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Structure of Tobacco Mosaic Virus A-protein in Low Ion Concentration at Alkaline pH.

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The size and shape of A-protein of tobacco mosaic virus coat protein (TMVP) were evaluated by means of small-angle X-ray scattering (SAXS) method using a synchrotron radiation source. The quantitative analysis of the SAXS data was made with model optimization assuming an isosceles triangular prism model. The results imply that the A-protein is composed of four single-layered subunits in lower ionic strength. Considering the difference of the A-protein structure in higher and lower ionic strength, the domain structure was proposed for a subunit which might be modeled as a thin isosceles triangular prism composed of four globular domain structure. These domains correspond probably to four helical regions in TMVP subunit, and seem to slightly rearrange their relative positions according to the external conditions. The A-protein of TMVP in low ion concentration is, therefore, formed by the rearrangement of domains. A slight rearrangement of domain positions in a subunit may result the formation of A-proteins of various shapes.

KEY WORDS: Tobacco mosaic virus/ Small-angle X-ray scattering/ Triaxial body model/ A-protein

INTRODUCTION

A tobacco mosaic virus (TMV) particle is approximated as a hollow cylinder of 300 nm in length with an inner diameter of 4 nm and an outer diameter of 18 nm. A particle is composed of about 2130 viral coat proteins (17,495 daltons each) protecting a single strand of ribonucleic acid. TMV coat protein (TMVP) can be readily isolated from intact virus by treating with acetic acid or at alkaline pH.

A-protein is the lowest state of aggregation of TMVP normally encountered. The predominant form in low temperature at alkaline pH gives rise to a sedimentation coefficient of about 4S. A-protein is not a discrete species but rather a rapidly interacting mixture of monomer (subunit), dimer, trimer, and diminishing amounts of larger aggregates(1). The degree of polymerization of TMVP A-protein probably reaches as high as of dodecamers(2). Models for the polymerization behavior of A-protein have been developed by Lauffer(3) and by Durham and Klug(4), where the smallest polymer present is a fan-shaped trimer with the long sides of the three cigar-shaped protein subunits in contact and with the long axes of the subunits all

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pointing toward a common axis(5). However, the structure of TMVP A-protein and its subunit was sledom discussed with sufficient details where only hydrodynamic data were available for the analysis of A-protein structure. The present SAXS data suffice the hydrodynamic data, and are capable of implying that the subunit of TMVP consists of four globular domains, and an A-protein is in turn formed with about four subunits with a possible rearrangement of these domains.

MATERIALS AND METHODS

Materials

TMV, Japanese common strain OM was purified with polyethylene glycol and by differential centrifugation from systemically infected leaves of tobacco (*Nicotiana tabacum* L. cv. Xanthi). TMVP was prepared by the acetic acid method. Samples of TMVP were dialyzed against 1.0 mM sodium phosphate buffer (PB) (pH 9.0) and 1.0 mM PB (pH 7.2) at 5°C, where TMVP is predominantly in A-protein state. Concentration of TMVP was determined spectrophotometrically using the absorbance value of 1.27 $ml.mg^{-1}.cm^{-1}$ at 282 nm. The molecular weight of the subunit is 17,500(6).

Small-Angle X-ray Scattering

SAXS experiments were performed with the optics and detector system of SAXES in the Photon Factory of the National Laboratory for High Energy physics, Tsukuba(7). A wavelength, λ, of 0.149 nm was used and the specimen-to-detector distance was about 1900 mm. The small-angle scattering from Q ($Q=4\pi \sin\theta/\lambda$, where 2θ is the scattering angle)$=1.3\times10^{-1}$ nm^{-1} to 3.35 nm^{-1} (the Bragg spacing equivalent to $d_B=4{,}80$ to 1.88 nm) was registered at 512 different angles by using the one-dimentional position-sensitive proportional counter with an effective length of 200 mm (Rigaku Denki Co. Ltd) (7). The counting time was 600 sec for each measurement. The net scattering intensities were calculated by subtracting the scattering intensities of a blank buffer solution from those of the protein solution. Since the scattered intensity J(Q) at smaller scattering angles is generally approximated in terms of an exponential function of the mean-square radius of gyration of a solute, the radius of gyration R_g can be evaluated from the initial slope of the straight line by plotting ln J(Q) against Q^2.

RESULTS AND DISCUSSION

SAXS data

SAXS experiments were performed with the solutions of TMVP in 1.0 mM PB (pH 7.2 or pH 9.0) at 5°C. The Guinier plot of TMVP solutions is approximately represented by a single straight line. The z-averaged radius of gyration was evaluated from the initial slope of these lines as shown in Table 1.

