Facilitated Diffusion

THE CASE OF CARBON MONOXIDE*

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SUMMARY

An application of a singular perturbation method to the case of carbon monoxide similar to that used previously for the case of oxygen (Murray, J. D., Proc. Roy. Soc. London B Biol. Sci., 178, 95 (1971)) shows that the absence of any observed facilitation of diffusion of this ligand by either hemoglobin or myoglobin in all of the experiments so far performed results from the very high affinity of both proteins for the gas. At every point in the solution both proteins were essentially at equilibrium with the gas but it was evidently impossible to reduce the pressure of carbon monoxide on the low pressure face of the solution sufficiently to remove a significant amount of the carbon monoxide from combination with the macromolecular carrier. The principle that a macromolecule can only function as a carrier under conditions in which its saturation with the ligand is incomplete in some region of the solution is quite general.

The role of myoglobin was originally thought to be to act as an intracellular molecular reservoir for the storage of oxygen. Although this role is undoubtedly an important one in certain cases, for example in various diving animals like the seal and the whale, where the concentration of myoglobin in the red muscles may be as high as 10%, it now begins to look as if a more general and widespread function of myoglobin might be to serve as a mechanism of intracellular oxygen transport. Indeed, there is strong evidence that myoglobin acts this way in a variety of muscles, such as the heart, which undergoes prolonged and repetitive activity and reaches a steady state as regards oxygen consumption (1).

An understanding of how myoglobin can function as an oxygen carrier has resulted from a study of the phenomenon of facilitated diffusion exhibited by solutions of both hemoglobin and myoglobin, as well as a number of other respiratory proteins. A recent analysis of the experiments in the light of the basic governing equation (2) confirms the earlier conclusion (3) that in the bulk of the solution the macromolecular carrier was everywhere at equilibrium with the dissolved oxygen. Under any conditions the carrier contributes directly to the transport of oxygen only in a region where the oxygen concentration is such that it is only partially saturated; its contribution in the region of higher oxygen concentration where its saturation is complete is an indirect one, resulting from its effect in steepening the concentration gradient of the dissolved oxygen.

It is striking and indeed at first sight somewhat puzzling that, in contrast to oxygen, carbon monoxide, which combines with hemoglobin and myoglobin at the same sites and in a very similar manner, shows no appreciable facilitated diffusion at all in any of the experiments. It is clear that the difference between the two ligands must somehow be related to differences in the kinetic and equilibrium constants which describe their reactions with the protein, whether hemoglobin or myoglobin. The dissociation velocity constant of carbon monoxide is of the order of 1000 times less than that of oxygen for both of these proteins and its affinity for myoglobin is about 40 times greater than that of oxygen; for hemoglobin it is about 250 times greater.

The present study represents an attempt to rationalize the profound difference in behavior between the two ligands, using a singular perturbation method. Although from a physiological point of view carbon monoxide is of far less interest than oxygen, nevertheless the analysis given here is important because of the light which it throws on the general principles involved in the intramolecular transport of any ligand by any macromolecular carrier, a subject of considerable significance for cellular biology.

THEORY

We consider the layer of solution through which the CO diffuses to be of thickness $l$ cm.¹ Let $c_0$ and $c_1$ be the CO concentrations in, say, moles cm⁻³ on the high pressure side ($x = 0$) and on the low pressure side ($x = l$) of the layer, respectively. We chose the $x$-axis to be perpendicular to the layer surfaces. Let $c$, a function of $x$, be the concentration of free CO anywhere within the solution and let $c_P$ be the concentration of the protein, $c_0$ and $c_1$ being the concentrations of CO bound to the two.

¹ All quantities will eventually be put in dimensionless form and so any consistent set of units will suffice.
which is uniform throughout the solution. Denote by \( Y \) (0 \( \leq \) Y \( \leq \) 1) the fractional saturation of the protein and by \( m \) the number of binding sites for the CO. Thus at any point \( mc_p Y \) moles cm\(^{-3}\) gives the concentration of bound CO.

