Communication

Sprayed Water Microdroplets Are Able to Generate Hydrogen Peroxide Spontaneously

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ABSTRACT: Ultrapure N_2 gas was bubbled through water, and the humidified output containing undetectable concentrations of ozone filled a closed chamber in which 18 MΩ-cm water was sprayed through a silica capillary to form microdroplets. Analysis of the collected microdroplets by NMR spectroscopy showed the presence of hydrogen peroxide at a concentration level ranging from 0.3 to 1.5 μM depending on the flow conditions. This was confirmed using a spectrofluorometric assay. We suggest that this finding establishes that when sprayed to form microdroplets, water has the ability to produce hydrogen peroxide by itself. When the N_2 gas is replaced by compressed air or O_2 gas, the concentration of hydrogen peroxide is found to increase, indicating that gas−surface interactions with O_2 in aqueous microdroplets promote the formation of hydrogen peroxide.

It has been reported that water microdroplets with diameters less than 20 μm contain hydrogen peroxide and that the H_2O_2 concentration increases as the microdroplet size is reduced to as small as 1 μm.[1] These observations were made with a pneumatic spray apparatus in which two coaxial capillaries are employed, the inner capillary containing water and the outer capillary containing compressed N_2, which serves as a nebulizing gas. The microdroplets were produced in an airtight chamber and collected below the spray nozzle. No voltage was applied to the microdroplets, and no catalyst was added to the water. Subsequent studies showed that the H_2O_2 produced in the water microdroplets that contained dissolved ketones or aldehydes could be used to promote the Dakin and Baeyer−Villager reactions.[2] It was also found that sprayed water microdroplets can act as a biocide.[3] It has been proposed that this might contribute to the seasonal variation of the transmission of viral respiratory infections.[4] A question was raised whether friction from the water flow might cause the appearance of the H_2O_2, but it was shown that H_2O_2 could also be generated by condensing water vapor from air on a cold surface.[5] Since then, Mishra and co-workers[6] reported that ozone can contribute to the observation of H_2O_2 in water microdroplets. However, they could not observe H_2O_2 in the absence of ozone, and they cast doubt on whether water microdroplets can form H_2O_2 spontaneously. The present study provides evidence that water microdroplets alone can generate H_2O_2. At an injection flow rate of 25 μL/min with a nebulizing gas of humidified ultrapure N_2 containing no detectable O_3, the concentration of H_2O_2 was determined by NMR spectroscopy[7,8] to be greater than 1 μM, which was confirmed using a spectrofluorometric assay.

Figure 1 presents a schematic of the experimental setup. Four different compressed gases were used to cause nebulization: ultrapure N_2 (99.999%); 2% O_2/98% N_2, compressed air, and pure O_2. All of the gases except compressed air were obtained from Praxair (San Ramon,
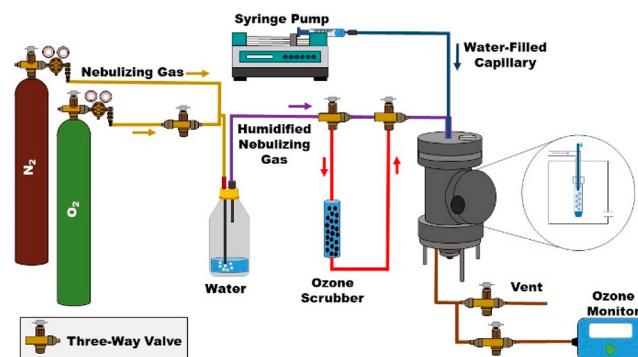

Figure 1. Aqueous microdroplet generation and collection apparatus.

