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Electrochemical Detection of MicroRNAs via Gap Hybridization Assay

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MicroRNAs have recently been associated with cancer development by acting as tumor suppressors or oncogenes and could therefore be applied as molecular markers for early diagnosis of cancer. In this work, we established a rapid, selective, and sensitive gap hybridization assay for detection of mature microRNAs based on four components DNA/RNA hybridization and electrochemical detection using esterase 2-oligodeoxynucleotide conjugates. Complementary binding of microRNA to a gap built of capture and detector oligodeoxynucleotide, the reporter enzyme is brought to the vicinity of the electrode and produces enzymatically an electrochemical signal. In the absence of microRNA, the gap between capture and detector oligodeoxynucleotide is not filled, and missing base stacking energy destabilizes the hybridization complex. The gap hybridization assay demonstrates selective detection of miR-16 within a mixture of other miRNAs, including the feasibility of single mismatch discrimination. Applying the biosensor assay, a detection limit of 2 pM or 2 amol of miR-16 was obtained. Using isolated total RNA from human breast adenocarcinoma MCF-7 cells, the assay detected specifically miR-21 and miR-16 in parallel, and higher expression of oncogene miR-21 compared to miR-16 was demonstrated. Including RNA isolation, the gap hybridization assay was developed with a total assay time of 60 min and without the need for reverse transcription PCR amplification of the sample. The characteristics of the assay developed in this work could satisfy the need for rapid and easy methods for early cancer marker detection in clinical diagnostics.

MicroRNAs (miRNAs) comprise a class of noncoding 18−25-nucleotide-long RNAs that can regulate the expression of genes in plants and animals by complementary binding to the 3′-untranslated region of mRNAs.[1] miRNA genes are transcribed in primary-miRNA transcripts (pri-miRNA), which are cleaved in the nucleus by the microprocessor complex Drosha/Pasha to a 60−70-nt stem−loop intermediate, known as miRNA precursor (pre-miRNA). Pre-miRNAs are exported from the nucleus into the cytoplasm by Exportin-5. In the cytoplasm, the stem−loop structure is recognized and cleaved by Dicer into a small, imperfect dsRNA duplex (miRNA:miRNA*). miRNA is recognized and assembled into ribonucleoprotein particles, a so-called RNA-induced silencing complex (RISC). miRNA guides the RISC complex to complementary 3′-untranslated regions of mRNA and induces mRNA degradation or represses their translation.[2,3]

Recently, it was demonstrated that miRNAs play an important role in cancer. The expression of miRNAs in cancer cells is dysregulated. Unique patterns of altered miRNA expression in cancer cells could serve as a diagnostic fingerprint.[4] Thus, miRNA expression levels in multiple primary and metastatic cancers was used to construct an miRNA classifier that identifies cancer metastases by their site of origin.[5] Two of the best-characterized tumor-suppressor miRNAs are miR-15 and miR-16. B-cell chronic lymphocytic leukemia (CLL) is the most common adult leukemia in developed countries and is associated with the loss of a chromosomal region. This region comprises miR-15 and miR-16, which are lost in around 70% of patients with CLL.[6] Thus, miRNAs may be good candidates as biomarkers in cancer and promise diagnostic, prognostic, and predictive information.[4,7]

Due to the exceptionally short length, very similar nucleotide sequences, and quite low expression levels, miRNA analysis requires improved miRNA profiling techniques.[8] Currently, miRNAs are predominantly detected with Northern blot, PCR, or microarray analysis.[9−14] These detection technologies are expensive and time-consuming and require well-trained scientists. Recently, several new miRNA detection methods based on alternative biosensing techniques, such as nanoparticle-amplified surface plasmon resonance imaging or bioluminescence detec-

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tion,[14,15] were reported. However, most of these methods require a chemical or enzymatic modification of target miRNA or the application of locked nucleic acids (LNAs) as capture probes owing to the short length of miRNAs. The instrumentation for optical signal readout is rather complex and expensive. Therefore, electrochemical biosensors have long been viewed as particularly attractive for bioanalysis because of their high sensitivity and selectivity, low detection limit, low cost, and ease of automatization.[16] Recently, two reports demonstrated the application of sensitive and selective direct electrochemical miRNA detection.[17,18]

