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Visible Photolysis and Amperometric Detection of *S*-Nitrosothiols

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Abstract

The concentration of *S*-nitrosothiols (RSNOs), endogenous transporters of the signaling molecule nitric oxide (NO), fluctuate greatly in physiology often as a function of disease state. RSNOs may be measured indirectly by cleaving the S–N bond and monitoring the liberated NO. While ultraviolet photolysis and reductive-based cleavage both decompose RSNOs to NO, poor selectivity and the need for additional reagents preclude their utility clinically. Herein, we report the coupling of visible photolysis (i.e., 500–550 nm) and amperometric NO detection to quantify RSNOs with greater selectivity and sensitivity. Enhanced sensitivity (up to 1.56 nA μM^{-1}) and lowered theoretical detection limits (down to 30 nM) were achieved for low molecular weight RSNOs (i.e., *S*-nitrosoglutathione, *S*-nitrosocysteine) by tuning the irradiation exposure. Detection of nitrosated proteins (i.e., *S*-nitrosoalbumin) was also possible, albeit at a decreased sensitivity (0.11 nA μM^{-1}). This detection scheme was used to measure RSNOs in plasma and illustrate the potential of this method for future physiological studies.

Keywords

S-Nitrosothiols; photolysis; nitric oxide; amperometry; electrochemical sensor

Introduction

Nitric oxide (NO) is intricately involved in a myriad of physiological processes (e.g., neurotransmission,[1] vasodilation,[2] and the immune response[3, 4]) despite its reactive nature and short lifetime in physiological milieu. As such, scientists across many disciplines have aimed to analytically investigate the expansive bioactivity of NO.[5] *S*-Nitrosothiols (RSNOs) have been determined to be highly important endogenous transporters of NO.[6, 7] Disease states (e.g., sepsis and asthma) characterized by abnormal NO levels also display similarly erratic (i.e., elevated or depressed) RSNO concentrations.[8] Although comparisons of RSNO concentrations between disease and non-disease states have been reported,[9–11] reliable methodology for quantifying basal levels of RSNOs in biological fluids remains unrealized. As such, the poor existing analytical methodology has resulted in a wide discrepancy of reported RSNO levels that span several orders of magnitude (i.e., nM–μM) in blood and plasma.[10]

To date, RSNO species are primarily measured indirectly by monitoring NO after S–N bond cleavage. Several methods exist for measuring NO including fluorescence,[5, 12]

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Supporting Information Available
Chemiluminescent data, representative amperometric responses to chemical and photolysis RSNO decomposition, and calibration curves. This material is available free of charge via the Internet at http://pubs.acs.org.

chemiluminescence,[13] and electrochemistry.[14] Similarly, varied approaches have been used to cleave S–N bonds, the most common being reduction and UV photolysis.[9–11, 15] While reductive cleavage is inherently encumbered by immobilization and/or the addition of external reagents (e.g., copper sources and reducing agents), UV photolysis is criticized for overestimating RSNO values due to the presence of endogenous interferents (e.g., nitrite, nitrate) that some have claimed convert to NO upon UV irradiation.[10, 16, 17] Often overlooked, RSNOs also exhibit a weaker absorbance in the visible region of ~550–600 nm. Sexton and coworkers have demonstrated that RSNOs may be homolytically cleaved to liberate NO via irradiation at either UV or visible maxima.[18] Although controversy exists regarding the selectivity of UV irradiation for RSNO species, Alpert et al. restricted photolysis to the visible range and successfully reduced chemiluminescent signal generated from nitrite during irradiation while still allowing for adequate RSNO sensitivity.[16]

The concept of coupling photolytic cleavage with electrochemical detection represents new analytical methodology for RSNO detection. Although previous electrochemical RSNO sensors have added or affixed redox reactive catalysts (e.g., copper nitrate and organoselenium species) and reducing agents (e.g., glutathione and cysteine) to NO permselective sensors, none have evaluated the use of photocatalytic cleavage to liberate NO.[19–26] In this work, we couple photolytic cleavage with an amperometric NO sensor to facilitate RSNO detection in physiological fluids (i.e., plasma) is described. The restriction of photolysis to the visible region circumvents the controversy surrounding the possible overestimation of RSNO values associated with UV photolysis and nitrite-derived NO.[16]

