Online Supplemental Information

Adaptable interaction between aquaporin-1 and band 3 reveals a potential role of water channel in blood CO_2 transport (Hsu et al.; 2017)

(1) ***To estimate the concentration of water molecules inside a RBC and how much water is used for blood CO_2/HCO_3^- conversion:***

<1-i> From the follows *(1-3):*

The concentration of pure water is 55.6 M.

93% of plasma is H_2O.

The average volume of a human RBC is ~86 fL (30% Hb, maximally 70% H_2O).

Each adult body contains ~2×10^{13} RBCs.

We calculated:

The concentration of water in plasma ($[H_2O]_{plasma}$) = 55.6 M × 93% = 52 M

The concentration of water inside an RBC ($[H_2O]_{in}$)

$$= \frac{86\times10^{-15}\ L}{1\ RBC}\times(70\%)\times\frac{1000\ g}{1\ L}\times\frac{1\ mol}{18\ g\ \mathrm{H2O}} = \mathbf{17\ \ M}$$

The number of water molecules inside an RBC:

$$\frac{86\times10^{-15}\ L}{1\ RBC}\times(70\%)\times\frac{1000\ g}{1\ L}\times\frac{1\ mol}{18\ g\ \mathrm{H2O}}\times\frac{6\times10^{23}}{1\ mol} = \mathbf{2\times10^{12}\ \ water\ \ \boldsymbol{molecules}/_{\boldsymbol{RBC}}}$$

<1-ii> Know that 13 moles of CO_2 are produced by a healthy adult per day, which is equivalent to 9 mmols of CO_2 produced per minute.

As maximally 90% CO_2 conversion is carried out by CAII inside RBCs, and 1 H_2O molecule is required for 1 CO_2 conversion, ~8 mmols of H_2O are required for CO_2 conversion per minute per adult.

In each RBC, the number of H_2O molecules required for CO_2 conversion per minute:

$$\frac{\left(8\ \ mmol\ \ H2O/_{minute\,*\,adult}\right)\left(6\times10^{23} molecules/_{mol}\right)}{2\times10^{13} RBCs/_{adult}} = \frac{\boldsymbol{2.4\times10^{8}\ \ H2O\ \ molecules}}{\boldsymbol{1\ \ RBC * minute}}$$

(2) To compare the reaction rates of intracellular CO_2 conversion in systemic arteries versus in capillaries:

Consider the reaction taken place inside RBCs in systemic circulation:

$$\mathbf{CO_{2(g)} + H_2O \underset{K_r}{\overset{K_f}{\rightleftharpoons}} HCO_3^-{}_{(aq)} + H^+{}_{(aq)}}$$

Forward reaction rate (R_f) = K_f $[CO_2]_{in}$ $[H_2O]_{in}$, where K_f is the forward reaction coefficient.
Reverse reaction rate (R_r) = K_r $[CO_2]_{in}$ $[H_2O]_{in}$, where K_r is the reverse reaction coefficient.

$[CO_2]_{in}$ or $[CO_2]_{out}$ refers to $[CO_2]$ inside or outside an RBC.

The reaction coefficient (K) primarily accounts for the extremely rapid enzymatic activity of CAII. Below we assessed whether AQP1-band 3 coupling could affect the reaction rate, or whether this coupling contributes to K_f.

In the calculation below, we only consider the forward reaction, which takes place when RBC circulate from systemic arteries to capillaries.

***<2-i> Know that** (1-3):*

P_{CO_2} in artery at 37°C ~40 mmHg

P_{CO_2} in systemic capillaries at 37°C ~46 mmHg

For each mmHg of P_{CO_2} at 37°C, there are 0.03 mmols of CO_2 dissolved per liter of plasma.

