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A model for the dimeric molecular structure of phytochrome based on small-angle X-ray scattering

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The dimeric molecular structure of pea phytochrome (monomer molecular mass 114 kDa) in the red-light-absorbing form was studied with reference to the structures of its molecular domains in a monomer (an N-terminal 59-kDa chromophoric domain and a C-terminal 55-kDa nonchromophoric domain) using small-angle X-ray scattering, and a 'four-leaved shape' model is proposed. The propriety of the model was confirmed by images of the molecule obtained by rotary shadowing electron microscopy.

Phytochrome; Small-angle X-ray scattering; Molecular structure; Domain structure

1. INTRODUCTION

Phytochrome is a noble photoreceptor chromoprotein in green plants for a variety of morphogenic and developmental responses to light [1,2]. Although the primary structure of phytochrome was recently determined [3], its tertiary structure is still obscure. It is difficult to apply X-ray crystallography to the studies of phytochrome, since it has not been crystallized due to its high molecular mass (about 250 kDa). The application of high-resolution NMR spectroscopy to polypeptides with a molecular mass of less than about 10 kDa is still limited [4]. Hence, SAXS [5] appears to be a useful technique for studying the structure of phytochrome in solution [6]. In the present study, the molecular structure of pea phytochrome (subunit molecular mass 114 kDa) in the red-light absorbing form [1] was studied by SAXS with reference to the structures of its molecular domains.

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Abbreviations: kDa, kilodaltons; *Rg*, radius of gyration; SAXS, small-angle X-ray scattering

2. MATERIALS AND METHODS

Phytochrome (subunit molecular mass 114 kDa) was prepared from etiolated pea (*Pisum sativum* cv. Alaska) seedlings as described in [7]. The 59-kDa tryptic fragment that contained the phytochrome chromophore was prepared as described in [8].

SAXS of the solutions of the samples was measured with small-angle X-ray scattering equipment for solutions at the photon factory of the National Laboratory for high energy physics in Tsukuba, Japan, as described in [7]. The acquired data were analyzed by a computer (ACOS 2000, NEC, Tokyo) and a personal computer (PC-9801 VM, NEC) using the software developed by one of the authors (M.N.). The constructed models were examined with respect to their fitting of the curve of $\ln(I(S)/I(0))$ vs S and the pair correlation function to the observed values, which were calculated as described in the literature [9]. $I(S)$ is the scattering intensity at the scattering vector S.

For rotary shadowing electron microscopy, phytochrome,

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dissolved in 1 M ammonium acetate (pH 7.1) containing 0.3 mM dithiothreitol and 50% glycerol, was sprayed onto freshly cleaved mica flakes, dried under vacuum, rotary shadowed with platinum at an angle 6°, and coated with carbon using a freeze fracture device (FD5A, Eiko Engineering Co., Mito) at room temperature. The resultant replica was observed under a Hitachi H 300 electron microscope. Samples were prepared under a dim green light [7].

3. RESULTS AND DISCUSSION

A molecular model of phytochrome in the red-light absorbing form was constructed, assuming that a phytochrome subunit consists of the 59-kDa N-terminal chromophoric polypeptide domain and the 55-kDa C-terminal polypeptide domain [10], both of which are approximated by ellipsoids of revolution or cylinders, and that the molecular structure of the former domain is approximated by that of the tryptic 59-kDa polypeptide for which an Rg of 38 Å was obtained in this study. The model was also constructed to incorporate the following three points: (i) the phytochrome molecule consists of two equivalent subunits with a two-fold rotational axis of symmetry [7]; (ii) the model molecule has an Rg of 54 Å [7]; (iii) the site of contact of the subunits is located in the 55-kDa nonchromophoric domain [11]. The shape and the dimensions of the 55-kDa nonchromophoric domain and the topological arrangement of the four domains in one molecule were varied systematically in order that the overall shape of the four correctly oriented domains could be approximated by the oblate ellipsoid with an axial ratio of 1:2.7 [6].

The best-fit model is shown in fig.1. The calculated curve of $\ln(I(S)/I(0))$ vs S for the model simulates well the observed curve in the S range from 0 up to 0.025 $Å^{-1}$ (fig.2). The molecule consists of two identical subunits (fig.1): upper subunit I and lower subunit II (dotted). Each subunit consists of an N-terminal 59-kDa chromophoric domain, A (I_A and II_A), approximated by an oblate ellipsoid with an axial ratio of 1:5 (half-axial length, 11 Å; equatorial radius, 55 Å) and a C-terminal 55-kDa nonchromophoric domain, B (I_B and II_B), approximated by a disc (height, 20 Å; radius, 35 Å). The domains A and B in a subunit are attached to each other at the edge of the ellipsoidal and flat plane with their rotational axes oriented in parallel. The subunits I and II make contact at the margin of the lower flat plane of I_B and the upper flat plane of II_B with their rotational axes oriented in parallel. The direction of the rotational axes and this contact plane are designated as the direction of the z-axis (upper positive) and the x–y plane, respectively. The molecular axis of the subunit I projected onto the x–y plane, which passes through the center of the circles for I_A and I_B, intersects with that of the subunit II at a crossing angle of 95°; the crossing point and the axis of symmetry in the x–y plane are determined as the origin of the coordinates and the x-axis (forward positive), respectively. The positive direction of the y-axis is the right side. The coordinates of the center of gravity for each body are (−23, 25, 30), (27, −30, 10), (27, 30, −10), and (−23, −25, −30) for I_A, I_B, II_A, and II_B, respectively, where the unit is 1 Å.