Experimental Section

Reagents and Materials

Methyltrimethoxysilane (MTMOS) was purchased from Fluka (Buchs, Switzerland). (Heptadecafluoro-1,1,2,2-tetrahydrodecyl)trimethoxysilane (17FTMS) was purchased from Gelest (Tullytown, PA). Glutathione (GSH), bovine serum albumin (BSA), dithiothreitol (DTT), and Antifoam B emulsion were purchased from Sigma Aldrich (St. Louis, MO). L-Cysteine-HCl-H_2O (Cys) was obtained from Pierce-Thermo Fisher Scientific (Rockford, IL.). Copper (II) nitrate was obtained from Acros Organics (Geel, Belgium). An X-Cite 120 Fluorescence Illumination System with a 120 W lamp was obtained from Lumen Dynamics (Mississauga, Ontario). The source was outfitted with a green filter at the end of the liquid wave guide to restrict the output from 500 to 550 nm. Distilled water was purified to 18.2 MΩ·cm with a Millipore Milli-Q Gradient A-10 water purification system (Bedford, MA). All other solvents and chemicals were analytical-reagent grade and used as received.

Preparation of NO-permselective Xerogel-Modified Electrode

Xerogel-modified electrodes were prepared according to Shin et al.[27] Briefly, platinum disk (2 mm diameter) electrodes sealed in Kel-F (total 6 mm diameter, CH instruments) were mechanically polished. A 20% 17FTMS, balance MTMOS (v:v) silane solution was prepared by dissolving 60 μL of MTMOS in 300 μL of ethanol. Sequential addition of 15 μL of 17FTMS, 80 μL of water, and 5 μL of 0.5 M HCl was followed by gentle agitation. The resulting solution was mixed for 1 h at room temperature. After the 1 h of mixing, 1.5 μL of solution was cast on the electrode surface and the electrode was gently rotated to ensure even coating. The xerogel was allowed to cure for 24 h at ambient conditions.

Preparation of *S*-Nitrosothiols

A modified procedure that has previously been reported for preparing low molecular weight RSNOs was used in this study.[23] Briefly, equal volumes of 5 mM thiol (i.e., GSH or Cys) in 120 mM sulfuric acid and of 5 mM sodium nitrite with 20 μM ethylenediaminetetraacetic

acid (EDTA) were mixed to promote nitrosation of the thiols. To prepare *S*-nitrosoalbumin (AlbSNO), a modified procedure reported by Stamler et al.[28] was used whereby an aqueous solution of BSA (200 mg mL^{-1}, ~3 mM) was prepared and mixed with 1.5 mM sodium nitrite in 0.5 M HCl for 30 min at room temperature. A less than stoichiometric amount of acidified nitrite was used to minimize nitrosamine formation on tryptophan residues of BSA.[29, 30] Resulting RSNO concentrations were verified via their UV absorption maxima at 335 nm (ε = 503, 586, and 3869 M^{-1} cm^{-1} for *S*-nitrosocysteine (CysNO), *S*-nitrosoglutathione (GSNO), and AlbSNO, respectively.[31]

Chemiluminescent Analysis of *S*-Nitrosothiols

Gas phase NO release from RSNOs was measured using a Sievers 280i Chemiluminescence Nitric Oxide Analyzer (NOA) (Boulder, CO). Calibration was performed with air passed through a Sievers NO zero filter and 26.8 ppm NO (balance N_2) gas. Samples were injected into 25 mL of deoxygenated pH 7.4 phosphate buffered saline (PBS) containing 500 μM diethylenetriaminepentaacetic acid (DTPA) to chelate trace copper and sparged with 80 mL min^{-1} N_2 stream. Additional N_2 was supplied to the reaction vessel to match the instrument collection rate of 200 mL min^{-1}. Nitric oxide analysis was performed in the dark at room temperature and by irradiating the reaction flask normal to its side with the light source at a distance of ~6 cm.

