FACILITATED TRANSPORT OF CO_2 ACROSS A MEMBRANE BEARING CARBONIC ANHYDRASE

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Received 23 January 1970

1. Introduction

We have already described some properties and some different mathematical aspects of the kinetics of membrane-bound enzymes [1–3]. Here, we show the mechanism of an interfacial enzymatic reaction resulting in facilitated metabolite diffusion through a membrane.

Several authors have reported that carbonic anhydrase facilitates CO_2 transport in a liquid phase [4–6]. The same effect is obtainable in the transfer of this gas through membrane-liquid interfaces giving a diffusion-reaction using bicarbonate as a transporter, especially when these interfaces become rate-controlling by concentration polarization. This is an example of the role of enzymes in lessening such an interface barrier.

The membranes used in our experiments were prepared by covering hydrophobic sheets (Silastic) on one or both faces with chemically combined, reticulated carbonic anhydrase. Neither liquid water nor ions (such as bicarbonate) can cross the hydrophobic film, but it is well known that gaseous CO_2 penetrates it quite easily.

2. Experimental

2.1. *Preparation of active films*

3 mg of purified carbonic anhydrase are dissolved in 2 ml of water. The enzyme is mixed to 1 ml of 2.5% glutaraldehyde solution in phosphate buffer 0.02 M, pH 6.8. The mixture is gently spread with a thin pipette on a "500-3 silastic" sheet (Dow-Corning). The reticulation of the proteins at 4°C lasts until the solvent is completely evaporated. The film is rinsed, in order to wash out glutaraldehyde and free enzyme molecules, until no more absorption of effluent water is observed at 280 nm.

The enzymatic activity of the membrane is tested according to already described procedures [7]. Blanks are made with membrane-bound inhibited enzymes.

2.2. *Determination of carbon dioxide transport*

The membrane was stretched between two 250 ml chambers.

Two different methods were used to measure CO_2 diffusion: the first used tracer $NaH^{14}CO_3$, which can be easily applied when the membrane is grafted with enzyme exclusively on the donor side. On this side, the chamber contained 0.025 M $NaHCO_3$ solution in 0.022 M, pH 7.35 veronal buffer. On the receiver side, it contained a 0.1 N NaOH solution in order to retain diffused CO_2. Radioactive bicarbonate was measured in a flow counter. This method is not applicable when the membrane is grafted with enzyme on both sides: the acceptor side would be too alkaline to allow full efficiency of the enzyme. Another technique using a pH-stat was preferred in that case, so that both chambers could be near the carbonic anhydrase optimum pH. They contained 0.01 M, pH 7.35 veronal buffer, and CO_2 diffusion was measured by the volume of NaOH 0.1 M added in order to keep the pH constant.

3. Results

When carbonic anhydrase is bound to the CO_2 *donor side* of the membrane, the CO_2 transport is increased 1.5 times as compared to ungrafted silicone in our experimental conditions. This factor reaches 2 when the enzyme is bound to *both sides*. The experimental results are summarized in fig. 1.

4. Discussion

Our aim is to illustrate briefly the mechanism of this facilitated transport and to underline its biological interest. The complete mathematical analysis will be described elsewhere.

Carbon dioxide transport in these systems is controlled by two different permeabilities:

- the permeability of the membrane, depending on its nature

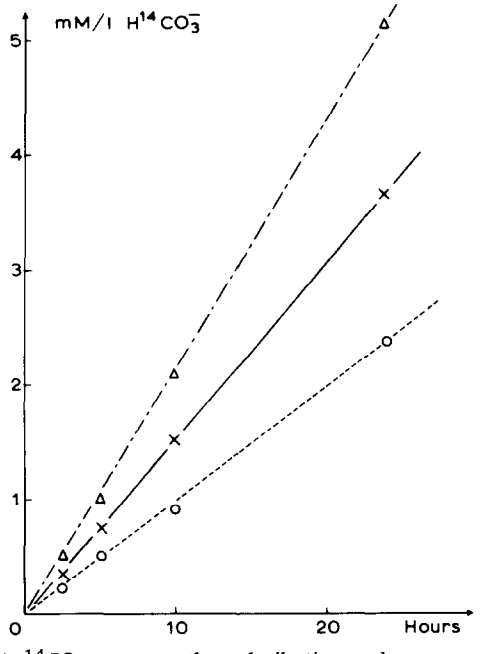


Fig. 1. $^{14}CO_2$ transport through silastic membrane.
- - - - - without enzyme
—— with one film reticulated carbonic anhydrase
-.-.-.-.- with a double film reticulated carbonic anhydrase

