THERMODYNAMIC PROPERTIES OF PURPLE MEMBRANE

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ABSTRACT We measured the density, expansivity, specific heat at constant pressure, and sound velocity of suspensions of purple membrane from Halobacterium halobium and their constituent buffers. From these quantities we calculated the apparent values for the density, expansivity, adiabatic compressibility, isothermal compressibility, specific heat at constant pressure, and specific heat at constant volume for the purple membrane. These results are discussed with respect to previously reported measurements on globular proteins and lipids. Our data suggest a simple additive model in which the protein and lipid molecules expand and compress independently of each other. However, this simple model seems to fail to describe the specific heat data. Our compressibility data suggest that bacteriorhodopsin in native purple membrane binds less water than many globular proteins in neutral aqueous solution, a finding consistent with the lipid surround of bacteriorhodopsin in purple membrane.

INTRODUCTION

Purple membrane functions as a light-driven proton pump in Halobacterium halobium (1). The membrane’s sole protein constituent is bacteriorhodopsin, and the pump involves the protein in a cycle that is initiated by light. This paper is a report of thermodynamic properties of the native membrane. The data reported in this paper represent the first comprehensive thermodynamic description of any native biological membrane. In addition, our data may be useful in interpreting purple membrane experiments in which temperature and/or pressure are varied.

METHODS AND MATERIALS

Purple membrane samples were obtained from several laboratories (see acknowledgements). A given sample was suspended in phosphate buffer (50 mM) at pH = 6.9, centrifuged at 100,000g for 30 min, and resuspended in fresh buffer by immersion sonication for 30 s while the sample was kept under nitrogen gas. We followed the purification procedure of Braiman and Mathies (2) by repeating the sonication and the centrifugation procedure at least two and usually three times. The final resuspension of the sample in the buffer was done so that a few milliliters of a 1–2% suspension of purified purple membrane was produced. This suspension was degassed under partial vacuum just before use.

For a purple membrane suspension made up only of purple membrane fragments and buffer, the suspension density, \( \rho \), can be expressed in terms of the apparent membrane density, \( \rho_{\text{mem}} \), the buffer density, \( \rho_{b} \), and the concentration, \( V_{\text{mem}} \):

\[
\rho = \rho_{\text{mem}} V_{\text{mem}} + (1 - V_{\text{mem}}) \rho_{b},
\]

where \( V_{\text{mem}} \) is the volume fraction of membrane in suspension.

Thermal Expansion Measurement

Differentiation of Eq. 1 with respect to temperature (T) yields an equation relating the expansivities of the suspension (\( \alpha \)), buffer (\( \alpha_{b} \)), and membrane (\( \alpha_{\text{mem}} \)):

\[
\alpha - \alpha_{b} = V_{\text{mem}}(\alpha_{\text{mem}} - \alpha_{b}),
\]

where \( \alpha = (-V \ln \rho / V T) \).

Measurement of the expansivity of the liquids was done in a vibrating quartz pycnometer built by two of us (C. J. Hardy and E. Gratton). We used distilled water and 1% NaCl for calibration. At any temperature between 5° and 30°C, we found that the linear relation, \( \Delta \rho = K \Delta T \), between density (\( \rho \)) and oscillation period (\( P \)) gave the same result as when we used the strictly proper Hooke’s Law relation (3), \( \Delta(P) = K' \Delta \rho \). The constant, \( K = (4.12 \pm 0.04) \times 10^{-6} \text{ g/ml \cdot ns} \), did not vary over the stated temperature range. All concentrations were measured by a dry weight method that is described later.

