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Collagen Membranes with Ribonuclease Inhibitors for Long-Term Stability of Electrochemical, Aptamer-Based Sensors Employing RNA

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Abstract

Electrochemical aptamer-based (E-AB) sensors offer advantageous analytical detection abilities on account of their rapid response time (sec to min), specificity to a target, and selectivity to function in complex media. Ribonucleic acid (RNA) aptamers employed in this class of sensor offer favorable binding characteristics resulting from the ability of RNA to form stable tertiary folds aided by long-range intermolecular interactions. As a result, RNA aptamers can fold into more complex three-dimensional structures than their DNA counterparts, and consequently, exhibit better binding ability to target analytes. Unfortunately, RNA aptamers are susceptible to degradation by nucleases, and for this reason, RNA-based sensors are scarce or require significant sample pretreatment before use in clinically-relevant media. Here, we combine the usefulness of a collagen I hydrogel membrane with entrapped ribonuclease inhibitors (RI) to protect small molecule RNA E-AB sensors from endogenous nucleases in complex media. More specifically, the biocompatibility of the naturally polymerized hydrogel with encapsulated RI promotes the protection of an aminoglycoside-binding RNA E-AB sensor up to 6 hours; enabling full sensor function in nuclease-rich environments (undiluted serum) without the need for prior sample preparation or oligonucleotide modification. The use of collagen as a biocompatible membrane represents a general approach to compatibly interface E-AB sensors with complex biological samples.

Graphical Abstract

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Note: The authors declare no competing financial interest.

Supporting Information

The electrochemical characterization of the photolithographically-patterned chip electrodes. This information will be available free of charge via the at <http://pubs.acs.org>

Keywords

Electrochemical Aptamer-Based Sensors; RNA aptamer; collagen I hydrogel; ribonuclease; ribonuclease inhibitor; tobramycin

INTRODUCTION

The promise of electrochemical aptamer-based (E-AB) sensors employing both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) sensing elements is growing rapidly resulting from their potential use in a myriad of point-of-care and clinical applications.¹ Applications are broad and range from the determination of the cancer biomarkers such as vascular endothelial growth factor,² and tumor necrosis factor (TNF),³ to monitoring small molecules like aminoglycoside antibiotics in human blood and serum.⁴ To date, the majority of E-AB sensors for detection in complex media described in the literature utilize DNA aptamers as the biological recognition element.⁵ DNA offers several advantages such as thermal stability, low immunogenicity, and cost-effectiveness.⁵ RNA aptamers, on the other hand, offer potentially better specificity in binding a target analyte a result of non-canonical regions on the oligonucleotide chain.^{6–9}

The use of RNA oligonucleotides as bio-recognition elements is limited by the relative abundance of ribonucleases that catalyze the hydrolysis of the P-O^{2'} bond of a nucleoside 2',3'-cyclic phosphodiester on the 3'-side and the cleavage of the P-O^{5'} bond of an oligonucleotide.¹⁰ Consequently, the number of sensors employing RNA as a recognition element is scarce and for employment in complex, biological media the sample must be pretreated or significant chemical modification to the nucleic acid is required.¹¹ There are several reported methods to overcome the disadvantageous instability of RNA. For example, Förster *et al.* demonstrated the usefulness of locked nucleic acids (LNAs), to build a nuclease-insensitive ricin-selective RNA aptamer.¹² This method required engineering of a ricin-selective aptamer modified with 2'-O-4'C-methylene- β -D-ribofuranose able to inhibit the cleavage of the P-O^{5'} bond. Unfortunately, the developed nuclease-resistant modification resulted in a significant decrease of the intrinsic helical parameters of the oligonucleotide thus reducing the binding affinity of the aptamer towards ricin.¹² In another example, Liu *et al.* engineered an RNA aptamer specific for tumor necrosis factor by replacing the non-bridging oxygens on the backbone of the oligonucleotide with sulfur, producing a phosphorothioate.³ This modification inhibits nuclease hydrolysis and cleavage