Thus,

$$P_{CO_2\,(artery)} \text{ at } 37^oC \sim 40 \text{ mmHg} \times \frac{0.03\ mM\ CO2\ dissolved\ in\ plasma}{mmHg} = 1.2 \text{ mM } CO_2$$

$$P_{CO_2\,(systemic\ capillaries)} \text{ at } 37^oC \sim 46 \text{ mmHg} \times \frac{0.03\ mM\ CO2\ dissolved\ in\ plasma}{mmHg} = 1.38 \text{ mM } CO_2$$

<2-ii> Know that the capillary transit time for erythrocytes is 1 – 3.5 sec, and the half-life ($T_{1/2}$) for inward CO_2 diffusion (or CO_2 uptake by RBCs) is 45 – 65 msec (4, 5). As diffusion is driven by concentration gradients across the cell membrane, the rapid $T_{1/2}$ for CO_2 diffusion into RBCs allows

$[CO_2]$ to reach an equilibrium before RBCs leave systemic capillaries. ***Thus, for simplicity of the calculation, we assume that*** **$[CO_2]_{in} = [CO_2]_{out}$**, ***and that CO_2 enters or leaves RBCs only by diffusion.***

$[CO_2]_{out}$ = 1.38 mM (inside a systemic capillary) or 1.2 mM (inside an artery)

When RBCs are in systemic capillaries:

$$R_{f\,(capillary)} = K_{f\,(capillary)}\,[CO_2]_{out}\,[H_2O]_{in} = K_{f\,(capillary)}\,(1.38\text{ mM})\,(17^{-}\text{ M})$$

When RBCs are in an artery:

$$R_{f\,(artery)} = K_{f\,(artery)}\,[CO_2]_{out}\,[H_2O]_{in} = K_{f\,(artery)}\,(1.2\text{ mM})\,(17\text{ M})$$

As RBCs enter from systemic arteries to capillaries, the change of the reaction rate for intracellular CO_2 conversion is:

$$\frac{R_{f(capillary)}}{R_{f(artery)}} = \frac{K_{f(capillary)}\;(1.38\;mM)(<\;17\;M)}{K_{f(artery)}\;(1.2\;mM)(17\;M)} \approx \text{max } 1.15\;\frac{K_{f(capillary)}}{K_{f(artery)}}$$

That is, if K_f does not account for osmotically-driven AQP1-band 3 coupling, then $\frac{K_{f(capillary)}}{K_{f(artery)}} = 1$, and the reaction rate would increase maximally 15% when RBCs enter the systemic capillary bed.

If AQP1 and band 3 are structurally and functionally coupled in systemic capillaries but less coupled or uncoupled in arteries, this shall result in larger $K_{f\,(capillary)}$ than $K_{f\,(artery)}$. Thus,

$$\frac{R_{f(capillary)}}{R_{f(artery)}} > 1.15$$

That is, the reaction rate will increase more than 15% when RBCs enter the systemic capillary bed.

(3) To further consider restricted erythrocyte sizes in systemic capillaries:

If $[H_2O]_{in}$ decreases ~10% (e.g. 17M→ 15M) when RBCs squeeze through systemic capillaries,

If AQP1-band 3 coupling does not involve in the reaction ($\frac{K_{f(capillary)}}{K_{f(artery)}} = 1$),

$$\frac{R_{f(capillary)}}{R_{f(artery)}} \sim 1 \times \frac{(1.38\ mM)(15\ M)}{(1.2\ mM)(17\ M)} = 1.015$$

So there is no change in the reaction rates of CO_2 conversion before or after red cells entering systemic capillaries.

Also, noticeably in the above example, a 10% drop in $[H_2O]_{in}$ results in 14% rate reduction for CO_2 conversion when RBCs circulate in capillaries. Further decrease in $[H_2O]_{in}$ would make $R_{f(capillary)}$ smaller than $R_{f(artery)}$. On the other hand, if transient AQP1-band 3 coupling supports intraerythrocytic CO_2 conversion, $\frac{K_{f(capillary)}}{K_{f(artery)}} > 1$, which could compensate the negative effect of erythrocyte volume restriction (or $[H_2O]_{in}$ reduction) on the forward reaction rate.

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