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Short communication

Carbon monoxide generation from hydrocarbons at ambient and physiological temperature: a sensitive indicator of oxidant damage?

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Abstract

This paper shows that a variety of carbon-containing materials (wool, cotton, wood, paper, latex, Tygon) release CO during incubation at ambient temperature. This CO production was enhanced by aerobic versus anaerobic incubation, increasing temperature, and exposure to fluorescent light. CO production from glucose solutions was enhanced by alkaline pH or prior boiling or autoclaving and reduced by the presence of superoxide dismutase or catalase. We conclude that a variety of materials are constantly undergoing oxidation at ambient or physiological temperature as evidenced by the release of CO. Measurements of this CO production could provide a simple, rapid and sensitive means of assessing oxidative damage.

1. Introduction

An enzymatically catalyzed breakdown of heme to biliverdin [1,2] is thought to be the sole source of carbon monoxide (CO) production in living organisms. In the course of measuring CO excretion to assess heme turnover in rats, the chow and bedding were found to liberate quantities of CO far in excess of the rat's endogenous production. This observation led us to investigate the possibility that other compounds similarly might be undergoing spontaneous oxidation as evidenced by the production of CO. In this paper we demonstrate that a wide variety of compounds are constantly liberating CO at ambient temperature and suggest that measurement of this CO formation could serve as a novel indicator of oxidant damage.

2. Materials and methods

Studies of CO release from test substances were carried out in 50-ml glass syringes lubricated with mineral oil and sealed with plastic stopcocks. The test material was placed in a 10-ml glass vial that rested on the plunger in a vertically maintained syringe. The syringe was purged with gas rendered CO-free via passage through a combuster (Cat-1 catalytic combustion filter; Trace Analytical, Menlo Park, CA, USA) and then filled with 44 ml of the gas. Incubations were carried out at 4°C, laboratory temperature (approximately 21°C) or 38°C, both in the dark

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or light (20–30 cm distant from a lamp containing two standard 15 W fluorescent bulbs).

Materials tested included pine wood, paper (white, bond), white cotton, wool, latex rubber, Tygon (tubing), polyolefin and glucose. Glucose was studied in the crystalline state or as a 5 g/dl solution in water or 0.2 *M* $NaPO_4$. In some studies, 420 units of catalase (Sigma, St. Louis, MO, USA) or 330 U of superoxide dismutase (SOD) (Sigma) were added to the reaction mixture. A unit of catalase decomposes 1 μmol of H_2O_2/min at pH 7 and 25°C. One unit of superoxide dismutase produces a 50% inhibition of the reduction rate of cytochrome *c* in a coupled system with xanthine and xanthine oxidase at pH 7.8 and 25°C.

Carbon monoxide was measured by gas chromatography (GC) using a 3 ft. × 1/8 in. (1 ft. = 30.48 cm, 1 in. = 2.54 cm) column packed with molecular sieve (type 5A, 40–60 mesh) and a reduction detector (Trace Analytical). The carrier gas was argon (40 ml/min), and the oven temperature 100°C. The lowest detectable concentration of CO (relative standard deviation ± 10%) was about 0.1 ppm. Comparison of selected CO measurements obtained with a Digilab Model FTS-40 Fourier transform infrared spectrometer agreed to within ± 10% of GC results.

3. Results

We initially attempted to measure CO production from various substrates in 60-ml plastic syringes (Beckton Dickenson, Franklin Lakes, NJ, USA). However, the syringe itself produced appreciable CO. For example, after 24-, 48- and 72-h incubations in the dark at 37°C, CO concentrations in the syringe were 0.9, 1.8 and 2.9 ppm, respectively. Comparable values for incubations under fluorescent light were 11, 28 and 39 ppm. Studies of the components of the plastic syringes placed in glass syringes demonstrated that CO primarily was derived from the polypropylene body of the syringe (0.036 μmol/24 h) rather than the rubber seal (0.0028 μmol/24 h).

Studies of oiled glass syringes showed CO concentrations of less than 0.15 ppm (0.00036 μmol) after 24 h of incubation. In all reported studies, CO production by a test substance was determined from the CO accumulating in the gas space of a glass syringe.

Fig. 1 shows the CO accumulation observed when a variety of common carbon containing compounds were incubated at ambient temperature and ambient partial oxygen pressure (P_{O_2}) either in the dark or under a fluorescent light. Each compound released some CO, although the rate of release per g varied widely between compounds. Exposure to light markedly enhanced the release of CO by most of these materials.

Fig. 2 shows that the rate of production of CO by these compounds during exposure to light increased as the incubation temperature was raised from 4°C to 21°C or 37°C. The substitution of an argon atmosphere for air markedly reduced but did not totally inhibit CO production (Fig. 3).