mechanisms of P-O bonds, but again requires complicated chemical modification of the aptamer. As an alternative to chemical modification, Ferapontova *et al.* demonstrated that a theophylline-selective RNA E-AB sensor exposed to previously-centrifuged (~3000 Da molecular weight-cutoff filter) blood serum sample exhibited a robust electrochemical signal.¹³ Jarczewska, *et. al.* demonstrated the usefulness of RNA aptamers to quantify the cancer biomarker urokinase plasminogen activator (uPA) in bovine serum albumin (BSA). Briefly, the substitution of the 2'-hydroxyl group of the ribose ring with a halogen (fluorine) allowed experimental measurements, inhibiting nuclease hydrolysis of the P-O bond of the nucleoside.¹⁴ The newly developed 2'-fluoro-pyridine RNA aptamer demonstrated nuclease resistant properties and improved the robustness of the ribonucleotide single-stranded sequence. All methodologies successfully enable RNA-based sensor function in nuclease-rich environments but require oligonucleotide redesign or time-consuming sample pretreatment. More recently, we demonstrated the usefulness of a polyacrylamide hydrogel membrane to passively protect an aminoglycoside-specific aptamer from nuclease activity in untreated serum.¹⁵ This method demonstrated an initial 30% signal electrochemical signal before stabilizing with the copolymerization of acrylamide and bisacrylamide and only provided protection for a short-time period.

In the present work, we demonstrate for the first time the use of a collagen hydrogel with ribonuclease inhibitor entrapped in the gel network to protect small molecule RNA-based E-AB sensors for at least 6 hours maintaining sensor function. To demonstrate this, an E-AB sensor we employed an engineered RNA sequence for the sensitive and specific detection of aminoglycoside antibiotics.¹⁶ Specifically, we find that the RNA-based sensors are protected by a collagen hydrogel formed in the presence of ribonuclease inhibitor (RI) with the sensors exhibiting no appreciable change in signal upon employment in unadulterated serum. The protection enables a quantitative titration directly in unadulterated serum representing the first demonstration of such in untreated serum with native RNA. Furthermore, we find that the collagen membrane does not appreciably affect the signaling abilities of the sensor, and thus the sensors respond quantitatively to the aminoglycoside antibiotic tobramycin. Given the generality and compatibility of forming collagen membranes, we believe this to be a general approach to protecting RNA-based sensors.

EXPERIMENTAL SECTION

Chemicals and solutions

Tris-2-carboxyethyl-phosphine (TCEP), 6-mercapto-1-hexanol (99%), Trizma base (2-amino-2-(hydroxymethyl)-1,3-propanediol, magnesium chloride (MgCl₂), sodium chloride (NaCl), tobramycin (Tob), ferrocene carboxylic acid, 97% (FCC), sulfuric acid (H₂SO₄), and 10X Tris-EDTA buffer, Dulbecco's modified Eagle's medium (DMEM), sodium hydroxide (NaOH), sodium acetate (NaOAc) Tetrabutylammonium hexafluorophosphate (TBAPF₆), ferrocene (FC) Fetal Bovine Serum (FBS), and Protector RNase Inhibitor were all used as received from Sigma-Aldrich. Hydrogen peroxide 30%, 95% ethanol, and 10x PBS buffer were used as received (Fischer Scientific). Collagen I from rat tail was used as received (Gibco). Ambion RNaseAlert QC System was used as obtained from Thermo Fischer Scientific. SP Sepharose Fast Flow was used as received from GE Healthcare Life

Sciences. All solutions were prepared using autoclaved, ultrapure water (18.0 MΩ cm at 25 °C) using a Biopak Polisher Millipore ultra-purification system (Millipore, Billerica, MA). The RNA aminoglycoside aptamer sequence (5'-HSC6-CUUGGUUUAGGUAAUGAG-MB-3' (D2 Sequence)¹⁶ was purified using dual-HPLC (Biosearch Technologies, CA) and used as received.

Electrode fabrication and characterization

The chip electrodes were fabricated on a 76.2 mm diameter borofloat glass wafer (WRS Materials, San Jose, CA) comprising 3 square working Au electrodes (4 mm²), one square Au quasi-reference electrode (4 mm²), and an Au counter electrode (21 μm²) (Figure S1). Chips were fabricated using standard photolithography techniques. Briefly, thin films of chromium and gold (50 and 1000 Å, respectively) were deposited using a six pocket Angstrom Electron Beam Evaporator. Wafers were spin-coated with Shipley 1813 (S-1813) photoresist solution and soft-baked immediately for 3 min at 90 °C. Samples were patterned using UV-lithography (Karl Suss MJB-3 mask aligner) with an in-house designed mask and developed using the Shipley CD-30 developer solution. Wafers were hard-baked at 150 °C for 5 min. Chromium and gold layers were etched with chromium (thin-film diamond (TFD) and Gold Etchant Type TFA solutions, respectively) to expose the electrode pattern.