Radii of gyration of TMVP aggregate estimated here (in 1.0 mM phosphate buffer) are considerably larger than the values for A-protein in the previous paper (7). Probably the aggregates in previous higher PB concentration of the 50 mM to

Table I. Radius of gyration and molecular dimension of isosceles triangular prism, with height 2A, base 2B and thickness 2C, optimized for A-protein.

pH	9.0	7.2
Rg (nm)	3.67	3.69
2A (nm)	10.46	11.00
2B (nm)	12.94	12.44
2C (nm)	2.28	2.26
AIC	−318	−316

100 mM are much compact owing to tight packing, and/or consist of proteins with lower degree of polymerization.

Though the radius of gyration R_g would indicate a size of a solute molecule, its exact shape could not be known from this single parameter. The shape of a solute molecule may be speculated by comparing the observed scattering curve with that calculated for various models of simple triaxial bodies.

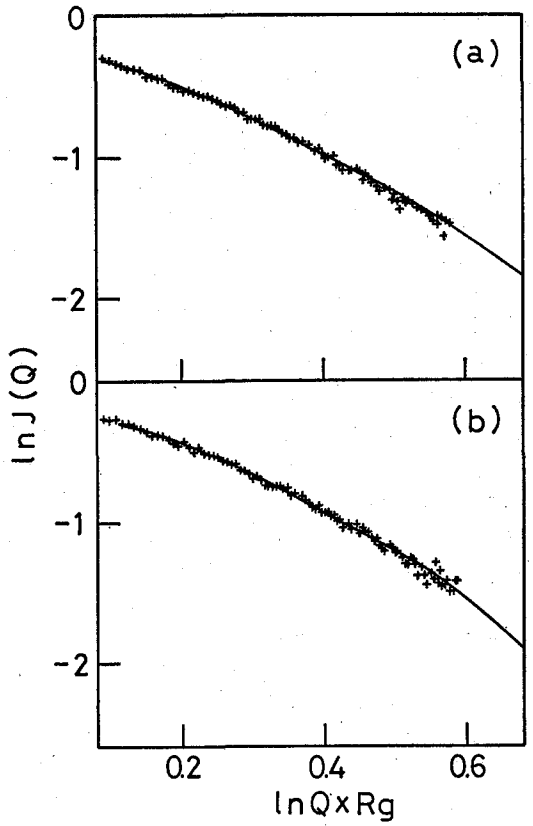

Fig. 1. Experimental scattering curves (++) for TMVP at 5°C with that calculated from an isosceles triangular prism model (solid lines). The protein concentration is 6.5 mg/ml in 1.0 mM PB at pH 9.0 (a) and pH 7.2 (b).

The crystallographic results(8) indicate each subunit (monomer) of TMVP being approximated by an isosceles triangular prism with height 2A, base 2B and thickness 2C. The scattering data were obtained with a simple triaxial body such as an isosceles triangular prism model(9,10) by the use of a non-linear least-squares fitting program SALS (provided by Data Processing Center, University of Tokyo) (11).

The results are summarized in Table 1 which exhibits the the optimized dimension of A-protein in terms of an isosceles triangular prism. Figure 1 displays the SAXS curves from the optimized isosceles triangular prism models (solid lines) with the experimental scattering curves. A good agreement was observed in most of the Q region between two curves.

Dimension of domain structures

According to SAXS and hydrodynamic measurements, bovine serum albumin consists of a covalently bonded trimer domain structure, with two spheres of radius 1.9 nm separately by one of radius 2.66 nm(12). Electron micrographs of human C1-inhibitor revealed a highly elongated molecule consisting of a globular domain structure having a diameter of 4.0 nm and a rod-like domain of length 33 nm and diameter 2.0 nm(13). These domain structures play a key role in respective physiological functions.

As shown in Table 1, thickness of the isosceles triangular prism model is about 2.2 nm in both cases. Since this value agrees well with the thickness assigned to a single subunit, the A-protein in low ionic strength seems to be composed of four single-layered subunits arranged in a fan shape. Other dimensions also are consistent with those evaluated earlier when considered a fan-shaped arrangement of four single-layered subunits.

Concerning the subunit of TMVP consists of two slewed helices (polypeptide number from N-terminal is 20–32 and 114–134) and two radial helices (polypeptide number is 38–48 and 74–88)(1), the TMVP subunit may be assumed to be constructed of four globular domains corresponding to these four helical regions. Here the molecular weight and a partial specific volume of subunit are assumed as 17,500 and 0.743 ml/g, respectively(6). One domian has a molecular weight of about 4,400. These domian structures are observed with Scanning Tunneling Microscope(14). These four domains are rather stable with respect to (helical) structures, but their relative positions will be rearranged according to the external condition. Thus a single-layer arrangement is probably preferred at low ionic strength rather than a double-layer arrangement usually observed.

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