INTERACTION OF IONOPHORES WITH BACTERIORHODOPSIN

A flash photometric study

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1. Introduction

Bacteriorhodopsin (bR) is organized in the cell membrane of *Halobacterium halobium* in patches (PM, purple membrane) [1] which can be isolated and studied in aqueous suspensions [2]. Illumination of a PM suspension initiates a photocycle which is associated with cyclic proton release and uptake [3]. The photocycle can be described by the scheme:

$$bR^{570} \rightarrow K^{590} \rightarrow L^{550} \rightarrow M^{412}\ (O^{660})$$

where the numbers refer to the approximate wavelength maxima of the transients [4]. In a conventional flash photolysis system, as in [5], the absorption band of bR^{570} centered at 570 nm, is temporarily bleached, while A_{412} and A_{660} max are observed. The appearance of the proton in the bulk phase is related to the formation of M^{412}.

If the rate of decay of M^{412} is slowed down it is also possible to study the interrelation between the photocycle and the protoncycle in the steady state, using continuous illumination of moderate light intensity. M^{412} was stabilized [6] by suspending PM in 4 M NaCl saturated with diethylether. Based on the observation [5] that high concentrations of valinomycin slow down the decay of M^{412}, also various ionophoric compounds were tried in order to increase in a controlled manner the steady state concentration of M^{412} [7,8]. It was found that the combined use of two ionophores, beauvericin (BV) and valinomycin (VL), cause a remarkable increment in the steady state concentration of M^{412} and in the light-induced acidification, compared to the effects obtained with VL alone. Many other ionophores which were tried proved to be ineffective and also BV alone had practically no effect. In this work an attempt was made to study by flash photolysis the mechanism by which the ionophores perturb the photocycle and to locate the step(s) at which BV exerts its effect.

2. Experimental

2.1. *Materials*

Valinomycin was a product of Sigma Chemical Co., St Louis, MO; beauvericin was a gift of Eli Lilly and Co., Indianapolis, IN.

2.2. *Methods*

For growth of *H. halobium* and isolation of PM the method in [2] was used with slight modifications. Flash photometric techniques were used, as in [5]. Decay profiles of 412 nm and recovery of the ground state chromatophore at 570 nm were analyzed and the half-life times for these parameters were calculated. Plots exhibiting biphasicity were analyzed by resolving into first-order exponentials graphically by the curve pealing method [5].

3. Results and discussion

To study the effect of the interaction of the iono-

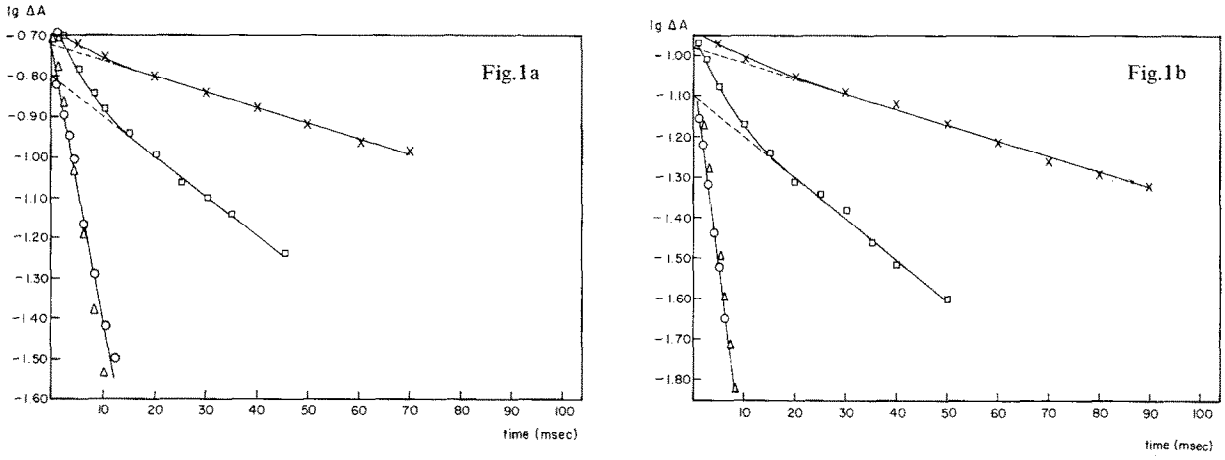

Fig.1. (a) Decay of the M^{412} transient; (b) regeneration of the bR^{570} chromophore. First-order plots. Basal assay medium: PM in a concentration equivalent to 4 μM bacteriorhodopsin was suspended in 1 M KCl. The pH was adjusted to 6.5. Additions: (○), none; (▽) 4 μg/ml of BV; (□) 16 μg/ml of VL; (×) 4 μg/ml of BV + 16 μg/ml of VL.

phores on bacteriorhodopsin photocycle, the decay and recovery of the above-mentioned transients were measured in the presence of VL or BV or when the two antibiotics were added simultaneously. The behaviour was compared to that of an aqueous PM suspension (control). Moreover, flash-induced ΔA, as a function of time, were measured over the ultraviolet and visible ranges.

