Rapid compressions in a captive bubble apparatus are isothermal

Wenfei Yan and Stephen B. Hall

Departments of Biochemistry and Molecular Biology, Medicine, and Physiology and Pharmacology, Oregon Health & Science University, Portland, Oregon 97239

Submitted 9 June 2003; accepted in final form 13 July 2003

Yan, Wenfei, and Stephen B. Hall. Rapid compressions in a captive bubble apparatus are isothermal. J Appl Physiol 95: 1896–1900, 2003. First published July 18, 2003; 10.1152/japplphysiol.00591.2003.—Captive bubbles are commonly used to determine how interfacial films of pulmonary surfactant respond to changes in surface area, achieved by varying hydrostatic pressure. Although assumed to be isothermal, the gas phase temperature \( T_g \) would increase by >100°C during compression from 1 to 3 atm if the process were adiabatic. To determine the actual change in temperature, we monitored pressure \( P \) and volume \( V \) during compressions lasting <1 s for bubbles with and without interfacial films and used \( P \cdot V \) to evaluate \( T_g \). \( P \cdot V \) fell during and after the rapid compressions, consistent with reductions in \( n \), the moles of gas phase molecules, because of increasing solubility in the subphase at higher \( P \). As expected for a process with first-order kinetics, during 1 h after the rapid compression \( P \cdot V \) decreased along a simple exponential curve. The temperature of the gas phase is not strictly known (6). The temperature of the subphase is routinely monitored and controlled, but the assumption that the gas and liquid phases are always isothermal remains untested. If compression of a bubble were adiabatic, without heat transfer across the interface, the temperature of the gas phase during an immediate increase in hydrostatic pressure from one to three atmospheres would rise by >100°C. For compressions of small bubbles at rates comparable to those on Langmuir troughs, which occur over several minutes, the assumption of isothermal conditions seems quite reasonable. Uncertainty increases with faster rates. Physiological compressions occur over a few seconds, and the unexpected behavior of films compressed at these and faster rates raises particular concern. Phospholipid monolayers in the fluid liquid-expanded phase, which collapse at the equilibrium spreading pressure of ~46 mN/m when compressed slowly, become kinetically trapped in a metastable structure if compressed fast enough to reach higher surface pressures (2, 18). Although an increase in temperature during a fast compression would seem likely to make a film more fluid, and therefore more prone to collapse, the extent to which temperature is unknown in these experiments complicates attempts to explain the unexpected metastability.

During a compression at the relatively low pressures used in standard experiments, a bubble should obey the ideal gas law. To determine the extent to which the bubble’s gas-phase temperature \( T_g \) deviated from its baseline value, we therefore monitored pressure \( P \) and volume \( V \) simultaneously during compressions lasting <1 s, beginning with the assumption that gas in the bubble would remain constant so that \( P \cdot V \) would be proportional to \( T_g \).

MATERIALS AND METHODS

Materials. Our experiments used ambient air and the following purified gases to form bubbles: helium (99.997% pure according to the supplier’s specifications; Byrne Specialty Gases, Seattle, WA), nitrogen (99.998%), and oxygen (99.997%). 1-Palmitoyl-2-oleoyl-phosphatidylcholine (POPC) was obtained from Avanti Polar Lipids (Alabaster, AL) and used without further purification or characterization. Agarose obtained from Sigma Chemical (St. Louis, MO) was adsorbed on the bubble’s continuous surface (7). Because the films are spread or adsorbed on the bubble’s continuous surface (17), they are isolated from the environment, and contamination that complicates longer experiments on troughs (10) is less likely. Saturated humidification of the gas phase results from the experimental configuration. Confine- ment of films by the continuity of the interface eliminates the need for artificial barriers. For studies at the high surface pressures that are of particular interest for pulmonary surfactant, the absence of these barriers represents a major advantage (17). The leakage or creep of films along or around artificial barriers (5) and the heterogeneous nucleation of collapse that may occur at these barriers are eliminated. For these several reasons, captive bubbles have achieved common use in studies of pulmonary surfactant (e.g., 2, 4, 11, 13, 15, 17, 19, 20).

Address for reprint requests and other correspondence: S. B. Hall, Molecular Medicine, M/C NRC-3, OHSU, Portland, OR 97239-3098 (E-mail: sbh@ohsu.edu).