We assume that the reaction of the protein with CO may be adequately described by two velocity constants \( k \) and \( k' \):

\[
\text{CO} + \text{Hb} \xrightleftharpoons[k']{k} \text{HbCO}
\]

This formulation applies exactly in the case of myoglobin, a one-site molecule; from a phenomenological point of view it also provides a reasonably good description of the kinetics of the combination of hemoglobin with CO except at very high degrees of saturation (\( Y \rightarrow 1 \)). In any case, if \( \rho \) denotes the rate of the reaction in the steady state situation, the one-dimensional equations governing the bound and free CO, respectively, are

\[
\frac{d}{dx} D_p \frac{dc}{dx} + \frac{d}{dx} D_\sigma \frac{d\sigma}{dx} = -\rho \quad \text{(2)}
\]

\[
\frac{d}{dx} D_p \frac{dc}{dx} = \rho \quad \text{(3)}
\]

where \( D_p \) and \( D_\sigma \), in cm\(^2\) sec\(^{-1}\) say, are the diffusion coefficients of the protein (here HbCO) and CO, respectively, which are independent of \( Y \), and \( \rho \) is the rate of Reaction 1 and is a function of \( c, \sigma, m, Y, k \), and \( k' \).

On the assumption that \( D_p, D_\sigma, c_p, \) and \( m \) are constants and that \( \rho \) is a given function, Equations 2 and 3 are two second order equations which require two boundary conditions each in \( x \) for \( c \) and \( \sigma \) for an exact solution to be obtained. Normally \( c \) and \( \sigma \) would have to be given at \( x = 0 \) and \( x = l \). There is some difficulty in specifying \( Y \), as has been discussed more fully by Murray (2). In the meantime we shall formally take

\[
c = c_0, \quad Y = Y_0 \quad \text{at} \quad x = 0
\]

\[
c = c_1, \quad Y = Y_1 \quad \text{at} \quad x = l
\]

(4)

If we add Equations 2 and 3, the \( \rho 's \) cancel and we are left with an equation which integrates immediately to give

\[
D_p \frac{d^2 c}{dx^2} + D_\sigma \frac{d^2 \sigma}{dx^2} (mc_p Y) = -\rho
\]

where the constant of integration \( F \) is the total (bound and free) flux of CO. Integrating Equation 5 again we get another constant of integration. Both constants are then evaluated in terms of the various parameters with the boundary values

Equation 4 to give

\[
D_p (c - c_0) + mc_p D_\sigma (Y - Y_0) = \frac{\pi}{l} \left[ D_p (c_1 - c_0) + mc_p D_\sigma (Y_1 - Y_0) \right]
\]

(6)

From Equations 5 and 6 the total flux \( F \) is given by

\[
F = D_p \frac{dc}{dx} + mc_p D_\sigma \frac{d\sigma}{dx} = \frac{\pi}{l} \left[ D_p (c_1 - c_0) + mc_p D_\sigma (Y_1 - Y_0) \right],
\]

(7)

At this stage if we could assign the values of \( Y_1, Y_0, c_0 \), and \( c_p \) together with those of all of the other constants, the total flux would be given immediately by Equation 7. As discussed by Murray (2) the specification of the \( Y 's \) is crucial in such problems. In fact he showed that they could not be assigned simply but had to satisfy certain relations, which were derived in a manner similar to that given below.

We are also interested in the individual distributions of \( c \) and \( \sigma \) throughout the interior of the layer and so we must specify the functional form of the reaction rate \( \rho \), which we take here to be

\[
\rho = k' mc_p (1 - Y)c - kmc_p Y
\]

(8)

If we now solve Equation 6 for \( Y \) in terms of \( c \) we get \( \rho \) as a function of the various constants and the variable \( c \) alone. By substituting the resulting expression for \( \rho \) into Equation 3 we obtain a single equation for the dependent variable \( c \):

\[
D_p \frac{d^2 c}{dx^2} = -[D_p c_0 + mc_p D_\sigma Y_0] \frac{k}{D_p} + \left[ \frac{kD_p}{D_\sigma} - k'mc_p \left( Y_0 - 1 + \frac{c_0 D_p}{mc_p D_\sigma} \right) \right] \frac{c}{l}
\]

\[
+ \frac{k}{D_p} \left[ D_p (c_0 - c) + mc_p D_\sigma (Y_0 - Y_1) \right] \frac{c}{l}
\]

\[
+ \frac{k'}{D_\sigma} \frac{c^2}{l}
\]

(9)

It should be remembered that the boundary conditions have already been incorporated into Equation 0.

