Biochimica et Biophysica Acta, 596 (1980) 352—358
© Elsevier/North-Holland Biomedical Press

BBA 78650

SOLUBILITY OF CARBON DIOXIDE IN LIPID BILAYER MEMBRANES AND ORGANIC SOLVENTS

S.A. SIMON [a] and JOHN GUTKNECHT [b]*

[a] *Departments of Physiology and Anesthesiology, Duke University Medical Center, Durham, NC 27710, and* [b] *Duke University Marine Laboratory, Beaufort, NC 28516 (U.S.A.)*

(Received June 25th, 1979)

Key words: Carbon dioxide; Partition coefficient; Solubility; Permeability; Organic solvent; Cholesterol; Phospholipid

Summary

Partition coefficients of carbon dioxide into lipid bilayers (liposomes) and organic solvents were measured as a function of temperature. The molar partition coefficient of CO_2 into liposomes of egg lecithin at 25°C was 0.95 (ml CO_2/ml lipid)/(ml CO_2/ml saline). The addition of an equimolar amount of cholesterol to the egg lecithin decreased the partition coefficient by about 25%. The partition coefficients for CO_2 into liposomes at 25°C were lower than the partition coefficients into octanol (1.3), hexadecane (1.5) and olive oil (1.7). The results are discussed in terms of the solubility-diffusion model of non-electrolyte transport through lipid bilayer membranes.

Introduction

Because of its physiological importance, carbon dioxide transport has been studied in many different systems, e.g., epithelia, single cells, lipid bilayers, soap films and monolayers. Although the CO_2 permeability (i.e., flux/concentration gradient) has been estimated for a variety of membrane systems, the molecular mechanisms of CO_2 transport are not well understood. One of several important factors controlling CO_2 permeability is the concentration of CO_2 in the membrane, usually expressed as a partition coefficient. Historically, partition coefficients for CO_2 into nonpolar liquids such as olive oil and hexadecane have been used to estimate CO_2 solubility in the hydrophobic region of biological membranes. However, recent work has shown that partition coefficients

* To whom correspondence should be addressed.

for small nonpolar solutes may be different in bulk liquids than in lipid bilayer and biological membranes [1,13,21].

In this study we measured the partition coefficient of CO_2 into egg lecithin and egg lecithin/cholesterol bilayers as a function of temperature. We also measured CO_2 partition coefficients into hexadecane and octanol, which are often used as models for the lipid region of biological membranes [2—4]. We found that the CO_2 partition coefficients range from about 0.5 to 2.0 and are about 50% smaller in lipid bilayers than in bulk solvents. The addition of cholesterol to egg lecithin in equimolar amounts decreases the partition coefficient by only about 25%.

Materials and Methods

Egg lecithin was purchased from Avanti Biochemicals and gave a single spot with thin-layer chromatography. Cholesterol and *n*-hexadecane (99 + %) were purchased from Applied Science and used as obtained. 1-octanol (99%) was purchased from Sigma Chemical Co. and used as obtained. The radioactive $^{14}CO_2$ was purchased as $NaH^{14}CO_3$ from New England Nuclear at a specific activity of about 60 Ci/mol.

Liposomes were made by drying the lipids under vacuum and then adding a solution of 0.1 M NaCl plus 0.01 M glutamic acid which was bubbled with nitrogen and titrated with NaOH to a pH of 4.0 at 20°C. The lipid/saline mixture was vortexed for about 1 min until a uniform dispersion was obtained. The final concentration of lipid was 75—200 mg/ml. Because the CO_2 partition coefficient is close to unity (see Fig. 1), we had to use a rather high volume fraction of lipid in the aqueous phase.

