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Light-scattering changes in the bacteriorhodopsin photocycle

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Light-scattering changes were found to accompany the bacteriorhodopsin photocycle in a suspension of purple membranes. A light-scattering signal comprises three phases, connected with (i) bR → M transition, (ii) transition of M to P intermediate (other names: N,R350 intermediate), (iii) P → bR transition. Light-scattering changes seem to result from changes in the purple membrane shape in response to the bacteriorhodopsin conformational changes during the photocycle.

Bacteriorhodopsin; Light scattering; Proton translocation; Purple membrane; (*Holobacterium halobium*)

1. INTRODUCTION

The M intermediate of the light-adapted bacteriorhodopsin photocycle was shown to pass into P (N, R350) intermediate relaxing to the initial bR form [1,2]. P intermediate formation is coupled to Schiff base reprotonation by a proton accepted from an inner proton-donor group, followed by subsequent proton re-uptake from the aqueous phase. In addition, in our experiments dealing with the P intermediate, the distance between the cuvette and the photomultiplier tube was found to affect the optical response of the sample. This effect accounted for a discrepancy between the difference spectra of P intermediate in [1] and [2]. We defined three phases in the light-scattering signal related to the bR photocycle. In our opinion, this may be due to changes in the purple membrane shape as a result of simultaneous protein conformational changes in the course of the photocycle.

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2. MATERIALS AND METHODS

Purple membranes were isolated from *Halobacterium halobium* ET1001 according to the standard procedure [3]. Flash-induced light-scattering signals in the light-adapted purple membrane suspension were recorded at a fixed wavelength with conventional flash photolysis apparatus supplied with two PM photocathodes of different size. The PM photocathode was located 50 mm from the sample. The diaphragm diameters were 24 and 5 mm. The light-scattering signal was obtained as a difference between the photoinduced optical changes measured at these diaphragm dimensions. The light-scattering signal amounted to about 10–20% of the whole absorbance changes at 335 nm. For photocycle excitation, a Quantel YG-481 ND-YAG Q-switched laser with frequency doublers (λ = 532 nm, $t_{1/2}$ = 15 ns, 10 mJ) was employed. Triton X-100 'Serva' and OSCh grade salts were used.

3. RESULTS AND DISCUSSION

Fig.1 shows 400 and 335 nm optical response transient kinetics and light-scattering signals of purple membrane at neutral pH (A and A′), at neutral pH in the presence of low Triton X-100 concentrations (B and B′) and at high pH (C and C′). According to our previous reports [2,3], the 335 and 400 nm wavelengths are of particular importance for measuring the photocycle events. At 400 nm, the M formation-decomposition is record-

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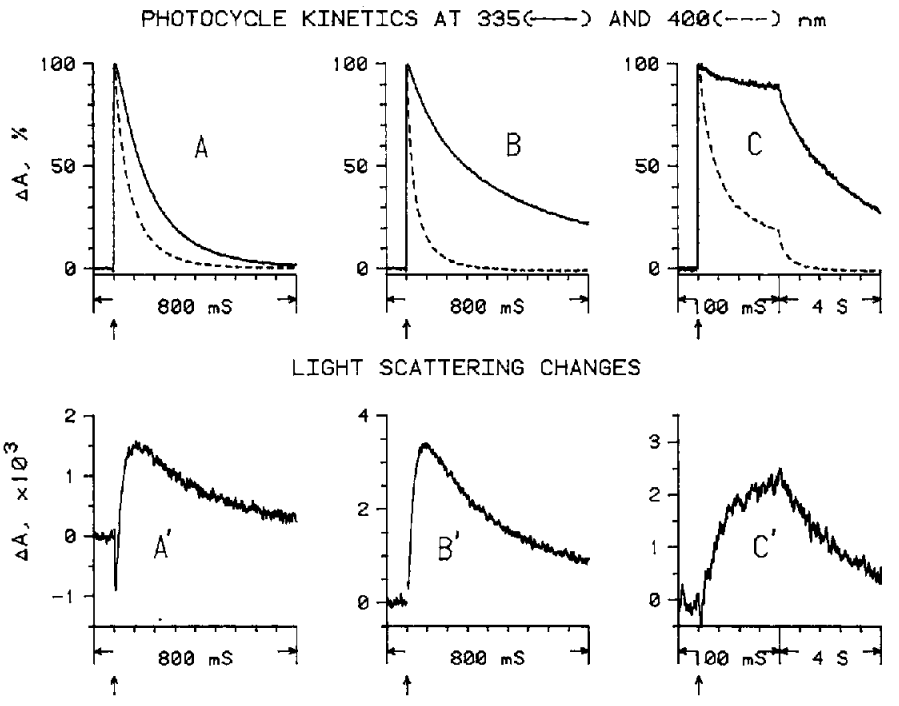