Previously, we described electrochemical detection of bacterial 16S rRNA using the thermostable reporter enzyme esterase 2 (ETS2) from *Alicyclobacillus acidocaldarius*. In our hands, EST2 as a reporter enzyme is superior to other similar reporter enzymes, such as alkaline phosphatase and horseradish peroxidase. This advantage includes the possibility for rational design of reporter enzyme−ODN conjugates, thermostability, and high sensitivity. Fragmented prokaryotic RNA was applied to immobilized capture oligodeoxynucleotide (ODN) in the presence of helper ODN and EST2-ODN detector conjugate.[19] We demonstrated the importance of continuous base stacking, in a coaxial helix formed by four hybridization components, for generating a significant signal. Here, we apply four-component hybridization for electrochemical detection of miRNAs and demonstrate the feasibility of this gap hybridization assay for sensitive and specific detection of synthetic miRNAs. In addition, we applied the developed method for parallel detection of miRNAs from a human breast adenocarcinoma cell line.

EXPERIMENTAL SECTION

Chemicals and Materials. DNA and RNA oligonucleotides were obtained from Biomers (Ulm, Germany). Sulfosuccinimidyl-4-(*N*-maleimidisomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) and tris(2-carboxyethyl)phosphine hydrochloride (TCEP) were from Pierce (Rockford, IL). MonoQ HR 5/5 was from Amersham Pharmacia Biotech (Freiburg, Germany). Tween 20, Triton X-100, octanethiol, bovine serum albumin (BSA), Luria−Bertani (LB) broth, and diethyl pyrocarbonate (DEPC) were purchased from Sigma-Aldrich (Taufkirchen, Germany). T4 polynucleotide kinase was from NEB (Frankfurt a. Main, Germany), and dNTPs were purchased from Roche (Mannheim, Germany). RNase-free DNase was from Qiagen (Hilden, Germany). Interface SCB-68 and software Labview 6.0 were from National Instruments (Munich, Germany). All reagents were analytically pure grade. All of the buffers used for miRNA work were autoclaved, and DEPC was treated to protect from RNase degradation.

p-Aminophenyl butyrate (pAPB) was synthesized as described by Wang et al.[20]

Table 1. Sequences of miRNAs Used in This Work

miRNA	sequence (5′ → 3′)[a]
miR-15	UAGCAGCACAUAAUGGUUUGUG
miR-16	UAGCAGCACGUAAAUAUUGGCG
miR-16_5′mismatch	UA**U**CAGCACGUAAAUAUUGGCG
miR-16_3′mismatch	UAGCAGCACGUAAAUAUUG**C**CG
miR-16_central mismatch	UAGCAGCACG**A**AAAUAUUGGCG
miR-16_5′overhang	UGCCUUAGCAGCACGUAAAUAUUGGCG
miR-16_3′overhang	UAGCAGCACGUAAAUAUUGGCGUUAAG
miR-21	UAGCUUAUCAGACUGAUGUUGA

[a] Single nucleotide mismatches are indicated as bold letters, whereas additional nucleotides are underlined.

Human breast adenocarcinoma cell line MCF-7 was kindly provided by Prof. Schobert (Universität Bayreuth, Germany) and was maintained as a monolayer culture in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (Biochrom KG Seromed, Germany) and streptomycin/penicillin (GIBCO, Germany). Cultures were grown at 37 °C in a humidified atmosphere of 5% CO_2/95% air.

Preparation of EST2-ODN Conjugate. Esterase−oligonucleotide conjugate (EST2-miR) was prepared as described.[20]

DNA and RNA Oligonucleotides. The 3′-thiol-modified DNA oligonucleotides for biochip fabrication are miR16-capture = 5′-ACGGTTCCCGAAGGCACATTCTCATTTTTTTTT−SH-3′, miR21-capture = 5′-TCTGCCCCCGAAGGGGACGTCCTATTTTTTTTT−SH-3′, and miR10a-capture = TTTGTCCCCGAAGGGAAAGCT-CTGTTTTTTTTT−SH-3′. All the capture probes contain a nine-nucleotide thymidin spacer at the 3′ end, which provides optimal immobilization and hybridization efficency.[19] Synthetic miRNA sequences were received HPLC-purified and dissolved in DEPC−H_2O. The miRNA sequences used in this work are listed in Table 1. 5′-Amino-modified detector oligonucleotide miR conjugated to EST2 is miR = 5′-NH_2−TTTTTT GGTTGCGC-TCGTTGCGGGACTTAACCCAACAT-3′.

miRNA Isolation. Total RNA or miRNA enriched fraction from human MCF-7 cells grown in six-well plates were extracted using a miRNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's recommended protocol. RNA concentration was determined by UV−vis spectrophotometry. RNA integrity was checked by TBE agarose gel electrophoresis analyzing integrity and relation of the 28S rRNA, 18S rRNA, and 5S rRNA bands.