To measure the expansivities of the liquids, we used the following technique. After the temperature of the filled pycnometer is allowed to stabilize for 4 h, the temperature, measured as the resistance across a calibrated platinum thermometer, and the period of oscillation for a single liquid, such as water, are first measured at a low temperature (~10°C). The temperature is then reset on the temperature controller to a higher temperature (~20°C) and the system is allowed to reequilibrate for 4 h. The resistance and period are now measured at the higher temperature. These pairs of measurements are repeated several times to signal average the measurement. From these data the slope of the period vs. resistance line, along with its standard error, is calculated. Similarly, the slopes for

\[\text{Here we provide justification for the use of straight line fits to period vs. temperature data sets: A priori the plots do not need to be linear. However, in developing a differential method for measuring the mean thermal expansivity of liquids, we noticed that plots of temperature (measured in ohms with a platinum thermometer) vs. period (of oscilla-}

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the buffer solution and for the purple membrane suspension are determined. \( \frac{\Delta P}{\Delta T} \) for the suspension and for the water solution are determined by comparing the buffer solution with the water solution, as follows:

\[
K \left[ \frac{\Delta P \text{(buffer)}}{\Delta T} - \frac{\Delta P \text{(water)}}{\Delta T} \right] = \frac{\Delta \rho}{\Delta T} \text{(buffer)} - \frac{\Delta \rho}{\Delta T} \text{(water)}.
\]

\( \Delta \rho \) (water) can be obtained from standard tables, so \( \Delta \rho \) (buffer) is determined. Similarly, the suspension’s \( \frac{\Delta \rho}{\Delta T} \) can be determined by comparing the suspension slope with the buffer slope or water slope.

The volume fraction of purple membrane in the suspension is determined by the weight fraction and pycnometrically measured values for the apparent density of the purple membrane (\( \rho_{\text{mem}} \)) and the suspension density (\( \rho_m \)) as follows:

\[
V_{\text{mem}} = \frac{\rho_{\text{mem}}}{\rho_m} \left[ \frac{C_1 - C_2}{1 - C_2} \right],
\]

where

- \( C_1 = \frac{\text{grams (membrane + buffer salts)}}{\text{grams suspension}} \)
- \( C_2 = \frac{\text{grams (buffer salts)}}{\text{grams (buffer solution)}} \)

Thermostated water flow through a fanned radiator gave a pycnometer temperature stability of \( >0.02^\circ\text{K} \). Typical temperature excursions during any particular period of measurement were \( <0.01^\circ\text{K} \).

To check our differential expansivity measurement, we also measured the apparent specific volume of purple membrane at 15°C and 25°C. The measurements were performed with a Mettler-Paradimter (Mettler Instrument Corp., Hightstown, NJ) in which a reference pycnometer is always filled with double-distilled water. Temperature fluctuations, which can be a large source of error in this type of measurement, are effectively filtered out of the data coming from the sample pycnometer, resulting in greater accuracy than that obtained from the sample pycnometer alone. The calibration and sample fluids were always introduced into the densimeter, and their periods measured, in the following order: N\(_2\) gas, double-distilled water, buffer, purple membrane suspension, buffer, double-distilled water, and N\(_2\) gas. N\(_2\) gas and double-distilled water were the same calibration fluids that were used to determine the instrumental constants that relate the period of oscillation of the sample pycnometer to the density of the sample (3). After obtaining the density data for the buffer and the purple membrane suspension, the weight fraction of the sample that is attributed to purple membrane was determined by a dry weight method: two weighed samples (each of buffer and suspension) were dried at 40°C, slowly under oil pump vacuum, until the dried samples had constant weight.

The measurements of the apparent specific volumes were made at 15°C and 25°C at two concentrations (~0.025 and 0.015, expressed as weight fractions). The latter sample was prepared by diluting a portion of the former sample with buffer. The reader is referred to reference 3 for further details concerning the calculations and error analysis for apparent specific volumes from liquid density data and dry weight data.

**RESULTS**

**Thermal Expansivity**

Table I gives the expansivity data from both the slope and the specific volume methods. Note that the values for the apparent specific volume near 15°C, as determined from suspensions with different concentrations of purple mem-

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568 BIOPHYSICAL JOURNAL VOLUME 46 1984
bran, differ by >1%. This error is mostly caused by the error in the weight fraction determination, which is ~1%. However, this concentration error contributes little to the error in the expansivity because the exact same weight fraction is used in the calculation of the apparent specific volumes at the higher and lower temperatures. The error in the expansivity is almost entirely a result of systematic and random errors in the period data from the pycnometer. The apparent expansivity of purple membrane from the most concentrated sample is $6.29 \times 10^{-4}$/°K. The value we obtain from the diluted sample is $5.62 \times 10^{-4}$/°K, with a weighted average of $6.04 \times 10^{-4}$/°K, which agrees with our differential (slope) measurement value of $(6.05 \pm 0.82) \times 10^{-4}$/°K. We obtained an expansivity of $5.75 \times 10^{-4}$/°K, for bleached membrane. For comparison, Table II gives the values for the expansivity of some elements, proteins, and compounds.

