between plot a and plot b clearly shows that a polycation-rich surface had been constructed by this ‘AMP-imprinting’ method. After 10 layers had been deposited, the frequency decrease of 2001 Hz was observed which corresponds to 320 Å in film thickness.

AMP imprinted in the multi-layer was removed by immersing the resonator into an acetate buffer solution (pH 5.5). An increase in resonance frequency (119 Hz) was observed after this treatment.

Frequency change in the AMP-imprinted and non-imprinted QCM systems

Fig. 3 shows the resonance frequency changes induced by the addition of AMP and its adenine analogues in the AMP-imprinted QCM system. The resonance frequency decreased in response to Ad addition (Fig. 3b). This change is attributed to the weight increase caused by the binding of Ad by the diol–boronate interaction. On the contrary, the resonance frequency was increased in AMP solution (Fig. 3a). Previously, we found that the polyion complex prepared in solution from polyanion **1** and polycation **2** in the presence of AMP shows swelling-shrinking phenomena which coincide with removal and re-binding of AMP.[11] In solution, we obtained a gel which is the charge-neutralised polyion complex containing AMP. Removal of anionic AMP from the polyion complex precipitate yielded the cation-excess polyion complex, which is swollen because of the enhanced hydrophilicity. It changes to the shrunken gel by the re-binding of AMP because the excess cationic charge is neutralised. It is reasonable to consider that the gel layer deposited onto the QCM resonator surface shows a similar swelling-shrinking behaviour to the polyion complex gel which is formed in solution. Therefore, the unique QCM response observed for AMP addition can be rationalised in terms of weight loss caused by the shrinking of the surface gel layer that offsets the weight gain arising from the AMP binding and overall induces a frequency increase. It was also confirmed that DAMP (which does not have the *cis*-diol moiety but does have the anionic phosphate group) and DAd (which has neither the *cis*-diol moiety nor the anionic phosphate group) hardly changes the resonance frequency (Fig. 3c and 3d). This supports the view that both the *cis*-diol-boronate and phosphate-polycation interactions are indispensable for the shrinking of the surface gel layer. We have plotted the frequency change values against AMP concentration (Fig. 4) and this showed that the detection limit for AMP is very low (~10 μmol dm^{-3}) and even more sensitive than a conventional biosensor system (~300 μmol dm^{-3}).[26] Our results exhibit a few potential advantages of the present system over the conventional system: *viz.*, i) the sensitivity is very high because the binding of a small amount of AMP is largely amplified into a shrinking phenomenon of the gel and ii) the selectivity is also very high because it responds only to targets having both a *cis*-diol moiety and an anionic group.

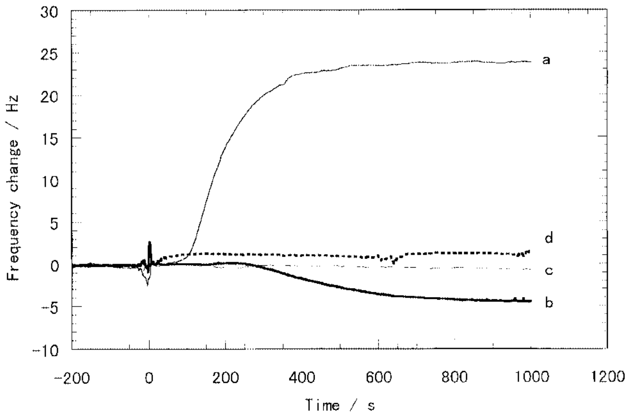


Fig. 3 *In situ* QCM frequency change for the AMP-imprinted system induced by the addition of (a) AMP, (b) Ad, (c) DAMP and (d) DAd. The final additive concentrations are 100 μmol dm^{-3}: 25 °C, pH 10.2 with 10 mmol dm^{-3} carbonate buffer.

