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Absence of kinetic barrier for transfer of protons from aqueous phase to membrane-water interface

H P MAITY† and G KRISHNAMOORTHY*
*Chemical Physics Group, †Solid State Physics Group, Tata Institute of Fundamental Research, Homi Bhabha Road, Bombay 400 005, India

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Abstract. The kinetics of interfacial proton transfer reaction is an important factor in proton transport across membranes. The following experimental system was designed in order to measure this kinetics. Sonicated liposomes having the protonophore SF6847 was suspended in Tris buffer. Application of a temperature jump (in ~ 3 μs) caused a drop in the aqueous phase pH which was subsequently sensed by the membrane-bound SF6847. The kinetics of this interfacial proton transfer reaction was monitored on μs timescales. The estimated bimolecular rate constant of 2×10^{11} M^{-1} s^{-1} for this process show that there is no kinetic barrier for the transfer of protons from the aqueous phase to the membrane-water interface.

Keywords. Proton transport; proton transfer; temperature jump; membrane-water interface; chemiosmotic hypothesis.

1. Introduction

Transport of protons across membranes is intimately involved in many membrane-based energy transducing systems (Mitchell 1979; Nicholls and Ferguson 1992). The overall transport process could be visualized as comprising of at least three steps: (i) proton transfer from the bulk aqueous phase (on one side of the membrane) to the membrane water interface, (ii) translocation of the proton across the membrane and (iii) proton transfer from the membrane-water interface to the bulk aqueous phase (on the other side of the membrane). Although the second process, namely the translocation across the membrane, has been shown to be rate-limiting in many situations (see for example, Kasianowicz *et al* 1987; Prabhananda andUgrankar 1991) proton transfer reactions at the interface has considerable significance in many processes. Some of these processes are as follows: (i) protonation/deprotonation reactions of membrane proteins, (ii) some transport systems where proton transfer at the membrane-water interface has been suggested as the rate-limiting step (Kovbasnjuk *et al* 1991; Levitt and Decker 1988; Riddell *et al* 1988) and (iii) lateral proton conduction along the membrane-water interface (Gabriel *et al* 1991; Rochel *et al* 1990). The last process is relevant in the localized chemiosmotic hypothesis (Williams 1988) which requires the interfacial proton transfer reaction to be slower than the rate of proton movement along the interface. Although rapid diffusion of protons on membrane surface has been suggested in some studies (Gabriel *et al* 1991; Heberle and Dencher 1990; Scherrer *et al* 1994; Alexiew *et al* 1995) there are indications to the contrary also (Polle and Junge

* Corresponding author (Fax: 091-22-215-2181; Email: GK@TIFRVAX.TIFR.RES.IN).

1989; Gutman *et al* 1992). Thus it is apparent that the question of the presence of kinetic barrier for proton transfer at membrane-water interface remains unanswered. With this view, we have now measured in a direct experiment, the rate of proton transfer from bulk aqueous phase to a probe bound at the membrane-water interface. Our results show that there is no kinetic barrier for the transfer of protons from bulk to the membrane-water interface.

2. Materials and methods

Egg phosphatidyl choline was obtained from Sigma Chemical Company (USA). Soyabean phospholipid was purified according to Kagawa and Racker (1971). The protonophore SF6847 was synthesized according to Horiuchi *et al* (1971). Phospholipid vesicles were prepared by sonication as described earlier (Krishnamoorthy 1986).

Temperature-jump (T -jump) experiments were performed using a home-made instrument (Prabhananda 1977) as described earlier (Ahmed and Krishnamoorthy 1990). T-jump experimental details are given in figure legends. The pK of SF6487 in the membrane phase was determined from pH titration of optical absorption spectrum (Ahmed and Krishnamoorthy 1990). The value obtained was 6·8 in the case of egg phosphatidyl choline vesicles.

