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Orientation of the chromophore transition moment in the 4-leaved shape model for pea phytochrome molecule in the red-light absorbing form and its rotation induced by the phototransformation to the far-red-light absorbing form

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Orientation of the chromophore transition moment in pea phytochrome (subunit molecular mass, 114 kDa) in the red-light-absorbing form (P_r) and its rotation induced by the phototransformation to the far-red-light-absorbing (P_{fr}) form was studied by measuring linear dichroism of oriented phytochrome prepared by a 'gel squeezing method'. The phytochrome in P_r showed a negative linear dichroism. On the contrary, the P_{fr} form showed positive linear dichroism. Assuming that the molecular axis for the two-fold rotational symmetry in the '4-leaved shape model' proposed recently for the dimeric molecular structure of the phytochrome [(1989) FEBS Lett. 247, 139–142], is oriented in parallel with the direction of the external constraint, the average azimuthal angle between the molecular axis and the transition moment of the chromophore was calculated as 59° or 121° for P_r and 52° or 128° for P_{fr}. These results explain well the polarotropic responses by phytochrome observed in plant organisms.

Phytochrome; Linear dichroism; Rotation of transition moment; Phototransformation; Oriented molecule; Gel squeezing

1. INTRODUCTION

Phytochrome [1,2], a photoreceptor in green plants in a variety of morphogenic and developmental responses to light, undergoes photoreversible transformation between P_r and P_{fr}. P_{fr} is believed to be the active form for inducing physiological responses. Recently, a '4-leaved shape model' was proposed for the dimeric molecular structure of pea phytochrome (subunit molecular mass, 114 kDa) based on small-angle X-ray scattering [3]. The model, however, lacks information regarding the chromophore. In the present study, the orientation of the chromophore in P_r and its rotation induced by the phototransformation to P_{fr} was studied by measuring LD of oriented phytochrome. The results were analyzed with reference to the model, which explains the reception of polarized light by phytochrome observed in plant organisms [4,5].

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Abbreviations: kDa, kilodaltons; P_r and P_{fr}, red-light-absorbing form and far-red-light-absorbing form of phytochrome; LD, linear dichroism

2. MATERIALS AND METHODS

Phytochrome (subunit molecular mass, 114 kDa; purity, more than 95%) was prepared from etiolated pea (*Pisum sativum* cv. Alaska) seedlings as described elsewhere [6].

Oriented samples of the phytochrome were prepared by the gel squeezing method [7]. Briefly, purified phytochrome was embedded in a 12% polyacrylamide gel (1 × 1 × 2.2 cm) in the presence of 50% glycerol, 50 mM Hepes and 1 mM Na_2EDTA, pH 7.8. The polymerized gel was compressed and uniaxially stretched. Compression ratio (CR) is defined as $(L_i - L_c)/L_i$, where L_i and L_c are the thickness of the gel in the initial and the compressed form, respectively.

LD was measured with a Hitachi 330 spectrophotometer with

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glass plate polarizers. $A_{\parallel}$ and $A_{\perp}$ are absorbance measured with the polarized plane parallel to and perpendicular to the stretching axis, respectively. Reduced dichroism, RD = $(A_{\parallel} - A_{\perp})/A_{iso}$ [8], was estimated as follows. A_{iso} for P_r was separately measured before stretching using unpolarized light, and that for P_{fr} was obtained using the equation: $A_{iso} = (A_{\parallel} + 2A_{\perp})/3$ [8] (see section 3). All the data were transferred to a microcomputer (Hewlett-Packard model 216). Baseline was corrected using the gel without phytochrome.

All the experimental procedures were performed under dim green safe light as described in [9].

3. RESULTS AND DISCUSSION

The phytochrome in P_r showed negative LD (fig.1A). Both the polarized absorption spectra $A_{\parallel}$ and $A_{\perp}$, according to the LD spectrum, of the phytochrome in P_r (fig.1A) were identical in shape to the spectrum measured by unpolarized light in the wavelength region from 500 to 700 nm. This indicates that the transition in this wavelength region comes from a single transition moment. Magnitude of the RD is proportional to CR (fig.2, line A), indicating that the phytochrome molecules orient when the gel is compressed and that the degree of orientation increases proportionally to the increasing CR. Maximum RD is estimated to be −0.33 by extrapolating the plots to CR = 1.0 as proposed in [10], where the gel is completely compressed and all the phytochrome is maximally oriented.

Since P_{fr} of the pea phytochrome (subunit molecular mass, 114 kDa) has been known to revert to P_r in the dark [11], LD for P_{fr} was corrected for this dark reversion as follows. $A_{\parallel}$ and $A_{\perp}$ were measured alternately several times after irradiation with saturating red light with a definite time interval. $A_{\parallel}$ and $A_{\perp}$ at absorption maximum of P_{fr} were plotted against time on a semilogarithmic scale, assuming first-order kinetics for the dark reversion. The $A_{\parallel}$ value at the time when $A_{\perp}$ was being measured, was obtained by multiplying by a correction factor determined from the plots. The LD value of P_{fr} thus obtained was positive in contrast to that of P_r (fig.1B). The maximum RD for P_{fr} was estimated to be 0.19 (fig.2, line B).