- the permeability of the boundary layers of the unstirred fluid on the donor and the acceptor side.

At equilibrium, the $(CO_3H^-)/(CO_2)$ ratio can be characterized by the Henderson-Hasselbach equation:

$$pH = pk + \log \frac{(CO_3H^-)}{(CO_2)}$$

But, when *equilibrium is destroyed locally* in the unstirred layer by departure of CO_2 through the membrane, three kinetic-processes appear; they are pictured in fig. 2.

1 - *CO_2 diffusion* from the stirred to the unstirred layer; then into the membrane; out of the membrane; into the second unstirred layer, then into the bulk solution.

2 - *Chemical decomposition* of HCO_3^- to replace diffused CO_2 (side 1) and transformation of CO_2 into bicarbonate ions (side 2).

3 - *Diffusion of HCO_3^-* from bulk solution to boundary layer to replace split HCO_3^- (side 1) and from boundary layer to bulk solution (side 2).

In each boundary layer, two different fluxes can be distinguished:

- bicarbonate fluxes J_{1C} and J_{2C} controlled by the two reactions $(CO_3H^- \rightarrow CO_2)$ in (1) and $(CO_2 \rightarrow CO_3H^-)$ in (2).

- carbon dioxide fluxes J_{1D} and J_{2D}, respectively on face (1) (donor) and (2) (receiver) of the membrane.

Inside the membrane, the resulting flux J (CO_2) is a sum of both preceding fluxes. At quasi-stationary state, one can write:

$$J_{1C} + J_{1D} = J(CO_2) = J_{2C} + J_{2D}$$

a) The *uncatalyzed* reversible reaction $CO_2 \rightleftharpoons HCO_3^-$ is rather slow [8]. If the membrane is very permeable to CO_2 molecules, one can practically neglect the J_{2C} reaction fluxes in the boundary layers. In these conditions, the only rate-determining steps of the overall CO_2 diffusion are the J_{1D} and J_{2D} CO_2 diffusions across these unstirred layers.

Actually, in absence of reactions, there can be no appreciable consumption or generation of bicarbonate near the membrane, so that (HCO_3^-) gradients will be small in these regions. Thus, J_{1C} and J_{2C} will be small, too.

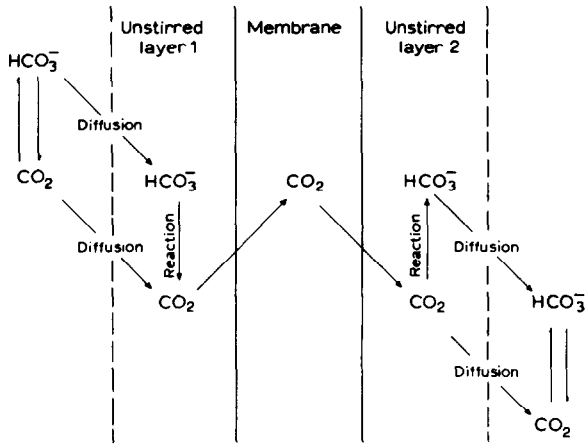
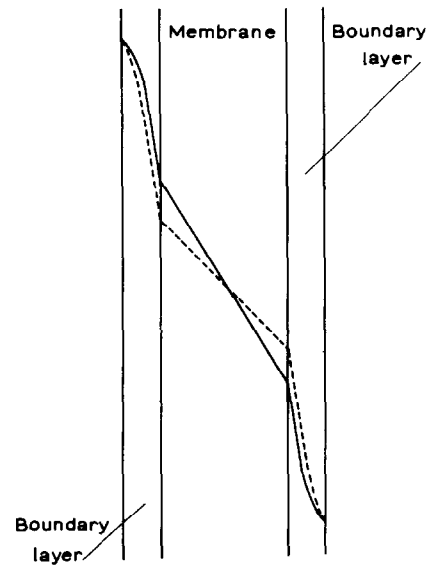


Fig. 2. Schematic mechanism of transport of dissolved CO_2 through a hydrophobic membrane.