Electrochemical Analysis of *S*-Nitrosothiols in Phosphate Buffered Saline

Electrodes were placed in a three-electrode configuration consisting of an Ag/AgCl reference electrode (3.0 M KCl, CH Instruments (Austin, TX)), a Pt counter electrode, and the xerogel-modified NO-selective working electrode. Sensors were polarized at 0.8 V (vs. Ag/AgCl) for ≥1 h and tested in 40 mL of deoxygenated PBS with DTPA (prepared via sparging with nitrogen during polarization) with constant stirring at room temperature. Electrooxidation currents were recorded with a CH Instruments 660A potentiostat. Analyses were run first in the dark while monitoring background current from photoelectric interference that was minimized by irradiating perpendicular to the electrode surface. This background was subtracted from amperometric responses to NO. Sensor calibration was carried out via a separate solution method for each concentration. The light intensity near the electrode surface as a function of the light source-electrochemical cell distance was measured at 550 nm using a Newport Model 840-C handheld optical power meter (Irvine, CA).

Electrochemical Analysis of *S*-Nitrosothiols in Plasma

Fresh, whole porcine blood was drawn into EDTA as an anticoagulant at a volume ratio of 9:1 (final EDTA concentration 5 mM). Platelet-rich plasma was obtained by centrifuging the blood at 2000×g for 30 min. Plasma was either analyzed as oxygenated or deoxygenated (with addition of 0.1% v:v Antifoam B emulsion and nitrogen sparging).

Results and Discussion

Analysis of Low Molecular Weight RSNOs

The xerogel-modified NO-selective sensor used in this study has been previously characterized.[27] In that report, sensor membrane fabrication was optimized (e.g., fluorosilane content of the membrane and thickness) to provide the largest permeability to NO while maintaining the greatest selectivity over common interferents (e.g., nitrite). A thickness of 9.6 μm was reported to yield the greatest response and thus this was used in the studies described herein. As the validity of the electrochemical sensor membrane for

accurate NO measurements was previously characterized (detection limit of ~100 pM NO), initial studies focused on evaluating the photolytic cleavage of RSNOs.

Conventional chemiluminescence NO detection was used to investigate the feasibility of measuring liberated NO from representative endogenous low molecular weight (LMW) RSNOs, *S*-nitrosoglutathione (GSNO) and *S*-nitrosocysteine (CysNO), after irradiation with visible light. Light from a 120 W mercury vapor arc lamp was filtered to 500–550 nm and proved sufficiently diffuse and of enough intensity (105 mW at the electrode surface for a light source distance of 6 cm) to generate appreciable response to RSNOs without undesirable sample heating. This configuration (Supporting Information, Figure S-1) triggered near constant levels of NO release from the RSNOs during the course of irradiation. Nitric oxide levels subsided when irradiation ceased and were observed to increase again upon re-irradiation illustrating the photoswitchable NO liberation typical of RSNOs.[6] As such, periods of visible irradiation resulted in controlled RSNO decomposition and NO generation.

Gas phase detection of NO is amenable to the indirect measurement of RSNOs because of the gaseous nature and low aqueous solubility of NO.[10] While more analytically challenging, the benefits associated with solution-based NO detection via electrochemistry include enhanced sensitivity, rapid response, inexpensive instrumentation, and potential for miniaturization and application to physiological milieu.[5, 14] With the use of an NO sensitive and selective sensor,[27] the amperometric response of LMW RSNOs during visible irradiation was examined by positioning the light source normal to the side of the electrode (i.e., perpendicular to the electrode surface) to minimize photoelectric emission into solution from the electrode surface and any ensuing electrochemical interference due to the generated photocurrent. Photoelectric emission and strategies for circumventing it have been described previously.[32, 33] Nevertheless, irradiation of the RSNO-containing oxygenated PBS solution resulted in a low but measureable amperometric signal (Supporting Information, Figure S-2) similar to that observed previously by others using electrochemical NO sensors in the absence of controlled irradiation to measure RSNOs in oxygenated solutions.[21, 22, 25, 26] As NO is expected to exhibit an extended half-life in aqueous solutions at sub-μM concentrations (due to the negligibly slow autooxidation of NO to nitrite),[34] alternative mechanisms were considered. Pronounced direct reaction of NO with oxygen has been shown to occur when bolus amounts of NO have been added to aerated solutions.[35, 36] In these instances, large local concentrations of NO are scavenged by oxygen before adequate mixing and dissipation. Likewise, the photolysis of RSNOs may similarly generate large local concentrations of NO that are readily scavenged upon reaction with oxygen. Mutus and coworkers also report that the photolysis of RSNO species in aerated aqueous solutions produces radicals from the reactions of the newly generated thiyl radical after homolytic cleavage of the S–N bond.[37, 38] For example, the reaction of NO and superoxide (resulting from the reduction of oxygen via a thiyl radical intermediate) has been shown to produce peroxynitrite.[37] With respect to superoxide, Hofler and Meyerhoff also noted poor RSNO sensitivity in oxygenated solutions for catalyst-based RSNO sensors that was attributed to superoxide generated from the reductive catalyst affixed to the sensing membrane.[26]

