Volume 229, number 2, 313–316 FEB 05663 March 1988

Counterions and the bacteriorhodopsin proton pump

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Received 11 January 1988

Theoretical and new experimental arguments are given to explain the reversal of photoelectric signals from purple membranes oriented and immobilized in gel due to the presence of TEMED. The continuous current induced by continuous illumination demonstrates a photoelement-like behaviour, the polarity of which is reversed by TEMED. The data render the counterion-collapse mechanism highly questionable.

Bacteriorhodopsin; Proton pump; (Purple membrane)

1. INTRODUCTION

The molecular mechanism of the light driven proton pump of bacteriorhodopsin (bR) from *Halobacterium halobium* [1] is not yet known in detail. Pertinent data obtained by different methods on the photocycle of bR are considered as important contributions towards understanding it.

Electric signals associated with the photocycle provide information about charge motion inside the protein [2]. In a recent paper we report the effect of diamines on the electric signal [3]. The effect of *N,N,N',N'*-tetramethylethylenediamine (TEMED) was followed in detail and it was found that the positive area of the electric signal (which is considered to be proportional to the number of translocated protons) turns into an equal but negative area at a TEMED/bR concentration ratio of ~1 at pH 6.9. The negative area can be abolished by increasing the TEMED concentration or by adding salt. The phenomenon has been interpreted as a reversal of the direction of the bR proton pump.

An alternative interpretation of the sign reversal of the electric signal was given recently by Marinetti [4]. According to this interpretation the protons released into the aqueous phase are picked up by TEMED molecules, resulting in a collapse of the counterion atmosphere near the highly negatively charged purple membrane (pm) surface. The shift of the first moment of the counterion distribution is calculated to be in the opposite direction to the proton pumping thus engendering a negative electric signal. Effects of TEMED and salt concentration changes as observed in [3] are also in accord with the calculations.

In this paper we present theoretical arguments and new experimental results to demonstrate that this alternative interpretation [4] is highly questionable.

2. THEORETICAL ARGUMENTS

The backward charge displacement derived from the calculations considering the 'counterion collapse' cannot account for the negative area of the displacement current measured by Tóth-Boconádi et al. [3]. The protons passing through the pm make a charge displacement of $d = +5$ nm (the thickness of the membrane [1]). If they continue in the forward direction, as assumed by Marinetti [4], the charge displacement arising from counterion collapse should be -10 nm in order to obtain the observed value $d = -5$ nm. The result of the calculation is only $d = -3.5$ nm, however.

The relaxation of the counterion collapse is left unmentioned in Marinetti's paper. Following his ideas one would expect a charge displacement due

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Published by Elsevier Science Publishers B.V. (Biomedical Division)

to this process in the direction of the proton extrusion. Such a phenomenon has not been observed in the kinetic measurements of Tóth-Boconádi et al. [3]. One could argue that the relaxation process is so slow that it does not produce signals with measurable amplitudes. It is known from the theoretical description of the displacement current signal (e.g. [5]) that amplitude is proportional to the rate constant $k = 1/\tau$ of the process. From the data in [3] one can estimate that a positive amplitude of 10% of the long living negative component could remain undetected which means that the time constant of the relaxation of the counterions should be >100 ms. This long process is, however, rather improbable and the new experiment reported in this paper (sections 3 and 4) excludes even this possibility.

An acceptable model must explain the existence of two negative components with an experimentally determined area ratio of 1:3 [3]. It is difficult to see how the counterion collapse mechanism could account for these facts.

3. MATERIALS AND METHODS

The pms used were obtained from *H. halobium* strain ET 1001 using standard procedures [6]. The concentration of bR was measured spectroscopically at 570 nm using an extinction coefficient of 6.3×10^4 $mol^{-1} \cdot l \cdot cm^{-1}$.

The measurements reported in [3] on pms oriented and immobilized in gel were repeated on pms oriented in suspension. The method is described in detail in [5]. The advantage of the suspension method is that the appearance of an effect of TEMED addition is prompt, i.e., without the complication caused by its diffusion into the gel as reported in [3].

