Comparison of carotenoid and bacteriochlorophyll calibrations of the membrane potential in chromatophores of *Rhodopseudomonas capsulata*

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An extensive comparison of bacteriochlorophyll and carotenoid absorbance changes shows that, under optimal conditions, these two intrinsic probes yield essentially the same value for the light-induced membrane potential. The bacteriochlorophyll changes appear to be more sensitive to ageing than the carotenoids. The effect of prolonged sonication on the diffusion potential-induced bacteriochlorophyll and the carotenoid changes suggests that this treatment decreases the effective thickness of the membrane.

Carotenoid absorbance change *Bacteriochlorophyll change* *Membrane potential* *Diffusion potential* *Photosynthesis*

1. INTRODUCTION

The measurement of transmembrane potential differences in photosynthetic organisms is relevant to the study of energy transduction because of the central role occupied by the electrochemical potential difference of protons across the membrane in the process of photophosphorylation ([1], reviews [2–4]). In photosynthetic bacteria the absorbance changes of carotenoids have long been a preferred probe for the measurement of transmembrane electrical potential differences [4,5]. In chloroplasts interpretation of the 515 nm change is more difficult [6]. Shifts in the long-wavelength bands of bacteriochlorophyll (Bchl) have also been documented [7]. They were reported to be analogous to those of the carotenoids [8–10] and also to be elicited via an electrochromic mechanism [11].

It has been suggested that carotenoids can also respond to localized fields in the chromatophore membrane [12] as well as in chloroplasts [13], at low temperatures. It also has been shown that the field-sensitive carotenoids and the Bchl responsible for the 850 nm band are located in different subunits of the light-harvesting pigment–protein complex [14,15]. This makes it interesting to compare calibrations of the light-induced absorbance changes of both the carotenoid and Bchl probes. Indeed, for chromatophores prepared from *Chromatium vinosum* the two pigments appeared to give different values for the light-induced field (unpublished results by Case and Parson cited in [5]).

In [16] we showed that the calibrations of light- and surface potential-induced Bchl absorbance changes are strongly dependent on preparation and measurement conditions. Here we present an extended analysis and comparison of the two probes. It is concluded that, under favourable conditions, carotenoids and Bchls yield the same value for the light-induced field.

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Abbreviation: DAD, diaminodurene

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2. MATERIALS AND METHODS

Cells of *Rhodopseudomonas capsulata* wild type were harvested in their log-phase and chromatophores devoid of any RbCl were prepared in the presence of 10% DMSO as in [17].

Absorbance changes were measured in a Cary 17 spectrophotometer. Saturating actinic illumination was delivered by a 200 W incandescent lamp provided with a 'DT Blau' (Balzer) broadband interference filter, a Schott BG18 glass filter and a perspex cuvette containing a 1 cm thick water layer as a heat filter. For measurements at 530 nm the photomultiplier was protected by a Schott GG 495 cut-off filter. To obtain a satisfactory signal-to-noise ratio for measurements in the bacteriochlorophyll region we used intensities of measuring light which had some actinic effect. We applied the same intensity of measuring light (as measured with a photocell, the wavelength dependence of which was calibrated with a thermopile), corrected for the difference in absorbance at 530 and for the efficiency of energy transfer between carotenoids and Bchl, which has been assumed to be 9% as in *Rps. sphaeroides* [18].

When carotenoid absorbance changes were monitored with this intensity of measuring light, application of saturating actinic illumination or diffusion potentials produced changes which were smaller by about 5 and 10%, respectively, as compared with those obtained with weak measuring light. To diminish the actinic effect further, the measuring beam was applied for only about 30 s before application of the diffusion potentials or actinic illumination. In the case of addition of RbCl to the medium, the chromatophores were allowed to equilibrate for 5 min with 0.1 μM valinomycin, which is the optimal concentration [17] for the application of the diffusion potential. To monitor the Bchl absorbance changes 837 nm was chosen as the measuring wavelength because at this wavelength the signal-to-noise ratio was much larger than at 850 nm while the signal was only about 20% smaller than the largest absorbance change measured at 850 nm (the wavelength located at the trough of the difference spectrum [16]).

As shown in [16] the diffusion potential-induced difference spectrum is the same as the light-induced one, thus allowing calibration of the light-induced Bchl changes. The same has been shown for the carotenoids [19]. Diffusion potential calibration was performed as in [17] with Na^+ chosen as the cation to compensate for the Rb^+-induced surface potentials. Administration of Rb^+ was at 5 different concentrations making a total cation concentration of 0.5 mM. An average was made for at least two experiments per concentration [16]. The calibration curve was calculated using linear regression analysis. The calibration slopes were multiplied by 1.15, a factor which corrects for the fact that the internal concentration of Rb^+ or K^+ was very low (see [17]). The surface potential-induced absorbance change was induced by addition of 10 mM $MgCl_2$ to the sample.