A crucial step in the procedure developed by Murray (4, 5) is the nondimensionalisation of the governing equations, which makes all relations independent of the choice of units. This is done here by writing

\[
c_l = c/c_0, \quad x_l = x/l
\]

\( c_0, x_0, \) and \( Y_1, \) of course, are now all pure numbers. If we now multiply both sides of Equation 9 by \( l^3/c_0 D_p \) we get the governing dimensionless equation for \( c_l \) as

\[
\frac{d^2 c_l}{dx_l^2} = (\alpha + \gamma x_l) + (\beta + \delta x_l) c_l + \lambda c_l^2
\]

(10)

where the dimensionless constants are given by

\[
\alpha = -\frac{k'c_0}{c_0 D_p} [D_p c_0 + mc_p D_\sigma Y_0]
\]

\[
= \frac{k'}{D_p} \left[ 1 + \frac{Y_0}{c_0 D_\sigma} \right] \left[ \frac{c_0 D_p}{mc_p D_\sigma} \right] \left[ \frac{Y_0}{c_0 D_\sigma} \right]
\]

(11)

\[
\beta = \frac{k'}{D_p} \left[ \frac{kD_p}{D_\sigma} - k'mc_p \left( Y_0 - 1 + \frac{c_0 D_p}{mc_p D_\sigma} \right) \right] \left[ \frac{c_0 D_p}{mc_p D_\sigma} \right] \left[ \frac{Y_0}{c_0 D_\sigma} \right]
\]

\[
\gamma = \frac{k'}{D_p} \left[ \frac{1 - c_0}{c_0} + mc_p D_\sigma (Y_0 - Y_1) \right]
\]

\[
\delta = \frac{k'}{c_0 D_\sigma}
\]

\[
\lambda = \frac{k'}{c_0 D_p}
\]

With \( c_l, x_l, \alpha, \beta, \gamma, \delta, \) and \( \lambda \) pure numbers it is clear that any consistent set of units may be used.

**Singular Perturbation Solution**

The values for the various constants which appear in Equation 11 under the conditions of the experiments are given in Table I.
They are based on data given by Whittenberg (1), and for $k$ and $k'$, by Gibson (6) and Brunori et al. (7). With these values of the $k$'s, the partition coefficient, ideally equal to $(k':k')_0$, is approximately 20 for Mb and 200 for Hb—the measured values are 40 and 250. Further, noting that $Y_0$ and $Y_1$ are $O(1)$, we obtain the approximate values of the constants in Equation 12 given in Table II. This shows that $\beta, \delta, \lambda$ are all very much larger than $\alpha$ and $\gamma$ by a factor $O(10^4)$. If we now introduce new quantities:

$$
\epsilon = 10^{-4}, \alpha = \epsilon^4a, \beta = \epsilon^6b, \gamma = \epsilon^4c, \delta = \epsilon^4d, \lambda = \epsilon^4s
$$

then $a$, $c$, and $f$ are all $O(1)$ while $d$ and $a$ are small as $O(10^{-9})$. Thus with the definitions in Equation 13, Equation 11 becomes

$$
\epsilon \frac{d^2y}{dx^2} = (b + a\delta)\epsilon + f\epsilon^2 + \text{small negligible terms } O(10^{-9})
$$