Electrochemical Aptamer-Based (E-AB) Sensor Fabrication

Before sensor fabrication, each chip electrode was immersed in piranha solution (70:30, H₂SO₄: H₂O₂) for 10 min (**Caution:** *Piranha solution reacts violently with most organic materials and must be handled with extreme care*) and then rinsed with autoclaved ultrapure water for 2 min. Prior incubation with the aptamer, the oligonucleotide sequence was reacted with 4 μL 10 mM TCEP for 1 h to reduce the 5'-disulfide bond on the chain. Following these steps, chip electrodes were incubated in a 400 nM RNA aptamer solution in autoclaved 20 mM Tris buffer (pH = 7.4) with 100 mM NaCl and 5 mM MgCl₂ for 1 h. Before use, the RNA aptamer stock was reduced in a solution of 10 mM TCEP. The electrodes were then passivated in a 4 mM 6-mercapto-1-hexanol solution for 1 h. Sensors were equilibrated in autoclaved 20 mM trizma buffer (pH = 7.4) with 100 mM NaCl and 5 mM MgCl₂ for 1 h prior to use.

Collagen matrix formation

Collagen hydrogels were formed using a previously described method.¹⁷ Briefly, a solution of 3 mg/mL collagen I from rat tail was mixed with DMEM, 1N NaOH, and water to complete a final collagen concentration of 2 mg/mL unless otherwise noted. 40 Units (U) of Protector RNase Inhibitor (RI) were added for a collagen concentration ranging from 1.5 to 2.5 mg/mL. The prepolymerized solutions were then dispensed onto the fabricated sensors in an in-house-designed cell and kept for 1h at 37°C. Once the hydrogel was formed, the new membrane-coated sensor was equilibrated and swelled for an hour with autoclaved 20 mM Trizma buffer (pH = 7.4) with 100 mM NaCl and 5 mM MgCl₂ for 1 h.¹⁸

Collagen I from rat tail purification

Pre-polymerized solutions of collagen were purified using Sepharose columns to test the effects of removing the need to add RI in the collagen. Briefly, small (2 mL) disposable chromatographic columns (Thermo Fischer Scientific) were packed using a 20% sulfopropyl (SP) Sepharose slurry. The columns were cleaned three times using 95% ethanol and ultrapure water for 1 h, and then degassed and equilibrated using 1x PBS for 45 min. A 20% slurry of SP Sepharose with 1x PBS buffer was equilibrated in the previously cleaned column for 30 min and washed 3 times after packing using the dissolving buffer. 500 μ L of collagen I from rat tissue with 5 μ L DMEM were eluted from the column by gravity. Columns were cleaned using 95% water and ethanol and stored for a maximum of 7 days in 0.2 M NaOAc in 20% ethanol.

Electrochemical measurements

Electrochemical measurements were carried out using CH Instruments 620D Electrochemical Workstation (CH Instruments, Austin, TX). The three-electrode configuration was composed of three working electrodes (WE), a pseudo-reference electrode (PRE), and the counter electrode all on the same chip as described above. Square wave voltammetry (SWV) was performed using a pulse amplitude of 25 mV with a constant frequency (100 Hz), with a step width of 1 mV.

RNase Activity Fluorescence Measurements

Fluorescence measurements were taken using Edinburg Instruments, UC920 Fluorometer. The excitation wavelength was fixed at 490 nm, and emission measurements were taken from 505 to 550 nm. Samples were prepared following the RNaseAlert Lab Kit Test protocol. Briefly, 45 μ L of the sample was mixed with 5 μ L RNaseAlert Buffer and incubated with the resuspended fluorescent substrate for 1 h at 37 °C. Fluorescent measurements were performed at room temperature.

RESULTS AND DISCUSSION

Nuclease Activity Causes Rapid Degradation of E-AB Signal

E-AB sensors provide a platform for diverse sensing applications including detection in complex media enabled by the specificity of the aptamer-target interaction and the selectivity of the electrochemical readout. The performance of this class of sensors is ultimately determined by the identity of the oligonucleotide sequence and the binding properties to target analyte. Currently, there are approximately 20 DNA aptamers reported in the literature in comparison to approximately 90 reported RNA aptamers.¹ Despite the overwhelming number of RNA aptamers, E-AB sensors employing RNA probes are scarce and have limited clinical applications because the oligonucleotide sequence is prone to degradation by exogenous factors.¹