5′ Phosphorylation of miRNA. After denaturation of synthetic miR-16 for 5 min at 70 °C, 0.6 nmol miR-16 was incubated in the presence of 100 μM ATP and 10 U T4 polynucleotide kinase in 100 mM Tris−HCl (pH 7.5), 10 mM $MgCl_2$, and 5 mM DTT at 37 °C for 30 min. Phosphorylated miRNA was purified by phenol extraction and ethanol precipitation. The RNA concentration was determined by UV−vis spectrophotometry.

In Vitro Transcription of Complementary miRNA Probe. miRNA probe consisting of complementary regions to capture, miRNA, and detector ODN was prepared by overlapping 30-nt oligonucleotides and in vitro transcription. Sequences of overlapping oligonucleotides are listed in Table S-1 (Supporting Information). PCR was performed with 25 pmol flanking primers and 25 fmol oligonucleotides in the presence of 1.25 U Taq DNA polymerase (Finnzymes, Espoo, Finnland) and 0.2 mM dNTPs in 10 mM Tris−HCl (pH 8.3), 1.5 mM $MgCl_2$, and 50 mM KCl.

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Amplification conditions were 1 cycle of 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s, and a final extension step at 72 °C for 5 min. For in vitro transcription, amplified PCR product with 5′ T7 RNA polymerase promoter sequence was used. In vitro transcription of 2.5 μg of PCR product was done in the presence of 5 U T7 RNA polymerase (Promega, Mannheim, Germany), 40 mM Tris−HCl (pH 8.1), 20 mM $MgCl_2$, 1 mM spermidine, 5 mM dithioerythritol (DTE), 0.01% Triton X-100, and 4 mM each NTP (Roche, Mannheim, Germany) and 80 U RNasin (Promega, Mannheim, Germany) for 4 h at 37 °C. DNA digestion was performed with 2U TURBO DNase (Ambion, Austin, TX) for 15 min at 37 °C, and the RNA was purified via ethanol precipitation. The quality of the RNA was controlled by agarose gel electrophoresis, and the RNA was quantified spectrophotometrically.

Electrochemical Biochip Measurement. Chip and instrumentation was used as described.[20] Briefly, chip modules were obtained from Heraeus Sensor Technology (Kleinostheim, Germany). Each chip (11 mm ×13 mm) contained 8 individual gold electrodes (diameter, 0.85 mm) with spaces of 2.0 mm to the next electrode positions. Four electrodes were used as reference electrodes and treated with $Ag/AgCl_2$ solution (Electrodag 6037 SS, Acheson Colloiden B.V., Scheemda, Netherlands). The voltammetric measurement principle was applied as described.[21] Briefly, the potential of the working electrode is pulsed (250 ms, +200 mV; 750 ms, −200 mV), and measuring phases and relaxation phases are alternately produced. For measurement, the printed circuit board of the chip was connected to a multipotentiostat device (Siemens CT PS6, Erlangen, Germany). The potentiostat was connected to a PC through a serial interface SCB-68 (National Instruments, Munich, Germany). Software Labview 6.0 was applied to control the potentiostat, collect the data, and plot figures.

The flow-through cell was built with a U-shaped silicon form with an internal volume of 10 μL (Figure S1, Supporting Information). A peristaltic pump from Amersham Pharmacia Biotech (Freiburg, Germany) was used for moving the various fluids.

Chip Preparation. The chips were treated with ethanol for 5 min, rinsed with H_2O, and dried under a filtered air stream.

The DNA monolayers on the electrode arrays were prepared by adding 0.2 μM 3′-thiol-modified capture DNA in 1 mM TCEP, 300 mM NaCl, and 10 mM sodium phosphate (pH 7.0) onto the individual electrodes. After incubation for 16 h, the electrodes were rinsed three times with 300 mM NaCl in 10 mM sodium phosphate (pH 7.0) to remove unbound DNA. Subsequently, each electrode was treated with 1 μL of 1 mM octanethiol for 1 h at room temperature.