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purified by extraction with organic solvent (1). High-purity chloroform and methanol (Burdick and Jackson, Muskegon, MI) were used as the spreading solvents for forming films of POPC. Water was distilled and then filtered through Macropure, Ultrapure DI, and Organic Free Cartridges from Barnstead Thermolyne (Dubuque, IA). The following reagents were purchased commercially and used without further purification or analysis: HEPES (GIBCO-BRL, Life Technologies, Grand Island, NY); CaCl₂•2H₂O (Baker, Phillipsburg, NJ); and NaCl (Mallinckrodt Specialty Chemicals, Paris, KY). All experiments used a subphase containing 10 mM HEPES, pH 7.0, 150 mM NaCl, and 1.5 mM CaCl₂ (HSC).

**Methods.** These studies used a pressure-driven captive bubble apparatus described previously (2, 14). The instrument measures the shape of an axisymmetric air bubble floating below an agarose ceiling during changes in hydrostatic pressure applied to the liquid subphase. The height and width of the bubble provide the basis for calculating its volume (9, 16). Applied hydrostatic pressure was provided by manually opening a valve as quickly as possible to a source of compressed gas (helium, nitrogen, or oxygen) at pressures up to 3.5 atm. Infusion through a custom-built needle with a closed tip and two orifices facing the sides of the chamber minimized turbulence and movement of the bubble. A needle valve in a fluid-filled line between the gas tank and the bubble chamber provided control of the rate at which pressure increased. Charge-coupled device (CCD) cameras monitored the profile of the bubble in the vertical axis to ensure the axisymmetric shape required for the analysis and in the horizontal axis to measure its height and width. Temperature of the subphase was monitored with a thermometer probe (YSI, Yellow Springs, OH) and regulated by a temperature controller (Cole-Palmer, Vernon Hills, IL) and heating pads (Minco, Minneapolis, MN) applied along the sides of the chamber. Measurements were recorded using programs constructed in LabView (National Instruments, Austin, TX).

Images acquired by CCD camera (Pulnix America, Mountain View, CA) were converted to digital format by frame grabber (PCI-1408, National Instruments) and transferred directly to memory in real time at the 30 Hz rate of the camera. Images were subsequently analyzed and volumes calculated from the dimensions of the bubble (9, 16). Hydrostatic pressures were measured by a transducer (Validyne, Northridge, CA) connected by a minimum length of a liquid-filled noncompliant tubing to the bubble chamber. The output of the pressure transducer was recorded via an analog-to-digital converter (PCI-1200, National Instruments) to computer memory.

The experiments required measurements of P and V as close to simultaneous as possible. Calculations of theoretical compressions for bubbles that strictly obey Boyle’s law showed that even small shifts in the time of the two measurements would produce large artifactual changes in P·V if they were incorrectly assumed to be coincident. To meet the rigorous requirements for measurements in real time, we used a separate computer to record each variable. Completion of each image on the framegrabber triggered a signal to the analog-to-digital converter on the second computer, which then recorded a reading from the pressure transducer. Each image, recorded every 33 ms, was therefore followed after ~10 μs by a single measurement of pressure.

We first used a bubble with a clean air/water interface to establish the behavior of the simplest system. We then determined whether the presence of spread films altered the basic behavior. Small aliquots, in the range of 0.10 μl, of POPC in chloroform:methanol (1:1) were deposited at the air/water interface to initial surface pressures <40 mN/m. Spreading solvent was removed by exhaustive exchange of the subphase while surface pressure was maintained at ~43 mN/m (3). Experiments on the captive bubble closely replicate isotherms previously established on Langmuir troughs for both dipalmitoyl phosphatidylcholine (3) and palmitic acid. Variation of the data is in all cases expressed as mean ± SD.

**RESULTS**

Measurements of pressure and volume during rapid compression of an air bubble with a clean air-water interface showed continuous changes over the course of ~0.5 s for each variable (Fig. 1). The data allowed precise estimates of the temperature change expected if the process were adiabatic. For an irreversible compression that occurred with no exchange of heat, the change in ΔU would be given by

\[ \Delta U = -W = \int_{V_1}^{V_2} P \cdot dV = n \cdot \bar{C}_v \cdot \Delta T \]

where W is the work done on the bubble, V₁ and V₂ are the initial and final volumes, n is for moles of gas, molar heat capacity Cᵥ at constant volume, and ΔT is a change in temperature (12). A common approximation for calculating the temperature change during such a compression is to assume that pressure increases instantaneously to its final value. For the actual compression, during which pressure changed at a finite rate, numerical integration of the measured pressure expressed relative to volume allowed calculation of the increase in temperature. For the compression in Fig. 1, for example, using the diatomic heat capacity to estimate Cᵥ for air, integration of the data showed that temperature would increase for an adiabatic process from 295.7 to 421.5°K.