RNA-based electrochemical, aptamer-based sensors rapidly degrade when immersed in 100% blood serum. The degradation of RNA is confirmed via the rapid loss in voltammetric peak current as a function of the time the sensor is immersed in serum (Figure 1). The SWV peak current is indicative of the surface concentration of the appended redox molecule (*i.e.*

methylen blue) to the electrode surface,^{19,20} which ranges from 10^{11} – 10^{12} aptamer molecules/cm² in a typical E-AB sensor.²¹ As RNA is cleaved via ribonuclease activity, the RNA-attached redox marker can diffuse from the surface into bulk solution causing a decrease in surface concentration and voltammetric peak current. Consistent with this, we observe a dramatic electrochemical signal decrease (>98% reduction in SWV peak current) when an E-AB sensor modified with an RNA aptamer for tobramycin¹⁶ is immersed in undiluted serum (Figure 1). Of note, this loss in signal is much larger than what is typically observed from the result of nonspecific adsorption of serum proteins when employing DNA aptamers.²²

The rapid degradation of RNA E-AB sensors is a result of nuclease activity. Serum contains high levels (~500 U/mL) of ribonuclease A as part of the controlled pancreatic degradation process of RNA.²³ With this in mind; we find that sensors employed in a 400 μ L sample of serum treated with 20 μ L x 40 U RI exhibit minimal signal degradation (Figure 1). RIs are ~50 kDa proteins that stoichiometrically bind (1:1) to RNases inhibiting nucleolytic activity.^{10,24} The observation of protected sensor elements in RI treated serum is consistent with previously published reports^{3,12,13,15,25} and is a clear indication that nuclease activity is the dominant cause of sensor failure.

Collagen Hydrogel Films Dramatically Improve RNA Sensor Stability in Serum

We employ a collagen-based perm selective membrane to protect RNA E-AB sensors from nuclease activity in complex media negating the need for sample pretreatment, the addition of exogenous reagents, or chemical modification to the RNA aptamer. E-AB sensors comprise single-stranded (ss) oligonucleotides (DNA or RNA) chemisorbed to a metal (commonly gold) electrode via gold-thiol self-assembled monolayer (SAM) chemistry.²⁶ These aptamer biorecognition elements are modified to contain a redox probe at the 3' distal end of the oligonucleotide and signaling is predicated on changes in the conformation of the surface-bound aptamer.²⁷ Due to the dependence of sensor signaling on aptamer conformation and flexibility, the aptamers need to be free to move in space to assure efficient electron transfer between the redox probe and the electrode surface. Collagen is one of the main components of the extracellular matrix (ECM) and one of the most abundant proteins in humans, which polymerizes into a natural three-dimensional hydrogel scaffold hypothesized to leave room for aptamer dynamics.^{28,29} Collagen matrices are commonly employed for cell culture,^{30–32} and microencapsulation of proteins or small molecules for drug delivery.³³ In light of this, in the present manuscript, we demonstrate the use of collagen for the protection of RNA sensing elements from nuclease degradation. However, in the long term, given the versatility of collagen in methods, for example, 3-D tissue culturing,³⁴ we believe the successful interfacing of collagen and E-AB sensors can provide a powerful hybrid sensor to investigate the cellular microenvironment.

The incorporation of a collagen film on the surface of an RNA E-AB sensor results in a polymer density-dependent change in signaling current. Specifically, sensors coated with a 1.5 mg/mL, 2.0 mg/mL, or 2.5 mg/mL collagen I hydrogel exhibited a decreased in voltammetric peak current of $23\% \pm 1\%$, $30 \pm 4\%$, and $68 \pm 1\%$, respectively (Figure 2). Literature suggests that average pore size in a 2 mg/ml collagen film is ~1 μ m in radius.³⁵ It

is important to note that pore implies a continuous opening and does not necessarily accurately represent a polymer mesh network expected with the collagen hydrogel, but as a first order approximation, it serves well. This pore size scales with the square root of polymer concentration and thus, over that concentration range we study, we do not expect an appreciable change in pore size about the nucleic acid aptamer. Combined with the observation that sensor performance is unimpeded regardless of what polymer concentration is used *vide infra*, we believe that there is likely little interaction between the collagen and aptamers. As such, the decrease in signal at higher polymer concentration is likely a result of the loss of electroactive surfaces area as the polymer coats the electrode surface. With increasing collagen concentration, we also observe a positive shift in peak potential for the reduction of methylene blue. As noted earlier, we are employing an Au quasi-reference electrode in the current experimental setup. Collagen carries a net positive charge and literature reports suggest the adsorption of collagen to polycrystalline gold results in a positive shift in the potential of zero charge of the electrode surface,³⁶ which includes our reference electrode. The observed shift in (pzc) potential is consistent with the positive shift in in pzc from increased collagen adsorption.