The low pH was used to insure that virtually all of the ^{14}C would be in the form of CO_2. The only other forms of CO_2 which are present in significant amounts at pH 4 are HCO_3^- (0.8%) and H_2CO_3 (0.3%) [5]. Both of these species are more polar than CO_2, so their partitioning into the membrane should not significantly affect the accuracy of our estimate for CO_2. Over the temperature

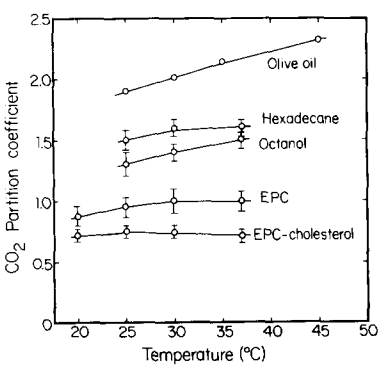

Fig. 1. Partition coefficients (K_p) for CO_2 into the hydrocarbon region of egg lecithin (EPC) and EPC/cholesterol (1 : 1) bilayers, octanol, hexadecane and olive oil as a function of temperature. The error bars indicate the standard deviations of 8—9 measurements. The olive oil data, from Battino et al. [10], have a precision of better than ±1.0%.

range of our experiments, the pH was always more than 1.8 pH units below the pK_a of the reaction, $CO_2 + H_2O \leftrightarrows HCO_3^- + H^+$, which is 6.1 at 25°C and 0.1 ionic strength [5]. Thus, the maximum ratio of HCO_3^-/CO_2 in our solutions was 0.02.

The method of measuring partition coefficients of volatile solutes in a solubility cell is described elsewhere [6]. Briefly, the cell [7] consists of four chambers whose liquid contents are separated from each other but connected through a gas phase. The four chambers are sealed with rubber stoppers through which samples may be extracted with a microsyringe. The temperature is controlled to ±0.2°C by a water jacket, and the apparatus is rotated continuously to insure mixing and reduce the equilibration time. In most experiments two of the chambers contained saline and two contained saline plus lipid. In others, octanol or hexadecane was substituted for the saline plus lipid.

The apparatus was flushed with argon prior to the addition of the experimental solutions. Then the solutions (0.7 ml each) were added to the four compartments and about 10 μCi of $Na^{14}CHO_3$ were injected into each solution. In the octanol and hexadecane experiments, $Na^{14}CHO_3$ was injected into the two saline solutions only, and equilibration was allowed to occur via the gas phase connecting the four compartments. A time of 2 h was needed to insure CO_2 equilibration among the saline, gas and lipid phases. In some experiments the partial pressure of CO_2 in the solubility cell was varied by varying the amount of $NaHCO_3$ added to the buffered saline solution.

Following the 2 h equilibration period, 70-μl samples were taken from the gas, the saline, and the lipid-saline (or organic) phases. The samples were injected into 10 ml of liquid scintillation cocktail (Aquasol, New England Nuclear) to which 1 ml of NaOH (0.1 M) was added to insure complete conversion of $^{14}CO_2$ into $^{14}CHO_3^-$. The samples were then counted in a liquid scintillation counter.

The partition coefficients (K_p) are expressed in molal units to facilitate comparison with the work of others. Thus, K_p is the solubility in lipid (ml CO_2/ml lipid) divided by the solubility in saline (ml CO_2/ml saline) at a constant temperature and partial pressure of CO_2. To provide a more realistic comparison between bilayers and organic solvents, we assumed that CO_2 partitioned only into the hydrocarbon region of the lipid bilayer. We assumed a hydrocarbon volume of 640 cm^3/mol for egg lecithin and 513 cm^3/mol for lecithin/cholesterol (1 : 1) [8]. We assumed the density of the lipid to be 1.0 g/cm^3 and the molecular weights of lecithin and cholesterol to be 787 and 386 g/mol, respectively. In order to estimate partition coefficients for CO_2 into the total lipid volume, our values should be multiplied by the factors 640/787 for lecithin and $(2 \times 513)/(787 + 386)$ for lecithin/cholesterol (1 : 1).