Regardless of the exact mechanism of oxygen limiting our observed amperometric response, deoxygenating the solution by sparging with nitrogen gas before analysis allowed significantly greater response during the irradiation period. As shown in Figure 1, a typical sensor response exhibited a linearly increasing current once irradiation was initiated. This response stabilized only after irradiation was ceased. The increasing current corresponded to a linearly increasing concentration of NO due to the near steady rate of RSNO decomposition as corroborated with the chemiluminescence data (Figure S-1). The benefit of such behavior is that the irradiation time may be used to tune the analytical utility of this

methodology. For example, longer irradiation periods may promote greater NO production thus enhancing the measured current and lowering the RSNO detection limits for this scheme. In contrast, shorter periods would yield smaller signals more rapidly (i.e., decreased analysis time). RSNO analysis employing this methodology is in turn customizable to meet the needs of a specific study. Although calibration was assessed using separate solutions for each RSNO concentration, multiple injections into the same solution enabled calibration of sensor response by plotting the change in current (i.e., not the additive current) during each irradiation period. This behavior is shown in Supporting Information, Figure S-3 whereby the enhanced rates of current production (i.e., increased slope of sensor response) due to the pseudo first-order kinetics of photolysis are evident within each irradiation period after subsequent injections.[6]

Throughout the entire period of irradiation examined (up to 7 min), the response to increasing LMW RSNO (i.e., GSNO and CysNO) concentration was linear (Supporting Information, Figure S-4). Periods greater than 7 min resulted in slight deviation from linearity, suggesting an upper limit where the amount of NO liberated from RSNOs is no longer at a steady rate and begins to diminish, as noted for the chemiluminescent data (Figure S-1). Likewise, the amperometric responses to GSNO and CysNO were comparable with GSNO giving a slightly higher sensitivity than CysNO (Table 1). This disparity in response is reflected in the compounds' molar absorptivities at 545 nm (ε = 17.2 and 14.9 M^{-1} cm^{-1} for GSNO and CysNO, respectively).[31] Nevertheless, the similar sensitivities illustrate the potential of this methodology to analyze multiple LMW RSNOs. While an irradiation period of 7 min facilitated the greatest sensitivity, 1 min of visible light exposure still provided linear response with suitable sensitivity (0.33 and 0.27 nA μM^{-1} for GSNO and CysNO, respectively). Of note, the sensitivities achieved with varying irradiation times were acquired with the same electrode. The reported electrochemical sensitivities are meant to illustrate tunability of the methodology. The innate sensitivity of the electrode to NO does not change during analysis. As shown in Table 1, theoretical detection limits (based on S/N =3) may be lowered with longer irradiation times, highlighting the interplay between analysis time and sensitivity when using visible photolytic cleavage. While the responses were linear for 0.16–5.0 μM RSNO throughout the irradiation period, improved linearity was noted at higher concentrations (0.6–5.0 μM RSNO). For example, the linearity from 0.16–5.0 μM was characterized with an R^2 = 0.9804 at 7 min of irradiation for CysNO, while an R^2 = 0.9916 was achieved for 0.6–5.0 μM.

The deviation from linearity at lower concentrations (i.e., 160 nM) suggests that feasible detection limits may be dependent on the light source intensity. Indeed, the total amounts of NO released during irradiation for CysNO and GSNO elucidated by chemiluminescence were ~0.07 and 0.11 mol NO per mol of RSNO, respectively. As each RSNO molecule binds one molar equivalent of NO, this amount corresponds to only 7 and 11% RSNO to NO liberation efficiency during the 7 min of irradiation for CysNO and GSNO, respectively. We predict that greater intensity sources would improve RSNO to NO conversion thus enhancing overall sensitivity, lowering detection limits, and extending the linear range. To explore the influence of light intensity on sensor response, the distance between the light source and the sample was varied from 6 to 136 cm, resulting in light intensities at the sample of 105–0.9 mW, respectively (Figure S-5). As expected, the response sensitivity was dependent on the apparent wattage, with lower light intensity resulting in a lower sensor response (current) at an equivalent irradiation times. We reason that even more intense sources would enable greater sensitivity. Unfortunately, such sources were not readily available for use in this study. The maximum achievable light intensity (105 mW) was thus utilized for all additional experiments.