The collagen membranes provide remarkable protection of the RNA sensing elements when sensors are employed in 100% undiluted serum. Regardless of the collagen concentration (1.5, 2.0, or 2.5 mg/ml), we find that all provide robust protection of the sensing elements. Specifically, we see no signal loss (voltammetric peak current) when the sensors are transitioned from buffer to serum, and this maintained for at least 50 min (Figure 3). This result is in direct contrast to what was observed when unprotected sensors are immersed in serum (Figure 1). Remarkably, we observe essentially no loss in signal over the course of 50 minutes of all sensors coated with collagen films indicating that ribonucleases are not reaching the sensor surface. Moreover, we find that this protection was afforded for up to at least 6 hours (see below).

In addition to excellent stability in serum, the newly-developed collagen/E-AB sensors respond to the aminoglycoside antibiotic tobramycin in 100% serum without sample pretreatment (Figure 4). It should be noted that sensor function is not possible in untreated serum and thus these data represent the first observation of native RNA aptamer function directly in untreated serum. All sensors were characterized at 100 Hz voltammetric frequency, for which sensors are employing the D2 aptamer exhibit “signal-off” behavior (Figure 4).¹⁶ As an example, the collagen-coated sensors exhibited a signal change of ~40% upon the addition of saturating amounts of tobramycin (1 mM). The binding curves are fit to a single site binding module (Langmuir Isotherm)^{37,38}, and we find sensors with 1.5 mg/mL, 2.0 mg/mL, and 2.5 mg/mL collagen films exhibited similar binding affinities (K_d) of $\sim 0.82 \pm 0.08 \mu\text{M}$. This is contrasted by the ~20% change in signal exhibited by the uncoated sensor *in buffer* with a K_d of $0.07 \pm 0.02 \mu\text{M}$ (Figure 4). Of note, while the sensors provide larger signal change when employed in serum compared to buffer, this observation is not unusual when using aptamer recognition elements.³⁹ More specifically, the media conditions such as the pH, salt composition, etc. have all been demonstrated to affect aptamer-target interactions so slight differences in ion concentration, pH, etc. between serum and our buffer can cause differences in sensor performance.

The Case for Encapsulated Ribonuclease Inhibitor

Before of the collagen hydrogel membrane, we treat all pre-polymerized collagen I solutions with ribonuclease inhibitor. We do this because collagen is isolated from animal tissue (rat tail) and is contaminated with an abundance of nucleases (see Figure 5). We find that without this pretreatment of the collagen, the sensors fail to perform or show no voltammetric signal after incubation with collagen (data not shown) indicating a loss of sensing monolayer. To confirm the presence and absence of nuclease activity in the untreated and treated collagen, we performed a fluorescence assay designed for detecting nuclease activity (RNaseAlert Lab Test Kit). In short, if nucleases are present and active, a fluorescently-labeled RNA strand is cleaved thus removing a fluorescently-labeled RNA from proximity of a linked quencher, resulting in an observable fluorescence signal at 520 nm.⁴⁰ We observe a significant fluorescence signal at 520 nm when the assay is run with collagen as received, confirming the presence of nucleases. When a 3.0 mg/mL pure polymerized collagen solution treated with 1 μ L x 40 U of RI was used, the fluorescence signal becomes negligible at 520 nm.

RI is present before the polymerization of the hydrogel membrane, it is likely it is still present in the hydrogel film. Furthermore, we believe that the RI may be adding a layer of active protection on the sensor surface. The observation that RNase inhibitors are in the collagen matrix indicates that the collagen film provides two layers of protection to the surface. The first is a nonspecific protection afforded by the size exclusion properties of the polymer mesh film. The second is the specific interaction between encapsulated RNase inhibitors and potentially and nucleases that can diffuse through the porous network. We previously reported the protection of RNA aptamer sensor surfaces with a polyacrylamide film, however, even with these films, we observed a reduction of ~30% current before stabilization.¹⁵ Here we find that the collagen-based membrane provides superior protection compared to polyacrylamide. However, if we employ the collagen I coated sensors incubated with serum at a pH ~ 10, a pH at which the RI should be inactivate, we observed a significant signal decrease (~30 %) over the time course of 6 hours, similar to the report by Schoukroun-Barnes *et al.* using a polyacrylamide membrane¹⁵ (Figure 6). Similarly, as an alternative strategy to eliminate nucleases from the collagen, we purified collagen using a Sepharose column.^{41,42} E-AB sensors coated with collagen I hydrogel purified by affinity chromatography did not withstand employment in blood serum (Figure 6). In summary, only collagen films prepared with RIs provided stable, long-term function in undiluted serum.