Isolated or synthetic RNA in 450 mM NaCl, 0.025% Tween 20, 1 mg/mL BSA, 25 mM EDTA, 30 mM NaH_2PO_4 (pH 7.4), 1 μg of each complementary RNA probe, and 0.2 μM EST2-miR was applied onto each electrode. Electrode arrays were then incubated at 65 °C for 20 min in a humidity chamber, and then the chip was kept at 20 °C for 5 min. After washing with 75 mM NaCl, 0.5 mM EDTA, 0.05% Tween 20, and 5 mM NaH_2PO_4 (pH 7.4) for 1 min at 25 °C, the chip was inserted onto a multipotentiostat. Background current reached steady state after flow through of 100 mM NaCl and 10 mM sodium phosphate (pH 7.0) for ~1 min. The detection of EST2 molecules, bound to the electrode by hybridization, was achieved by delivering the substrate pAPB through the flow chamber at a flow rate of 250 μL/min. After the flow was stopped, the EST2 activity was measured as a change of current intensity (dI) per time (dt) within the first 5 s. In the initial linear phase, this value is proportional to the amount of immobilized RNA on the particular position.[20]

RESULTS AND DISCUSSION

The schematic principle of miRNA detection is shown in Scheme 1. The key feature of this method is generation of a continuous base stacking between immobilized capture ODN, miRNA, EST2-ODN conjugate, and complementary RNA probe. In the presence of a particular miRNA, this four-component hybridization brought the reporter enzyme EST2 into the vicinity of the gold electrode. Hydrolysis of substrate *p*-aminophenyl butyrate (pAPB) to electroactive *p*-aminophenol (pAP) resulted in a detectable electrical signal. An amplification of electrochemical signal is provided, first, by the high turnover of enzymatic reaction and, second, by redox recycling of quinoneimine to pAP.[22] Due to application of thermostable reporter enzyme, hybridization among all four components could be performed in one step.[19] In contrast, in the absence of miRNA, the hybridization is destabilized because a 22-nt gap is generated between the capture and detector ODN. This interrupted base stacking results in destabilizing of the detector ODN hybridization and the absence of electrochemical signal. The stability of the nucleic acid hybridization events is determined by two essential parameters: namely, base pairing between complementary strands and stacking between adjacent bases. Base stacking proved to be the main stabilizing factor in the DNA double helix.[23,24] Furthermore, in the presence of base stacking, the hybridization association rate constant is higher than without base stacking.[25] In addition to base stacking, resolving of the secondary structure of complementary RNA probes is essential for an efficient gap-hybridization reaction and stabilization of the complex involving capture and detector ODN.[26]

For a proof-of-principle, capture ODNs for miR-16 complementary RNA probe were immobilized on three electrodes, whereas one electrode was treated with no capture ODN. Subsequently, all gold electrodes were treated with an octanethiol solution to remove unspecifically bound DNA capture probes and prevent unspecific binding of nucleic acids or proteins on the gold surface.[27] Hybridization between a complementary RNA probe and EST2-miR detector conjugate was performed in the presence or absence of miR-16 (Figure 1). Without miRNA, all electrodes with a capture probe specific for miR-16 showed only a minor signal, whereas the blank electrode produced no signal (Figure 1A). However, in the presence of miR-16, the three electrodes with

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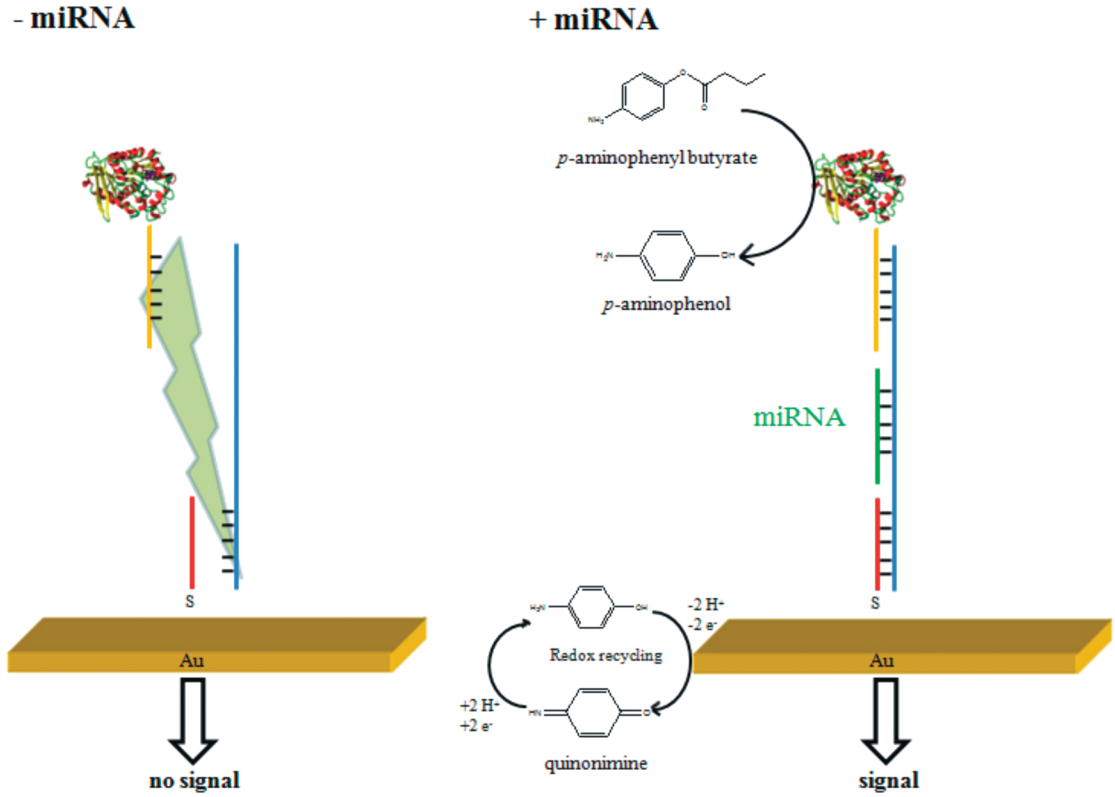
Scheme 1. Electrochemical Detection of Gap Hybridization among Immobilized Capture ODN (red), miRNA (green), EST2-miR Conjugate (yellow), and Complementary RNA probe (blue)