The superior sensitivity of the NO-permselective electrode warranted the investigation of its coupling with reductive-based RSNO cleavage for comparison. The influence of copper (II) nitrate, varied reducing agents (e.g., DTT and GSH), and their concentrations (1–10 μM) on the measurement of GSNO in PBS was thus evaluated employing similar xerogel-modified electrodes. While signal (i.e., current) was observed upon injection of copper nitrate with and without DTT into solutions of GSNO, the use of GSH as a reducing agent proved most efficient in converting Cu(II) to Cu(I), the required active species for RSNO decomposition, and yielded the most rapid response (Figure S-6). The observed signal using Cu(II) (without added reducing agent) may be attributed to trace thiol in the RSNO solution generating active Cu(I).[39] Experiments were performed with Cu(II) opposed to directly utilizing Cu(I) as Cu(I) compounds are generally insoluble in water.[40] After systematically examining the concentration dependency of these reagents (Figure S-7), it was found that injecting a freshly mixed solution of 10 μM copper nitrate and 10 μM GSH generated the greatest and most rapid GSNO response. As might be expected, the copper-mediated reductive cleavage yielded larger currents than visible photolysis due to the visible light only weakly cleaving the S–N bond while the reductive pathway employed a large concentration to afford the greatest response by near complete RSNO to NO conversion.[24] Nevertheless, the benefit of visible photolysis is the avoidance of additional reagents. As such, visible photolysis was the focus hereafter.

Analysis of Macromolecular RSNOs

Nitrosated proteins, particularly nitrosated serum albumin (AlbSNO), constitute a majority of RSNO species in circulation.[41] Thus, RSNO analysis of physiological fluids would benefit from a simple method capable of quantifying nitrosated macromolecules. Of note, the utility of previous electrochemical RSNO sensor designs has only been demonstrated for nitrosated proteins in the presence of additional cysteine that promotes transnitrosation (i.e., direct transfer of the nitroso moiety) between the protein and LMW thiol.[25] The subsequent amperometric response is attributed to CysNO formation/detection. Thus, the ability to measure nitrosated macromolecules is lacking when using these earlier methodologies.

Similar to the LMW RSNO species, the amperometric response to AlbSNO during irradiation was investigated as a function of the RSNO to NO liberation protocol (Figure S-8). While detection of AlbSNO was achieved with visible photolysis, the sensitivity was greatly reduced compared to the LMW RSNOs (Table 1). In addition, the theoretical detection limit was also much greater, partly explaining the large variability and limited linearity observed. Chemiluminescent analysis of AlbSNO corroborated this hypothesis with only 1.3% conversion of RSNOs to NO during the 7 min irradiation period (Figure S-9). Reduced AlbSNO to NO conversion efficiency has been reported previously.[10] While AlbSNO exhibits a greater molar absorptivity (3869 M^{-1} cm^{-1}) at 545 nm,[31] the unusual stability of the protein-based S–N bond relative to LMW RSNOs has been well-characterized and is evidenced by a greater resilience to decomposition under physiological conditions (i.e., an extended half-life).[6, 28] The cause of this enhanced stability has been ascribed to conformational protection of the nitrosocysteine group as it lies within a hydrophobic crevice of the protein isolated from solvent.[42] The conformational restriction may also promote radical recombination between NO and the thiyl radical after irradiation, a phenomenon that has been reported to enhance RSNO stability in other macromolecules.[43] Unlike the response for LMW RSNOs (plateauing after ~7 min of light irradiation), the signal observed upon irradiating AlbSNO solutions continued to increase slightly at longer irradiation times (Figure S-9) that may prove analytically useful if analysis time is not an important criterion. Although not as sensitive, the ability to directly measure AlbSNO without a transnitrosation intermediate step to LMW RSNOs sets this strategy apart from previously published electrochemical macromolecular RSNO detection schemes.[21–25]