Gel samples prepared as in [3] were used for measuring the continuous current generated by continuous illumination. The light source was a 250 W tungsten lamp (Carl Zeiss, Jena) filtered by a glass filter transmitting above 550 nm. The irradiance after the filter was 3×10^{-2} $W \cdot cm^{-2}$. The photocurrent was measured by a Keithley 417 picoampere meter with internal resistances of 10^6 Ω and 10^4 Ω for measuring in the s and ms range, respectively. Because the capacitance of the platinized electrodes was $\sim 10^{-3}$ F, the time constants were 10^3 and 10 s, respectively.

4. RESULTS AND DISCUSSION

Electric signals associated with the photocycle of bR were recorded with a 360 μM bR suspension for a series of TEMED concentrations. In fig.1 the results obtained without and with a TEMED concentration of 400 μM are shown. In the absence of TEMED the usual electric signals are observed [5], showing positive long living components, while in the presence of TEMED the long living components are negative. The areas of the signals versus TEMED concentration are given in fig.2. The positive area turns to an equal but negative one at a TEMED/bR ratio of ~1.2, however, at higher TEMED concentrations the original positive area is reestablished, as was observed with gel samples [3].

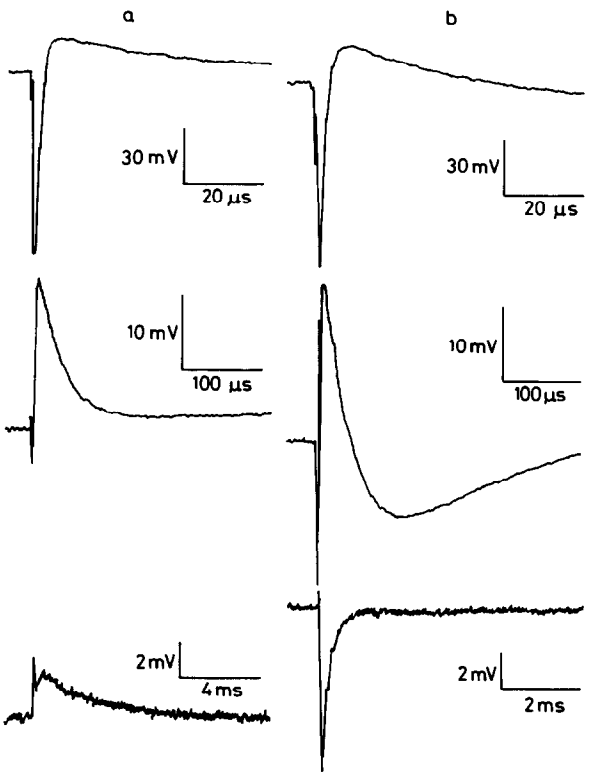

Fig.1. Electric response signal of pms oriented in suspension. bR concentration, 360 μM. (a) Untreated pms, pH 6.5; (b) TEMED concentration 400 μM, pH 6.9. Room temperature.

The data show a reversal of the electric signal also at 400–450 μM TEMED which is seemingly beyond the applicable range of the counterion-collapse mechanism (see table 2 of [4] where a negligible effect is already present at 300 μM TEMED). These data show that the TEMED effect is determined by the ratio of TEMED to bR concentrations and not by the absolute concentration of TEMED in the solution as was assumed in [4].

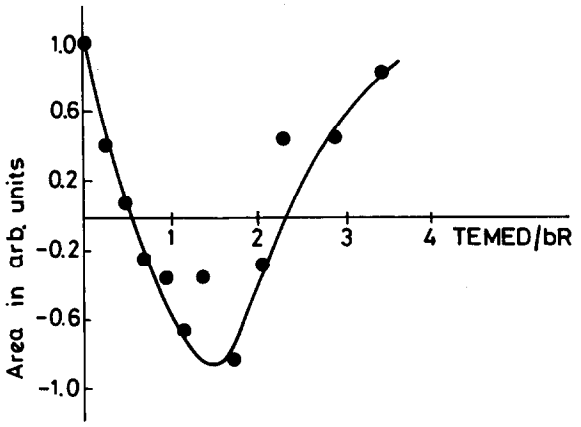

Fig.2. Dependence of the area of the electric signal on TEMED concentration. Data normalized to zero TEMED concentration. pH range 6.5–8. Room temperature.