During the rapid compression, however, the product P·V actually fell (Fig. 1). Because for an ideal gas,
concluded that the kinetics of mass transfer, which had little effect on the change in $P_{V}$ phase with dissolved gas before the experiment also the solubility constant (Fig. 3). Saturation of the subcourse of the rapid compression correlated poorly with $P_{V}$, but inconsistent with a decreased $T_g$. If mass transfer occurred at a finite rate, further equilibration of gas between the two phases after the compression would cause $n$ and $P_{V}$ to continue falling. In contrast, if the compression somehow cooled the bubble, subsequent equilibration of the two phases would cause $T_g$ and $P_{V}$ to rise. The magnitude of the initial change in $P_{V}$ was consistent with the known solubility, expressed by Henry's law, of air in water at the higher pressure. Given sufficient time, 60% of a 160-$\mu$l air bubble would dissolve in the ~2.5-ml subphase. In all respects, a decrease in $n$ reasonably explained the fall in $P_{V}$ observed during the rapid compression.

Temperature might nonetheless increase, although to a lesser extent than the opposing decrease in $n$. The behavior of temperature could be evaluated most easily if the change in dissolved gas could be eliminated. Experiments using gases with different solubilities, however, showed that the decrease in $P_{V}$ over the course of the rapid compression correlated poorly with the solubility constant (Fig. 3). Saturation of the subphase with dissolved gas before the experiment also had little effect on the change in $P_{V}$ (Fig. 3). We concluded that the kinetics of mass transfer, which would be distinct from the equilibrium solubility, dominated the initial change in $n$.

We instead determined the variation of $n$ as a function of time ($t$), and then used that information to estimate $T_g$. To a first approximation, gas should dissolve in the subphase according to first-order kinetics. $n$ and $P_{V}$ when $T_g$ is constant should therefore follow a simple exponential function of $t$. Measurements of $P_{V}$ generally fit well to exponential curves (Fig. 2). We assumed that after the rapid compression, the temperature of the two phases would equilibrate in <10 min. The fitted equations $[P_{V}(t)] = [P_{V}]_0 e^{-kt}$, obtained during measurements between 10 and 60 min after the compression, therefore were used to describe $P_{V}$ at the constant temperature of the subphase ($T_s$). The expression

$$n(t) = \frac{[P_{V}]_0 e^{-kt}}{RT_s}$$

provided the variation of $n$ in the bubble at all times, independent of the actual $T_g$. These expressions for $n(t)$ then allowed determination of the actual $T_g$ according to

$$T_g = \frac{P_{V}}{n(t)R}$$

Because processes of adiabatic heating and heat transfer might have different kinetics, temperature might exceed its final value during the course of a compression. We therefore measured $P_{V}$ after partial compressions to intermediate pressures as well as after the full compression to >3 atm. Values of $\Delta T = T_g - T_s$ differed from 0 by <2°C for 2.5 atm and by ~1°C at 1.8 and 3.1 atm (Fig. 4).
These experiments using bubbles with clean surfaces left open the possibility that the presence of an interfacial film would alter the results. Surface films slow mass transfer across air/water interfaces (8), and they might also change rates of heat transfer. Rapid compression, however, produced a decrease in P/V for bubbles coated with a monomolecular film of POPC (10.4 ± 0.7%) that was slightly larger than for bubbles with clean surfaces (9.5 ± 0.4%) (Fig. 5). At the low surface tensions produced by the compressed films, the shape of the bubbles was much flatter and the surface area significantly larger. These differences might have produced more rapid equilibration of gas across the interface, and the slightly larger initial decreases in n and P-V. The films in these experiments reached surface pressures well above the equilibrium spreading pressure of ~46 mN/m but sustained these surface pressures for prolonged periods, demonstrating their relative metastability. The presence of the POPC films therefore had only a minor effect on transfer of either mass or heat from the captive bubble, and transformation of the fluid films to a metastable form occurred with minimal change in temperature.

**DISCUSSION**

These studies used the variation of P·V to monitor temperature in captive bubbles during compressions that are fast relative to rates available on Langmuir troughs. This approach for determining the bubble’s temperature provided significant advantages over two alternatives. Temperature probes for the gas phase have response times >2 s that are unacceptably slow for the processes considered here. Calculations of heat transfer to provide estimates of Tg involve uncertainties because of convection and changes in the bubble’s shape that are unacceptably large. Although complicated by changes in n at different pressures, measurements of P and V, both of which are accessible at appropriate rates, provided the most reasonable approach for determining Tg.