CA). The gases were bubbled through water to remove possible impurities, such as O_3, before filling an airtight stainless steel chamber. An ozone scrubber (model CDU-30, Oxidation Technologies, LLC, Inwood, IA) that could be turned on or off was also added. The water was deionized ACS reagent grade, ASTM D1193 Type 1, having a resistivity of >18 MΩ-cm, a conductivity of <0.056 μS/cm, and a total organic carbon compound content of <50 ppb. All of the tubing was made of copper to prevent contamination. The water flowed through a borosilicate capillary with an inner diameter of 100 μm and an outside diameter of 350 μm. The amount of 700 μL of microdroplets was collected in a 50 mL Falcon tube, which was placed inside the airtight chamber that was operated at higher pressure than the outside atmosphere
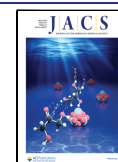
to prevent any outside contamination from flowing back into the chamber. The chamber was evacuated and filled three times with each gas before sample collection. The distance between the sprayer outlet and the bottom of the Falcon tube was about 10 cm. The ozone level of the gas leaving the chamber was monitored using an ozone monitor (model nanO3, Ohio Lumex Co., Inc., Cleveland, OH). The ozone concentration in the ultrapure N_2 was found to be at or below the detection limit of this device (3 ppb or less according to the manufacturer).

The sealed quartz NMR tube was taken a to Bruker 600 MHz Avance III NMR spectrometer equipped with a noncryogenic probe. It was operated in a manner closely following the procedure of Bax and co-workers.[7,8] The contents of the Falcon tube were transferred to 5 mm quartz NMR tubes. In a typical sample, a 2-N-(morpholino)-ethanesulfonic acid (MES) stock solution (60 μL, 50 mM in D_2O) was added to the analyte (540 μL), followed by adjustment of the pH to within ± 0.05 pH units of the desired value (6.06) using a glass electrode. The temperature was set at 2 °C. Data were typically acquired with an acquisition time of 0.01 s, a delay of 0.05 s between scans, 4000 points, and a spectral width of 20 kHz over 20 000 scans. Figure 2 shows a comparison of the NMR spectra between a water standard having a known H_2O_2 concentration (5 μM) and the collected microdroplets.

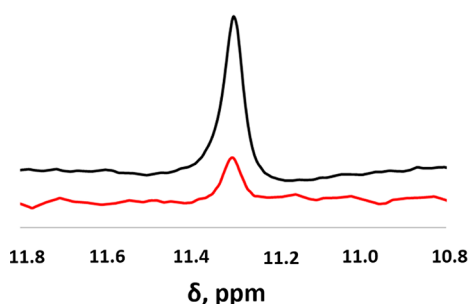**Figure 2.** NMR spectra of (black) 5 μM hydrogen peroxide and (red) water microdroplets nebulized by ultrapure humidified N_2 gas at an injection rate of 25 μL/min.

We also measured the presence of H_2O_2 using a spectrofluorometric assay (MAK165, Sigma-Aldrich, St. Louis, MO) that has a linear dynamic range of 0.1 to 10 μM. For this purpose, we added 50 μL of microdroplets (or standards) and 50 μL of the master mix (including peroxidase enzyme and red substrate in assay buffer) to each well of a 96-well fluorescence microplate and measured the fluorescence intensities (λ_{ex} = 540 nm and λ_{em} = 590 nm) using a fluorescence microplate reader. We tested and verified this procedure against our NMR measurements. We found that the concentration of H_2O_2 depended on the flow rate of water to form microdroplets (see Figure 3), which may influence the microdroplet size distribution. It also alters the collection time, which is expected to increase the apparent H_2O_2 concentration because the evaporation rate of H_2O is faster than that of H_2O_2. The results are presented in Figure 3, where the error bars represent one standard deviation. Clearly, H_2O_2 is being generated in water microdroplets in the absence of ozone.

