Short Communication

Apparent Bicarbonate Uptake and Possible Plasmalemma Proton Efflux in *Chara corallina*

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ABSTRACT

It is shown that the apparent uptake of bicarbonate by cells of *Chara corallina* could be the result of a proton efflux coupled to extracellular production of CO_2 from bicarbonate, with CO_2 being taken up by the cell. The theoretical results presented here show that the influx of CO_2 across the plasmalemma can be much greater than previously thought, if there is a large efflux of protons across the plasmalemma, and that, if this occurs, there would be a much steeper gradient of pH near the cell surface than previously thought possible.

It has been claimed that carbon for photosyntheses will cross the *Chara corallina* plasmalemma in the form of HCO_3^- rather than CO_2 (5, 6, 10). The purpose of this communication is to show that extracellular chemical production of CO_2 coupled to a proton efflux can proceed at a much greater rate than previously thought (5) and be much more difficult to detect than previously thought. It will be shown that the pH gradient near the cell surface will be much steeper than previously thought possible, if there is a large plasmalemma proton efflux and if the cell is a strong sink for CO_2.

A plasmalemma proton efflux will produce CO_2 through the reaction: $H^+ + HCO_3^- \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O$ (1, 2, 5, 11, 12). When this reaction takes place sufficiently near the cell surface, most of the CO_2 produced will reach the cell surface. Carbon flux into the region near the cell surface from the bathing medium would be carried by HCO_3^- diffusion. Electrical forces can be ignored (3, 4, 8).

The essential question is: how high can the proton concentration be near the cell surface? The greater the proton concentration, the greater will be the rate of extracellular CO_2 production. At steady state, the following equation can be written by continuity:

$$-D_H \partial^2[H^+]/\partial x^2 = k_f[H^+][HCO_3^-] - k_b[CO_2] \qquad (1)$$

where D_H is the diffusion coefficient for protons in an aqueous medium. The right-hand side of equation 1 is the net rate of CO_2 production, with $k_f = 9 \times 10^4$ 1 mol^{-1} s^{-1} and $k_b = 4.3 \times 10^{-2}$ s^{-1} (12). This equation is an excellent approximation for pH $\geq$ 6 (1, 2, 5, 11, 12). The values of the reaction coefficients given by different workers agree within 20%.

The back-reaction rate, $k_b[CO_2]$, can be much smaller than the forward rate, if the cell surface is a strong sink for CO_2, over a distance from the cell surface which a CO_2 molecule has a high probability of diffusing before "back-reacting." If this distance, x_f, is conservatively estimated as that which the molecule could diffuse in one-tenth of its mean lifetime before back-reacting (with "lifetime" being the exponential time constant = k_b^{-1}), then

$x_f = (0.2 D_{CO_2} k_b^{-1})^{1/2} = 90 \mu m.$

The equilibrium value of $[HCO_3^-]$ is a function of total carbon concentration and pH (5, 12) and would decrease by a factor of 3.2 as pH decreases from 8 to 6, for a fixed total carbon concentration. However, the physical situation under consideration is not one of equilibrium with a uniform pH, but one of a nonequilibrium steady state with a possibly steep pH gradient. If the pH changes sharply over a distance of the order of the x_f calculated above (which is also comparable to the cells' intrinsic unstirred layer), then the $[HCO_3^-]$ gradient required to drive the measured carbon influx would give less than 0.1 mM change in concentration over that distance. Based on these considerations, if it assumed that $k_f[HCO_3^-]$ varies much less than $[H^+]$, equation 1 can be solved under the approximation that $k_f[HCO_3^-]$ is constant and, for distances less than x_f from the cell surface (so that the back reaction can be ignored);

$$[H^+] = [H^+]_{x = 0} \exp(-x/\lambda) \qquad (2)$$

where $\lambda = (D_H/k_f[HCO_3^-])^{1/2}$. For $[HCO_3^-] = 0.3$ mM, this gives $\lambda = (10^{-4}$ cm^2 $s^{-1}/27$ $s^{-1})^{1/2} = 19 \mu m$; for $[HCO_3^-] = 1$ mM, $\lambda = 10$ μm. This means that pH could change by 1 unit over a distance of less than 44 μm. Thus, there can be a sharp drop in proton concentration near the cell surface that would probably not be detected by the electrodes used to measure surface pH (4, 6). The physical meaning of this mathematical result is that the extracellular flux of protons will fall off exponentially with distance from the plasmalemma (and, hence, $[H^+]$ will also fall off exponentially) because the net reaction rate is proportional to $[H^+]$ under the assumption that $k_f[HCO_3^-]$ is constant.

