Biochimica et Biophysica Acta, 546 (1979) 183—186

BBA Report

BBA 41315

ORIENTATION AND LINEAR DICHROISM OF THE REACTION CENTERS FROM *RHODOPSEUDOMONAS SPHAEROIDES* R-26

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(Received October 25th, 1978)

Key words: Linear dichroism; Reaction center; Orientation; (Rhodopseudomonas sphaeroides)

Summary

Linear dicroism of chromatophores and isolated reaction centers from the photosynthetic bacterium *Rhodopseudomonas sphaeroides* strain R-26 was studied using a novel technique of orientation. The results are discussed in view of the reaction center structure and its position in the membrane. The advantages of the new orientation technique are also outlined.

Linear dichroism (LD) spectroscopy of photosynthetic pigments can reveal their role in energy transfer and charge separation processes. Only little information was found in the literature regarding the LD studies of macromolecular objects [1] due to the difficulties of preparation of oriented samples [2]. In a recent publication, Rafferty and Clayton [3] described an orientation technique in dried gelatin films. This technique yielded an efficient orientation of reaction center particles from *Rhodopseudomonas sphaeroides* strain R-26 with dichroic effects about 100 times greater than those found in earlier studies [1]. However, the orientation in dried gelatin films restricted the pH to 5.5—4.5, and required low relative humidity of the sample [3]. We studied the LD of chromatophores and reaction center particles in water environment with neutral pH using a novel orientation technique.

The chromatophores and reaction center particles were isolated from the cells of *Rps. sphaeroides* strain R-26 by a modification of the method of Feher and Okamura [4,5] using the non-ionic detergent lauryl dimethylamine oxide

Abbreviation: LD, linear dichroism.

and ion-exchange chromatography on DEAE-cellulose. The buffer suspension of chromatophores or reaction center particles was mixed with the components of the polyacrylamide gel (acrylamide, 10—15% (w/v); *N,N'*-methylenebisacrylamide, 0.3—0.5% (w/v); glycerol, 50% (v/v)). The samples were polymerized by the addition of 0.03% (v/v) *N,N,N',N'*-tetramethylethylenediamine and 0.05% (w/v) ammonium persulfate. Spectral characteristics of the chromatophores and reaction centers packed in the polyacrylamide gel did not differ from those in buffer suspensions. After polymerization, the gel was squeezed in a home-made cuvette so that the sample stretched and its cross-section reduced approximately 2-fold. During the stretching of the sample, the pores of the gel also stretched. We propose that asymmetric (oblong) macromolecules packed in the gel align with their long axes predominantly parallel to the direction of stretching, while the spherical membranes are squeezed into ellipsoids.

Absorption measurements were made with a SF-18 dual-beam recording spectrophotometer (LOMO, Leningrad, U.S.S.R.) modified for operation in the 415—950 nm spectral region. The details of the apparatus will be published elsewhere. We recorded absorption spectra $A_{\parallel}$ and $A_{\perp}$ with linearly polarized light with electric vector parallel and perpendicular, respectively, to the direction of the stretching of the sample.

Figs. 1 and 2 show typical absorption spectra $A_{\parallel}$ and $A_{\perp}$ and calculated spectra of the degree of polarization $P = (A_{\parallel} - A_{\perp})/(A_{\parallel} + A_{\perp})$ of oriented chromatophores and reaction centers at room temperature. We have studied about a dozen samples from three preparations and observed good reproducible dichroic effects. The exact values of P depend on the degree of squeezing. For a given sample, we calculated P with an accuracy about ± 0.01 due to high optical quality of the samples. The spectra shown in Fig.1 are much the same as those obtained by Vermeglio and Clayton [6], who studied LD of chromatophores from the same bacterium and used spreading and air-drying tech-

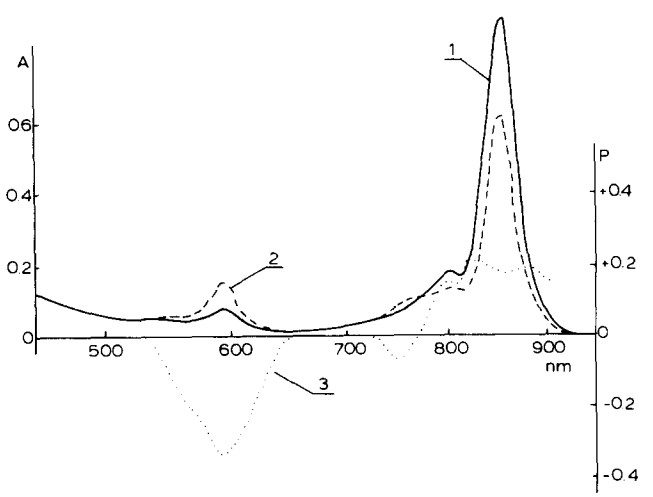

Fig.1. Absorption and degree of polarization spectra of oriented chromatophores from *R. sphaeroides*, strain R-26. 1, $A_{\parallel}$; 2, $A_{\perp}$; 3, *P*.