We also investigated the effect of using different nebulizing gases and whether visible-light radiation affected the rate of H_2O_2 production. The ozone concentrations were measured
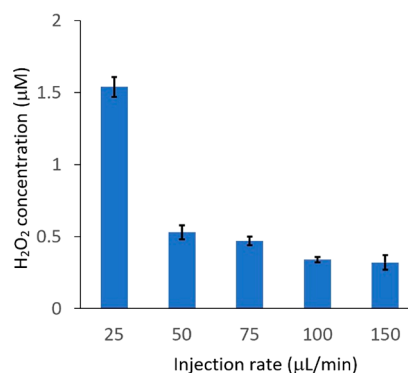

Figure 3. Dependence of the H_2O_2 concentration as a function of the flow rate of ultrapure N_2 nebulizing gas.

for different nebulizing gases with the ozone scrubber off. For all of the gases except compressed air, we found that the ozone concentration was close to the limit of detection. For compressed air, the ozone concentration was 3.2 ppb with the scrubber off and at or below the limit of detection with the scrubber on. With each gas at a flow rate of 100 psi, we found the H_2O_2 concentration to be 0.49 $\pm$ 0.05 μM for N_2, 0.69 $\pm$ 0.05 μM for 2% O_2/98% N_2, 1.12 $\pm$ 0.02 μM for compressed air (21% O_2/78% N_2), and 2.00 $\pm$ 0.05 μM for O_2. The factor of 4 increase in the H_2O_2 concentration in going from N_2 to O_2 suggests that adsorption of O_2 at the gas−water interface of the aqueous microdroplets markedly enhances H_2O_2 production. This finding corrects what was reported in ref 1. The H_2O_2 concentration was also measured with the room lights on or off. Within the uncertainty of our measurements, the concentration values were the same, indicating that H_2O_2 production is not driven by ambient radiation.

In many previous studies, the quantitative detection of trace amounts of H_2O_2 has been based on recording the fluorescence or colorimetric intensity of various compounds exposed to the solution containing hydrogen peroxide. However, these detection schemes can suffer from chemical interference or incomplete reactions. NMR detection allows direct, interference-free, quantitative measurements of H_2O_2 down to submicromolar levels.[7,8] In the study by Mishra and co-workers[6] using fluorometric detection with an assay whose limit of detection was 250 nM, the H_2O_2 concentration was demonstrated to increase from 2 to about 5 μM in a microdroplet spray as the ozone concentration was increased from 10 ppb to about 10 ppm. This is a remarkable finding and shows the importance of ozone in the formation of H_2O_2 in water microdroplets. The statement was also made that in the absence of ozone gas, H_2O_2 does not form in water microdroplets. We reach an opposite conclusion, as we measured the formation of H_2O_2 at a lower O_3 concentration level than was reported for their ozone study. Indeed, at the lowest O_3 concentration reported by Mishra and co-workers[6] (~10 ppb), they observed that the H_2O_2 concentration increased compared with the next lowest concentration of O_3 (~17 ppb), but no explanation was offered for that observation.

It is certainly to be wondered how water microdroplets themselves can generate H_2O_2. Nebulization of water leads to charge separation and the production of negatively and positively charged microdroplets from contact electrification at the water−air and water−wall interfaces. Contact electrification may account for all or part of the H_2O_2 formed