Since the experimental data were obtained from different preparations of chromatophores, the standard deviations (SD) for all the calculated parameters were quite large. For this reason we excluded some of the most deviating results (less than 20% of the data). This decreased the SD for the majority of the parameters by at least a factor of 2, but did not alter the average value of the parameters to any significant extent.

3. RESULTS

Comparing the calibration values of the light-induced membrane potential as measured by Bchl absorbance changes and as measured by the carotenoid absorbance changes, we observed that the probes each depended in a different way on the growth conditions of the bacteria, storage of the chromatophores and some treatments we applied to the chromatophores [16]. The discrepancies we noticed between the two probes were mostly due to variations in the Bchl calibration values: those obtained with the carotenoid absorbance changes were remarkably constant. We therefore extended these measurements and made a statistical analysis. A summary of this analysis is shown in table 1.

The most important result is in the column which contains the ratio of the two calibration values ($\Delta\psi^{l}$Bchl/$\Delta\psi^{l}$car) as a function of various treatments. Except for the treatment which induces some artificial ageing in the chromatophores, it should be noticed that the ratios are very close to unity, which indicates that, under optimal conditions, the two ways of calibrating the membrane

Table 1

Diffusion potential calibrations of light-induced membrane potentials for different storage conditions or treatments of chromatophores of *Rhodopseudomonas capsulata* wild type

Exps	ΔA^{l}_{Bchl}	ΔA^{l}_{car}	ΔA^{d}_{Bchl}	ΔA^{d}_{car}	$\Delta\psi^{l}_{Bchl}$	$\Delta\psi^{l}_{car}$	$\Delta\psi^{l}_{Bchl}/\Delta\psi^{l}_{car}$
Ice	10.5 ± 0.8	24 ± 3	1.7 ± 4	4.2 ± 4	325 ± 50	290 ± 25	1.09 ± 0.7
N_2	9.1 ± 0.9	22 ± 3	1.7 ± 1	3.9 ± 3	280 ± 37	290 ± 35	1.01 ± 0.08
r.t.	9.9 ± 1.2	22 ± 3	1.2 ± 3	3.7 ± 9	430 ± 95	315 ± 60	1.35 ± 0.04
Glut	9.8 ± 1.2	22 ± 3	1.9 ± 2	4.1 ± 5	310 ± 10	320 ± 13	0.97 ± 0.06

Measuring medium: 10 mM Mops, pH 7, 4.5 mM NaOH, 50 mM NaCl. For the experiments with actinic illumination 100 μM DAD and 10 mM Na-ascorbate were added; for the diffusion potential experiments 0.1 μM valinomycin was added. [Bchl] ~7 μg/ml. Ice, chromatophores stored on ice; N_2, chromatophores stored on liquid nitrogen; r.t., same as N_2 but exposed for 30 min to room temperature; Glut, same as N_2 but stock solution treated for 20 min with 0.1% glutaraldehyde and thereupon stored on ice. Values of all the parameters, except the ratio of bacteriochlorophyll to carotenoid calibration, are presented in arbitrary units

potential are equivalent. The discrepancy between the calibration ratio of the aged chromatophores (1.35) and the controls (1.01) is largely due to the 40% decrease in response of the Bchl probe to diffusion potentials ΔA_{Bchl}, while its response to the electrical field created by actinic illumination ΔA^{l}_{Bchl}, did not change significantly. Mild fixation with glutaraldehyde makes the calibration ratio insensitive to this kind of ageing. This is also true when the glutaraldehyde-treated chromatophores were exposed for 20 min to room temperature. In this case, it is also the diffusion potential-induced Bchl absorbance change, being restored to its normal value, which makes the difference: for the glutaraldehyde-fixed chromatophores the light-induced Bchl absorbance change is also unaltered. Thus, it seems as if some structural damage occurred in the aged chromatophores.

To test further the involvement of membrane structure in the response of the electrochromic probes we studied the influence of the duration of sonication used for the isolation of the chromatophores. Table 2 summarizes the results obtained with two different sonication regimes. In this case we can also see that perturbation of the membrane structure caused by the longer duration of sonication increases the Bchl/car calibration ratio. In these experiments, however, the change in calibration ratio is caused not only by a decrease in sensitivity of Bchl to diffusion potentials (ΔA^{d}_{Bchl} dropped from 1.7 to 1.4), as shown above for the ageing effect, but also by a concomitant increase in sensitivity of the carotenoids to the diffusion potential application (ΔA^{d}_{car} rose from 4.75 to 5.75). Also here the light-induced absorbance changes of the two electrochromic probes were remarkably constant.