Fi. 3. Partial pressure profiles of CO_2
—— in the presence of carbonic anhydrase
- - - - without enzyme

If CO_2 flows even more easily through the membrane than through the donor boundary layer, this layer will be *depleted*; if evacuation of CO_2 is slow from the receiving boundary layer, *accumulation* of CO_2 appears there. These two combined effects result

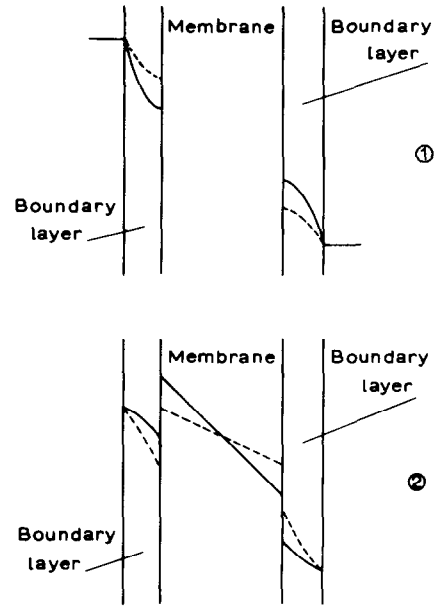


Fig. 4. Concentration profile of bicarbonate ions (1) and of CO_2 (2)
—— in the presence of carbonic anhydrase
- - - - without enzyme
(Concentration scale of bicarbonate ions (1) has been reduced).

in an important *concentration polarization* of CO_2, with a corresponding deformation of the concentration gradient inside the membrane and the boundary layers.

b) *Carbonic anhydrase* can increase several thousand times the conversion rate of $CO_2 \rightleftharpoons HCO_3^-$ (dehydration on the side 1 and hydration on the side 2). This effect steepens the HCO_3^- gradients in the boundary layers. A better feeding and evacuation of the membrane surfaces results from it. Simultaneously it increases the CO_2 concentration gradient inside the membrane.

The greater the $(HCO_3^-)/(CO_2)$ ratio at equilibrium, the more important are the increases of J_{1C} and J_{2C} bicarbonate fluxes due to the presence of the enzyme, and the more, the membrane linked anhydrase increases the total flux of CO_2 across this membrane. This effect is already appreciable at pH 7.35 where there are 10 times as many bicarbonate ions as CO_2 molecules at the temperature of our experiments (25°C). It increases when temperature rises.

Concentration profiles and partial pressure profiles are shown in quasi-stationary state conditions (figs. 3 and 4) for an uncatalysed diffusion (without enzyme) and a facilitated diffusion (with carbonic anhydrase).

The mathematical investigation of the concentration profile will be found in a later publication.

5. Conclusion

The described process is a facilitated CO_2 transport, but it is not actually an active transport, as long as no energy is spent to run up electrochemical-potential gradients. When the boundary layer is a limiting factor, the modification of the concentration profiles inside it can give rise to a facilitated transport. This is the case when the membrane is more permeable to the molecule than the surrounding liquid phase. This actually happens in numerous physiological and cytological systems.

Such processes may give rise to *selective transport mechanisms* when the boundary layer determines the flow of different molecular species across the membrane but where only one of them is metabolized. In this case, the modification of enzyme activities can regulate fluxes and selectivities by acting on facilitated transport.

The efficiency of such an enzyme located at the interfaces is greater than if distributed in the bulk of the liquid phase, for the described mechanism is far more active at the interface than inside the thoroughly mixed liquid phase.

References

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