Table 1 summarizes the results of studies of glucose incubated with air at 37°C in the dark. The crystalline sugar yielded negligible CO as did a distilled water solution of glucose. Glucose dissolved in 0.2 *M* phosphate buffer demonstrated increasing CO release with increasing pH. Experiments at pH 7 showed that when the glucose solution was boiled for 4 min or autoclaved for 15 min, CO production was enhanced

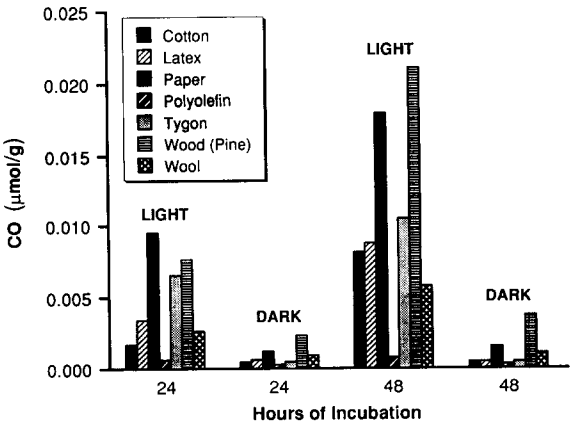

Fig. 1. Production of CO by various materials incubated at ambient temperature and P_{O_2}, either in the dark or under fluorescent lighting.

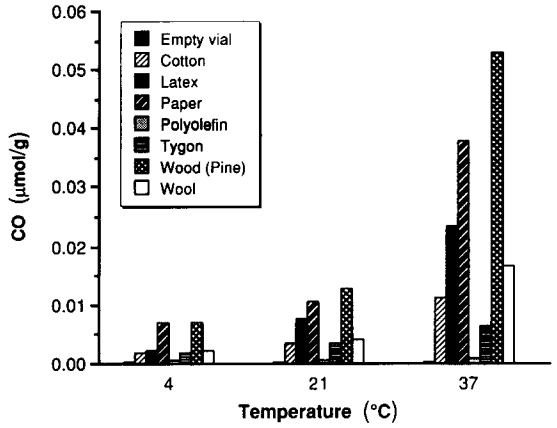

Fig. 2. Influence of temperature on production of CO by various materials during exposure to ambient P_{O2} and fluorescent lighting.

by about 50-fold and 150-fold, respectively. The addition of superoxide dismutase (330 units) or catalase (420 units) to the boiled or autoclaved glucose solutions reduced, but did not eliminate, CO production. When boiled glucose was incubated anaerobically, CO production was undetectable. Production of CO was roughly doubled when glucose was incubated in the light as opposed to the dark (data not shown).

Studies with a commercial source of sterile glucose for oral use (5% Glucose Water, Ross Labs.) demonstrated sizable CO production (0.14 μm g^{-1} 24 h^{-1}) when a 1:1 dilution in pH 7.4 phosphate buffer was incubated in the light.

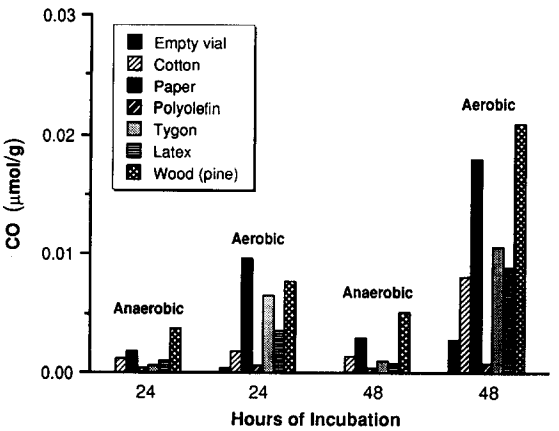

Fig. 3. Production of CO by various materials at ambient temperature under fluorescent lighting when incubated aerobically (ambient P_{O2}) or anerobically.

4. Discussion

Accurate measurement of CO production from substrates required an incubation system that produced minimal quantities of this gas. Plastic syringes released appreciable CO, particularly when exposed to light, a phenomenon previously attributed to dissolution of atmospheric CO from the plastic [3]. However, this CO seemingly must reflect production by the syringe since the CO concentration in our syringes reached values (39 ppm) which were far in excess of atmospheric CO concentration (<2 ppm). Glass syringes lubricated with mineral oil and sealed with stopcocks leaked at a negligible rate and produced minimal CO, and the CO production from various substrates was therefore assessed via the release of CO into the gas space of glass syringes.

