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PHOTOELECTRIC EFFECTS IN BACTERIAL CHROMATOPHORES

COMPARISON OF SPECTRAL AND DIRECT ELECTROMETRIC METHODS

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Generation of photoelectric potential in chromatophores of *Rhodopseudomonas sphaeroides* has been measured (i) spectrophotometrically, using electrochromic shift of carotenoid absorption band or (ii) electrometrically, by means of two electrodes separated by a collodion film covered on one side with chromatophores. A 15 ns laser flash was used to induce a single turnover of photosynthetic reaction centers. It was found that results obtained by both methods are similar in (i) direction of electric vector (the chromatophore interior positive) and (ii) redox titration curves ($E_m = 10$ mV). The magnitudes of the photopotential were about 60 and 25 mV, when monitored with spectral and electrometric techniques, respectively. In both cases, the rise times of the photopotentials were faster than time resolution of the techniques used. Decay of the response of carotenoids was found to be slower than that in the collodion film system. The addition of ubiquinone Q_{10} into the decane solution of asolectin used to impregnate the collodion film led to slowing down of the decay. The carotenoid response decay could be accelerated by FCCP or *o*-phenanthroline. In the latter case, the shape of the decay curve coincides with decay of the photopotential measured in the collodion film system. It is suggested that decane extracts secondary ubiquinone from chromatophores attached to the collodion film. Such an unfavorable effect can be strongly decreased by added ubiquinone

Introduction

In some photosynthetic bacteira, energization of the chromatophore membrane is accompanied by a shift of absorption bands corresponding to carotenoids. A similar shift can be obtained when diffusion potential of K^+ in the presence of valinomycin is imposed across this membrane. Agents collapsing membrane potential inhibit the carotenoid shift [1]. These observations were considered as pointing out that carotenoid shift monitors formation of transmembrane electric potential difference in chromatophores [2]. However, it was found later that a carotenoid shift of the same kind can arise due to changes in the surface potential [3] as well as to formation of intramembrane dipoles [4,5]. Thus an uncertainty appeared in interpretation of carotenoid shift as a transmembrane potential probe. Nevertheless, this method is

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Abbreviations: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; Mes, 4-morpholineethanesulfonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; $\Delta\psi$, transmembrane electric potential difference; E_m, the midpoint redox potential; E_h, the ambient redox potential measured with platinum and Ag|AgCl electrodes.

still used very often, mainly due to its high time resolution. For instance in *Rps. sphaeroides* chromatophores, it was found that a short laser flash inducing a single turnover of photosynthetic redox chain gives rise to the carotenoid shift which is so fast ($\tau_{1/2} < 100$ ns) that it could be attributed only to the primary events in the reaction centers [6–8].

In our group, an electrometric method of measuring the membrane-linked electrogenesis has been developed [9–11]. In this case, chromatophores were adsorbed on one of the two surfaces of the collodion film impregnated with decane solution of phospholipids. In such a system, any translocation of charges occurring through the chromatophore membrane or inside this membrane if they are not parallel to its plane, can be measured with two electrodes immersed into electrolyte solutions separated by the film. This method seems to be as good as the carotenoid probe for measuring the fast electrogenic events (its time resolution is 50 ns) and it is better than the carotenoid probe in that respect that one can discriminate between charge translocations directed across and along the membrane. One more advantage consists in the fact that the collodion film technique can be used to study systems lacking electrochromic pigments.

In this paper, we have compared both the above-mentioned methods in experiments on one and the same object, i.e., *Rps. sphaeroides* chromatophores.

Methods and Materials

Chromatophores of *Rhodopseudomonas sphaeroides* (wild-type strain R2) were isolated as described previously [12]. Light-induced spectral shift of carotenoids was measured at 523 nm as described previously [13]. Carotenoid shift induced by K^+ diffusion potential was monitored with an Aminco Chance DW-2 double-wave spectrophotometer. To optimize the experimental conditions, effects of surface potential, internal K^+ level and valinomycin concentration upon carotenoid response were taken into account according to Symons et al. [14]. Electrometric measurement of the photoelectric effect was carried out according to Drachev et al. [10]. Chromatophores were added to the electrolyte solution on one side of the collodion film impregnated with decane solution of asolectin. Electrolyte solution comprised 0.1 M NaCl/2 mM $MgSO_4$/0.05 M Tris-HCl (pH 7.55). Electric potential difference between two Ag|AgCl electrodes separated with the collodion film was monitored with a Data Lab 905 transient recorder connected with a computer, Nova 3D. Redox titration of electrical and spectral responses was carried out under aerobic conditions as described previously [11]. Bacteriochlorophyll concentration was estimated using a molar extinction coefficient at 850 nm equal to 95 000 [15].