An average azimuthal angle, θ, between the direction of gel stretching axis and the transition moment of the chromophore, was calculated using the maximum RD and the following equation. RD of the molecules with uniaxial orientation around

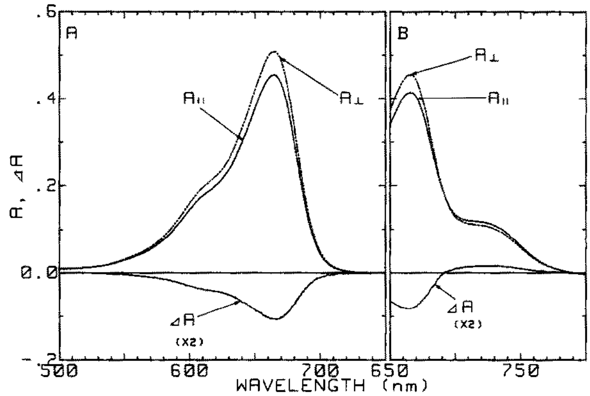

Fig.1. Linear dichroism of oriented pea phytochrome (subunit molecular mass, 114 kDa) in P_r (A) and P_{fr} (B) in compressed 12% polyacrylamide gel at 15°C at pH 7.8. Compression ratios are 0.5 for both A and B. ΔA is magnified by two. $A_{\parallel}$ for P_{fr} is corrected for the effect of dark reversion. For details, see the text.

the z-axis parallel with the direction of the external orienting constraint (the direction of the gel stretching) can be expressed as $3S_{zz}(3\cos^2\theta - 1)/2$, where S_{zz} is a diagonal element of the orientation matrix [8], and equals 1 at the maximum compression. θ was calculated as 59° or 121° for P_r and 52° or 128° for P_{fr}, respectively.

The dimeric phytochrome molecule has been shown to have an axis of two-fold rotational symmetry, which is defined x-axis in the 4-leaved shape model [3]. It is most probable that the phytochrome molecules in the gel orient with this C_2 symmetry axis parallel with the direction of the external orienting constraint (the direction of stretching axis, z-axis) as suggested for the dimeric reaction center of photosynthetic bacteria [12]. To simplify the situation, the x- and z-axes in the 4-leaved shape model are redefined as z- and x-axes, respectively, in the present analysis (see fig.3A). Based on this assumption, θ could be interpreted as the average azimuthal angle between the new z-axis of the model and the transition moment of the chromophore in the chromophoric domains [3].

Phytochrome, which is responsible for polarotropism, is proposed to attach to biological membranes and rotate on the membranes around the axis vertical to the membrane plane [13,14]. A binding site to membranes is proposed in the 4-leaved shape model, which is on the exposed side

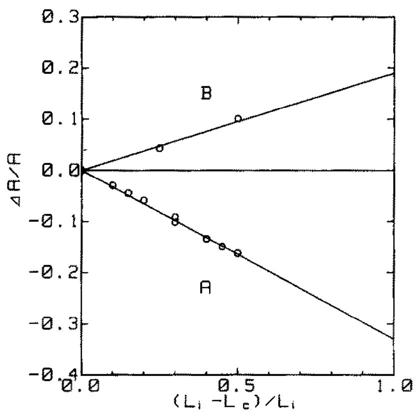

Fig.2. Dependence of reduced dichroism (RD) of oriented pea phytochrome (subunit molecular mass, 114 kDa) in compressed 12% polyacrylamide gel at 15°C at pH 7.8 calculated at the absorption maximum of P_r (A) and P_{fr} (B), on the compression ratio (CR). For details, see the text.

of the attached two non-chromophoric domains [3]. The binding at this site makes the *z*-axis (C_2 symmetry axis defined as *x*-axis in the 4-leaved shape model) perpendicular to the plane of the host membrane, suggesting that the molecules rotate around this axis (fig.3A). The uniaxial rotation can be substituted by the uniaxial orientation around the *z*-axis (fig.3B). Under these conditions, the maximum physiological response to polarized light has been calculated to occur in two ways depending on the azimuthal angle (θ) between the *z*-axis and the average transition moment of the chromophore [14]. When θ is larger than a critical angle, the so-called 'magic angle', the maximum physiological response is brought about by light with the polarized plane parallel with the host membrane. Smaller values of θ than the magic angle give the maximum response to light with the polarized plane perpendicular to the membrane. This magic angle is 54.7° or 125.3°. The obtained θ of 59° or 121° for P_r and 52° or 128° for P_{fr} agree with these values, explaining the observations that red light and far-red light are most effective when they are supplied parallel with and perpendicular to the membrane surface, respectively, in the polarotropic response by phytochrome [4,5]. This, in turn, is in agreement with the properties of the '4-leaved shape model'.