C. corallina "acid-band" pH, under experimental conditions, is in the range of 7.5 to 8.5 (4, 6, 10). It can easily be shown, based on the preceding considerations, that

$$J_{CO_2, x = 0} = -(D_H k_f[HCO_3^-])^{1/2}[H^+]_{x = 0}. \qquad (3)$$

For pH = 6 at the cell surface, and $[HCO_3^-]_{x = 0} = 1$ mM, this gives $J_{CO_2, x = 0} = 95$ pmol cm^{-2} s^{-1}, which is comparable to measured carbon flux in the acid band (5–7). That this flux can be maintained by CO_2 produced within x_f of the cell surface can be shown by integration of the CO_2 production rate over that distance. The foregoing ignores the cell wall Donnan potential. This is valid because any effect on $J_{CO_2, x = 0}$ resulting from an increase in $[H^+]$ in the cell wall would be cancelled by an opposite effect on $[HCO_3^-]$. The effect of plasmalemma surface potential on transport can also be ignored (8).

The possibility raised in this communication that CO_2, rather than HCO_3^-, carries carbon across the plasmalemma could be tested by (*a*) measurements of pH gradients at the cell surface with a precision better than 5 μm (compared to the precision of 20 to 100 μm available now; this measurement would perhaps be easier

at lower [HCO_3^-], for which λ would be larger), and (b) by precise carbon uptake kinetic studies, in which the kinetic behavior predicted by equation 3 is compared to experimental data. Equation 3 predicts that carbon flux is proportional to [HCO_3^-]$^{1/2}$ at low carbon concentrations, with a saturation at high carbon concentration that will be imposed by the photosynthetic system. Such kinetic studies will be extremely difficult, because [H^+]$_{x\,=\,0}$ must be measured and controlled by the experimenter to cancel the effect of a change in proton efflux that would be likely to occur when [HCO_3^-]$_{x\,=\,0}$ is changed, and [HCO_3^-]$_{x=\,0}$ will have to be calculated using numerical computer methods (4, 8, 9) since it cannot be directly measured or analytically calculated. In OH^- diffusion experiments with the cell surrounded by an unstirred layer produced by agar to a distance of 4 mM (4, 6, 8, 9), numerical computer results from the same calculations used to describe OH^- diffusion (4, 8, 9) show that [HCO_3^-] at a distance of 50 μm from the cell surface would be drawn down by less than 0.25 mM relative to the background level, for a carbon influx of 100 pmol cm^{-2} s^{-1} maintained for 30 min. In carbon uptake experiments, agar is usually not used (5-7, 10) and the draw-down of HCO_3^- at 50 μm from the cell surface would be less as a result of convective mixing in the region beyond the cell's intrinsic unstirred layer. The draw-down discussed here would add to the [HCO_3^-] gradient close to the cell surface of approximately 0.1 mM/100 μm.

Whether the proton pump model can account for all of the kinetic data on carbon assimilation by C. corallina (5-7, 10) remains to be seen. For example, apparent competitive inhibition of HCO_3^- uptake by CO_3^{2-} (7) could result from the higher rate coefficient for the reaction of CO_3^{2-} with H^+, compared to the rate coefficient for the reaction of HCO_3^- with H^+. The results presented here show that the plasmalemma influx of CO_2 can be much greater than previously thought if there is a large plasmalemma efflux of protons and that, if this is occurring, there would be a much steeper gradient of pH near the cell surface than previously thought possible.

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