Detection of carbon monoxide from biological tissue using difference frequency generation in periodically-poled lithium niobate near 4.6 μm

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ABSTRACT

A mid-infrared gas sensor using difference frequency generation was developed to measure trace levels (~ ppb) of biological carbon monoxide (CO). A periodically-poled lithium niobate crystal is pumped by a continuous wave Ti: Sapphire laser and a compact diode pumped Nd:YAG laser operating at 864.86 and 1064.6 nm, respectively. The strong infrared transition R(6) at 2169.2 cm^{-1}(4.61 μm) is chosen for convenient CO detection without interference from other gas species. Carbon monoxide is collected and flowed into a multipass cell with an effective optical path length of 18.3 m. Using such an experimental arrangement, we detected the generation of CO at levels of 30 ppb during a 30 min period from living vascular smooth muscle cells (1×10^7) in basal state.

Key words: gas detection, laser spectroscopy, vascular smooth muscle cells

1. INTRODUCTION

Recent work indicates that carbon monoxide (CO) produced by heme oxygenase can play a role as a physiological messenger similar to nitric oxide[1]. Since the CO production from biological tissue is extremely small (~ 100 ppb)[2], the measurement technique of the CO concentration has been limited to gas chromatography. Although this method is sensitive, it cannot measure the CO concentration directly, and requires several time consuming intermediate steps of chemical reactions (about 15 min). Infrared absorption spectroscopy using a difference frequency generation (DFG) technique[3] is an attractive alternative approach for the detection of biological CO at the ppb level in real-time. Furthermore, simple absorption measurements can detect CO directly. Unlike in gas chromatography, we avoid the addition of any chemicals which react with CO in order to determine the concentration. This work demonstrates the use of a novel sensitive, selective mid-infrared gas sensor capable of detecting biological CO.

2. MATERIALS AND METHODS

2.1. Sensor configuration

A schematic of DFG-based sensor is shown in Fig. 1. A periodically poled lithium niobate (PPLN) crystal was pumped by a convenient pseudo tunable high power diode laser (ie, a cw single-frequency Titanium: Sapphire ring laser) operating at 864.86 nm with an output power of 1.5 W and a compact diode-pumped, non-planar monolithic ring Nd:YAG laser operating at 1064.6 nm with an output power of 750 mW. The output of both lasers is coupled into a singlemode fiber using a free space coupler with coupling efficiency of 60 %. Fiber polarization controllers were used to set the polarization states for both input beam to be linear and vertical. The two fiber coupled pump sources were combined into a single mode fiber using a 850/1080 nm fiber wavelength division multiplexer (WDM) as an effective beam splitter. A single-AR coated achromatic 10 mm focal length lens was used to image the beam spot from the output fiber end into a 19 mm long PPLN crystal, which contained eight gratings with periods of 22.4 μm to 23.1 μm in increments of 0.1 μm. A grating period of 22.8 μm was required for DFG at 4.61 μm which corresponds to a suitable CO absorption line [R(6)] without interference

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from other gases. The crystal temperature was controlled to 23°C for maximum spectral quasi-phasematching. The mid-IR DFG output was collimated by an uncoated 50 mm focal length CaF_2 lens and the residual pump beams were blocked by an AR coated Ge filter. The DFG radiation at 4.61 μm was measured to be 15 μW. A part (20%) of the IR output was deflected by a coated ZeSe beamsplitter and monitored by a liquid-nitrogen-cooled InSb detector. The beam transmitted through the beamsplitter was focused into a multi-pass absorption cell which provided an 18.3 m optical path length with 90 passes through a compact sample volume of 400 cm^3. The cell was connected to a vacuum manifold that included a pressure gauge and a 21-G needle for collecting the sample gas. After exiting the cell, the IR beam was collected by an off-axis parabolic mirror and focused onto a second liquid-nitrogen-cooled InSb detector. The detectors had a noise-equivalent power (NEP) of 0.8 pW $(Hz)^{-1/2}$. For balanced detection of CO in ambient air the IR beam path to the first detector was set to be equal to the beam path outside the multi-pass cell to the second detector.

High resolution wavelength scans of the Ti: Sapphire laser were performed by tilting a thin etalon by means of a computer controlled galvanometer. For absorption measurements, a scan range of 7 GHz was adequate to monitor a single CO peak at a pressure of 100 Torr. The DFG signal detected after the multi-pass absorption cell was filtered and amplified with a lock-in amplifier. The data was digitized and transferred to a laptop computer using a 16-bit A-D card. The data was analyzed with LabVIEW 5.0 -Software (National Instruments, Inc.). Segments of CO absorption were removed from the base line that matches the estimated base width of CO Lorentzian absorption peak. An eighth order polynomial was then fitted to the remaining baseline to approximate 100% transmission at the CO peak. In addition, a Lorentzian lineshape was fitted to the CO absorption line[3].