A wide variety of common non-biological substances (cotton, wood, paper, Tygon, latex) were found to liberate CO when incubated at ambient temperature and P_{O2} (see Fig. 1). The rate of this CO release was diminished during anaerobic incubation, enhanced with increasing temperature (4°C to 37°C), and increased by exposure to fluorescent light. Each of these findings supports the concept that the CO release reflected an oxidative process, rather than desorption of CO dissolved in the test materials. The influence of light indicates that photo-oxidation, presumably via singlet oxygen, was an important mechanism in the CO production.

Additional studies were carried out with glucose (see table), a well-defined, biologically important compound. During incubation at ambient P_{O2} and at 37°C in the dark, little or no CO production was observed with glucose in the crystalline state or in distilled water. With dissolution in PO_4 buffer, CO production was very dependent upon pH, increasing linearly as pH was increased from 5 to 9. Boiling a glucose solution markedly enhanced the subsequent CO

Table 1
Production of CO from glucose (5 g/dl) incubated at ambient P_{O_2} in the dark at 37°C

Form	pH	Heat treatment	Antioxidant	CO (μmol g^{-1} 24 h^{-1})
Crystalline		No	None	Not detectable[a]
In distilled water		No	None	Not detectable
In PO_4 buffer	5.0	No	None	Not detectable
In PO_4 buffer	6.0	No	None	0.0063
In PO_4 buffer	7.0	No	None	0.022
In PO_4 buffer	8.0	No	None	0.046
In PO_4 buffer	9.0	No	None	0.23
In PO_4 buffer	7.0	Boiled	None	1.38
In PO_4 buffer	7.0	Boiled	SOD	0.38
In PO_4 buffer	7.0	Boiled	Catalase	0.30
In PO_4 buffer	7.0	Autoclaved	None	4.1
In PO_4 buffer	7.0	Autoclaved	SOD	2.1
In PO_4 buffer	7.0	Autoclaved	Catalase	1.5

[a] Not detectable indicates CO production of <0.2 nmol above that observed in control syringe.

production, and even greater CO release was observed with autoclaving. Autoclaving has been demonstrated to alter the structure of glucose [4], and the increased CO from the heated compound presumably indicates that the altered molecule was more susceptible to oxidant damage. The appreciable CO production observed with a commercial glucose solution used in patient care similarly suggests that this glucose had been altered by the sterilization process.

Production of CO by boiled glucose was totally eliminated when an argon atmosphere was used in place of air. The low, but measurable, CO production observed during anaerobic incubation of the non-biological compounds (Fig. 1) may reflect the inability to adequately deoxygenate these compounds because of their solid state or the involvement of different types of oxidative reactions. The addition of either superoxide dismutase or catalase to the glucose solution reduced the release of CO, suggesting that superoxide and hydrogen peroxide played roles in the CO release.

We conclude from the CO generation in the above studies that a wide array of compounds undergo spontaneous oxidation at ambient or physiological temperature. The biomedical literature contains only a few references to such CO release. Vreman et al. [5] reported that light exposure induced CO production from a variety of compounds (including glucose) in the presence of the photosensitizer, tin protoporphyrin. Wolff and Bidlack [6] reported that CO was released during lipid peroxidation. In addition, CO production at ambient temperature has been reported during the auto-oxidation of phenols [7] such as L-3,4-dihydroxyphenylalanine [8]. Lastly, we observed that mouse feces and urine produced CO via a non-enzymatic reaction [9].

In the biomedical literature, most references to CO production concern the release of this gas during the heme oxygenase catalyzed breakdown of heme to biliverdin. The finding that CO production rather accurately reflects heme breakdown [1,2] suggests that the non-specific CO production observed in the present study is inhibited by body antioxidants. The present study demonstrates that accurate assessment of the CO production resulting from heme turnover requires extreme care in order to exclude artifacts resulting from non-specific CO production by the materials employed in the collection of the gas samples.

We propose that CO production might serve as a novel indicator of oxidant damage. In our assay system, the production of 0.2 nmol of CO could be differentiated from background CO. In the case of glucose, this amount of CO reflected

the oxidation of about $1/10^7$ of the carbon molecules. With minor modifications of the assay system, the sensitivity of the technique could be enhanced by several orders of magnitude. In addition, the method is technically very simple. The low solubility of CO in heme-free liquids results in the quantitative accumulation of CO in the gas space over the solution. The untreated gas space is directly injected onto the GC column, and CO elutes in about 2 min with no interference from other gases. Thus, measurement of CO release could provide a simple, rapid and sensitive means of quantitatively assessing oxidative damage to a wide variety of compounds.

Acknowledgements

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