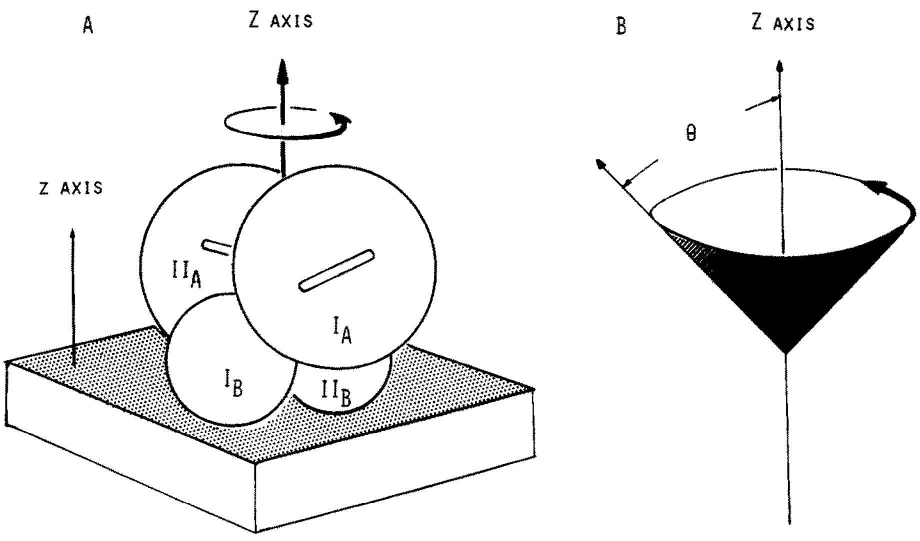

Fig.3. (A) Schematic illustration for the orientation of the chromophore transition moment in the '4-leaved shape model' of pea phytochrome (subunit molecular mass, 114 kDa) in P_r [3], which is attached on a membrane and rotates around its C_2 symmetry axis, *z*. The *x*- and *z*-axes in the original model are redefined as *z*- and *x*-axes in the present analysis. I_A and II_A are the N-terminal 59-kDa chromophoric domains in the subunit I and II, respectively. I_B and II_B are the C-terminal 55-kDa nonchromophoric domains in the subunits I and II, respectively, which are responsible for the membrane binding site. The bar in I_A and II_A represents the transition moment of the chromophore. *z*-axis is normal to the membrane surface, which coincides with the *z* axis and also the stretching axis in the present analysis. (B) The average azimuthal angle, θ, between the *z* axis and the transition moments, θ are 59° or 121° for P_r and 52° or 128° for P_{fr}, respectively. The 'magic angle' is 54.7° or 125.3°. For the details see the text.

The situation is illustrated in fig.3.

The observed rotation angle between P_r and P_{fr} was 7° or 173°, which is smaller than those obtained with oat large [15] and intact [16] phytochromes (32° and 30°, respectively) using a photoselection technique on non-oriented immobilized molecules. This discrepancy may result from the uniaxial orientation of the molecules [8] and the different measuring technique. The small rotation angle which we obtained suggests that the angle between the plane formed by the z-axis and the P_r transition moment and the plane in which the transition moment rotates, is relatively close to 90°.

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REFERENCES

[1] Smith, H. (1975) Phytochrome and Photomorphogenesis, McGraw-Hill, London.

[2] Furuya, M. (ed.) (1987) Phytochrome and Photoregulation in Plants, Academic Press, Tokyo.

[3] Tokutomi, S., Nakasako, M., Sakai, J., Kataoka, M., Yamamoto, K.T., Wada, M., Tokunaga, M. and Furuya, M. (1989) FEBS Lett. 247, 139–142.

[4] Haupt, W., Mortel, G. and Winkelnkemter, I. (1969) Planta 88, 183–186.

[5] Kadota, A., Wada, M. and Furuya, M. (1982) Photochem. Photobiol. 35, 533–536.

[6] Tokutomi, S., Kataoka, M., Sakai, J., Nakasako, M., Tokunaga, F., Tasumi, M. and Furuya, M. (1988) Biochim. Biophys. Acta 953, 297–305.

[7] Abdourakhmanov, I.A., Ganago, A.O., Erokhin, Y., Solov'ev, A.A. and Chugunov, V.A. (1979) Biochim. Biophys. Acta 546, 183–186.

[8] Norden, B. (1978) Applied Spectroscopy Reviews 14, 157–248.

[9] Yamamoto, K.T. and Furuya, M. (1983) Plant Cell Physiol. 24, 713–718.

[10] Dorssen, R.J., Vasmel, H. and Amesz, J. (1986) Photosynthesis Research 9, 33–45.

[11] Yamamoto, K.T. and Smith, W.O. (1981) Plant Cell Physiol. 22, 1149–1158.

[12] Breton, J. and Nabedryk, E. (1987) in: The Light Reactions (Barber, J. ed.) pp.159–259, Elsevier, Amsterdam.

[13] Björn, L.O. (1984) Physiol. Plant. 60, 369–372.

[14] Sugimoto, T., Ito, E. and Suzuki, H. (1987) Photochem. Photobiol. 46, 517–523.

[15] Sundqvist, C. and Björn (1983) Physiol. Plant. 59, 263–269.

[16] Ekelund, N.G., Sundqvist, C., Quail, P.H. and Vierstra, R.D. (1985) Photochem. Photobiol. 41, 221–223.