**Adiabatic Compressibility**

A value for $\beta_{mem}^s/\beta_{water}^s$ of $0.506 \pm 0.034$ was obtained for the purple membrane at 25°C. Within the uncertainty, the value was constant over the frequency range 600 KHz–10 MHz. Using a value for $\beta_{water}^s$ of $4.55 \times 10^{-5}$ atm (8), for the membrane we obtain $\beta_{mem}^s = (2.30 \pm 0.15) \times 10^{-5}$/atm. For comparison, Table III gives $\beta^s$ values of other materials.

**Apparent Specific Heat at Constant Pressure**

The values obtained for the apparent specific heat of the purple membrane are 0.484, 0.504, and 0.500 cal/°K · g.

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**Table I**

**EXPANSIVITY DATA**

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>A. Slope data (as in Eq. 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\chi_\Omega$</td>
<td>$V_{mem} = 0.0216$</td>
</tr>
<tr>
<td>water: 826.8 ± 1.2</td>
<td>$C_1 = (7.988 \pm 0.001) \times 10^{-3}$</td>
</tr>
<tr>
<td>buffer: 837.2 ± 0.2</td>
<td>$C_2 = (3.3280 \pm 0.003) \times 10^{-2}$</td>
</tr>
<tr>
<td>suspension: 843.5 ± 1.4</td>
<td>$\sigma_{mem} = (6.05 \pm 0.82) \times 10^{-4}$/°K</td>
</tr>
</tbody>
</table>

**B. Apparent specific volume data**

<table>
<thead>
<tr>
<th>Weight fraction</th>
<th>$T$ (°C)</th>
<th>$\rho$ (g/ml)</th>
<th>$\rho_a$ (g/ml)</th>
<th>Apparent specific volume (ml/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02511</td>
<td>15.43</td>
<td>1.00904</td>
<td>1.00496</td>
<td>0.8350</td>
</tr>
<tr>
<td>(native)</td>
<td>25.29</td>
<td>1.00679</td>
<td>1.00280</td>
<td>0.8402</td>
</tr>
<tr>
<td>0.01458</td>
<td>5.10</td>
<td>1.00882</td>
<td>1.00603</td>
<td>0.8055</td>
</tr>
<tr>
<td>(native)</td>
<td>15.23</td>
<td>1.00769</td>
<td>1.00496</td>
<td>0.8101</td>
</tr>
<tr>
<td>0.01582</td>
<td>15.43</td>
<td>1.00766</td>
<td>1.00497</td>
<td>0.8268</td>
</tr>
<tr>
<td>(bleached)</td>
<td>25.29</td>
<td>1.00545</td>
<td>1.00281</td>
<td>0.8315</td>
</tr>
</tbody>
</table>

$\sigma_{mem} = 6.04 \times 10^{-4}$/°K

$\sigma_{bleached} = 5.75 \times 10^{-4}$/°K.