Results

Initially we measured Henry's constant (K_H), which relates the partial pressure of the gas (p) to its molar fraction in water (x_w), i.e., $K_H = p/x_w$. We found that K_H was independent of p_{CO_2} over the range of 0.08—7.1 mm Hg. The values of K_H at different temperatures are shown in Table I. Similar results were obtained with either heating or cooling, as would be expected if K_H were

TABLE I

HENRY'S CONSTANT (K_H) AND OSTWALD SOLUBILITY COEFFICIENT (α_w) FOR CO_2 IN NaCl (0.1 M) PLUS SODIUM GLUTAMATE BUFFER (0.01 M, pH 4) AT FOUR DIFFERENT TEMPERATURES

Temperature (°C)	Henry's constant (K_H) * (10^6 mm Hg)	Ostwald coefficient (α_w) * (ml CO_2/ml saline, pCO_2 = 760 mm Hg)
20	1.16 ± 0.06	0.86 ± 0.05
25	1.32 ± 0.05	0.78 ± 0.03
30	1.61 ± 0.06	0.65 ± 0.03
37	1.84 ± 0.07	0.58 ± 0.03

* Results are presented as the mean ± S.D. of 20 measurements.

independent of p_{CO_2}. Table I also shows the Ostwald CO_2 solubility coefficient for water (α_w) at four different temperatures. The Ostwald coefficient is defined as the ml of gas dissolved per ml of solvent at 1 atm pressure. To obtain α_w, we used Henry's constant and extrapolated p_{CO_2} to 1 atm. Our values for α_w agree within 4% with values published for CO_2 in 0.12 M NaCl [9]. This agreement confirms that CO_2 in our system is at equilibrium and is predominantly in the gas form.

Fig. 1 shows CO_2 partition coefficients for egg lecithin, lecithin/cholesterol (1 : 1 molar ratio), octanol and hexadecane. Also shown for comparison are the partition coefficients for CO_2 into olive oil, calculated from the data of Battino et al. [10]. The values of K_p were rather insensitive to temperature over the range of 20 to 37°C, which is due to the similar temperature dependencies of CO_2 solubility in water and lipid. Furthermore, the K_p values for the bulk liquids were all higher than the values for lecithin and lecithin/cholesterol bilayers. Finally, the addition of cholesterol to lecithin in equimolar amounts reduced K_p by only about 25%. (The K_p values for lecithin/cholesterol at a 2 : 1 molar ratio are not shown because they were indistinguishable from the values for a 1 : 1 molar ratio.) The values of K_p were also found to be independent of p_{CO_2} over a range of 0.08—7.1 mm Hg.

Fig. 2 shows the Ostwald coefficients (α) for CO_2 into lecithin, lecithin/cholesterol (1 : 1), octanol, hexadecane and olive oil [10] at different temper-

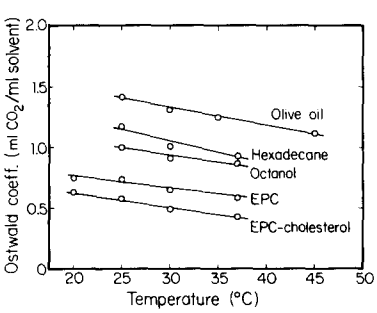

Fig. 2. Ostwald solubility coefficients (ml CO_2/ml solvent at p_{CO_2} = 760 mm Hg) in egg lecithin (EPC) bilayers, EPC/cholesterol (1 : 1) bilayers, octanol, hexadecane and olive oil [10] as a function of temperature.

atures. The Ostwald coefficient was calculated from the equation, $\alpha = \alpha_w K_p$. Our value of α for hexadecane at 25°C agrees with that of Blank [11]. Our values of α in liposomes at 37°C are about 50% of the value reported for red cell ghosts, i.e., 1.0 ± 0.4, calculated from the data of Power and Stegall [12].

Discussion

Partition coefficients for CO_2 are important for understanding the mechanisms of CO_2 transport through membranes. We have shown that the partition coefficient for CO_2 into the hydrocarbon region of lipid bilayers is about 50% lower than the K_p into several organic solvents (Fig. 1). The differences between bilayers and organic solvents would be slightly larger if we used the molar volumes of lecithin and lecithin/cholesterol rather than the hydrocarbon volumes (see Materials and Methods). Results qualitatively similar to ours have been obtained for noble gases [13].