The effect of the duration of sonication on the carotenoids was less marked (an increase from 5.05 to 5.45) in another set of experiments where the

Table 2

Diffusion potential calibrations of light induced membrane potentials for different sonication regimes

Exps	ΔA^{l}_{Bchl}	ΔA^{l}_{car}	ΔA^{d}_{Bchl}	ΔA^{d}_{car}	$\Delta\psi^{l}_{Bchl}$	$\Delta\psi^{l}_{car}$	$\Delta\psi^{l}_{Bchl}/\Delta\psi^{l}_{car}$
Ice 2 × 30 min	11.1	29	1.7	4.75	380	360	1.06
2 × 100 min	10.6	28	1.4	5.75	435	290	1.50
N_2 2 × 30 min	9.6	23	2.0	4.25	300	330	0.98
2 × 100 min	9.5	21	1.5	5.25	380	240	1.60

Conditions as in table 1

sonication time was increased from 2 × 30 min to 2 × 90 min. The overall results of the two sets of experiments were very similar, however.

4. DISCUSSION

As is obvious from an examination of table 1, calibrations of the light-induced absorbance changes, under optimal conditions, yield essentially the same value whether carotenoid or Bchl absorbance changes are monitored. This result seems to substantiate the fact that both carotenoid and Bchl absorbance changes can be used as an indicator of membrane potential. This is especially relevant for studies in those bacteria (such as *Rhodospirillum rubrum*) in which the carotenoid absorbance changes are not pronounced enough to enable estimation of the membrane potential.

Here we report peak values of the membrane potential (as calibrated with the carotenoid absorption band shift) of 280 ± 30 mV. Most of the discrepancy with [17] can be explained by the difference in measuring conditions. To avoid interference with absorbance changes in the infrared produced by reaction centre oxidation we added DAD and ascorbate to the medium, which gives about 10% higher membrane potentials as checked with light-induced carotenoid absorbance change. Moreover, we observed that, in the presence of a sufficiently high background of ions used to 'screen' the surface charge, the diffusion potential is significantly smaller, up to 25%, than in the absence of these ions (Swysen et al., unpublished). This is explained by the finite permeability of the cations in question and is even valid for choline chlorides. These observations, in fact, alleviate part of the criticism against the calibration method we proposed [4,17]. The two instances where we observed variations in the Bchl/car calibration ratio are most likely caused by some perturbation of the chromatophore membrane structure. This is substantiated by the fact that the ageing effect induced by incubation of the chromatophores at room temperature is prevented by mild glutaraldehyde fixation. It is quite surprising, however, that this structural damage affects only the diffusion potential-induced absorbance changes and not at all those elicited by actinic illumination. The fact that prolonged sonication increases diffusion potential-induced carotenoid absorption band shifts is equally surprising, but gave us a hint as to what the explanation may be: it seems as if this treatment decreases the thickness of the effective electrical capacitor of the membrane, since the same diffusion potential elicits a larger carotenoid shift. The electrical potential difference is the same, but the intramembrane field seems to increase. Earlier experiments that we have repeated on chromatophores of *Rps. sphaeroides* could also be explained by this phenomenon: in those cases we observed that, preparing chromatophores with different sonication regimes from 2 × 30 min to 2 × 120 min, the light-induced wavelength shift per 100 mV (as calculated in [20]) increased from 0.7 to 1.2 nm (unpublished). The decrease in Bchl sensitivity upon sonication could be explained by putting the field-sensitive area of the Bchl molecule more to the outside of the membrane, more or less at the level where the effective capacitor plate would be situated, so that if the effective membrane thickness decreases, the Bchl response also diminishes. A different location of the B-850 Bchl and carotenoid probes in the membrane is in line with our current knowledge of the light-harvesting complex composition [14]. The ion sensitivity of the light-induced response of both probes to duration of sonication is still feasible with regard to this hypothesis, since the structural alteration discussed above need not influence the light-induced charge separation.

It is difficult, however, to envisage how the field-sensitive carotenoids which seem to lie close to the membrane surface [15] are more an integral part of the membrane low-dielectric core than is B-850. Precise data on the structure of the light-harvesting complex should shed more light on this issue.

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