3. Results and discussion

The experimental design is shown in figure 1. Unilamellar vesicles having the protonophore SF6847 was suspended in Tris (tris hydroxymethylaminomethane)

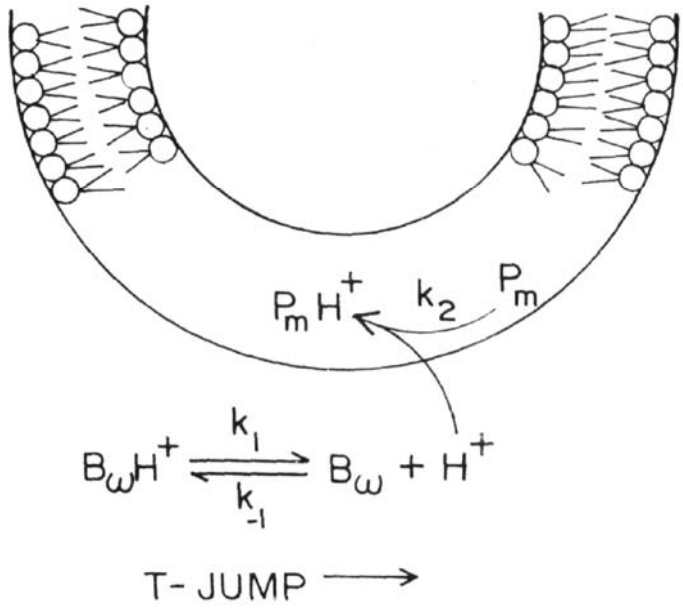

Figure 1. Scheme for T -jump induced increase in the concentration of bulk phase protons and subsequent interfacial reaction with the proton transporter, SF6847 bound to the membrane (P_m). $B_w H_+$ represents protonated Tris buffer.

buffer. SF6847 (3,5-di-tert-butyl-4-hydroxybenzylidenemalanonitrile) is a very efficient protonophore which transports protons by a carrier mechanism (Krishnamoorthy 1986; Terada 1981; Krishnamoorthy and Ahmed 1992). The concentration of the vesicle and SF6847 were adjusted such that the membrane-water partition of SF6847 was largely (> 90%) in favour of the membrane phase (Krishnamoorthy 1988; Ahmed and Krishnamoorthy 1990)· Application of a T- jump (~ 5°C) generated a pH jump to a lower value in the aqueous phase with a time constant of 2·5 μs (Krishnamoorthy 1986). The pH jump is due to the large negative temperature coefficient of pK of Tris buffer (– 0·031/°C). The drop in bulk phase pH is then sensed by SF6847 located in the membrane. Although the exact location of membrane bound probes is still unclear (Das *et al* 1993) it is very likely that protonation of SF6847 involves interfacial proton transfer reaction. Since the pH of membrane-bound SF6847 is 6·8 (see §2) the rapid protonation event could be monitored via changes in the pH dependent absorption spectrum of SF6847.

Figure 2A shows some typical results of experiments described above. Following the application of T-jump, the transmittance (at 465 nm) increased in an exponential manner with a time constant (τ) of ~ 19 μs. This increase in transmittance could be ascribed to a decrease in the concentration of the deprotonated form of SF6847 which has absorption maximum at 465 nm. That the increase in the transmittance is due to a decrease in the concentration of the deprotonated SF6847 caused by a drop in the bulk pH and not by any other process was shown in the following way. Replacement of Tris buffer in the medium by phosphate buffer caused a very significant reduction in the amplitude of the relaxation process (figure 2B). Phosphate buffer has a substantially smaller temperature coefficient of pK (–0·003/°C) and hence the T-jump induced pH jump will be reduced by a factor of 10 when compared to Tris buffer. The proton transfer reactions could be represented as

$$B_w H^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B_w + H^+ \tag{1}$$

$$P_m + H^+ \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} P_m H^+, \tag{2}$$

where B_w and P_m refer to deprotonated forms of the Tris buffer (in the aqueous phase) and SF6847 (in the membrane phase) respectively· The first reaction is a homogeneous one and is coupled to the second reaction which represents the proton transfer at the membrane-water interface. The T jump causes a larger perturbation of the first equilibrium resulting in increased concentration of H^+. By assuming (see latter for justification) that this reaction equilibrates faster than the second, we can derive (Bernasconi 1976) the following expression for the exponential time constant τ associated with the shift in the second equilibrium,

$$\frac{1}{\tau} = k_2 [H^+] + \frac{k_2 [P_m](K_1 + [H^+])}{K_1 + [H^+] + [B_w]} + k_{-2}, \tag{3}$$

where $K_1 = k_1/k_{-1}$. The relaxation traces (figure 2A) were analysed according to the