2.2. Cell Culture and gas collection

Rat aortic smooth muscle cells were cultured serially in minimum essential medium supplemented with 10% fetal calf serum and were plated in T75 culture flasks having a volume of 250 cm^3. When the cells reached confluence, flasks containing about 1 x 10^7 cells were divided into two groups. One group of flasks contained cells which was not treated with any chemicals (control group), while the other contained cells treated with 20 μM hemin 8 hours prior to the CO measurement (hemin group). Hemin is a stable form of heme which serves as a substrate for the heme oxygenase (HO) catalyzed formation of CO, described by the following reaction.

$$\text{Heme} \xrightarrow{\text{HO}} \text{CO} + \text{Biliverdin}$$

The HO-mediated degradation of heme is the primary mechanism for the production of cellular CO.

The flask containing cells and medium was sealed and then placed on a heating pad to keep the medium temperature constant to 37°C. Two 21-G needles connected to the flask were connected to the multi-pass cell and to a gas cylinder with pure nitrogen gas, respectively. The gas inside the flask was removed and displaced with the pure nitrogen gas. Then the following procedures were repeated every 30 minutes to measure the cultured-cell-produced-CO continuously: (1) A vacuum inside the multipass cell was generated by the vacuum pump. (2) The flask containing gas, which should be a mixture of pure nitrogen and a certain amount of the CO produced from the biological samples was pumped into the multi-pass cell until inside pressure of the cell reached a level of 100 Torr. (3) The loss of gas within the flask, approximately 54 cm^3, was refilled with pure nitrogen to maintain the inside pressure of the flask constant to ambient level. (4) CO in the multipass cell was measured using DFG based IR absorption spectroscopy.

3. RESULTS

Figure 2 shows a CO spectrum of the R(6) transition at 2169.198 cm^{-1} at room-temperature and 100 Torr pressure. The measurement time is 2 min. The width of the fitted Lorentzian curve was estimated using the theoretically predicted Lorentzian width from HITRAN 96. The Lorentzian-lineshape fit to the data has a FWHM of 1.77 x 10^{-2} cm^{-1}, and yields a fitted magnitude of the peak absorbance of 2.33 x 10^{-2}. The CO mole fraction computed from the absorption peak is 236 +/- 10 ppb. The error corresponds to the root mean squared fit residuals.

A time trace of the measured CO within the flask containing the vascular smooth muscle cells for a duration of 180 min is

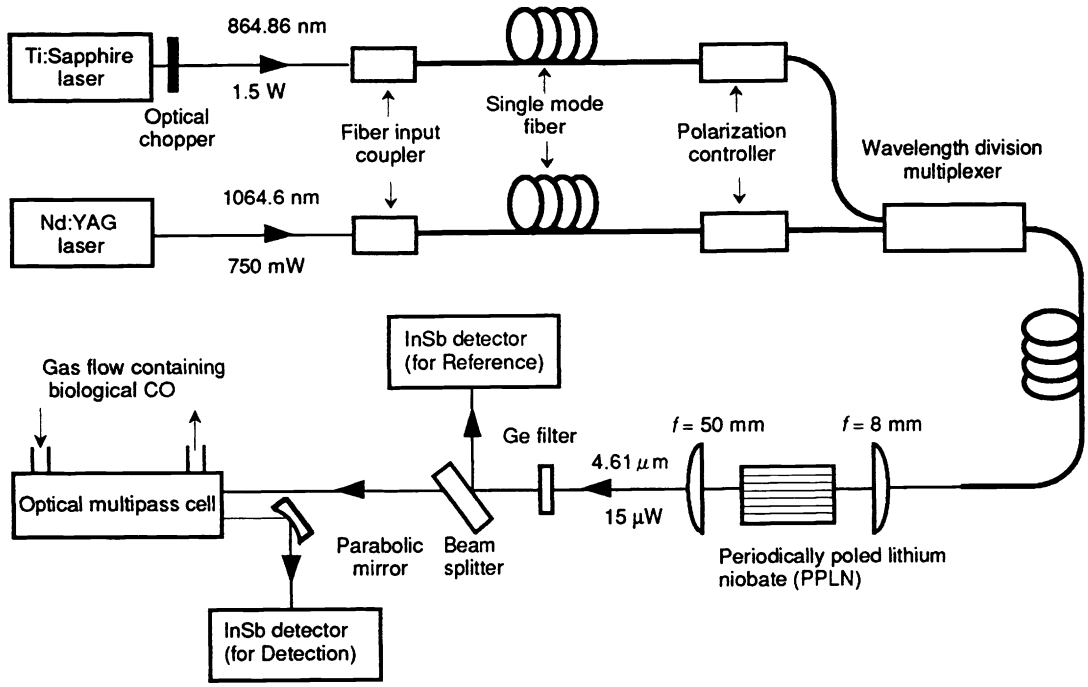

Fig. 1 Optical arrangement of the DFG-based sensor for CO detection.