The solubility and partition coefficients for CO_2 into octanol, olive oil and hexadecane are similar within a factor of 1.5 (Figs. 1 and 2) despite the different polarities of these three solvents. This nondiscrimination is due to the fact that CO_2 is a linear triatomic molecule with no permanent dipole moment. In contrast to CO_2, more hydrophilic and hydrophobic molecules may distribute very differently among these three solvents. For example, the partition coefficients for urea and glycerol are 5000 to 7000 times higher in octanol than hexadecane, and for water the difference is about 800-fold [3,4]. These differences undoubtedly reflect the solute's ability to form hydrogen bonds with octanol but not hexadecane. In contrast, the K_p for a very hydrophobic molecule such as hexane is lower in octanol than in bulk hydrocarbon [1].

Carbon dioxide is unusual among gases in that it distributes in roughly equal concentrations (v/v) among gas, water, organic solvents and lipid bilayers, both with and without cholesterol (Table I, Fig. 2 and Refs. 5 and 9). Consequently, the solubility coefficients for CO_2 in various animal tissues are similar to those for lipid bilayers and biological membranes, despite the fact that the tissues contain water, salt and protein [9]. In the red cell membrane the partition coefficient for CO_2 at 37°C is about 1.6 [12], roughly twice the value for a lecithin/cholesterol bilayer (Fig. 1). This value is surprisingly high in view of the large amount of protein and boundary lipid, both of which are expected to lower the solubility of gases. For example, the partition coefficient for butane into the red cell membrane is about 28% of that into a lecithin/cholesterol bilayer [21].

The molecular mechanism(s) of non-electrolyte translocation through lipid bilayers are not well understood [3,14]. Small non-electrolytes whose partition coefficients into hydrocarbon range from about 10^{-5} to 10^{-1} seem to permeate by a solubility-diffusion mechanism [3]. If CO_2 transport occurs by this mechanism, then

$$P = \frac{K_p \times D}{d} \tag{1}$$

where P is the permeability coefficient (cm/s), D is the average intramembrane

TABLE II

CO_2 PERMEABILITY COEFFICIENTS AND APPARENT CO_2 DIFFUSION COEFFICIENTS IN THREE TYPES OF MEMBRANES

Membrane	Permeability coefficient (cm/s)	Diffusion coefficient * (10^{-7} cm^2/s)	Reference
Hexadecyltrimethylammonium bromide bilayer **	7.8	49	15
Egg lecithin/cholesterol/decane bilayer	0.35	2.2	16
Mammalian erythrocyte	0.36 ***	1.1	12,17,18

* Calculated from Eq. 1, assuming $d = 5 \cdot 10^{-7}$ cm and $K_p = 0.8$ for the two lipid bilayers and 1.6 for the erythrocyte membrane [12].

** This is a 'Perrin first-order black film' separating two gas phases. The film is formed from hexadecyltrimethylammonium bromide in 0.1 M NaBr.

*** This is the average of two approximations based on data of Roughton [17] (0.15 cm/s) and Forster (0.58 cm/s), assuming that CO_2 goes through the lipid part of the membrane. Direct measurements of CO_2 permeability in erythrocytes have not been accomplished.

diffusion coefficient (cm^2/s), d is the membrane thickness (cm), and K_p is the average partition coefficient.

Table II shows CO_2 permeabilities through a bilayer of hexadecyltrimethylammonium bromide (7.8 cm/s), a bilayer of lecithin, cholesterol and decane (0.35 cm/s) and a mammalian red cell membrane (0.36 cm/s). Table II also shows apparent intramembrane diffusion coefficients (cm^2/s), calculated by Eqn. 1: hexadecyltrimethylammonium bromide, $49 \cdot 10^{-7}$; lecithin/cholesterol/decane, $2.2 \cdot 10^{-7}$, and the red cell, $1.1 \cdot 10^{-7}$. In the red cell membrane, the value for CO_2 diffusion is comparable to Solomon's [19] estimate for methanol, i.e., $0.5 \cdot 10^{-7}$ cm^2/s.