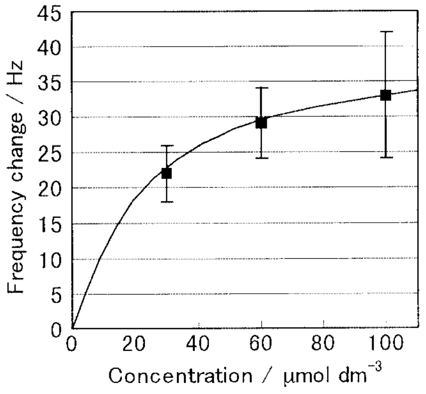


Fig. 4 Plots of frequency change *vs.* AMP concentration in the AMP-imprinted system: 25 °C, pH 10.2 with 10 mmol dm^{-3} carbonate buffer. The plots are the average values of three samples. The reproducibility is shown by the error bars.

The frequency response of the non-imprinted QCM system is

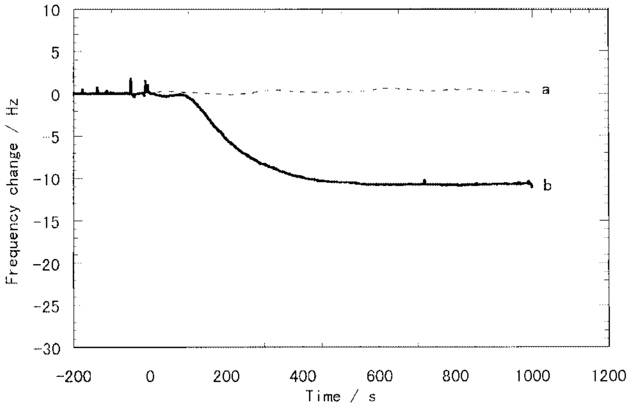


Fig. 5 *In situ* QCM frequency change for the non-imprinted system induced by the addition of (a) AMP and (b) Ad. The final additive concentrations are 100 μmol dm^{-3}: 25 °C, pH 10.2 with 10 mmol dm^{-3} carbonate buffer.

shown in Fig. 5. It responded to neutral Ad but did not to anionic AMP. In this case, the surface polyion complex layer should be charge-neutralised and more hydrophobic than that of the AMP-imprinted system. Probably, the relatively hydrophobic Ad can enter into the film layer while the binding of AMP bearing a hydrophilic phosphate group is thermodynamically unfavourable.

Conclusion

The present study demonstrates that the 'molecular-imprinting' technique developed in a polyion complex system is reproducible in multi-layers deposited by an alternating adsorption sequence on a gold-coated QCM resonator. In addition, the results imply that QCM is a convenient tool to estimate the 'molecular-imprinting' efficiency. We believe that this is a rare example of a successful combination of the 'molecular-imprinting' technique with a QCM system which renders a facile and sensitive detection of AMP possible. Undoubtedly, this methodology has a wide scope of future applications.

Experimental

Synthesis of polymers

Polyanion **1** was synthesised as reported.[11] Polycation **2** was purchased from Aldrich.

Preparation of QCM resonators

An AT-cut 9 MHz quartz crystal (USI system, Japan), each side of which was coated with a gold electrode of area 16 mm^2, was exposed to a 1 mmol dm^{-3} 3-mercaptopropionic acid–ethanol solution for 24 h, followed by rinsing with water and drying with nitrogen. Polyelectrolyte adsorption was then performed as follows. The quartz crystal was immersed in a polycation solution for 10 min, followed by washing with water and drying with nitrogen. This polycation-coated substrate was then exposed to a polyanion solution for 10 min, followed by washing with water and drying with nitrogen. This procedure was repeated until 14 layers for the non-imprinted system and 10 layers for the AMP-imprinted system had been adsorbed. The *in air* QCM frequencies were measured at each adsorption step. All these experiments were conducted at room temperature.

Removal of AMP from the AMP-imprinted polyion complex layer

The QCM substrate was immersed into an acetate buffer solution (pH 5.5 with 90 mmol dm^{-3} CH_3COONa and 10 mmol dm^{-3} CH_3COOH) at room temperature for 20 min, followed by washing with water and drying with nitrogen.

Frequency change in the AMP-imprinted and non-imprinted QCM systems

The QCM measurements in aqueous solution were possible only when one side of the crystal was sealed in a Teflon casing. The resonator was equilibrated with 10 cm^3 of 10 mmol dm^{-3} carbonate buffer solution (pH 10.2) in a glass vessel at 25 °C, and then a small amount of 100 mmol dm^{-3} AMP (or its analogue) was added to the solution.

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