from condensed water vapor on a cold surface.[5] The surface charges of microdroplets induce an electric double layer (EDL)[9−11] and cause microdroplets to act as electrochemical cells.[12] In this sense, the anode is the negatively charged periphery of the water microdroplet, and the cathode is the positively charged species in the interior of the water microdroplet. The existence of hydroxyl radicals ($\cdot$OH) in water microdroplets was demonstrated previously.[13] It seems that the $\cdot$OH must come from the loss of an electron from the hydroxide anion (OH^-). We suggest that H_2O_2 arises from recombination $\cdot$OH at the water microdroplet periphery. The standard reduction potential of $\cdot OH/OH^-$ appears to lie between 2.10 V at pH 0 and 1.25 V at pH 14.[14] If we assume that this potential acts over 5 Å, this corresponds to an electric field of $2-4 \times 10^7$ V/cm. Recently, Head-Gordon and co-workers[15] calculated that the electric field alignments along free O−H bonds at the air−water interface are $\sim 1.6 \times 10^7$ V/cm larger on average than that found for O−H bonds in the interior of the water microdroplets. This estimate is in good agreement with the measured electric field strength of a water microdroplet in oil.[16] It seems then that the electric field at the gas−water interface is sufficiently strong that it can assist OH^- to release its electron. It also seems likely that the $\cdot OH/OH^-$ reduction potential is lowered as a result of OH^- crowding at the gas−water interface.[17] Where the electron released from OH^- goes remains an unsettled question at present. It is probable that the electron released in "spontaneous" oxidation of OH^- anions would be neutralized by the net positive charges carried by a subpopulation of microdroplets. Some other possibilities are impurities at the gas−water interface, charging of the walls surrounding the water flow, and electron solvation.

The electron may well attach to O_2 molecules incident on the water microdroplet surface to form superoxide anion (O_2^-), which, again, is on its way to forming H_2O_2 by more than one possible reaction. This mechanism might account for the large increase in H_2O_2 concentration when the N_2 nebulizing gas is replaced by air (which contains 21% O_2) or pure O_2. Many mysteries about the chemical characteristics of water microdroplets remain to be explored and understood, but it does seem clear that water microdroplets can exhibit remarkable reduction powers, such as the reduction of doubly charged ethyl viologen (EV^{+2}) to EV^{+1}[18] and the recent report of the formation of pyridine anions and $\cdot$OH when a solution of pyridine dissolved in water was sprayed without the application of any external electric field.[19]

In conclusion, ozone in contact with water microdroplets is an important way of generating H_2O_2, but water microdroplets are capable of generating H_2O_2 without the presence of ozone.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Lee, J. K.; Walker, K. L.; Han, H. S.; Kang, J.; Prinz, F. B.; Waymouth, R. M.; Nam, H. G.; Zare, R. N. Spontaneous Generation of Hydrogen Peroxide from Aqueous Microdroplets. Proc. Natl. Acad. Sci. U. S. A. 2019, 116 (39), 19294−19298.

(2) Gao, D.; Jin, F.; Lee, J. K.; Zare, R. N. Aqueous Microdroplets Containing Only Ketones or Aldehydes Undergo Dakin and Baeyer−Villiger Reactions. Chem. Sci. 2019, 10 (48), 10974−10978.

(3) Dulay, M. T.; Lee, J. K.; Mody, A. C.; Narasimhan, R.; Monack, D. M.; Zare, R. N. Spraying Small Water Droplets Acts as a Bacteriocide. QRB Discovery 2020, 1, No. e3.

(4) Davidse, A.; Zare, R. N. Effect of Relative Humidity in Air on the Transmission of Respiratory Viruses. Mol. Front. J. 2021, 05 (01n02), 5−16.

(5) Lee, J. K.; Han, H. S.; Chaikasetsin, S.; Marron, D. P.; Waymouth, R. M.; Prinz, F. B.; Zare, R. N. Condensing Water Vapor to Droplets Generates Hydrogen Peroxide. Proc. Natl. Acad. Sci. U. S. A. 2020, 117 (49), 30934−30941.

(6) Gallo, A., Jr.; Musskopf, N. H.; Liu, X.; Yang, Z.; Petry, J.; Zhang, P.; Thoroddsen, S.; Im, H.; Mishra, H. On the Formation of Hydrogen Peroxide in Water Microdroplets. Chem. Sci. 2022, 13 (9), 2574−2583.

(7) Kakeshpour, T.; Bax, A. NMR Characterization of H_2O_2 Hydrogen Exchange. J. Magn. Reson. 2021, 333, 107092.