Results and Discussion

In Fig. 1A, the calibrating curve for carotenoid response is shown. Varying the concentration of KCl added to the chromatophore suspension in the presence of valinomycin, we obtained different K^+ diffusion potentials. In Fig. 1B and C, one can see photoelectric response to a 15 ns laser flash measured by the carotenoid and electrometric methods, respectively. Both techniques indicate that positive charging of the chromatophore interior occurs. The magnitude of the photopotential was found to be higher when measured by the spectral than by the collodion film method (60 versus 25 mV, respectively). Unfortunately, we failed to calibrate the magnitude of electrometric responses. As shown previously, the measured photopotential amplitude was less than or equal to the potential built up across the chromatophore membrane. The kinetics of $\Delta\psi$ generation measured by this method were recorded without distortions [9,13]. In both cases, kinetics of the photopotential generation was faster than time resolution of the methods. Under our experimental conditions, almost the full extent of photoelectrogenesis was apparently due to charge separation between bacteriochlorophyll dimer and primary quinone. As it follows from the antimycin affect shown in Fig. 2, the millisecond phase due to quinone oxidation was missing when measured by the collodion film method and was slight in carotenoid response. The magnitude of the microsecond electrogenic phase arising from the reduction of bacteriochlorophyll by cytochrome c_2 was less than 5% of the whole responses measured by the two methods (not shown). This may be explained

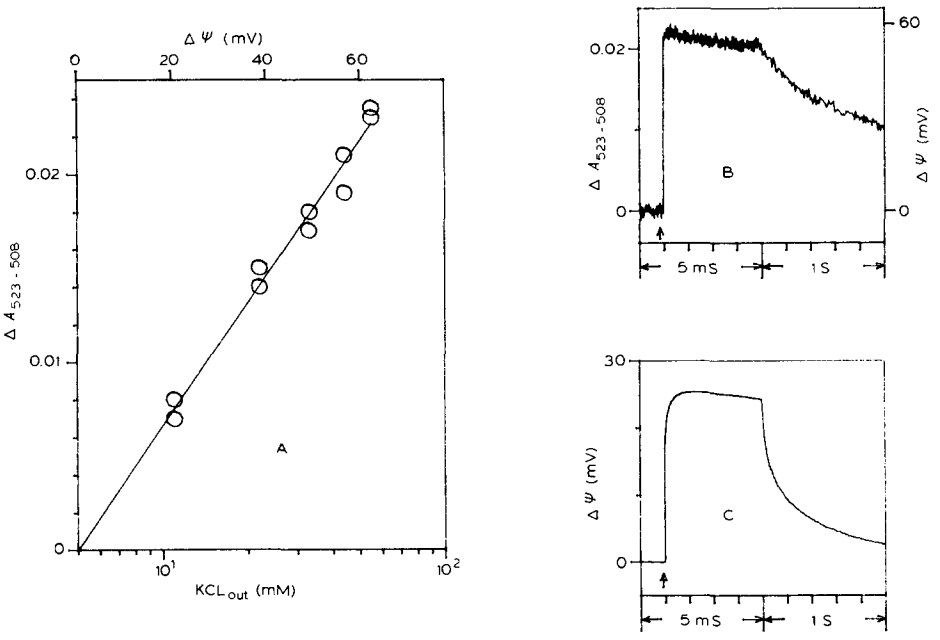

Fig. 1. Kinetics of the laser flash-induced carotenoid shift and of photopotential in the chromatophore-collodion film system. (A) Calibration of the carotenoid shift by K^+ diffusion potential. Chromatophores (25 μM bacteriochlorophyll) were incubated in 2 mM $MgSO_4$/5 mM KCl/100 mM NaCl/50 mM Tris-HCl (pH 7.55)/20 nm valinomycin. Different concentrations of KCl were added and a 532–509 nm absorption shift was measured. The value of $\Delta\psi$ across the membrane of the chromatophores was obtained from the Nernst equation. (B) Carotenoid shift induced by 15 ns laser flash. Chromatophores (25 μM bacteriochlorophyll) were incubated in 2 mM $MgSO_4$/100 mM NaCl/50 mM Tris-HCl (pH 7.55). The trace is an average of 100 flashes. (C) Direct measurement of the laser flash-induced electric potential generation in the system 'chromatophores-collodion film'. Incubation mixture as in B. In both B and C, upward deflection of the curve corresponds to positive charging of the chromatophore interior.

by cytochrome c_2 extraction in the course of chromatophore isolation.

As one may see in Fig. 1, the half-time of decay of the photopotential was 1 s for the carotenoid response and about 10-times faster for the electrometric measurement.