capture ODN specific for miR-16 complementary probe produced a significant electrochemical signal after stoppage of substrate flow (Figure 1B). The blank electrode showed no signal, which revealed no unspecific binding of EST2-ODN conjugate to the gold electrode. This is in accordance with previous reports presenting a dramatic decrease in the signal when no perfect base stacking was generated for bacterial 16S rRNA[19,28] or bioprocess-relevant marker gene detection.[29]

Specificity of this electrochemical gap hybridization assay was first tested with three miRNA sequences (miR-15, miR-16, and miR-21). miR-15 and miR-16 are structurally closely related. Deletion of these miRNAs results in B-cell chronic lymphocytic leukemia (CLL).[6] MiR-21 is also overexpressed in many tumors and contributes to myocardial diseases.[30] The sequence alignment of these miRNAs revealed a strong similarity between the 5′ region (seeding region) of miR-15 and miR-16. The seeding region is important for binding to 3′ UTRs of the mRNA target. Because miR-15 and miR-16 have identical targets, they consist of a homologous 5′ region. However, in the 3′ region, the sequences differ markedly. Compared to miR-15 and miR-16, miR-21 consists of a different 5′ and 3′ sequence (Figure 2A). Different mixtures of every 200 nM miR-15, miR-16, and miR-21 were hybridized on a biochip with capture ODNs specific for miR-16. In the absence