(8) Kakeshpour, T.; Metaferia, B.; Zare, R. N.; Bax, A. Quantitative Detection of Hydrogen Peroxide in Rain, Air, Exhaled Breath, and Biological Fluids by NMR Spectroscopy. Proc. Natl. Acad. Sci. U. S. A. 2022, 119 (8), e2121542119.

(9) Zilch, L. W.; Maze, J. T.; Smith, J. W.; Ewing, G. E.; Jarrold, M. F. Charge Separation in the Aerodynamic Breakup of Micrometer-Sized Water Droplets. J. Phys. Chem. A 2008, 112 (51), 13352−13363.

(10) Lin, S.; Xu, L.; Wang, A. C.; Wang, Z. L. Quantifying Electron-Transfer in Liquid-Solid Contact Electrification and the Formation of Electric Double-Layer. Nat. Commun. 2020, 11 (1), No. 399.

(11) Lin, S.; Chen, X.; Wang, Z. L. Contact Electrification at the Liquid−Solid Interface. Chem. Rev. 2022, 122 (5), 5209−5232.

(12) Chamberlayne, C. F.; Zare, R. N. Microdroplets Can Act as Electrochemical Cells. J. Chem. Phys. 2022, 156 (5), 054705.

(13) Lee, J. K.; Samanta, D.; Nam, H. G.; Zare, R. N. Micrometer-Sized Water Droplets Induce Spontaneous Reduction. J. Am. Chem. Soc. 2019, 141 (27), 10585−10589.

(14) Stein, G. Standard Electrode Potential of the OH−Ion. Correlation with Absorption Spectrum and Energy of Solvation. *J. Chem. Phys.* **1965**, *42* (8), 2986−2986.
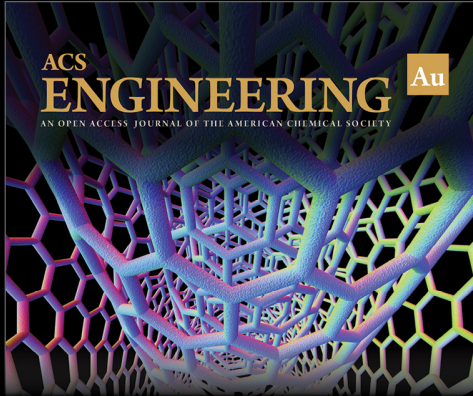
(15) Hao, H.; Leven, I.; Head-Gordon, T. Can Electric Fields Drive Chemistry for an Aqueous Microdroplet? *Nat. Commun.* **2022**, *13* (1), 280.

(16) Xiong, H.; Lee, J. K.; Zare, R. N.; Min, W. Strong Electric Field Observed at the Interface of Aqueous Microdroplets. *J. Phys. Chem. Lett.* **2020**, *11* (17), 7423−7428.

(17) Vogel, Y. B.; Evans, C. W.; Belotti, M.; Xu, L.; Russell, I. C.; Yu, L.-J.; Fung, A. K. K.; Hill, N. S.; Darwish, N.; Gonçales, V. R.; Coote, M. L.; Swaminathan Iyer, K.; Ciampi, S. The Corona of a Surface Bubble Promotes Electrochemical Reactions. *Nat. Commun.* **2020**, *11* (1), 6323.

(18) Gong, C.; Li, D.; Li, X.; Zhang, D.; Xing, D.; Zhao, L.; Yuan, X.; Zhang, X. Spontaneous Reduction-Induced Degradation of Viologen Compounds in Water Microdroplets and Its Inhibition by Host−Guest Complexation. *J. Am. Chem. Soc.* **2022**, *144* (8), 3510−3516.

(19) Zhao, L.; Song, X.; Gong, C.; Zhang, D.; Wang, R.; Zare, R. N.; Zhang, X. Sprayed Water Microdroplets Containing Dissolved Pyridine Spontaneously Generate Pyridyl Anions. *Proc. Natl. Acad. Sci. U. S. A.* **2022**, *119* (12), e2200991119.