Orientation of purple membrane in combined electric and magnetic fields

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Abstract The orientation of purple membrane in gels for photoelectric measurements is relatively poor, when they are prepared with the standard technique of applying a DC electric field and rapid polymerization. We have improved it by adding a high magnetic field (17.5 T) and increasing the viscosity of the membrane suspension. This process has resulted so far in a 3-fold increase of the photoelectric signals obtained. The magnetic susceptibility of purple membrane was determined.

Key words: Bacteriorhodopsin; Orientation; Electric field; Magnetic field; Electrooptics; Photoelectric signal; Magnetic susceptibility

1. Introduction

Bacteriorhodopsin (bR) is the simplest proton pump known in biological systems and one of the best characterized membrane proteins [1]. It is obtained in the form of membrane fragments, the purple membrane (pm), which contains bR as the only protein in a two-dimensional crystalline lattice. Most information about intramolecular processes during bR's cyclic photoreaction, the photocycle, which translocates the proton across the membrane, has been obtained by timeresolved absorption and vibrational spectroscopy [2]. Important information about the proton pumping process can also be gained by photoelectric measuring techniques [3,4]. Both methods are based on the creation of an asymmetry in originally random suspensions of pm using an orienting electric field. The electric asymmetry of the sample can be fixed by gel formation [4]. The gel technique has the advantage that the resulting preparations are very stable, the medium can be changed repeatedly, and simultaneous electrical and optical measurements are easily accomplished [5]. The sustained application of an electric field necessary during polymerization, however, decreases orientation of pm because of electrophoretic disordering effects [6]. The poor orientation becomes a serious problem, when photoelectric signals of bR must be measured under extreme conditions, such as very high ionic strengths. During an investigation on the specificity of ion transport by bR we had to work at molar salt conditions near pH 0 and experienced great difficulties in measuring the slow components of the bR photoelectric signals, which were crucial for our arguments [7].

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Abbreviations: bR, bacteriorhodopsin; pm, purple membrane; TEMED, tetrametyl-etylenediamine; α, electric polarizability; χ, magnetic susceptibility; E, electric field strength; H, magnetic field strength; n, refractive index; ELMA, method for orientation of membrane fragments by a combined use of electric and magnetic fields.

To continue the work, we had to improve the technique and have tried a combination of electric and magnetic fields. Magnetic field also induces a parallel ordering of the membrane sheets [8], which can be maintained nearly perfectly during polymerization [9]. However, since the magnetic dipole in contrast to the electric is an induced dipole, the asymmetric membranes are randomly facing in opposite directions. We shall use the term alignment for this type of order. If, however, an orienting electric field acts on the membrane sheets, subsequent application of a high magnetic field should considerably improve the degree of orientation, and, afford larger photoelectric signals and a better signal-to-noise ratio.

2. Materials and methods

Purple membrane-containing wild-type bR was prepared from *Halobacterium salinarium* strain ET 1001 using standard procedures [10]. The average diameter (0.6 μm) of the membrane fragments was determined by electron microscopy. Suspensions containing 50 mM pm, 15% acrylamide, 1% tetramethyl-etylenediamine (TEMED) and 0.3% ammonium persulfate were filled into cuvettes of 20 × 15 × 10 mm containing platinized Pt electrodes. In some cases 1% agarose, cooled down from 30°C to 10°C for gel formation, was used instead. An electric field of 20 V/cm was applied for preorientation. A magnet with a magnetic field of up to 17.5 T (MPI High Magnet Laboratory in Grenoble) was used to increase the orientation.

Magnetic birefringence [11] and photoelectric measurements [4] were used to check the alignment of pm and improvement in the orientation, respectively. While electric signals are proportional to the orientation of pm, magnetic birefringence measures the degree of alignment of pm by the magnetic field. As has already been recognized, the theoretical formulas describing magnetic birefringence can be directly adapted from those of electric birefringence when the alignment in the electric field is entirely due to and induced dipole mechanism [11]. Substituting the electric polarizability, α, with the magnetic susceptibility, χ, and the electric field strength, E, with the magnetic field strength, H, in the expression derived by O'Konski, and using a low-field-strength approximation according to Shah [12], the normalized birefringence can be expressed by neglecting the higher-order terms, as follows:

$$\Delta n/\Delta n_{sat} = (2/15) \times (\chi_1 - \chi_2) \times H^2/2kT + ... \quad (1),$$

where indices 1 and 2 refer to the directions parallel with and perpendicular to the symmetry axis of the disk-shaped membranes.