Since $\epsilon << 1$, Equation 14 belongs to the singular perturbation class of equations which have been discussed at length by Murray (4, 5) and as applied to this specific equation by Murray (2). Basically the appearance of the small parameter $\epsilon$ multiplying the highest derivative implies that, as a first approximation away from the boundaries $x_1 = 0$ and $x_1 = 1$, the solution is given by simply neglecting the $O(\epsilon)$ term in Equation 14. However this reduces the second order differential equation to a zeroth order one with the resulting loss of the ability to satisfy two boundary conditions. Thus we would normally not expect to be able to satisfy the boundary conditions of the exact problem with the approximate solution given by ignoring the $\epsilon$ terms completely. The singular perturbation procedure shows that, in this class of problem, in general, there are two very thin regions of thickness $O(\epsilon^2)$, or in dimensional form $O(\epsilon^2) \Delta$ where here is $O(10^{-3} \text{ cm})$, where the approximate solution from Equation 14 with $\epsilon = 0$ is made to satisfy the boundary conditions by joining it onto the rapidly changing solution holding in these thin regions. In the specific situation here however the thin regions are so thin that the solution can hardly be expected to change significantly within them. We are thus led, by the built-in biological restrictions, to conclude that the reduced problem is essentially one and Equation 18 implies that $Y_1$ is essentially one and Equation 18 implies that $Y_1 = Y_0$ exactly. It should be noted that the value obtained for $Y_1$ is independent of $c_1$ here.

The implication of Equations 17 and 18 is that there is no facilitated CO transport, the protein being saturated completely with Equations 12 and 13, the first part of Equation 16 applied to the second part of Equation 15 gives

$$
C_a /C_0 = \frac{1}{f} (b + \epsilon)
$$

When Equations 17 and 18 are expressed in terms of $\beta, \lambda, \delta$ using Equation 13 and the results are combined with Equation 12, we find, on taking account of the numerical values listed in Table I, that Equation 17 implies that $C_a$ is essentially one and Equation 18 implies that $C_a = C_0$ exactly. It should be noted that the value obtained for $Y_1$ is independent of $c_1$ here.

The second part of Equation 16 gives

$$
C_a /C_0 = \frac{1}{f} (b + \epsilon)
$$

which is of course the solution which would be obtained if there were no hemoglobin-CO complex formed and simple diffusion alone prevailed.

It should perhaps be mentioned that the Solution 19 is obtained by ignoring terms which are small compared to unity. Since the small terms on the right of Equation 14 are $O(10^{-3})$ the error in the solution cannot be more than a few ($<5$) per cent.

The singular perturbation method as applied to this particular class of equation reduces the problem to that of solving a quadratic equation as distinct from the original one of solving a nonlinear second order differential equation. In the special case considered here the quadratic equation becomes a linear algebraic one. Further the method provides, in general, values

<table>
<thead>
<tr>
<th>Table I</th>
<th>Hemoglobin</th>
<th>Myoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m_p$ (moles cm$^{-2}$)</td>
<td>$1.2 \times 10^{-4}$</td>
<td>$1.2 \times 10^{-4}$</td>
</tr>
<tr>
<td>$D_p$ (cm$^2$ sec$^{-1}$)</td>
<td>$2.5 \times 10^{-7}$</td>
<td>$4.4 \times 10^{-7}$</td>
</tr>
<tr>
<td>$l$ (cm)</td>
<td>$4$</td>
<td>$4$</td>
</tr>
<tr>
<td>$D_0$ (cm$^2$ sec$^{-1}$ in 20% solution)</td>
<td>$2.2 \times 10^{-4}$</td>
<td>$2.2 \times 10^{-2}$</td>
</tr>
<tr>
<td>$k$ (sec$^{-1}$)</td>
<td>$9 \times 10^{-4}$</td>
<td>$9 \times 10^{-4}$</td>
</tr>
<tr>
<td>$k'$ (cm$^2$ mole$^{-1}$ sec$^{-1}$)</td>
<td>$8 \times 10^{-4}$</td>
<td>$1.7 \times 10^{-4}$</td>
</tr>
<tr>
<td>$c_0$ (mole cm$^{-3}$ at 100 mm Hg)</td>
<td>$2 \times 10^3$</td>
<td>$5 \times 10^4$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table III</th>
<th>CO</th>
<th>$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>$0.04$</td>
<td>$0.10$</td>
</tr>
<tr>
<td>Mb</td>
<td>$0.02$</td>
<td>$0.07$</td>
</tr>
</tbody>
</table>

* This notation means “of the order of.”
for the saturations $Y_0$ and $Y_1$ for which it is difficult to assign values a priori. Here using Equation 19 in connection with Equation 6, $Y = 1$ everywhere.