One major difference among these membranes is the cholesterol content, which is zero in the hexadecyltrimethylammonium bromide bilayer but approximately equimolar with phospholipid in the other two membranes [16,20]. Another difference may be a higher water content of the hexadecyltrimethylammonium bromide film, in which as much as 30% of the membrane surface area may be occupied by 'aqueous pores' [15]. Thus, in hexadecyltrimethylammonium bromide films the permeability to eight different gases correlates better with water solubility than with oil solubility [15]. Our results show that the presence of cholesterol reduces only slightly the partition coefficient and solubility for CO_2 in lipid bilayers (Figs. 1 and 2). Furthermore, the solubility of CO_2 in water is similar to that in lipid (Table I and Fig. 2). Thus, we infer that the difference in order of magnitude between the CO_2 permeabilities of the hexadecyltrimethylammonium bromide bilayer and the other two membranes is due primarily to a difference in the intramembrane diffusion coefficients, as suggested by the values shown in Table II.

Acknowledgements

This work was supported by USPHS grants HL 12157 and ES 01908. We thank L.A. Koro and A. Walter for helpful advice and assistance.

References

1 Simon, S.A., Stone, W.L. and Busto-Latorre, P. (1977) Biochim. Biophys. Acta 468, 378–388
2 Seeman, P. (1972) Pharm. Rev. 24, 583–655
3 Finkelstein, A. (1976) J. Gen. Physiol. 68, 127–135
4 Wolosin, J.M., Ginsburg, H., Lieb, W.R. and Stein, W.D. (1978) J. Gen. Physiol. 71, 93–100
5 Edsall, J.T. and Wyman, J. (1958) Biophysical Chemistry, Vol. 1, Chapter 10, Academic Press, New York
6 Stone, W.L. (1975) J. Biol. Chem. 250, 4386–4390
7 Wishnia, A. and Pinder, T.W., Jr. (1966) Biochemistry 5, 1534–1542
8 Small, D.M. (1967) J. Lipid Res. 8, 551–557
9 Handbook of Respiration (1958) (Dittmer, D.S. and Grebe, R.M., eds.), W.B. Saunders, New York
10 Battino, R., Evans, F.D. and Danforth, W.F. (1968) J. Am. Oil Chem. Soc. 45, 830–833
11 Blank, M. (1962) J. Phys. Chem. 66, 1911–1920
12 Power, G.G. and Stegall, H. (1970) J. Appl. Physiol. 29, 145–149
13 Katz, Y. and Simon, S.A. (1977) Biochim. Biophys. Acta 471, 1–15
14 Dix, J.A., Kivelson, D. and Diamond, J.M. (1978) J. Membrane Biol. 40, 315–342
15 Princen, H.M., Overbeek, J.Th.G. and Mason, S.G. (1967) J. Colloid Int. Sci. 24, 125–130
16 Gutknecht, J., Bisson, M.A. and Tosteson, D.C. (1977) J. Gen. Physiol. 69, 779–794
17 Roughton, F.J.W. (1959) Progr. Biophys. Chem. 9, 55–104
18 Forster, R.E. (1969) in CO_2: Chemical, Biochemical and Physiological Aspects (Forster, R.E., Edsall, J.T., Otis, A.B. and Roughton, F.J.W., eds.), pp. 275–284, National Technical Information Service, No. NASA SP-188, Washington, DC
19 Solomon, A.K. (1974) Biochim. Biophys. Acta 373, 145–149
20 Rouser, G., Nelson, G.J., Fleischer, S. and Simon, G. (1968) in Biological Membranes, Physical Fact and Function (Chapman, D., ed.), pp. 5–69, Academic Press, New York
21 Miller, K.W., Hammond, L. and Porter, E.G. (1977) Chem. Phys. Lipids 20, 229–241