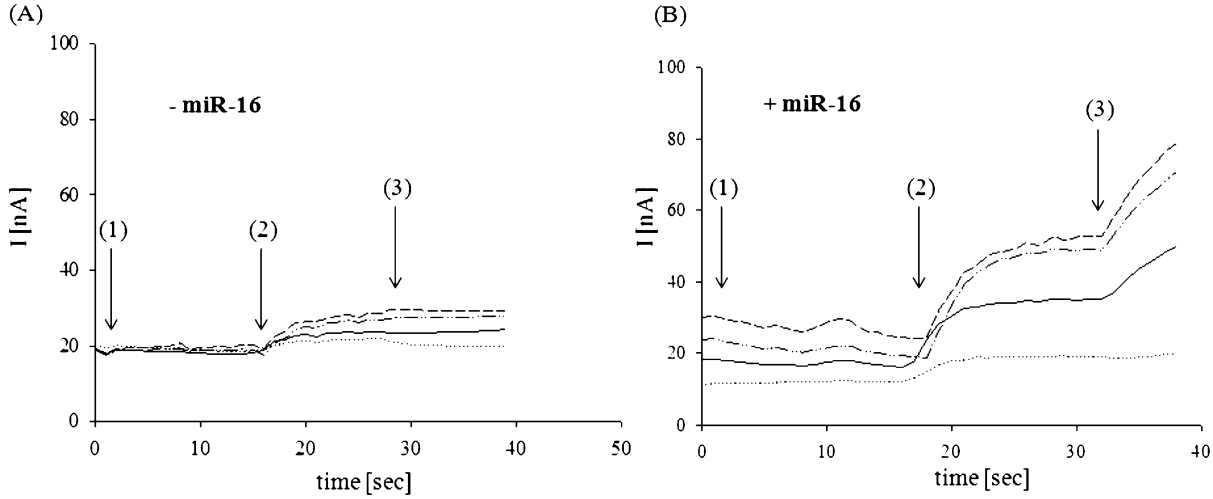


Figure 1. Electrochemical signals in the absence (A) and in the presence of 200 nM miR-16 (B) via gap hybridization assay. Particular electrodes were loaded with miR-16 capture (---, —, - • • -) or only with octanethiol, blank (•••). The current course vs time diagrams are shown. Buffer flow through the hybridization chamber (1) was followed by addition of substrate pAPB (2). After reaching a plateau of current, flow was stopped and the change in the current vs time was determined (3).

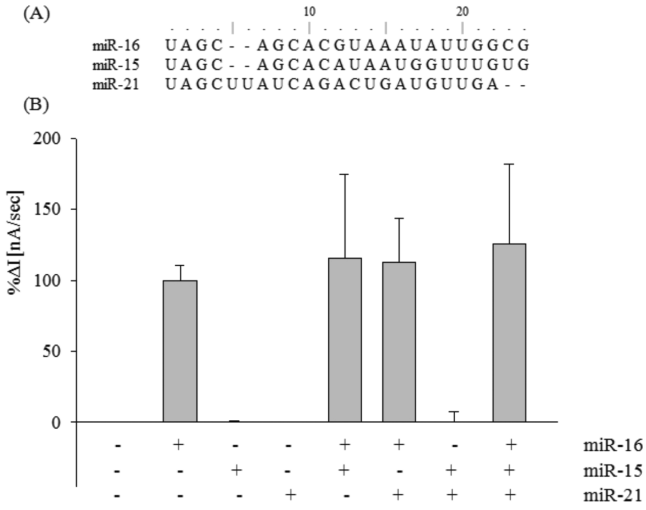


Figure 2. Selectivity of miRNA detection. (A) Sequence alignment of three tested miRNAs. (B) Electrochemical signals obtained for miR-16 alone or with different mixtures of miRNAs. All miRNAs were applied at a 200 nM concentration for the gap hybridization assay. The electrochemical signal resulting after hybridization with 200 nM miR-16 was set as 100% ($n = 3$).

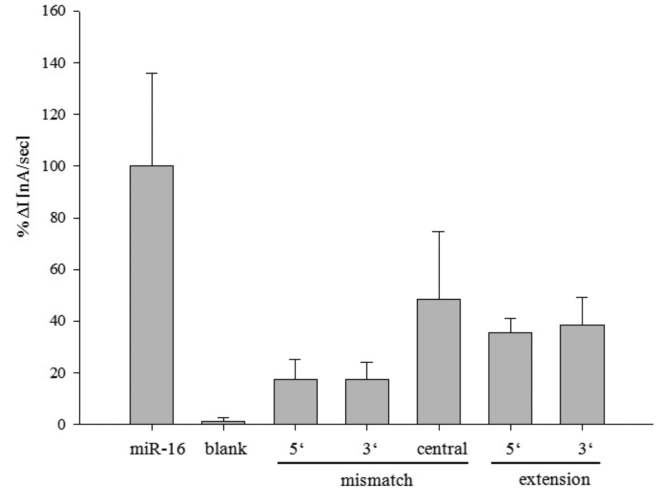


Figure 3. Electrochemical detection of miR-16 and miR-16 with terminal or central mismatches and miR-16 with terminal 5 nucleotide extensions. miR-16 (200 nM) artificially mismatched or extended miRNAs were applied for gap hybridization assay. The electrochemical signal resulted after hybridization with 200 nM miR-16 was set as 100% ($n = 3$).

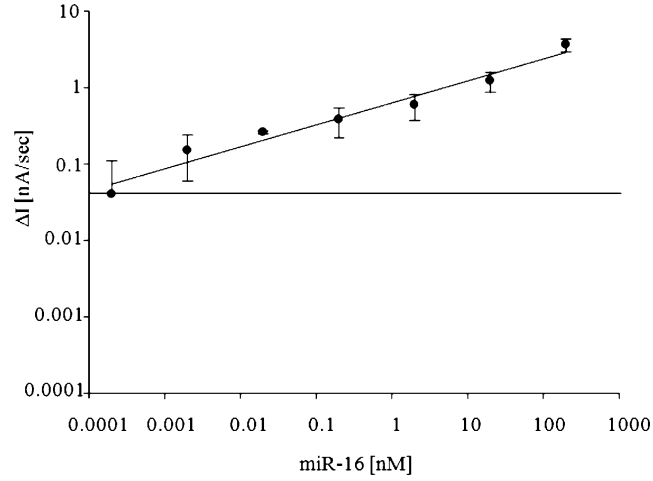


Figure 4. Sensitivity of electrochemical miR-16 detection. Linearity is $R^2 = 0.97$; the horizontal line threshold value for determination of detection limit (blank +3SD) is indicated ($n = 3$).