Profiles of the M^{412} decay and bR^{570} regeneration (log ΔA versus time) for PM suspended in 1 M KCl were plotted in fig.1a,b. The rates of decay of the M^{412} and recovery of the bR^{570} were appreciably slowed down by VL in accordance with the data in the literature. BV alone was found to be rather ineffective. The data points of the sample containing BV fall on the plot representing the control. However, though ineffective alone, BV markedly enhanced the inhibitory effect of VL causing further stabilization of the photointermediate M^{412} and slowing down the recovery of the bR^{570}.

Note that in the presence of either VL or VL + BV the kinetics of regeneration of the bR^{570} and that of the decay of the M^{412} are biphasic. Biphasic kinetics were observed for the decay of the phototransients in PM, containing high concentrations of VL [5]. Biphasic kinetics have also been found for PM in water suspensions [9] where two first-order decay processes were resolved having half-lives of < 1 ms and several ms. In water suspension, however, the amplitude of the fast process is very small, thus it is not observed for the control in fig.1a,b.

The rate constants of the decay of the M^{412} and regeneration of bR^{570} and the transients' half-lives were measured in 1 M KCl suspensions. The half-lives of the transients were increased by almost a factor of 10 by an addition of 10–16 μg/ml of VL and were further increased by a factor of almost 3 in the presence of BV (4 μg/ml). The constants measured in 1 M KCl suspension are summarized in table 1. The overall rate is somewhat slower in water suspension (not shown) than in 1 M KCl.

Phototransient spectra of PM in 1 M KCl containing both VL (16 μg/ml) and BV (4 μg/ml) were drawn at 2 ms, 50 ms and 100 ms after the flash (fig.2). This was done by plotting the difference in absorbance, point by point, for various wavelengths before and after the flash. The phototransient spectra are comparable to the phototransient spectra of PM without the antibiotics [5,10], except that the life-time is very much extended. The O^{660}, believed to be involved in a minor separate route of regeneration to bR^{570}, is not observed in the spectra, being quenched by 10 μM VL [5]. A rather pronounced negative shoulder, similar to that observed in the PM in the water suspension [5,10], persists at 480 nm. It can be concluded that the same transients with unshifted maxima are opera-

Table 1
Rate constants and half-lives of bR phototransients

Additions	μg/ml	M^{412}				bR^{570}			
		k_2 s^{-1}	t_2 ms	k_1 s^{-1}	t_1 ms	k_2 s^{-1}	t_2 ms	k_1 s^{-1}	t_1 ms
None		223.0	3.1			153	4.5	–	–
Valinomycin	16.0	23.8	29.2	192.0	3.6	23.0	30.2	225.0	3.1
Valinomycin	4.0								
+ beauvericin	16.0	8.85	78.3	78.0	8.9	9.0	77.3	96.0	7.2

Basal assay medium as in fig.1. For other details see section 2

tive in the photocycle with and without antibiotics. It is noteworthy that VL which effectively perturbs the photocycle and BV which can act only in conjunction with VL are both neutral depsipeptides which form positively charged complexes with cations [11]. The difference between the two is mainly in the ring size. These rather stringent structural requirements point to the possibility that a specific interaction takes place between the hydrophobic complexes and the bacteriorhodopsin protein.

The results also indicate that the antibiotics affect the photocycle, mainly in the stage of the decay of the M^{412} and the rate of the photoactivated formation of the M^{412} (via the L^{550} intermediate) is not appreciably changed. Similarly, it was reported [12] that, while the degree of hydration of thin layers of purple membrane determines the relaxation time of the M^{412} decay, the rate of the formation of the intermediates leading to the M^{412} is practically unaffected by changes in hydration.

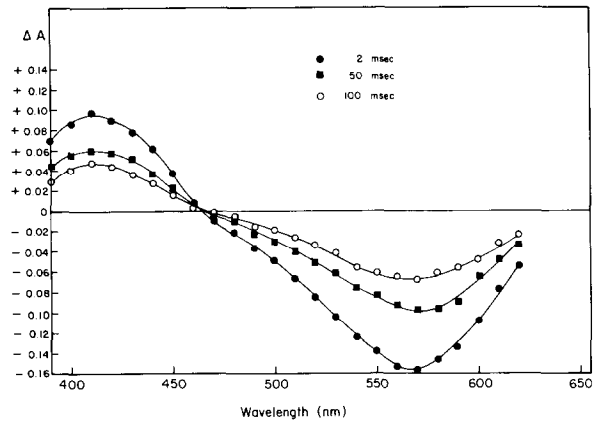

Fig.2. Transient spectra of bacteriorhodopsin. Basal assay medium (see fig.1) + 4 μg/ml BV + 16 μg/ml VL. The time which passed between the light flash and the recording of the spectrum is indicated on the figure. For other details see section 2.

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