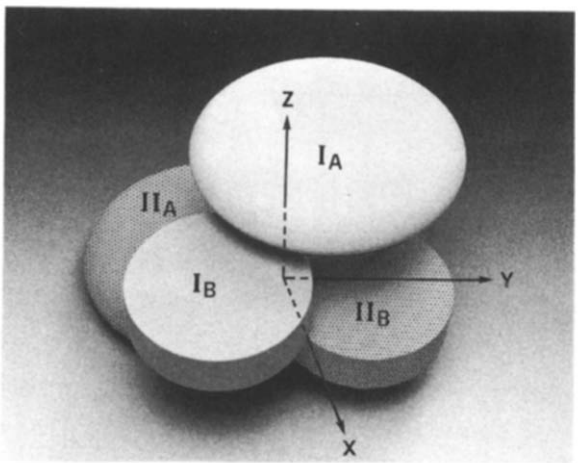

Fig.1. A model for the dimeric molecular structure of pea phytochrome (subunit molecular mass 114 kDa) in the red-light absorbing form, in 100 mM potassium phosphate and 1 mM Na_2EDTA, pH 7.8, at 7°C, as proposed from the results of SAXS. For details, see the text.

Images of the phytochrome molecule in the red-light absorbing form were observed by rotary shadowing electron microscopy. They showed heterogeneity indicating that the molecules attached to the mica flakes in various manners. The images which showed intraparticular structure can be classified into three groups, i.e., dimeric, trimeric, and tetrameric as shown in the top, middle and bottom lines of fig.3, respectively. These images occurred in the ratio of 1.0:0.74:0.31, respectively. The proposed model in fig.1 seems like a four-

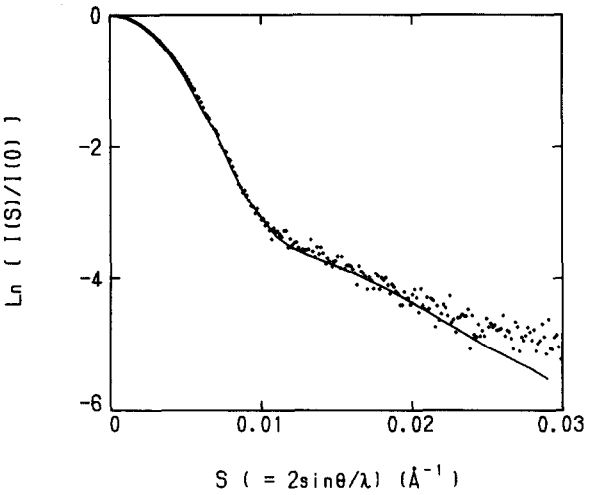

Fig.2. The curve of $\ln(I(S)/I(0))$ vs S calculated from the model of pea phytochrome as shown in fig.1 (solid line) and that of the observed results (dots). $I(S)$ is the scattering intensity at scattering vector S.

leaved clover when looked at from the positive direction of the z-axis (tetrameric images). By rotary shadowing, domain II_A (fig.1) of such molecules may be buried in the deposit of platinum on the mica flake to some extent. Furthermore, domains I_B and II_B may be joined together by the deposit in some cases. Such molecules may show trimeric images. Dimeric images may be obtained when the molecule is viewed in the x–y plane. In particular, the right-side-slanting image (right two images in the top line, fig.3) may be obtained when the molecule is seen from the negative direction of the x-axis. Thus, the proposed model was well supported by the images obtained by electron microscopy.

It has been the subject of controversy whether the double-dumbell-like images observed with the negatively stained phytochrome are artefacts [12] or not [13,14]. The present model puts an end to the controversy about the shape of the phytochrome molecule. The molecular dimensions of the model correspond to those obtained from the Stokes radius of phytochrome [15] and its molecular shape explains the nonglobular behaviour of phytochrome molecules during steric-exclusion chromatography [7,11,16] and sedimentation equilibrium centrifugation [11,17]. The model proposes the existence of a possible binding site (on the exposed side of the two attached discs)

Fig.3. Rotary shadowing electron microscopy of pea phytochrome (subunit molecular mass 114 kDa) in the red-light absorbing form (× 150000). For details, see the text.

to biological membranes, which is presumed to be located in the 55-kDa nonchromophoric domain [18] and to be involved in the reception of polarized light by phytochrome in some organisms [19,20]. The proposed model will be, thus, of great help in efforts to understand the molecular mechanism of light-signal transduction by phytochrome.

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