of any miRNA, there was, as shown in Figure 1A, no electrochemical signal produced. In the presence of only miR-15, miR-21, or mixtures thereof, the biochip showed only minor background signals. There is no cross-hybridization of miR-15 or miR-21 with the miR-16 complementary RNA probe. In the presence of mixtures of miR-16 and one or two other miRNAs, the mean signal is comparable to the electrochemical signal in the presence of only miR-16. A specific interaction of miR-16 can also be achieved in a mixture of different miRNAs.

To further test the specificity of the gap hybridization assay, miRNAs were designed with a 5′, 3′, or a central mismatch as compared with miR-16 (Table 1). In the absence of any miRNA, no electrochemical signal was generated. Synthetic miR-16 with terminal mismatches revealed after hybridization a signal of around 20%, as compared with the signal after hybridization with a complementary miR-16 (100%). However, a central mismatch has a weaker influence on the signal strength as compared with the terminal mismatches and produced a signal of ~50% compared to the signal after hybridization with complementary miR-16 (Figure 3). In the future, optimization of mismatch discrimination can possibly be achieved by introducing modified nucleotides in the complementary RNA probe.

Pre-miRNA containing an entire miRNA sequence may disturb mature miRNA detection.[31] Therefore, we elongated the 5′ or 3′ terminus of miR-16 with 5 nt of pre-miR-16 and tested the hybridization of these elongated miRNAs. Compared to the signal after hybridization with complementary miR-16, they produced an electrochemical signal of around 40% (Figure 3). The elongation of miRNAs resulted in a thermodynamically more stable secondary structure, as compared with mature miRNA, whose hybridization efficiency is obviously lower when compared to mature miRNA.[32]

The perfect fitting of miRNA in the gap between capture and detector DNA influences the hybridization of miRNA. This was confirmed by the fact that 5′ phosphorylated miRNA gave a slightly higher signal (2.88 ± 0.50 nA/s) as compared with unphosphorylated miR-16 (2.23 ± 0.46 nA/s). This result demonstrated that the 5′ phosphorylated sticky ends of miRNA contribute to the stabilization of the DNA/RNA association. Similar stabilizing effects were reported for DNA assemblies due to hydrogen bonds

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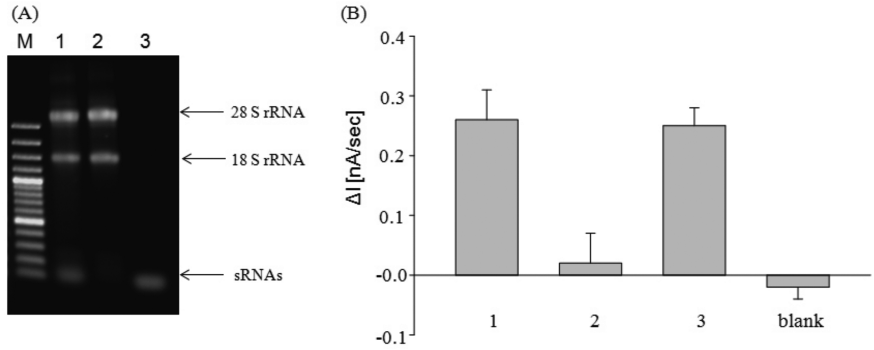

Figure 5. Electrochemical detection of miR-16 isolated from MCF-7 cells. (A) 1.2% agarose gel electrophoresis of isolated total RNA (lane 1), enriched >200 nt RNA (lane 2), and enriched <200 nt RNA (lane 3) are shown. M is a DNA marker for size determination. (B) Electrochemical signals of fractions 1, 2, and 3 shown in A were determined with electrochemical gap hybridization assay (B). Blank is the signal obtained with hybridization buffer in the absence of RNA ($n = 3$).

between 3′-hydroxy groups and 5′ phosphate groups at nick positions.[33]