Our results show that for bubbles with and without interfacial films compressed from 1 to >3 atm at the fastest rates that we could achieve, the product n·Tg decreases. This finding makes it most unlikely that the compression is strictly adiabatic. Temperature would increase by ~45% during such a process, requiring that n would fall by >70% to achieve the observed reduction in n·Tg. This change in n exceeds the expected increase in dissolved gas at the higher pressure. The drop in n·Tg therefore effectively rules out the full increase in Tg expected for an adiabatic process.

Heat exchange could still be incomplete. Temperature would increase, although less than for an adiabatic compression and by a smaller factor than the

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![Fig. 4. Estimated difference between the temperature in the two phases during the initial 10 min after rapid compressions. Change in temperature (ΔT) = Tg - Ts, where Tg, the subphase temperature, was measured, and the gas phase temperature (Tg) in the bubble was obtained from Tg = P-V/n(t)-R. n(t) is estimated from back extrapolation of the curves fit at t > 10 min to P-V versus t (Fig. 2). Data are the means of at least three experiments in each case. Symbols represent the means ± SD at specific times.](image)

**Fig. 4.** Rapid compression of an air bubble coated with a monomolecular film of 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC).

A: characteristics of the bubble during the rapid compression. Split time scale shows the intervals before, during, and after the rapid compression. B: characteristics of the interfacial film during and after the rapid compression.

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![Fig. 5. Estimated difference between the temperature in the two phases during the initial 10 min after rapid compressions. Change in temperature (ΔT) = Tg - Ts, where Tg, the subphase temperature, was measured, and the gas phase temperature (Tg) in the bubble was obtained from Tg = P-V/n(t)-R. n(t) is estimated from back extrapolation of the curves fit at t > 10 min to P-V versus t (Fig. 2). Data are the means of at least three experiments in each case. Symbols represent the means ± SD at specific times.](image)
decrease in n, explaining the fall in n-T_g. Our estimates of n place an upper limit on the change in temperature of 2°C (Fig. 4). This value represents a conservative estimate. Given the minimal change in temperature, the two phases are likely to equilibrate in much <10 min. If the exponential curves used to estimate n(t) are fit to a broader range of P-V measurements that begin sooner after completing the rapid compression, ΔT falls. With the use of the full set of P-V data for 2.5 atm, for instance, the estimated changes in temperature are ~0.6°C less than the values shown. For most applications of the captive bubble apparatus to studies concerning pulmonary surfactant, changes in temperature of 2°C would have marginal importance. Because the actual changes are likely to be less, we consider any increases in temperature during compression to be insignificant.

Our findings of course are subject to several assumptions and approximations. Some of these are unimportant. Differences between the behavior of real gases, expressed by Van der Waals’ equation, and ideal gases are small, ~0.2%, at the pressures considered here, so that the use of the ideal gas equation introduces minimal error. Interfacial tension, which causes an increase in hydrostatic pressure in the gas relative to the subphase that is ignored by our calculations, would for a spherical bubble of 160 μl with a clean surface cause a change of <0.0005 atm. Although we have not formally considered the condensation of water vapor to liquid at the higher pressure, the thermal effects of that process and the compression itself would be additive and so included in our estimates of temperature. The use of the horizontal profile to calculate V during the rapid compression assumes that the bubble is axisymmetric. Although the vertical profile is not monitored during these fast changes, the values of V obtained at the end of compressions, when we could show that the bubble is axisymmetric, to different intermediate pressures agree with values obtained at different P during a continuous compression. Perhaps the most important assumption is that the dissolution of gas in the subphase at the end of the fast compression follows first-order kinetics. Given the close fit of the data to the predicted behavior, this assumption seems at least reasonable.

Our results are limited to the rates used in our experiments. At some point, faster compressions presumably will produce significant deviations from isothermal conditions. For rates that are pertinent for pulmonary physiology, however, and that are sufficient to transform the fluid films containing extracted calf surfactant (2) or individual phosphatidylcholines (2, 18) to metastable structures, previously expressed concern that compressions might be adiabatic (6) appear unfounded.

The authors thank Ted G. Laderas for initial efforts at real-time programming.

DISCLOSURES

These studies were supported by funds from the American Lung Association of Oregon and National Heart, Lung, and Blood Institute Grant HL-60914.

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