At least two reasons may be regarded responsible for the faster decay in the chromatophores attached to the collodion film. (i) Attachment per se may decrease the electric resistance of the chromatophore membrane. (ii) As previously discussed [9], decane used as a solvent for phospholipids impregnating the collodion film may extract ubiquinone dissolved in the chromatophore membrane, thereby arresting the normal way of the primary quinone oxidation and hence promoting electron transfer from primary quinone back to chlorophyll (see also Ref. 16).

Both effects can be induced also in chromatophore suspension under conditions of the carotenoid shift measurement, the former by adding a protonophorous uncoupler and the latter by adding *o*-phenanthroline which inhibits electron transfer from primary to secondary quinone. As it is clear from Fig. 2A, *o*-phenanthroline at the concentration used, mimics exactly the effect of the attachment of chromatophores upon photoresponse decay. On the other hand, uncoupler FCCP fails to do this.

Such relationships, as well as some other observations made in our group [9], suggest that something is wrong with the free quinone pool of chromatophores associated with the collodion film, a fact which is not surprising because of the presence of decane in the system. The addition of ubiquinone Q_{10} into the decane solution of asolectin (used for the impregnation of the collodion film) leads to slowing down of the photoelectric

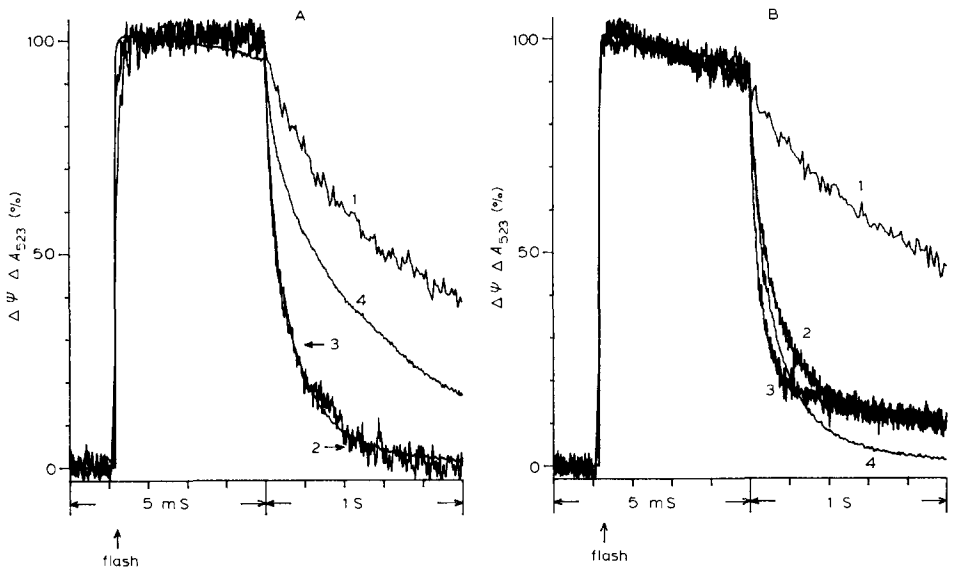

Fig. 2. Comparison of the photopotential decays measured with the collodion film system and with the carotenoid probe under different conditions. (A) 1, 2, carotenoid shift; 3, 4, chromatophore-collodion film system; 2, 20 mM *o*-phenanthroline was added; 4, the asolectin solution (used for the impregnation of the collodion film) was supplemented with 10 mg ubiquinone Q_{10} (Sigma) per 1 ml *n*-decane. (B) 1–3, carotenoid shift; 4, chromatophore-collodion film system. 1–4, 1 μM antimycin was added. 2 and 3, $2 \cdot 10^{-7}$ M and $5 \cdot 10^{-7}$ M FCCP were added. Incubation mixture and bacteriochlorophyll concentration as in Fig. 1B. All curves were normalized by amplitudes.

potential decay, which is obviously due to a partial reconstitution of the secondary quinone pool (Fig. 2A, curve 4).

Very fast rise of the photopotential revealed by both methods indicate that in both cases oxidoreduction between the primary electron donor and the primary quinone is responsible for almost the full extent of electrogenesis, since other light-induced electron transfer reactions in the chromatophore redox chain are slower than the response observed.

It was interesting to reveal whether redox states of the primary quinone are identical in chromatophores in suspension and in chromatophores immobilized on the collodion film. To this end, redox titrations of the photoelectric potential was carried out in these two systems. As it is shown in Fig. 3, the change in redox potential of the medium from +100 mV to −100 mV completely inhibits photoelectric responses measured by both methods used. Both methods gave identical E_m values (pH 6.8–6.9) equal to +10 mV which is close to E_m of the primary quinone, estimated in *Rps. sphaeroides* chromatophores [17].