CONCLUSION

In conclusion, we demonstrate the ability of a collagen I hydrogel membrane with entrapped ribonuclease inhibitors enables long-term stability of RNA-based E-AB sensors in nuclease-rich environments (undiluted, unaltered serum) with unimpeded sensor performance for at least 6 hours. The sensor protection is achieved while still allowing for quantitative titration of target tobramycin binding partner without any sample pre-treatment or nucleic acid chemical modification – something never achieved using E-AB sensors with RNA. While we have previously reported the use of a polyacrylamide hydrogel to protect RNA-based sensors, we find here that collagen pretreated with RI provides superior protection. There is

much literature precedence to support the idea of active, hydrogel-encapsulated proteins and enzymes,^{43–45} and we believe that the encapsulated inhibitor is providing an added layer of active protection of the sensor surface. Given the generality of E-AB sensor platforms, a large number of RNA aptamers, and the generality of collagen gel formation, we believe this approach should apply to the field of E-AB sensors for the detection of small molecules.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Electrochemical, aptamer-based sensors using RNA-based recognition elements are not stable when employed in unadulterated blood serum. (Left) Voltammetric peak current, an indication of viable RNA molecules on the electrode surface, dramatically decreases when the sensor is transitioned from 100 mM Tris buffer to 100% bovine serum within 10 min of exposure. (Right) Conversely, sensors employed in serum treated with 20 μ L of ribonuclease A (RNase A) inhibitor exhibit relatively stable signal compared to the ~98% signal loss in untreated serum. All data points and error bars are the averages and standard deviation of at least three independently fabricated sensors respectively.

Figure 2.

The incorporation of a protective collagen membrane causes a polymer concentration-dependent yet a steady decrease in current. Specifically, incorporation of a 1.5 mg/mL membrane causes a voltammetric peak current decrease of $23\% \pm 1\%$; a 2.0 mg/mL lead to a $30\% \pm 4\%$ decrease, and a 2.5 mg/mL film caused a reduction in peak current of $68\% \pm 1\%$.

Figure 3.

The collagen film dramatically improves the long-term stability of the RNA aptamer on the surface of the electrode when it is in contact with 100% undiluted serum. The aptamer sensors coated with collagen films of 1.5 mg/mL, 2.0 mg/mL, and 2.5 mg/mL all show virtually no change in voltammetric signal. More specifically, regardless of the collagen concentration, all exhibit minimal signal degradation (<3%) over the course of 50 minutes. All data points and error bars are the averages and standard deviation of at least three independently fabricated sensors respectively.

Figure 4.

The incorporation of collagen hydrogel provides for the first time the quantitative employment of an RNA-based E-AB sensor in undiluted, untreated serum. (Left) The E-AB sensors for tobramycin is a “signal-off” sensor, quantitatively indicating the presence of tobramycin with a decrease in voltammetric peak current. Data shown is representative of a sensor protected with a 2.0 mg/ml film. (Right) All RNA E-AB sensors coated with different collagen membranes demonstrated a similar response towards tobramycin. All data points and error bars are the averages and standard deviation of at least three independently fabricated sensors respectively.

Figure 5.

The ribonuclease inhibitor withstands the chemical conditions required for collagen film formation. Using a commercial RNase activity assay, we find collagen type I, isolated from rat tail, contains intrinsic nucleases activity as indicated by the emission peak at ~520 nm. Treatment of the collagen sample with the addition of 40 U of RNase inhibitor inhibits nuclease activity. Finally, treating a collagen sample with an inhibitor that has undergone a pH cycle to pH ~10 (a pH at which the inhibitor loses activity) back to pH 7 still inhibits nuclease activity, indicating the inhibitor can withstand the conditions of collagen gel formation.

Figure 6.

The presence of RNase inhibitor in the collagen film provides further protection to the E-AB sensor surfaces. Sensors coated with a collagen I membrane with encapsulated RI demonstrated stable signal for up to 6 hours when employed in unadulterated serum. When collagen coated sensors are run in serum at pH 10, a pH at which the RI is not active, the sensor fails to remain stable. Similarly, a sensor coated with collagen I native protein previously purified using ion exchange chromatography on a Sepharose column failed to remain stable in serum.