no more visibly appears until oscillations commence. As the system begins to oscillate, white polymer precipitate is observed. However, small amounts of polymer do form, as can be seen from Figure 1a in which the transmittance slowly decreases during the inhibition period.

The polymerization does not occur continuously but rather in a stepwise manner, in phase with the oscillations in the cerium(IV) concentration. Figure 1b shows that the polymer appears as the cerium(IV) is reduced, but while the cerium is being oxidized, the amount of polymer remains constant. This observation is consistent with experiments which indicate that radical intermediates' concentrations oscillate. Försterling et al. measured oscillations in [$BrO_2^•$] on the micromolar level.[14] Venkataraman and Sørensen[15] measured oscillations in the malonyl radical concentrations on the nanomolar level.

To eliminate the possibility that the precipitation process is itself periodic, acrylonitrile polymerization was initiated by cerium(IV)/$H_2SO_4$ and malonic acid. Figure 2 shows that the polymer appears monotonically as the cerium is reduced. This experiment also indicates that malonyl radicals formed by the oxidation of malonic acid[15-19] can initiate polymerization. The same experiment was performed using bromomalonic acid synthesized according to the procedure of Försterling et al.[20] Polymer formed, but much more slowly than with malonic acid.

We determined that the initiation is not from $BrO_2^•$ radicals formed during the oxidation of cerium. Using cerium(III)/$H_2SO_4$ and bromate solution of the same concentrations as with the Ce(IV) experiments, acrylonitrile did not polymerize. Also, cerium(IV) does not react at a significant rate with acrylonitrile.

The inhibition period is proportional to the amount of acrylonitrile initially present in the system up to 1.0 mL, and then it reaches a plateau. The addition of bromide or bromomalonic acid shortens the inhibition period. If acrylonitrile is added after 21 min, no inhibition period occurs. This suggests that acrylonitrile is scavenging bromomalonyl radical, interfering with the production of bromide, and preventing the system from switching to the reduced state. After 21 min, enough bromomalonic acid has accumulated that scavenging by acrylonitrile does not prevent bromide release.

The central problem is why little polymer is observed after the initial burst even though Ce(IV) is present with malonic acid. We believe that polymerization is started initially by malonyl radicals formed as the Ce(IV) is reduced. The Ce(III) is reoxidized in an autocatalytic process involving $HBrO_2$ and $BrO_2^•$.[21] The $BrO_2^•$ radical can terminate growing polymer chains via disporportionation, thus stopping the polymer from growing to a sufficient length to precipitate while regenerating $HBrO_2$. The rate of disproportionation is much greater than the rate of polymer chain termination because the concentration of $BrO_2^•$ ($\sim 10^{-6}$ M) is a thousand times greater than the polymer chain concentration ($\sim 10^{-9}$ M, assuming all of the malonyl radicals initiate polymerization). Support for this scenario was provided by adding acrylonitrile 1.6 min after the cerium was added, when $BrO_2^•$ would be present. No polymer was observed.

The appearance of polymer while oscillations continue in the standard BZ reaction is not a general property of vinyl monomers. The addition of other monomers such as methyl methacrylate and styrene stops oscillations completely unless the $[H_2SO_4]_0 \geq 3$ M, but butyl acrylate behaves similarly to acrylonitrile.

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## Hybridization of Nucleic Acids Immobilized on a Quartz Crystal Microbalance

Yoshio Okahata,*[1a] Yuka Matsunobu,[1a] Kuniharu Ijiro,[1a] Masayuki Mukae,[1b] Akira Murakami,[1b] and Keisuke Makino[1b]

*Department of Biomolecular Engineering Tokyo Institute of Technology Ookayama, Meguro-ku, Tokyo 152, Japan Faculty of Textile Science, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606, Japan*