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**Table II**

**THERMAL EXPANSIVITY OF SELECTED MATERIALS**

<table>
<thead>
<tr>
<th>Material</th>
<th>$-\frac{\partial \ln \rho}{\partial T}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>$1.25 \times 10^{-4}$</td>
<td>(19)</td>
</tr>
<tr>
<td>DMPC</td>
<td>$8.8 \times 10^{-4}$</td>
<td>(9)</td>
</tr>
<tr>
<td>Olive oil</td>
<td>$7.19 \times 10^{-4}$</td>
<td>(17)</td>
</tr>
<tr>
<td>Purple membrane (10°–20°C)</td>
<td>$6.05 \times 10^{-4}$</td>
<td>(This work)</td>
</tr>
<tr>
<td>BSA</td>
<td>$4.99 \times 10^{-4}$</td>
<td>(18)</td>
</tr>
<tr>
<td>Human apotransferrin</td>
<td>$4.53 \times 10^{-4}$</td>
<td>(10)</td>
</tr>
<tr>
<td>Water (10°–20°C)</td>
<td>$1.5 \times 10^{-4}$</td>
<td>(17)</td>
</tr>
<tr>
<td>Copper</td>
<td>$5.02 \times 10^{-5}$</td>
<td>(17)</td>
</tr>
<tr>
<td>Iron</td>
<td>$3.56 \times 10^{-5}$</td>
<td>(17)</td>
</tr>
<tr>
<td>Diamond</td>
<td>$3.43 \times 10^{-4}$</td>
<td>(19)</td>
</tr>
</tbody>
</table>

at 15, 25, and 35°C, respectively, having an estimated uncertainty of ±0.045 cal/°K · g.

**DISCUSSION**

A simple model that describes some of our results is one in which the apparent membrane density is expressed as a volume-weighted average of the protein density and the lipid density:

$$\rho_{mem} = \rho_{pro} V_p + \rho_{lipid} (1 - V_p),$$

where $V_p$ is the volume fraction of bacteriorhodopsin in the membrane. Differentiation with respect to temperature or pressure gives:

$$\frac{\partial \ln \rho_{mem}}{\partial X} = V_p \left( \frac{\partial \ln \rho_{pro}}{\partial X} + (1 - V_p) \frac{\partial \ln \rho_{lipid}}{\partial X} \right),$$

where $X$ is either the temperature or the pressure.

Consider Eq. 5 as a model for thermal expansion, i.e., with temperature as the variable of differentiation. We used published diteradecanoyl phosphatidylcholine data at 20°C as typical lipid data (9), i.e., $\rho_{lipid} = 1.06$ g/ml and $\partial \ln \rho_{lipid}/\partial T = -9.3 \times 10^{-5}$ g/ml°C. A value of $\partial \ln \rho_{pro}/\partial T = -6.5 \times 10^{-4}$ g/ml°C was chosen as a typical value for

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**Table III**

**ADIABATIC COMPRESSION OF SELECTED MATERIALS**

<table>
<thead>
<tr>
<th>Material</th>
<th>$\beta = -\frac{\partial \ln \rho}{\partial P}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentane (20°C)</td>
<td>$1.58 \times 10^{-4}$</td>
<td>(8, 17)</td>
</tr>
<tr>
<td>Ether (20°C)</td>
<td>$1.49 \times 10^{-4}$</td>
<td>(8, 17)</td>
</tr>
<tr>
<td>Acetone (20°C)</td>
<td>$8.86 \times 10^{-4}$</td>
<td>(8, 17)</td>
</tr>
<tr>
<td>Water (20°C)</td>
<td>$4.61 \times 10^{-4}$</td>
<td>(8, 17)</td>
</tr>
<tr>
<td>DMPC</td>
<td>$4.34 \times 10^{-4}$</td>
<td>(9, 11)</td>
</tr>
<tr>
<td>Purple Membrane (25°C)</td>
<td>$2.30 \times 10^{-5}$</td>
<td>(This work)</td>
</tr>
<tr>
<td>BSA</td>
<td>$1.05 \times 10^{-5}$</td>
<td>(12)</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>$4.67 \times 10^{-6}$</td>
<td>(12)</td>
</tr>
<tr>
<td>Mercury (20°C)</td>
<td>$3.55 \times 10^{-6}$</td>
<td>(8, 17)</td>
</tr>
</tbody>
</table>
proteins (10). To find plausible values for \( V_p \) and \( \rho_{pen} \), we solved Eq. 4, subject to the constraints that \( \rho_{men} \) is equivalent to our measured value of 1.22 g/ml, that the purple membrane is 75% protein by weight (1), and that \( \rho_{lipid} = 1.06 \) g/ml. As a result, Eq. 4 gives values for \( \rho_{pen} = 1.28 \) g/ml and for \( V_p = 0.71 \). Inserting these values into Eq. 5 gives a predicted value of \( \alpha_{men} = 6.15 \times 10^{-4} / \text{K} \), which agrees well with our measured value of \((6.05 \pm 0.82) \times 10^{-4} / \text{K}\).