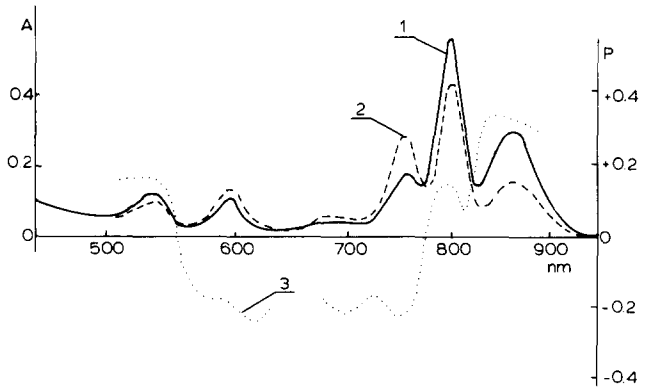

Fig. 2. Absorption and degree of polarization spectra of oriented reaction centers from *R. sphaeroides*, strain R-26. 1, $A_{\parallel}$; 2, $A_{\perp}$; 3, *P*.

niques of orientation [7]. Our spectra also agree with earlier results reported by Breton [7]. The agreement of signs and magnitudes of dichroic effects in characteristic absorption bands with those available [6,7] leads us to a conclusion that the orientation of chromatophores by our technique is as effective as that by spreading and airdrying techniques [6,7], and that the planes of chromatophore membranes in our samples are predominantly parallel to the stretching direction.

Fig. 2 shows that isolated reaction center particles are also effectively oriented by our technique. The highest value of the degree of polarization $P = +0.33$ at 865 nm is about 100 times greater than that reported by Penna, Reed and Ke [1] and is similar to the results of Rafferty and Clayton [3]. It follows that the reaction center particles have a significant asymmetry of shape, presumably, an oblong shape. The Q_y transition dipole of bacteriochlorophyll corresponding to the 865 nm absorption band lies almost parallel to the stretching direction. We propose that this dipole makes a small angle with the long axis of the oblong reaction center particle. The comparison of spectra shown in Figs. 1 and 2 leads us to conclude that the reaction center particles are oriented in the chromatophore membranes and lie predominantly parallel to the membrane plane.

We have observed significant dichroic effects in all characteristic absorption bands of reaction centers in visible and near infrared. The dip in the spectrum of the degree of polarization near 810 nm [6] indicates the presence of an absorption band with a different orientation of the transition moment. Our results reveal qualitative agreement with those reported in [3], though the exact values of dichroic effects (summarized in Table I) show certain difference.

Table I shows that in the 865, 597 and 535 nm absorption bands the dichroic effects obtained with two orientation techniques are very similar. This is not the case for the 799 and 754 nm bands. We have obtained a 2-fold smaller degree of polarization in the 799 nm band and a 2-fold greater degree of polarization in the 754 nm band compared to the values reported by Rafferty and Clayton [3]. We ascribe the difference to a change of the conformation state of reaction centers in dried gelatin films. The short-wave-length shift of

TABLE I

DEGREES OF POLARIZATION $P = (A_{\parallel} - A_{\perp})/(A_{\parallel} + A_{\perp})$ FOR ORIENTED REACTION CENTERS FROM *RHODOPSEUDOMONAS SPHAEROIDES* R-26 AT ROOM TEMPERATURE (300 K)

The values of P are calculated from the dichroic ratios $A_{\parallel}/A_{\perp}$ [3]

Wavelength (nm)	Degree of polarization	
	Orientation in polyacrylamide gel	Orientation in dried gelatin films [3]
865[a]	+0.33	+0.35
799	+0.15	+0.26
754	—0.23	—0.12
597	—0.20	—0.26
535	+0.16	+0.19

[a] Absorption maximum shifted to 848 nm during the preparation of the dried film [3].

the 865 nm absorption band observed in [3] may also result from this change.

The samples oriented in stretched polyacrylamide gel are conserved in a medium that contains about 40% of buffer solution. Changing the buffer, one can adjust pH, ionic strength and other important characteristics of the environment. The polyacrylamide gel does not form chemical bonds with polypeptide chains [8] and does bot bind water [9]. The polyacrylamide gel is transparent in the 280—1350 nm spectral region that permits optical studies of various biological macromolecules, especially those containing chromophore groups.

The exact values of angles between the transition dipoles and the "long axis" of the reaction center particle can be calculated basing on statistics of distribution of macromolecules in the stretched sample. This work is in progress now.

We thank Prof. G. Feher, Prof. W.W. Parson and Dr. R.K. Clayton for their kind gifts of the mutant culture R-26 of *Rhodopseudomonas sphaeroides.*

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