Fig.3 shows that the continuous photocurrent measured on pms oriented and immobilized in gel under continuous illumination is quantitatively reversed in the presence of TEMED. The figure also contains the response to flash excitation in order to demonstrate that the orientation of the gel was the same in both cases: the first negative signal, which corresponds to the *trans-cis* isomerization [5], does not change direction, only the long living components corresponding to the long paths of proton migration in bR.

The time-resolved rise and decay of the continuous photocurrent are shown in fig.4. A multiexponential fit to the decay part of the curves

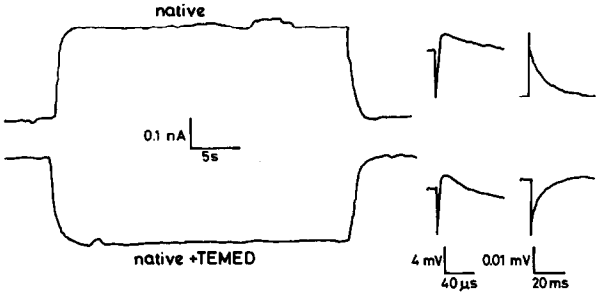

Fig.3. Continuous current during continuous illumination of pms oriented and immobilized in gel. The direction of the current is reversed in the presence of TEMED. 360 μM bR, 250 μM TEMED, pH 7, at room temperature. The transient signals are also shown in two different time and amplitude ranges.

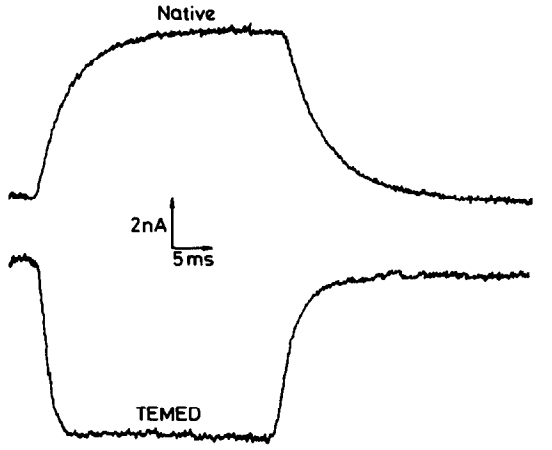

Fig.4. Time-resolved rise and decay of the continuous current. The light (λ > 550 nm from tungsten lamp, irradiance 0.5 W/cm^2) was chopped by a fast shutter (opening time, 0.8 ms), the signals were amplified by a home-made current amplifier and registered by a transient recorder. 32 repetitions.

yielded the same time constants as those characteristic of the slowest components of the photoelectric signal measured by flash excitation, both in native and in the TEMED-treated sample (2.5 ms, 7.1 ms and 1.1 ms, 4.7 ms, respectively). The rise of the stationary signal, however, is slightly faster than its decay, because of the excitation process.

These results exclude the possibility of an unseen long living relaxation of the counterion collapse, because in this case the negative TEMED signal could not be oppositely equal to the positive one in the absence of TEMED. Figs 3 and 4 also demonstrate that the displacement current caused by light on bR can be taken as a photoelement, the direction of which is reversed by adding TEMED in a concentration of ~1 TEMED/bR.

5. CONCLUSION

Given the above theoretical and experimental evidence we feel that the 'counterion-collapse mechanism', although an attractive explanation of the experimental findings of Tóth-Boconádi et al. [3] and may save the unidirectionality of the bR proton pump, is not acceptable. The imperative for keeping the original explanation, the reversal

of the proton pump, is now even stronger because of the photoelement nature of the illuminated bR demonstrated by data shown in figs 3 and 4.

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