Now consider Eq. 5 as a model for adiabatic compression. We use \((\partial \rho_{lipid}/\partial P_p) = 4.60 \times 10^{-3} \) g/ml · atm (11), and 0.15 \( \beta_{water} \) for the adiabatic compressibility of bacteriorhodopsin, treating bacteriorhodopsin as a typical globular protein (12). Eq. 5 now yields \( \beta_{men}^S = 1.74 \times 10^{-3} / \text{atm} \) for the membrane, which compares to our measured value of \((2.30 \pm 0.15) \times 10^{-3} / \text{atm}\).

This deviation between the model and the measurement is expected. The value for the compressibility of protein that we used in our model is typical for charged, hydrated globular proteins. The apparent adiabatic compressibility of globular proteins has been shown to be greatly influenced by the bound water (13). Conditions that release the bound water (e.g., appropriate pH changes) yield higher apparent compressibilities for globular proteins than conditions that tend to bind water to charged groups on the protein. The fact that our measured compressibility is greater than the simply modeled value is thus expected because of the absence of bound water on those surfaces of the bacteriorhodopsin molecules that are in contact with the lipid molecules.

The fact that the simple additive models that we have constructed give predictions that agree so much with our data suggests that the lipid and protein components of the purple membrane act independently in thermal expansion and adiabatic compression. Other evidence that supports the simple additive model for thermal expansion is that, within the errors of our expansivity measurements, native and bleached membrane have the same expansivity. The existence of the 2-D lattice did not measurably affect \( \alpha_{men} \).

Wilkinson and Nagle (14) reported an average value of \(-0.50 \) cal/°K · g for the apparent specific heats of lipids in bilayer suspensions at temperatures 20° below and above the main transition temperature. Assuming additivity in specific heats for the protein-lipid mixture gives a value of \(-0.50 \) cal/°K · g for the bacteriorhodopsin in the membrane, a value that is 50% larger than the average value for globular proteins given by Privalov and Khechinashvili (15).

Presently, we do not understand why this apparent specific heat for the purple membrane is unusually large. We can speculate that differences in the structure or amount of bound water between bacteriorhodopsin and the globular proteins discussed in reference 15 may account for the large apparent specific heat of the membrane. Furthermore, it is not completely clear that our data do not agree with the data in reference 15 because we do not know the error in our specific heat measurement. We only estimated it from the dispersion in our three measurements.

The determination of the apparent ratio of the specific heat at constant pressure to the specific heat at constant volume can, a priori, be accomplished via either of two calculations using the data that have been presented in the Results section of this paper. One can calculate the apparent values for the density, adiabatic compressibility, specific heat at constant pressure, and expansivity of the purple membrane, and use these values in the following equation to calculate the apparent isothermal compressibility of the membrane:

\[
\beta_{mem}^T - \beta_{mem}^S - \frac{T \alpha_{mem}^2}{C_p \rho_{mem}}.
\]

One then just calculate:

\[
\gamma_{mem} = \frac{\beta_{mem}^T - \beta_{mem}^S}{\beta_{mem}^S} = \frac{C_p^mem}{C_p^mem}.
\]

Alternatively, one has, for a given purple membrane suspension

\[
\beta_{T}^p = \beta_{S}^p + \frac{T \alpha_{mem}^2}{C_p \rho_{mem}}.
\]

Similarly, one has, for the buffer:

\[
\beta_{T}^b = \beta_{S}^b + \frac{T \alpha_{mem}^2}{C_p \rho_{buf}}.
\]

Subtracting these two equations yields:

\[
\beta_{T}^p - \beta_{T}^b = (\beta_{S}^p - \beta_{S}^b) + T \left[ (\alpha_{mem}^2/C_p \rho_{mem}) - (\alpha_{mem}^2/C_p \rho_{buf}) \right].
\]