Thus, the above data in combination with results of other studies carried out in this group [9,18,19] point to the following advantages of the collodion film system as compared with the carotenoid shift probe.

(i) The former method is much more sensitive than the latter. In Figs. 1B and 2, the carotenoid response curve represents an average of 100 measurements and nevertheless is still much more noisy than the corresponding curve in the collodion film system, although it represents a result of the single measurement. In fact, the sensitivity difference is as large as several orders of magnitude, i.e., very much larger than the difference in the photopotential magnitude which is 1.5–2.5-times lower in the collodion film system.

(ii) The collodion film method is much more universal than measurement of the carotenoid shift which occurs in chloroplast and certain photosynthetic bacteria only. Our method was found to be applicable to any type of proteoliposome studies, as well as to membrane particles from bacteria, mitochondria and chloroplasts, bacteriorhodopsin membrane sheets, etc. [9–11,13,18,19].

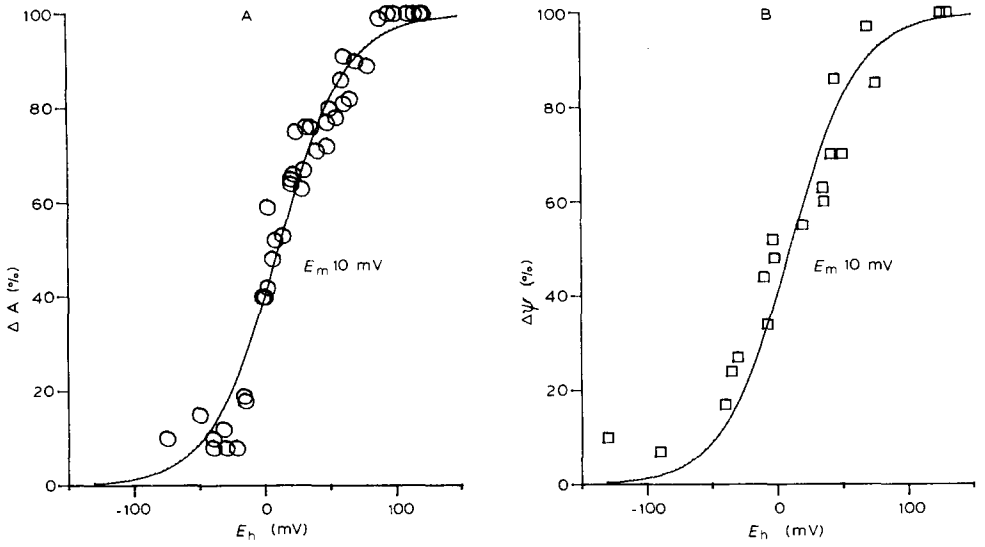

Fig. 3. Redox titration of photoelectric responses of the *Rps. sphaeroides* chromatophores, measured with carotenoid probe (A) or with collodion film system (B). Chromatophores (25 μM bacteriochlorophyll) were suspended in 17 mM Tris-HCl, 17 mM Hepes, 17 mM Mes, pH 6.9 for (A) and 6.8 for (B). 0.1 mM *N,N,N',N'*-tetramethyl-*p*-phenylendiamine, 0.1 mM 3-methyl-1,4-naphthoquinone, 30 μM phenazine methosulfate, 30 μM phenazine ethosulfate, 0.1 mM 1,4-naphthoquinone sulfonate, 0.1 mM 2-methoxy-1,4-naphthoquinone and 0.4 mM 2-hydroxy-1,4-naphthoquinone were used as mediators.

(iii) The system developed in this group is more direct than the spectral probe. Dealing with such complicated systems as biological ones, we always should regard the direct method as much more desirable than any indirect method. For instance, a carotenoid shift may report about charge displacement not only across but also along the membrane. Moreover, it is difficult to exclude that factors other than electric field can affect the carotenoid spectrum.

As to disadvantages of the collodion film method, the effects of the solvent (decane) should be taken into account. This is apparently not critical for such systems studied in our group as (bacterio)chlorophyll – primary acceptor, bacteriorhodopsin, visual rhodopsin, cytochrome oxidase, transhydrogenase, H^+-ATPase and H^+-pyrophosphatase. However, reactions requiring diffusable quinones such as Q-cycle mechanism seem to be injured, most probably by decane, when vesicles are immobilized onto the collodion film. In such cases, carotenoid probe rather than collodion film should be used. Unfortunately, it cannot be applied to the mitochondrial Q-cycle so that fast kinetics of electrogenic reactions in this system still cannot be measured. Nevertheless, as follows from Fig. 2A (curve 4) this drawback of the collodion film method probably may be improved by the addition of suitable quinones.

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