Strong orientational ordering of the near-infrared transition moment vectors of light-harvesting antenna bacterioviridin in chromatophores of the green photosynthetic bacterium *Chlorobium limicola*

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The direction of the transition moments of chlorosome pigments in chromatophores of the green photosynthetic bacterium *Chlorobium limicola* was studied by linear dichroism. Orientation of chromatophores was achieved by stretching a polyacrylamide gel in which they were packed. It was shown that in each individual chromatophore the Q_y transition moment vectors of the whole chlorosome bacterioviridin are parallel to each other and are practically ideally oriented along the chlorosome long axis. The exact value of the angle α between the bacterioviridin transition moments and the long axis of the chlorosome is calculated to be $\alpha = 0°$, the mean square deviation being 7°.

Linear dichroism *Bacterioviridin orientation* *Green bacteria* *Bacterial photosynthesis* *Chromatophore*

1. INTRODUCTION

It has been shown theoretically that the photosynthetic unit (PSU) structure should be strongly optimized in vivo to operate with a 90% quantum yield of primary charge separation in the reaction center (RC) [1]. The basic principles of the structural organization of an optimal model's PSUs have been considered by us in [2–5]. The mutual orientation of the transition moment vectors of PSU molecules is a major factor making the optimization of energy transfer from the antenna to the RC possible [4,5]. It was shown that in optimal model PSUs these vectors are parallel to each other and to either the long or short axis of an 'elementary' PSU. It is possible that in some natural PSUs the co-operative effect of several optimizing factors ensures high efficiency without requiring an 'ideal' orientational ordering of these vectors. It has been shown that, in vivo, there is at least partial orientational ordering of transition dipoles [6]. We believe that if an ideal orientational ordering of transition dipoles in vivo occurs, then it would be advisable to investigate the ordering in large and efficient PSUs, for which the requirements for their structure optimization are more rigorous than those for small PSUs [1]. This is why the green sulfur bacteria were chosen for study, as their light-harvesting antenna is an order of magnitude larger than that of purple bacteria, and several-fold greater than that of higher plants. PSUs of green sulfur bacteria contain about 1000 bacterioviridin (BVr) molecules and about 80 bacteriochlorophyll *a* (BChl *a*) molecules (per RC P840) [7,8], with their main near-infrared absorption peaks at 730–750 and 810 nm, respectively. At the same time, as shown by us previously for *Chlorobium limicola*, its PSU is very efficient: energy transfer from light-harvesting BVr superantenna to BChl *a* antenna takes place within

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20–50 ps with an efficiency >95% [9], and that from BChl *a* to RCs within 20–60 ps with an efficiency >92% [8,10]. Our aim was to investigate dipole orientations in *C. limicola* BVr superantenna in the so-called chlorosomes, rod-shaped structures containing all the cell BVr (about 10000 BVr molecules per chlorosome [7]).

2. MATERIALS AND METHODS

Cultivation of *C. limicola* cells and isolation of chromatophores were performed as in [8]. A chromatophore is the photoactive chlorosome-membrane complex whose absorption spectrum does not differ from that of the whole cell. The direction of the transition moments of pigments was studied by linear dichroism. Orientation of chromatophores was achieved by uniaxially stretching a polyacrylamide gel in which they were packed [11]. Absorption spectra were measured at room temperature with a Specord M40 spectrophotometer (Karl Zeiss, Jena). The absorbances of the measuring light polarized parallel ($A_\parallel$) and perpendicular ($A_\perp$) to the direction of sample stretching (i.e. to the orientation axis) were measured in the region 600–900 nm. Data were analyzed proceeding from statistics on distribution of rod-shaped particles in the stretched sample [11–14]. If the sample deformation is symmetric with respect to the z-axis, then $l'_x = l_x/\sqrt{N}$; $l'_y = l_y/\sqrt{N}$; $l'_z = l_z N$, where l_x, l_y, l_z, l'_x, l'_y, l'_z are the sample dimensions before and after deformation, respectively, and N is the degree of sample deformation. The dependence of the degree of dichroism, $P = (A_\parallel - A_\perp)/(A_\parallel + A_\perp)$, on the angle, α, between the transition moment vector and the long axis of the rod-shaped particle in a stretched polymer, for a given degree of sample deformation, N, is described by the following set of equations [12]:

$$P(\alpha,N) = \frac{(3\cos^2\alpha - 1)\cdot(3T(N) - 1)}{3 - \cos^2\alpha + T(N)\cdot(3\cos^2\alpha - 1)} \quad (1)$$

$$T(N) = \frac{N^3}{N^3 - 1}\left(1 - \frac{\arctan\sqrt{N^3 - 1}}{\sqrt{N^3 - 1}}\right) \quad (2)$$

3. RESULTS AND DISCUSSION

The shape of the individual chromatophore was examined in a Hitachi H-12 electron microscope and found to be rod-like. From an average of about 50 images of electron micrographs of *C. limicola* chromatophores the length and diameter of the rod-shaped chromatophores were estimated to be 160 and 35 nm, respectively, in agreement with data of other authors [15]. It is well established that the maximal dimension of chromatophores of all known green bacteria is determined by the dimension of the long axes of their chlorosomes [7,15]. Therefore, the linear dichroism spectra yield information about the orientation of the oscillators relative to the long axis of the chlorosome of each individual chromatophore (this long axis is parallel to the cell membrane [15]).