We have measured all the quantities on the right side of Eq. 10. We can therefore calculate the apparent isothermal compressibility of the membrane via its defining equation:

\[
\beta_{mem}^T - \beta_{mem}^S = V_{mem} (\beta_{mem}^T_{mem} - \beta_{mem}^S).
\]

where \( V_{mem} \) = volume fraction. Now one proceeds as before to calculate the ratio of specific heats:

\[
\gamma_{mem} = \frac{\beta_{mem}^T_{mem}}{\beta_{mem}^S}.
\]

Note that these two ways of performing the calculation give different results, because neither the density, the expansivity, nor the specific heat at constant pressure enters into Eq. 8 linearly. We believe that the latter type of calculation (i.e., Eq. 10) is the correct one because the equation of the form of Eq. 8 is true only for a well-defined thermodynamic system in equilibrium (16). Purple membrane fragments suspended in an aqueous buffer are not well-defined systems because of their interactions with the solvent. In particular, they do not have well-defined masses.
or volumes, but only apparent densities. In contrast, the suspension and buffer are well-defined systems.

For these reasons, we performed the second type of calculation for the purple membrane suspensions and buffers. Because the specific heat, expansivity, and sound velocity measurements were all performed on suspensions that had different purple membrane concentrations (but contained the same buffer), it was necessary to normalize all the $\rho_{\text{mem}}$ values to the same concentration, which can be chosen arbitrarily. We chose to use the weight fraction of 0.02701 for convenience. For this concentration, we found the following values, expressed in cgs units:

$$\beta^S - \beta^L = - (5.05 \pm 0.08) \times 10^{-13}$$

$$\alpha = (2.183 \pm 0.017) \times 10^{-4}/\theta K$$

$$\sigma = (2.43 \pm 0.02) \times 10^{-4}/\theta K$$

$$C_p^L = (4.14 \pm 0.02) \times 10^7$$

$$C_p^P = (4.09 \pm 0.02) \times 10^7$$

$$\beta^T - \beta^L = - (4.20 \pm 0.09) \times 10^{-13}$$

$$\beta^T = (4.53 \pm 0.05) \times 10^{-11}.$$

We finally arrive at

$$\beta^T_{\text{mem}} = (2.67 \pm 0.18) \times 10^{-5}/\text{atm}.$$

This yields an apparent value of:

$$\gamma_{\text{mem}} = \frac{\beta^T_{\text{mem}}}{\beta^T} = 1.16 \pm 0.08.$$

Hence, the apparent value for the specific heat at constant volume is

$$C_v^{\text{mem}} = \frac{C_v^P}{\gamma_{\text{mem}}} \times .504 = 0.434 \text{ cal/g/°K}.$$

The apparent values for thermodynamic properties of the purple membrane at 25°C are summarized below

$$\rho_{\text{mem}} = 1.22 \pm 0.01 \text{ g/ml}$$

$$\alpha_{\text{mem}} = (6.05 \pm 0.82) \times 10^{-4}/\theta K$$

$$\beta_{\text{mem}} = (2.30 \pm 0.15) \times 10^{-5}/\text{atm}$$

$$\beta_{\text{mem}} = (2.67 \pm 0.18) \times 10^{-7}/\text{atm}$$

$$C_p^P = (0.50 \pm 0.05) \text{ cal/g/°K}$$

$$C_p^P = (0.43 \pm 0.06) \text{ cal/g/°K}.$$

In conclusion, we wish to stress that the thermodynamic quantities that we have reported are apparent values, i.e., there may be contributions from the bound water in addition to contributions that are intrinsic to the lipid and protein molecules. We did not specifically study the contributions of the bound water to any of our reported quantities, and we stress the importance of understanding such contributions before attempting to make hypotheses concerning the contributions from the protein and lipid molecules.

We thank Drs. W. Stoeckenius, R. Lozier, T. Ebrey, K. Nakanishi, and V. Balogh-Nair for providing us with the enormous amounts of purple membrane that were required to develop and complete this study. Special thanks go to Chung-ho Chang for the bleached sample, to Dr. Michael B. Weissman for his invaluable assistance in the data analysis, and to Dr. Benjamin Gavish for having proposed this experiment.

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