3. Results and discussion

Since it is known, that the alignment of pm in the magnetic field depends on the size of the fragments and their concentration [8], we first checked alignment as a function of magnetic field strength with our preparation at a concentration from which we would expect a good electric signal with the gel technique. The measuring cell with the membrane suspension was introduced into the magnet, the field switched on and slowly increased to 17.5 T. The in situ birefringence measurement shows that, in the case of wild-type pm, saturation was

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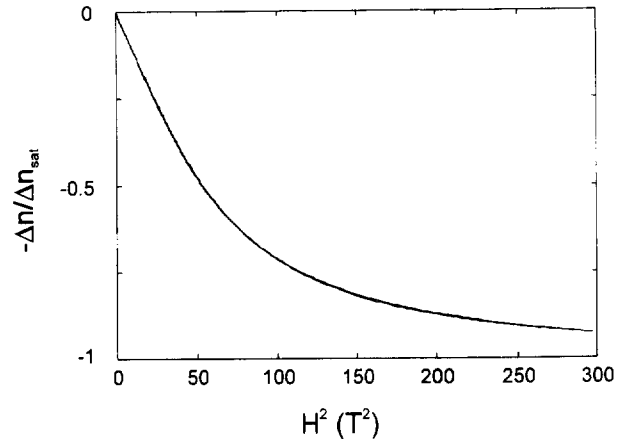

Fig. 1. Normalized magnetic birefringence of pm suspension (OD_{575} = 1.6, pH = 7, distilled water) as a function of field strength squared (H^2). The field strength was increased from 0 to 17.5 T and then returned to 0 with a constant speed of 0.1 T/s.

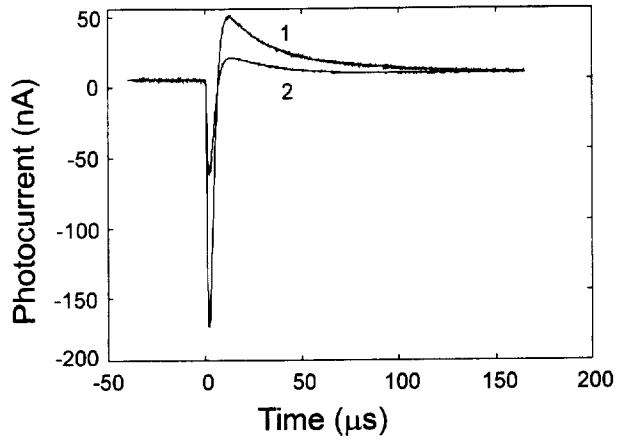

Fig. 2. Comparison of photocurrents from bR-containing gels prepared by the ELMA-technique (1) and the standard technique (2).

approached with the maximal available field (Fig. 1). This fact gave us the chance to derive a characteristic magnetic susceptibility value of pm, determined from the initial slope of the curve according to (1): $\chi_1 - \chi_2 = 7.41 \times 10^{-22}$ J/T^2. When calculating with the average membrane size, this susceptibility corresponds to an average molar value (per mol bR) of 1.68×10^{-2} J/(mol × T^2), which reasonably supports previous results (1.2×10^{-2} J/(mol × T^2)) [8], taking into account the effect of membrane size distribution [6].

In the second type of experiments the pm was oriented with the combination of electric and magnetic fields ('ELMA' method). The time for polymerization was adjusted to be approximately 4 minutes. This time interval was devided into three periods. In Period 1, a 20 V/cm electric field was switched on, in Period 2 the sample was introduced fast into the 17.5 T field, and in Period 3 the electric field was switched off and the magnetic field alone was acting on the polymerizing membrane suspension. The gels showed the same birefringence, as that was measured in suspension, indicating that the alignment of pm in the gel was close to perfect. However, the photoelectric signal compared to gels oriented only by electric fields as a control, indicated that orientation of the membrane fragments increased only by 30%. We attributed this result to a flip-flop effect, i.e. random reorientation of the membrane fragments by rotational diffusion during Period 3. Since, according to the Debye theory, the rotational correlation time linearly increases with the viscosity of the medium, we added Ficoll 400 (to a final concentration of 5%) to the membrane suspension, or used an ultra-low-melting-point agarose gel instead of polyacrylamide in a next set of experiments. The time schedule of the orientation was adjusted to 20 s, 10 s and 40 s for periods 1, 2 and 3, respectively, and the polymerization time was decreased to 70 s. Photoelectric signals of pm in these gel samples gave a 3-fold-increased amplitude, as compared to the ones prepared by the 'traditional' method (water suspension, electric field only) as it is shown in Fig. 2. This increase constitutes a considerable improvement for the kinetic measurement of photoelectric signals of bR, in cases where the basic limitation is the poor signal-to-noise ratio.

4. Conclusions

It is established that the combined application of electric and magnetic fields is a feasible approach to obtain improved oritentation of membranes for photoelectric measurements, and extends the application range of the gel method. It has also facilitated the development of a novel method, which allows recording of intramolecular charge motions accompanying the bR photocycle in all the three space dimensions [13]. However, optimization of the technique and the application of still higher magnetic fields will be necessary to realize the full potential of this method.

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