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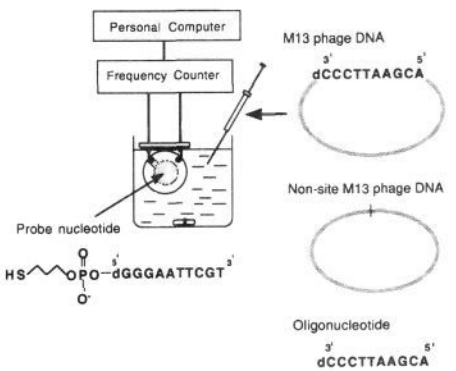
Current methods of gene analysis are dependent upon the ability to detect specific DNA sequences in a heterogeneous mixture. The immobilization of DNA on a solid support has been used to separate complementary DNAs from solution by procedures such as the affinity capture method[2,3] and the sandwich hybridization method[4] using radioisotope-labeled or hapten-labeled DNA. These conventional methodologies have some difficulties: (i) pre- and posttreatments are required to modify DNAs with probes or proteins and to analyze hybridizations, respectively; (ii) it takes a relatively long time to analyze the results; and (iii) it is difficult to detect quantitatively the absolute amount and the time course of hybridization.

In this communication, we propose a new methodology to detect in situ, quantitatively, the one-to-one hybridization between the oligonucleotides immobilized on Au electrodes of a quartz crystal microbalance (QCM) and target M13 phage DNAs in aqueous solutions from the frequency changes of the QCM. QCMs are known to provide very sensitive mass measuring devices because their resonance frequency decreases upon the increase of a given mass on the QCM on a nanogram level.[5,6]




The QCM employed is a crystal of commercially available 9-MHz, AT-cut quartz on which Au electrodes were deposited on both sides (area: 16 mm$^2$ × 2). The QCM was connected to

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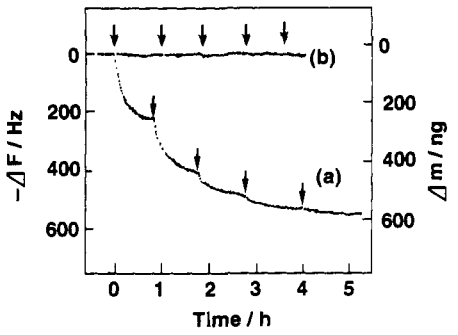


**Figure 1.** Typical frequency changes of 10-mer nucleotide (106 ng, 35 pmol) immobilized QCM, responding to each addition (120 ng, 0.05 pmol) of (a) M13 phage DNA and (b) nonsite M13 phage DNA in a 10-mL aqueous solution at 50 °C.

a handmade oscillator designed to drive the quartz at its resonance frequency in aqueous solutions.[6,7] The frequency changes were followed by universal frequency counter (Iwatsu Co., Tokyo, Japan, Model SC 7201) attached to a microcomputer system (NEC Co., Tokyo, Japan, Model PC 9801). Calibration showed that a frequency decrease of 1 Hz corresponded to a mass increase of 1.05 ng on the QCM electrode (16 $mm^2$) both in aqueous solutions and in air.[6,7]

We have prepared the 10-mer deoxynucleotide having a mercaptopropyl group at the 5'-phosphate end by the phosphoramidite method[8] as a probe DNA, whose sequence was complementary with the *Eco*RI binding site of single-stranded M13 phage DNAs (7249 base pairs, MW = $2.4 \times 10^6$). Nonsite phage M13 DNAs (7239 base pairs) were also prepared in which the complementary 10 nucleotides were removed. The QCM was immersed in an aqueous solution of the probe DNA with an SH group (1500 ng in 5 mL) for 20 min at 25 °C.[9] The amount of the probe nucleotide immobilized was calculated to be 106 ± 10 ng (35 pmol) on both sides (16 $mm^2 \times 2$) of the electrodes. This value was calculated to be ca. 8% coating coverage of the Au electrode surface of the QCM.