**PHYSICAL INTERPRETATION**

The analysis just given is in every respect formally the same as that given previously for the case of oxygen (2). In both cases the fact that we can neglect the second derivative in the governing Equation 9 implies in view of Equation 2 and 3, that the velocity of the reaction $\rho$ in Equation 8 is negligible and hence that the system is everywhere essentially at equilibrium. For both ligands the reduction of the basic second order non-linear differential equation to a simple algebraic one in which the parameters are constrained in such a way as to meet the boundary conditions, results in the two relations:

$$\alpha + \beta + \gamma = 0$$

$$\alpha + \gamma + (\beta + \rho)C_1/C_0 + \lambda(C_1/C_0)^3 = 0$$

which are obtained from Equation 11 on setting the left side equal to zero and putting $x_1 = 0$, $c_1 = 1$, and $z_1 = 1$, $c_1 = C_1/C_0$, respectively. The profound difference of behavior of the two ligands stems from the fact that in the case of oxygen all parameters $\alpha$, $\beta$, $\gamma$, $\delta$, and $\lambda$ are of the same order of magnitude, namely $O(10^3)$, whereas in the case of carbon monoxide the two constants $\alpha$ and $\gamma$ are reduced from $O(10^3)$ to $0(10)$ due primarily to the greatly reduced values of kinetic dissociation constants which lead to greatly increased value of the affinity constants. This makes it possible to neglect $\alpha$ and $\gamma$ in Equation 20 and it is for this reason that $Y$ remains essentially equal to unity everywhere, as will be seen by substituting Equation 19 into Equation 6 and setting $\alpha$ and $\gamma$ equal to zero.

From a physical point of view the implication of this is that at the low pressure side of the layer the pumping system used in the experiments was inadequate to maintain the pressure of carbon monoxide at a sufficiently low value to remove the ligand to any significant extent from the carrier molecule. When we look at the situation from this point of view the difference in the behavior between oxygen and carbon monoxide is to be rationalized as a result of the much greater affinity of the protein for carbon monoxide than for oxygen.³

³The values of $p_1$ (the pressure required to half-saturate the carrier molecule) for the two gases are given in Table III.

myoglobin compared with 0.85 for hemoglobin. Myoglobin has an approximately 14-fold greater affinity for oxygen than hemoglobin.

Although the singular perturbation method employed in this analysis is only applicable under the special condition that the dimensionless coefficient of the second order derivative in the basic Equation 14 is small in comparison with the other terms, nevertheless the general principles that emerge from the analysis are applicable to any ligand and any macromolecular carrier. Foremost among these is the principle that the carrier molecule can only make a significant contribution when its ligand affinity is such in relation to the ambient ligand activity that it is only partially saturated with ligand in some part of the system. This follows from Equation 7, where it will be seen that if $Y_1 = Y_0$, which in general will be true only if $c_1 = c_0$, or if, at sufficiently high values of $c$, $Y_1 \rightarrow Y_0 \rightarrow 1$, or, at sufficiently low values, $Y_1 \rightarrow Y_0 \rightarrow 0$, then the macromolecule will not contribute to the flux. Another related principle is that only if the life-times of the liganded and unliganded molecules, which are determined by the values of $k$ and $k'$, are sufficiently short in relation to the time required for the molecule to diffuse across the distance $l$, which is given by $V/D_p$, will there by any facilitation. This second principle, which like the other is intuitively more or less obvious, is confirmed by Equation 12 where it will be seen that each of the Greek letter constants is proportional to one or another of the dimensionless ratios $k_1 p_1 D_p$ or $k' p_1 c_1 D_p$. When these become sufficiently small the right hand side of Equation 11 vanishes and $c$ becomes linear in $z$. In this case it is possible that $Y_0 \rightarrow Y_1$ even when $c_0 \neq c_1$. The situation may be thought of in terms of relaxation times.

In conclusion it might be pointed out that myoglobin, in view of the particular value of its affinity for oxygen, is well adapted to play a role in the transport of oxygen under conditions prevailing within the cells. On the other hand hemoglobin, with a lower oxygen affinity, is well adapted to its function of carrying oxygen from the lungs to the tissues, operating over a higher range of oxygen pressures.

**REFERENCES**

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