shown in Fig. 3. Carbon monoxide production was observed in both the control and the hemin treated cells. The CO production of the hemin treated cells was significantly larger than that of the control cells. If it is assumed that the CO generation rate r (ppb/ 30 min) is constant during the entire time period of 180 min, we can describe the CO concentration within the flask as a function of time $C(t)$, that satisfies the following equation:

$$C(t) = r + C(t-30)\,(V_{flask} - V_{loss})/\,V_{flask} \quad (t \geq 30 \text{ min}, C(0) = 0 \text{ ppb}) \tag{1}$$

where V_{flask} represents the flask volume of 250 cm^3, and V_{loss} represents the gas volume which is delivered to the multipass cell during every filling and procedure, estimated to be 54 cm^3. Substituting these figures into Eq.(1) results in:

$$C(t) = 4.63\, r\, (1 - 0.784^{t/30}) \tag{2}$$

We applied Eq. (2) as a fitting line to the data shown in Fig. 3 to obtain each value of r. As a result, the r of the control cells was 28.5 +/- 4.0 (ppb/ 30 min) and r of the hemin treated cells was 42.6 +/- 8.8 (ppb/30 min). Substituting these values into gas equation; $n = PV/RT$ where n is number of moles; P is the pressure inside the flask (1.01×10^5 N/m^2); V is the volume of the flask (2.50×10^{-4} m^3); R is the gas constant (8.31 $J \cdot K^{-1} \cdot$ mol); T is temperature in the flask (310 K), we obtained 280 +/- 40 (pmol/ 30 min) and 418 +/- 86 (pmol/ 30 min), respectively.

4. DISCUSSION

We have detected trace levels of CO production from biological cells using DFG-based mid-infrared absorption spectroscopy. The present study is the first report to demonstrate the detection of CO from living vascular smooth muscle cells (VSMCs) .

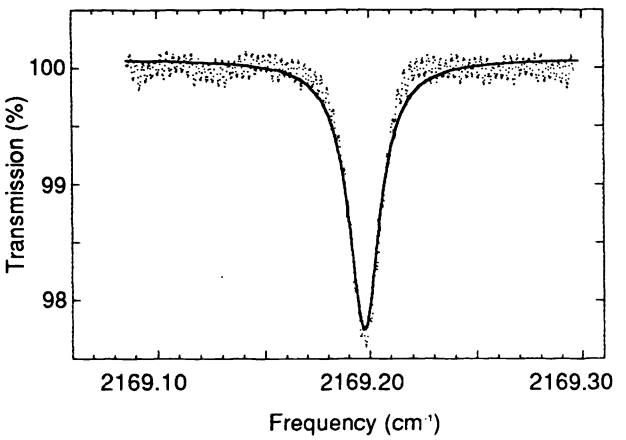

Fig. 2 Spectrum of the R(6) transition at 2169.198 cm^{-1} of CO in room-temperature at 100 Torr pressure. The dots represent the measured data, a 5 sweep average. The solid trace is a fit to a Lorentzian profile.

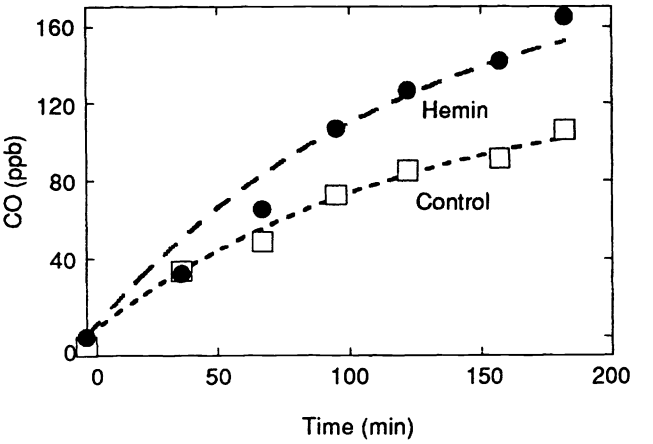

Fig. 3 CO production from vascular smooth muscle cells over a 3 h period. The fitting line can be expressed by $Y = 4.63\ r\ (1\text{-}0.784^{x730})$.