Fig.1 shows typical absorption spectra $A_\parallel$ and $A_\perp$ and the calculated spectrum of the degree of dichroism, P. We have studied about a dozen samples from 3 preparations and observed a well-reproducible dichroic effect. We calculated P values with an accuracy of 1–2%. Fig.1 shows that in the sole BVr absorption region in the near-infrared region (710–770 nm) the P values are constant with an accuracy ± 0.01. At wavelengths $\lambda <$ 710 nm and $\lambda >$ 770 nm BVr is not the only absorbing oscillator [7–10]. Fig.1 shows that in these regions the degree of dichroism varied significantly; this indicates the presence of absorption bands with different orientations of the transition

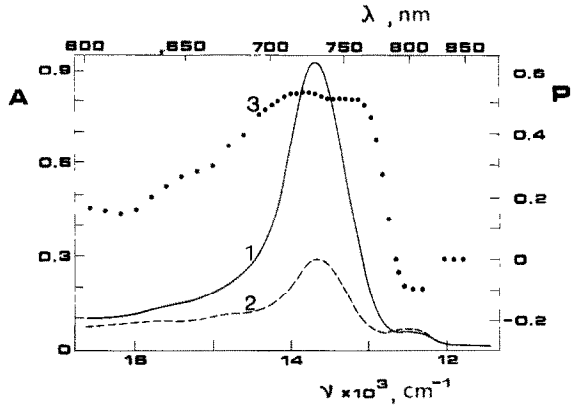

Fig.1. Room-temperature absorption and degree of dichroism spectra of oriented chromatophores of *C. limicola*. (1) $A_\parallel$, (2) $A_\perp$, (3) $P = (A_\parallel - A_\perp)/(A_\parallel + A_\perp)$. Spectra measured for $N = 1.98$.

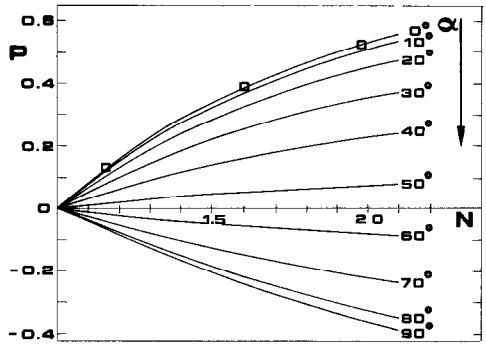

Fig.2. Theoretical dependences of the degree of dichroism P on the degree of sample deformation N for 10 α values, shown near the respective curves, $P(N)$. (□) Regions of $P(N)$ values with experimental $P(N)$ values taking into account the accuracy of P and N measurement. The experimental $P(N)$ value for $N = 1$ (i.e. for unstretched sample) is equal to $P(1) = 0$. Experimental $P(N)$ values measured at the BVr absorption maximum of oriented chromatophores of *C. limicola* for 3 N values: 1.16, 1.60, 1.98.

moments. Therefore, the exact values of the α angles between the transition dipoles and the long axis of chlorosome may be calculated only for the main fraction of BVr absorbing at 710–770 nm. If the shape of the test particles and the statistics of their distribution in the stretched sample are known, it would be sufficient to measure the degree of dichroism for a given degree of sample deformation to determine α [6,12]. To examine the adequacy of the theory employed we measured the P values for 3 different values of N: 1.16, 1.60 and 1.98. We measured N values with an accuracy of about 1%. Fig.2 shows the parametric family of theoretical $P(N,\alpha)$ curves calculated with eqns 1 and 2, and the experimental $P(N)$ values obtained from $A_{\parallel}$ and $A_{\perp}$ spectra for 3 values of N. It is clearly seen that the model of rod-like particles well describes the orientation of chromatophores of *C. limicola* in the gel. This provides additional, independent proof of the rod-like shape of chromatophores investigated. The mean value of α was calculated to be $\alpha = 0°$, the mean square deviation being 7°.

Thus, in each individual chromatophore of *C. limicola*, the Q_y transition moment vectors of light-harvesting superantenna bacterioviridin (about 10000 BVr molecules [7]) are essentially parallel to each other and practically ideally oriented along the chlorosome long axis. This ideal orientation is one of the optimizing factors ensuring the highest rate of heterogeneous energy transfer measured by us in [9]. Our result is the first experimental demonstration of the ideal ordering of the Q_y transition moment vectors of pigments in light-harvesting systems in vivo.

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