Detection in Plasma

Investigating the efficacy of RSNO detection in physiological fluids is necessary to evaluate the potential of this methodology for biologically-relevant studies (e.g., the study of RSNO fluctuations in blood, plasma).[8] Initial efforts to detect RSNOs in 40 mL of whole blood proved problematic (i.e., no appreciable signal), ostensibly due to an inability of the light to penetrate the sample and scattering/absorbance from red blood cells. Previous studies have shown RSNO photoinstability in blood for samples exposed to ambient light in vessels with large surface area to volume ratios (i.e., surgical butterfly tubing), suggesting photolytic cleavage and detection is feasible if adequate light penetration is achieved.[44–46] Nevertheless, the greater translucency of plasma represents a more suitable matrix for ex vivo analysis. Similar to PBS, oxygenated plasma showed little response during irradiation. In contrast, irradiating a fresh sample of deoxygenated plasma for 7 min led to a current response of 0.19 nA that may be ascribed to endogenous RSNO species. Subsequent additions of CysNO to calibrate the sensor response in plasma resulted in a sensitivity of only 0.07 nA μM^{-1}, illustrating diminished analytical performance in this physiological matrix (Figure 2 and Table 1). Other researchers have noted similarly diminished sensitivity in physiological fluids, attributing such response to rapid scavenging of NO by proteins (e.g., cell-free hemoglobin).[10, 14, 47] In this instance, the slight opacity of plasma (compared to PBS) may also diminish sensitivity. Nevertheless, the response for this configuration was linear (R^2 = 0.9949) with a limit of detection of 0.17 μM for 7 min of irradiation. Due to disparate sensitivity to LMW and protein RSNOs, determination of the endogenous levels of RSNOs in plasma using this approach remains convoluted at this time. However, the lessened sensitivity to AlbSNO in PBS and further reduced sensitivity in plasma indicates that visible photolysis may prove useful for selective measurement of LMW RSNOs in physiological fluids. Indeed, injections of AlbSNO into plasma did not result in any appreciable increase in sensor response. Based on this knowledge and the sensitivity in plasma, the calculated amount of endogenous LMW RSNO species in the porcine plasma sample was determined to be ~3 μM. Comparative study of analytical methodologies is still necessary to confirm this concentration as it is at the higher end of the previously reported range (i.e., 1 nM to 10 μM) of plasma RSNO levels.[10]

Conclusions

The analytical methodology developed in this work represents the first coupling of photolytic cleavage with electrochemical RSNO detection. The sensitivity to LMW RSNOs achieved after 7 min of irradiation surpasses that of recently reported RSNO electrochemical sensors,[22, 25] in part due to the use of an analytically superior NO-permselective electrode. The studies described in this work were not designed for a clinical goal, but rather to demonstrate the utility and response tunability. Both the light source and corresponding electrode materials may be optimized further to aid in the detection of RSNOs when using this method for a specific application. For instance, the use of higher intensity sources may increase the observed signals while more narrow visible light filters may lead to increased selectivity for specific RSNOs. Miniaturized needle type microelectrodes and the use of fiber optics may extend the use of this methodology to in vivo analysis.

To broaden the analytical utility of this method, the RSNO to NO conversion efficiency should be improved to enhance the signal and shorten the associated response time. Beyond greater light intensity, this may be facilitated by using microfluidic device technology for which smaller sample volumes will be more completely irradiated.[48] Furthermore, the narrow channels and corresponding thin sample cross sections may also decrease sample opacity and red blood cell scattering, facilitating RSNO analysis in whole blood.[44–46] Studies are currently underway in our laboratory to integrate NO-permselective electrodes within microfluidic devices.