Fig.1. Kinetics comparison of the laser-induced optical changes at 400 (---) and 335 nm (——) in the purple membrane suspension (A,B,C) with the light-scattering changes (A',B',C'). The vertical arrow indicates the moment of the laser flash. The sample contained: 12 μM bacteriorhodopsin; (A and B) 1 M NaCl, 50 mM phosphate buffer, pH 7; (B) the sample is supplemented with 0.015% Triton X-100; (C) 50 mM K^+-borate-phosphate buffer, pH 10.2. Everywhere, t = 4°C. In (A,B,C) the light-scattering contribution is reduced to minimum.

ed. The 335 nm wavelength, being characteristic of the β-band in the 13-*cis* bR absorption spectrum, was used to measure the decay of the P intermediate.

At neutral pH the light-scattering signal of the purple membrane suspension displays a complicated three-phasic character. The signal kinetics does not depend on the measuring wavelengths. The first phase (fig.1A') is attributed to the light-scattering decrease. This phase includes a component with a rate identical with that of M formation (not shown). The second phase of larger amplitude has the opposite direction (light-scattering increased), and its rate approaches both the fast component in the M intermediate relaxation process and P intermediate formation at this temperature ($\tau_{1/2}$ = 20–40 ms). The final third phase is a backward reaction towards the zero level. Its one-exponent fitting appears 1.5–2 times slower ($\tau_{1/2}$ = 250–350 ms) than the P intermediate relaxation.

There is striking similarity between (i) the two phases of the final light-scattering signal and (ii) the formation-relaxation of the P intermediate, when conditions are favourable for P intermediate appearance, i.e. at high pH or at neutral pH in the presence of low Triton X-100 concentrations. Triton X-100 (fig.1B) accelerates the 400 nm relaxation process and slows down relaxation at 335 nm, thus accelerating M intermediate decay and producing a stabilizing effect on the P intermediate. Under these conditions, the second phase of the light-scattering signal (fig.1B) grows in amplitude and accelerates to 10–20 ms. The relaxation phase appears in good agreement with the decay process at 335 nm, i.e., the P intermediate decomposition and recovery of the initial form. In this case the first phase connected with the M formation is not detectable. At high pH (fig.1C and C'), the scattering increase phase also follows the fast M decomposition, while the relaxation phase – the decay of P intermediate.

The light-scattering signals disappear after careful sonication of purple membranes or when the membranes are solubilized with Triton X-100. Amplitude of the second phase is considerably reduced, if purple membranes are treated with the proteolytic enzyme papain (not shown). This treatment produced no significant effect either on the photocycle kinetics, or on the proton translocating ability of bacteriorhodopsin.

We assume that the most probable reason for the light-scattering changes in the photocycle is some change in the membrane shape. One may speculate that the shape transitions may be somehow related to the potential generation during the photocycle, appearing in the same time scale. However, the electric phases concerned with the M formation and M→P transition are unidirectional (see [4]), while the respective light-scattering phases are opposite in sign. Additionally, we observed that the buffer concentration does not affect the light-scattering signal, rather indicating that the latter is indifferent to the local pH changes in the purple membrane-water interface [4]. More probably the shape transition of purple membranes may be accounted for by the bR conformational changes during the photocycle (see also [5–10]). The most pronounced phase in the light-scattering signal is responsible for the M→P transition, especially when the experimental conditions favour P accumulation. This process, though closely related to Schiff base protonation, also includes the bR conformational transition involving the water exposed part of the protein.

In this connection, it is worth mentioning that the involvement of bR conformational changes during the photocycle was assumed on the basis of experiments, where the M decay (i.e. M→P transition) was retarded by the increase of viscosity [11], hydrostatic pressure [12,13] or by different cross-linking reagents [5,14] and metal cations [14,15]. The latter may produce pH-dependent complexes with the amino acid residues. Such complexes stabilize the protein structure and prevent conformational transitions [15].

NOTE ADDED IN PROOF

When the article was already prepared for publication we became aware about the results obtained by J. Czégé on the light-scattering changes in the bR photocycle and its relation to the purple membrane bending [Acta Biochim. Biophys. Hung. (1987) 22, 463–478].

However, relation of these changes to P intermediate formation-decomposition was not studied.

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