There are several studies that have reported the detection of biological-cell-produced CO but all experienced experimental difficulties in measuring CO due to limited CO production (Table 1). The reported CO production rates with heme range from 0.75 - 4.4 x 10^2 pmol/ 30 min, which agrees with our results although the tissue and/or material are different from those in this work. Treatment with heme is known to induce HO, which may result in an increase of CO. The observed increment of CO production by heme treatment was approximately 1.5 times of the control in this study. The ratio of the increment is comparable with results from other studies using VSMCs[1]. Even in the control cells, which were not exposed to heme, we detected basal CO production of 280 pmol/ 30 min. There was no observation of basal CO production from VSMCs in previous research. However, it may exist because endogenous heme is produced even at basal state without the addition of exogenous heme[4]. Furthermore, VSMCs express HO mRNA and HO protein at basal state[5] suggesting the existence of CO production at basal state. Independent of the HO pathway, CO is also known to be produced from a source different from heme through a microsomal lipid peroxidation[6]. Basal CO production from brain tissue, 8.6 x 10^2 pmol/ 30 min[4], is larger than our results. This discrepancy may be due to a difference in the HO type. Type 2 HO, a constitutively expressed isoform, is highly expressed in the brain even in the basal state, while weakly expressed in the VSMCs[7]. Type 1 HO, an inducible isoform, is predominant in the VSMCs, but the expression is weak in the basal state. The basal CO production is smaller compared to a basal production of nitric oxide (NO) by vascular endothelial cells. A chemiluminescent method revealed that the basal NO production in freshly harvested endothelial cells (10^7 cells) lies in the range from 1.5 to 13.2 nmol/30 min[8], which is approximately 5 - 50 times of the present basal CO production in VSMCs.

Carbon monoxide can be detected by several methods, such as infrared absorption, colorimetry, electrochemical methods based on selective membranes, gas chromatography, and radioisotope counting. However, these methods except gas chromatography and radioisotope counting are not capable of detecting trace levels of CO less then 1 ppm. There are currently three kinds of techniques based on gas chromatography for detecting sub-ppm levels of CO. One technique uses a flame ionization detector[9]. CO is converted to methane by a methanator and then measured by the detector. The detection limit is 10 pmol CO/ 330 µl incubate volume[10], corresponding to 750 ppb. Another technique is to use a helium ionization detector, which potentially has CO sensitivity of 3 ppb. However, the carrier gas must be purified of water, oxygen, nitrogen, carbon dioxide to a level below the ppb range. Hence, the detection limit for CO increases to 70 ppb in the presence of ambient air and humidity[11]. The third technique employs atomic absorption by Hg vapor which results from the chemical decomposition of HgO by CO[2][12] and results in a high sensitivity; 1- 10 pmol/ 2 ml volume[13],

Table.1 CO production rates from biological tissue

CO production rate (x 10^2 pmol/ 30 min)/ Condition	Tissue/ Material / Amount	Method	Reference
4.4 /with Heme 14 /with Heme+NADPH	Rat aorta/ homogenate/ 1 mg protein	Gas chromatography with photometric detector	[12]
11 /with Heme + NADPH	Rat aorta/ homogenate/ 1 mg protein	Gas chromatography with flame-ionization detector	[9]
8.6	Rat olfactory bulb/ culture cells/ 33 mg protein (presumably 10^7 cells)	Radioisotope	[4]
0.75-2.8/with Heme 15 -28 /with Heme+NADPH	Guinea pig brain/ homogenate/ 1 mg microsome protein	Gas chromatography with photometric detector	[2]
2.8 4.2 /with Heme	Rat vascular smooth muscle/ cultere cells/ 10^7 cells	DFG-based mid-infrared spectroscopy	Present results

corresponding to 12 - 125 ppb. This method is also affected by water, oxygen, and carbon dioxide. The method using radioisotope (^{14}C)[4] is characterized by its high sensitivity, estimated to be at sub-ppb levels for CO. The intrinsic measurement processes is complicated since preincubation of biological cells with ^{14}C requires more than 5 hours. It is difficult to avoid a contamination by $^{14}CO_2$. Although the present method is a simple absorption measurement, it has a high sensitivity (~20 ppb) and a relatively fast analysis time response(~ 120 s). Moreover, this method does not suffer from interference by ambient air and water vapor, because we use a strong ro-vibrational absorption line at 2169.2 cm^{-1}, which is free of interference by absorption peaks of other gas species. In summary we report that continuous CO concentration measurements of biological tissue can be conveniently obtained by a tunable mid-IR gas sensor. This technique can be also applied to other biomedical relevant gases specifically nitric oxide[14].

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