A thorough comparative investigation must still be undertaken with the use of these and previously reported devices[19–26] to more accurately determine the true basal levels of RSNOs in blood. Careful attention to sample handling and preparation are obviously critical to the validity of such measurements.[10, 11, 49] Once determined, the future application of such devices for point-of-care diagnostics and prognostics may be achieved and guide clinical intervention and disease outcome.[8]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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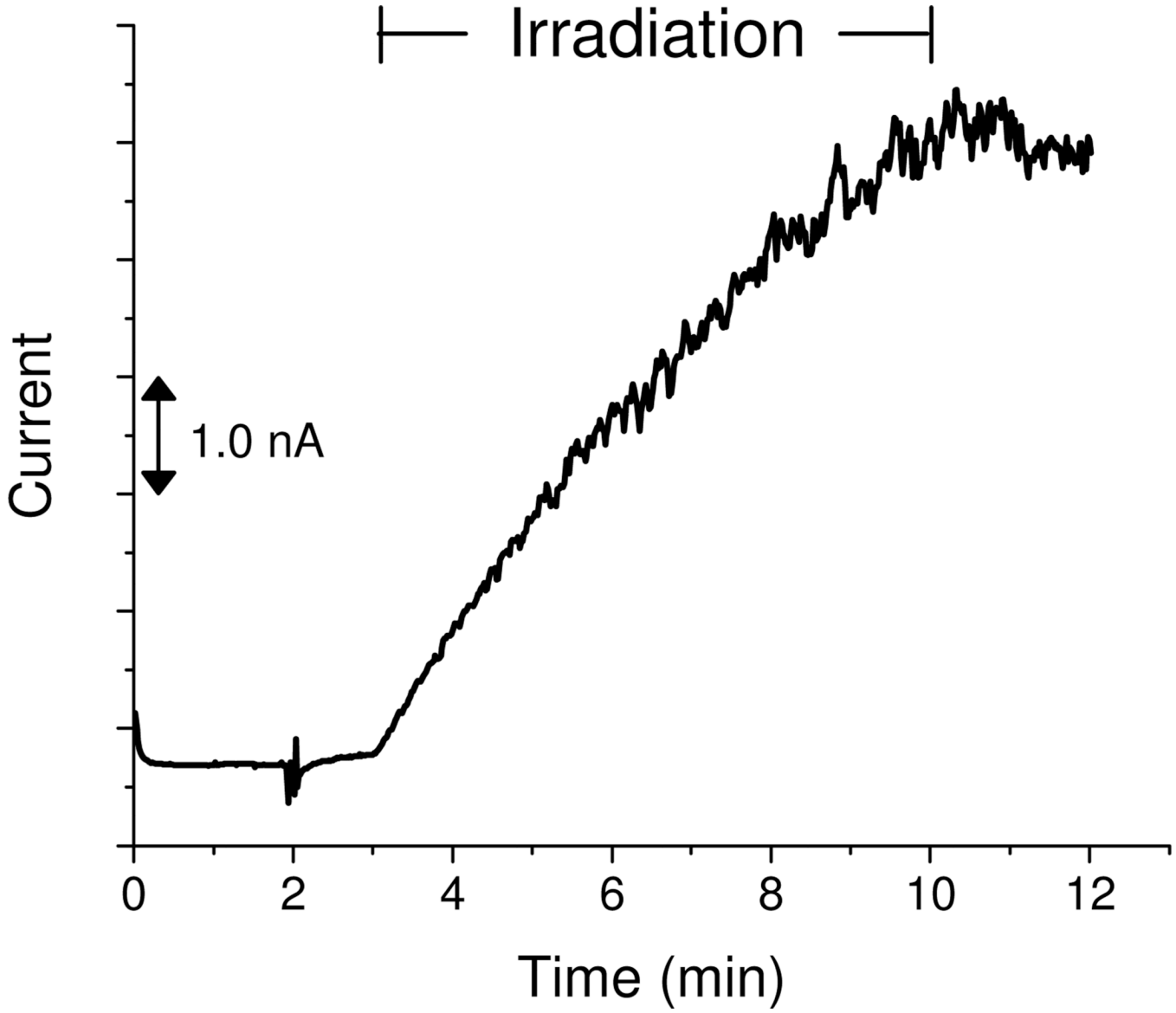

Figure 1.
Representative amperometric response to 5.0 μM GSNO in deoxygenated PBS. The 7 min period of irradiation with visible light is noted between 3 and 10 sec.