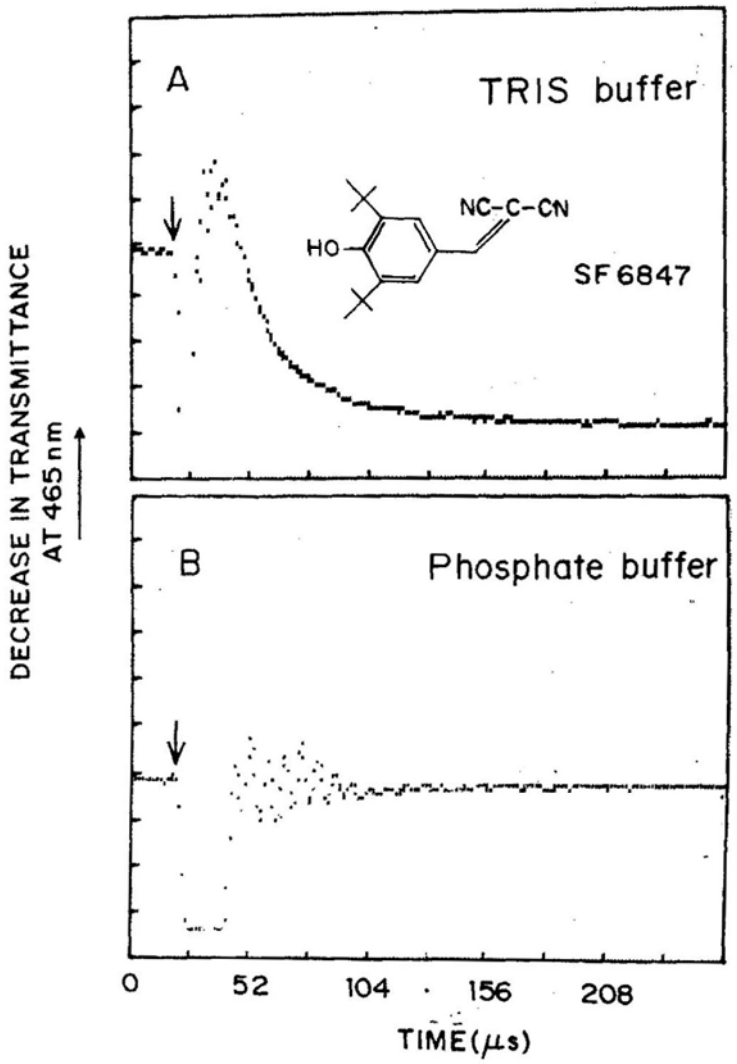

Figure 2. Typical traces of changes in transmittance at 465 nm following T-jump in a vesicle suspension having SF6847. Egg phosphatidyl choline vesicles were suspended in 150 mM KCl, 30 mM buffer, pH 7·5 to a final lipid concentration of 2 mg/ml· The suspension had 15 μM of SF6847· The buffer was Tris in **(A)** and KH_2PO_4 in (**B**). The arrows mark the time of application of T jump (~ 5°C) by discharging 17 kV through the sample. The initial oscillations in the signal are probably due to cavitation effects. The heating time constant was ~ 2·5 μβ and the instrument response time constant was 0·2 μs.The measured relaxation time constant (A) is 19 μs. Each trace is an average of five experiments.

above equation using the values of pK of bound SF6847 and Tris to get the bimolecular rate constant k_2 associated with the binding of protons to the membrane-bound SF6847. The analysis gives a value of 2×10^{11} M^{-1} s^{-1} for k_2. Similar results were obtained in the case of soybean phospholipid vesicles also (data not shown). This value is in the range of the rate constant associated with

diffusion controlled processes in aqueous solutions (k~$1\times10^{10}M^{-1}s^{-1}$ –2×10^{11} M^{-1} s^{-1}) (Hague 1971). Since the rate of interfacial proton transfer reaction obtained here is also diffusion controlled, one could question the validity of the assumption of faster equilibration of reaction (1) which is also diffusion controlled, in deriving the equation (3). However, in our experimental system $[B_w] \simeq 1000\ [P_m]$ and hence our assumption that the reaction (1) would equilibrate faster than reaction (2) would be justified.

What is the implication of k_2 having the value in the range of diffusion-controlled reaction rate constants? The answer is that this shows clearly the absence of any kinetic barrier for the transport of protons from the bulk aqueous phase to membrane-water interface· Similar result has been obtained by Kotlyar *et al* (1994) in mitochondrial membranes using laser pulse-generated pH jumps· However, our experimental system produces step perturbation in pH [unlike the laser pulse method (Kotlyar *et al* 1994) which generates pH pulses] which makes the mathematical analysis less complicated and hence more reliable·

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