Figure 1 shows typical time courses of frequency changes of the probe nucleotide-immobilized QCM responding to each addition of M13 phage DNA or nonsite M13 DNA as a target DNA to distilled water (10 mL) at 50 °C. In the case of the complementary M13 DNA, the frequency decreased with time, responding to each addition, and it was saturated at $-\Delta F = 560 \pm 10$ Hz (mass increase of 590 ± 10 ng) at a concentration of 600 ng (0.25 pmol) in 10 mL. At the saturation point, all DNAs added in the solution bound to the probe nucleotide on the QCM, and an M13 phage DNA was calculated to hybridize with ca. 0.5 mol % of probe nucleotides on the QCM. The minimum concentration needed to detect M13 phage DNA was observed to be 1 pM (25 ng in 10 mL) in the solution. The nonsite M13 phage DNA hardly interacted with the probe DNA on the QCM at 50 °C (see curve b in Figure 1).

After the hybridization of the probe nucleotide on the QCM with M13 phage DNA at 20 °C, the temperature of the solution was increased gradually at the rate of 10 °C/min. The frequency of $-\Delta F = 560 \pm 10$ Hz due to the hybridization increased discontinuously between 55 and 65 °C and reverted to the original value of the probe-immobilized QCM ($\Delta F = 0$ Hz) above 70 °C. This phenomenon occurred reversibly when the temperature was decreased to 20 °C. These results indicate that the hybridization on the QCM was melted (separated) with increasing temperature in the range 55-65 °C, and $T_m$ of the hybridization was determined to be 60 °C.

**Table I.** Hybridization Amounts between the Probe-Immobilized QCM and Various 10-mer Nucleotides in Solution at 25 °C[a]

| nucleotides[b] | $\Delta m$/ng | % hybridization[c] |
|---|---|---|
| 3'dCCCTTAAGCA5' | 380 ± 10 | 100 |
| 3'dCCCTTAAGGG5' | 350 ± 10 | 92 |
| 3'dTGCTTAAGCA5' | 350 ± 10 | 92 |
| 3'dCCCTAAAGCA5' | 125 ± 10 | 31 |
| 3'dCCCTGAAGCA5' | 100 ± 10 | 26 |
| 3'dCTGCTACGGG5' | 0 | 0 |
| 3'dAGCCGTACCC5' | 0 | 0 |

[a] The probe nucleotide of HS-5'PdGGGAATTCGT3' was immobilized on the QCM with a relatively large amount (847 ng, 280 pmol, ca. 65% coating coverage of electrodes) in order to get high sensitivity for the detection of low molecular weight oligonucleotides, compared with M13 phage DNA. [b] Concentration: 400 ng (130 pmol) in 10 mL. The complementary sequences with the probe are shown with underlines. [c] Indicates how much of the nucleotide in the solution bound to the probe on the QCM.

The QCM technique could be applied for the detection of hybridization with various 10-mer oligonucleotides as target DNAs. Hybridization amounts obtained from frequency changes are summarized in Table I. In the case of the totally complementary 10-mer nucleotide, all target nucleotides added in the solution bound and hybridized on the QCM. The oligonucleotides having more than eight continuous complementary base pairs could also bind with the probe on the QCM. This hybridization was not stable, since the frequency reverted to the original value when the QCM moved to the new aqueous solution. Oligonucleotides having five continuous and complementary base pairs partially bound with the probe, but the hybridization was not stable.

In summary, the oligonucleotide-immobilized QCM will become a new useful tool to detect specific DNAs by the weight of the direct hybridization in solutions without any pre- or posttreatments and without nonspecific bindings of DNAs and proteins. We emphasize that this method is also useful to study molecular level kinetic understanding of base-pair hybridizations in oligonucleotides.

(7) Calibration was performed by the deposition of polymer or lipid cast film[6a] or Langmuir-Blodgett lipid film[6c] on the QCM, and the constant obtained was consistent with the Sauerbrey equation constant[5] both in air and the aqueous phase.

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## Complexation of Benzamidinium by a New Family of Artificial Receptors

Thomas W. Bell* and Vincent J. Santora

*Department of Chemistry*
*State University of New York*
*Stony Brook, New York 11794-3400*

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In comparison to numerous reports relating the structures of drugs to their interactions with natural recognition sites,[1,2] there are relatively few examples of the complexation of drugs (e.g., barbiturates) by *synthetic* receptors.[3] Benzamidine groups are attractive targets for complexation studies because they are found

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