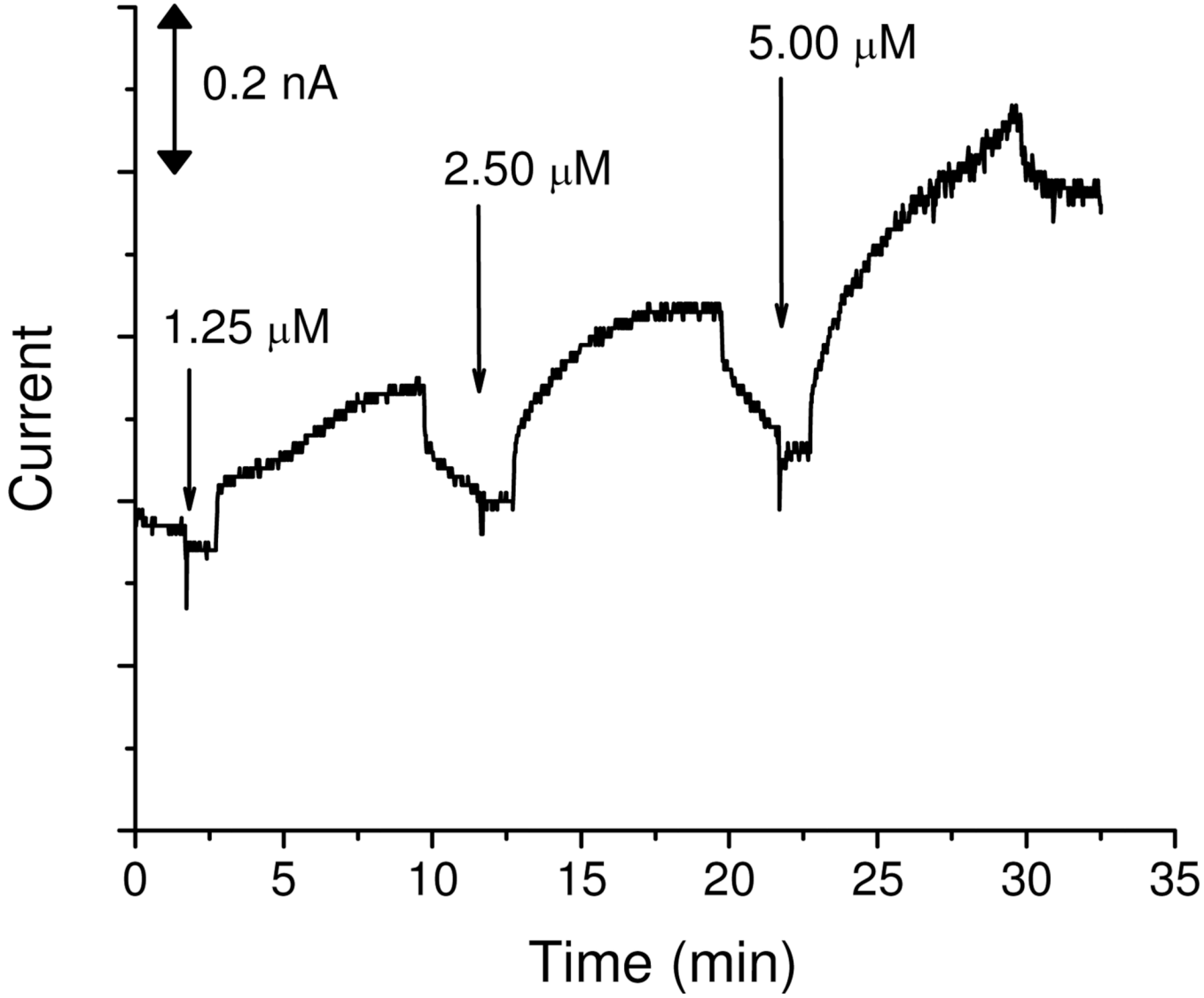

Figure 2.
Representative amperometric response to injections of CysNO in deoxygenated plasma followed by 7 min periods of irradiation with visible light. Of note, the small current response illustrates the presence of photoelectric interference that is baseline subtracted prior to analysis.

Table 1

Effect of irradiation time on amperometric sensitivity and limit of detection to a range of *S*-nitrosothiol species.

	Sensitivity (nA μM^{-1})			Limit of Detection (μM)		
	Irradiation Time (min)					
RSNO species	**1**	**4**	**7**	**1**	**4**	**7**
CysNO (PBS)	0.27	0.92	1.31	0.18	0.05	0.04
GSNO	0.33	1.08	1.56	0.14	0.04	0.03
AlbSNO	0.02	0.08	0.11	2.66	0.61	0.42
CysNO (plasma)	0.03	0.05	0.07	0.40	0.24	0.17