In addition to selectivity, sensitivity is an important parameter for a biosensor. Sensitivity of the assay for detection of miRNA was tested by the method depicted in Figure 4. We measured the electrochemical response after hybridization with different dilutions of miR-16. Figure 4 shows a correlation of the signal intensity with the concentration of miR-16. A linear relationship was found in the concentration range between 200 and 0.002 nM miR-16. With EST2 as the reporter enzyme and gap hybridization of miRNA, we reached a detection limit of around 2.0 pM, which represents 2.0 amol miRNA (Figure 4). The detection limit was determined as the signal mean value, which corresponds to the blank value (−0.02 nA/s) plus three times the standard deviation of the blank (0.02 nA/s). This detection limit is much better than using Northern blot analysis or some fluorescence-based methods and comparable to other electrochemical methods (Table S2, Supporting Information). Along with this high sensitivity, the presented assay is rapid, because the hybridization event and enzymatic labeling are achieved within one step. Furthermore, no labeling of miRNA is necessary, and no LNAs or PNAs have to be used as capture probes.[18,34]

To demonstrate performance of the developed assay in the detection of miR-16 in real samples, we performed electrochemical biochip measurement with RNA isolated from human breast adenocarcinoma cell line MCF-7. For this study, we used total RNA, enriched small RNA (<200 nt), or enriched long RNAs (>200 nt). Integrity and efficiency of enrichment of different RNA species was analyzed by agarose gel electrophoresis (Figure 5A). The different RNA samples were analyzed by gap hybridization on an electrochemical biochip with capture probes specific for miR-16. As a negative control, a sample without RNA was used for hybridization (blank), which resulted in no electrochemical signal (Figure 5B). After hybridization with a total RNA sample and with an enriched small RNA fraction, a significant electrochemical signal was generated. However, in the presence of the enriched long RNA fraction, only a minor background signal was detected. It should also be noted that in the presence of the total RNA, the signal for miR-16 was nearly identical to the signal after hybridization with enriched a small RNA fraction, indicating no effect on the hybridization kinetics or specificity in a large excess of other RNAs.

The use of synthetic capture probes and a miRNA-complementary RNA probe enables the possibility to generate an addressable biochip.[35] Furthermore, we designed a universal EST2−detector conjugate for hybridization with a complementary RNA probe, allowing a one-step hybridization reaction.[19] Therefore, we tested the multiplex detection of three miRNAs, miR-16, miR-21, and miR-10a, isolated from MCF-7 cells on a biochip immobilized with miR-16-, miR-21-, or miR-10a-specific capture probe, respectively. To test cross-hybridization or unspecific binding to the gold electrode, one electrode was treated with no capture probe. After gap hybridization of the total RNA isolated from the MCF-7 cells, the blank electrode and the electrode with capture specific for miR-10a showed no or only background signal (−0.01 ± 0.03 nA/s and 0.01 ± 0.03 nA/s). However, the electrodes with capture probes specific for miR-16 and miR-21 produced a significant signal (0.38 ± 0.08 nA/s and 0.80 ± 0.21 nA/s; $n = 3$). Real-time analysis of the expression levels of pre-miRNAs in MCF-7 cells revealed that miR-16 is an average expressed miRNA, whereas miR-21 is a highly expressed miRNA.[11] However, miR-10a expression was not detected in MCF-7 cells. The expression levels measured with gap hybridization and electrochemical detection confirmed that miR-21 is more highly expressed than miR-16 in MCF-7 cells.

CONCLUSIONS

We have developed a rapid, specific, and sensitive gap hybridization assay for microRNAs based on enzymatic and redox recycling amplification of an electrochemical signal. EST2 as a reporter enzyme is superior to most other reporter enzymes because of the possibility to site-specific modification with ODNs. Furthermore, EST2 is a thermostable enzyme enabling the reporter enzyme to be present during hybridization reactions at elevated temperatures. This allows the here-described one-step, four-component, hybridization assay established for miRNA detection. The gap hybridization method was applied for sensitive and selective determination of miR-16. Furthermore, miR10a, miR-16, and miR-21 expression levels in MCF-7 cells were determined.

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The biosensor assay revealed that miR-21 is more highly expressed than miR-16 in cancerous cells. The need for sensitive cancer detection of early markers in cancer diagnosis is immense and can be potentially fulfilled by the establishment of electrochemical nucleic acid hybridization-based biochips operating in an instrument that allows automatic operation. The possibility of enlarging the number of electrodes on a biochip would favor a fast and inexpensive method to monitor miRNA expression levels of cancer patients near bedside. Therefore, such a tool would be valuable in clinical diagnostics and drug discovery.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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