Solution X-ray Scattering Study of Reconstitution Process of Tobacco Mosaic Virus Particle Using Low-Temperature Quenching

Yuzuru Hiragi and Yoh Sano

The reconstitution process of tobacco mosaic virus (TMV) was investigated by the solution X-ray scattering measurements with the synchrotron radiation source using low-temperature quenching. TMV assembly in an aqueous solution is completely stopped below 5℃. The TMV assembly was traced by the small-angle X-ray scattering (SAXS) measurements at 5℃ on a series of solutions prepared by low-temperature quenching after incubation either at 15, 20 or 25℃ appropriate interval between 0 and 60 min. The SAXS results were analyzed by the Guinier plot, the Kratky plot and the distant distribution function. In order to account the time course of SAXS profiles in terms of the elongation of TMV assembly, a model calculation was performed by applying Glatter's multibody method. The simulated model functions support the conclusion that the incubation of the RNA and protein of TMV began to reconstitute TMV instantly after mixing, proceeded steeply to a long rod.

Key words: Solution X-ray scattering/ Tobacco mosaic virus/ Reconstitution/ Quenching

Tobacco mosaic virus (TMV) is one of the well-characterized plant viruses, and consists of single stranded RNA long surrounded by a single type of coat protein of subunits. It forms a hollow cylinder of a length 3000Å, inner diameter of 40Å, and an outer diameter of 180Å. The TMV particles can be reconstituted *in vitro* from its constituents under physiological conditions in two steps of nucleation and elongation. Real-time observation of the elongation process of the assembly may be attainable by using time-resolved (TR) small-angle X-ray (SAXS) scattering measurements. Fortunately, TMV assembly in aqueous solution can be stopped below 5℃ (1). If a reaction mixture of TMV-RNA and TMV-protein kept at 25℃ is quenched into ice water, this quenched mixture maintains the assembly as long as it is kept at 5℃. We can trace the TMV reconstitution process by carrying out SAXS measurements on a series of reaction solutions quenched at 5℃ with appropriate time intervals. We refer this method as a low-temperature Quenching. SAXS method is available at the Photon Factory in KEK, Japan.

Tobacco mosaic virus, Japanese common strain OM was

MOLECULAR BIOLOGY AND INFORMATION —Biopolymerstructure—

Scope of research

Our research aims are to elucidate structure-function relationships of biological macromolecules, mainly proteins, by using phisicochemical methods such as spectroscopic and X-ray diffraction methods. The following attempts have been mainly made in our laboratory for that purpose. (1) Peptide secondary or supersecondary structures in aqueous or hydrophobic environments are studied to get a principle of protein architecture, employing various spectroscopic methods. (2) X-ray diffraction studies on protein structures in crystal and in solution are carried out by crystallographic and/or small-angle X-ray scattering techniques to elucidate structure-function relationships of proteins.


Prof
TAKAHASHI
Sho
(D Sc)


Assoc Prof
HATA
Yasuo
(D Sc)


Instr
HIRAGI
Yuzuru
(D Sc)


Instr
FUJII
Tomomi


Assoc Instr
AKUTAGAWA
Tohru

Students
HISANO, Tamao (DC)
MATSUMOTO, Tomoharu (DC)

propagated in inoculated leaves of *Nicotiana tabacum* L. cv Xanthi. Leaves harvested were homogenized with 100 mM phosphate buffer (pH 7.0) containing 0.1% (v/v) thioglycolic acid. The virus was collected by two cycles of differential centrifugation. RNA was isolated by phenol/bentonite extraction; coat protein was isolated by the acetic acid method.

After mixing each 5 ml of RNA and protein solution in a tube at 5℃, the tube was immersed into a thermostat at either temperature of 15, 20, or 25℃. At an appropriate time interval between 0 and 60 min, each 0.5 ml of the reaction solution was subtracted from the tube and was quenched in ice water below 5℃ as quick as possible. The elongation reaction was completely stopped by quenching at 5℃.

SAXS experiments were carried out at 5℃ with the optics and detector system of SAXES installed on the 2.5 GeV storage ring in the Photon Factory, KEK, Tsukuba, Japan. Scattering intensities were registered with wave length of 1.49Å in the range $0.013Å^{-1} < Q < 0.355Å^{-1}$, where Q denotes the amplitude of the scattering vector equal to $4\pi\sin\theta/\lambda$ and 2θ is the scattering angle. Specimen chamber was kept at 5℃ throughout the experiments in order to prevent the elongation reaction. Net scattering intensities were calculated by subtracting the scattering intensities of a blank buffer solution from those of the assembly solution.

The TMV assembly solutions observed contain different degrees of elongation, and thus each radius of gyration Rg evaluated from SAXS measurements corresponds to the z-average radius of gyration Rg,z.

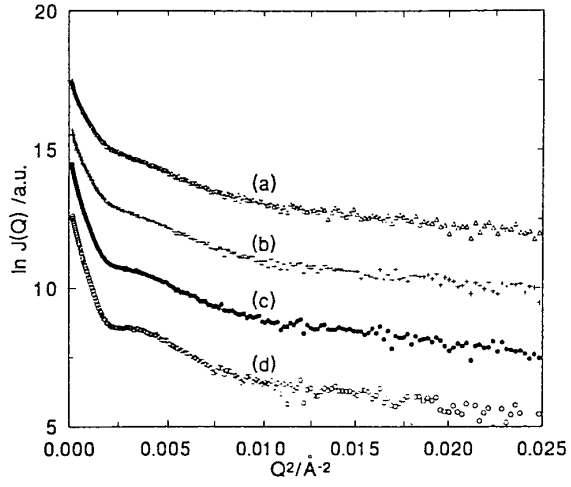

Figure 1. Guinier plots derived from the TMV assembly process. The TMVP solution was quenched at (a) 0 min, (b) 2 min, (c) 8 min, and (d) 25 min after mixing RNA at 20℃.

Fig.1 shows the Guinier plots of a series of TMV assembly samples as an example, quenched at 0, 2, 8 and 25 min after mixing at 20℃. In a low Q range a straight line was depicted in each Guinier plot, and its slope yields Rg,z. An initial slope increases with incubation time at 20℃, and a peak at around $Q^2=0.003Å^{-2}$ becomes steeper. The value of Rg,z(TMVP+RNA) increases more rapidly at 20℃ with the incubation time, compared to that at either 15℃ or 25℃, while the value of Rg,z(TMVP) hardly changes with time.

The Kratky plot is more sensitive than the Guinier plot to the changes in polymer chain configuration. Fig.2 shows the time variation of Kratky plots during TMV assembly at 20℃, where the SAXS data are obtained from the solutions quenched at 1 min, 5 min, 11 min and 25 min. A pronounced increase is observed in the first peak at around $0.002Å^{-1}$, whereas the second peak is almost invariant at about $0.07Å^{-1}$. A similar time variation in the Kratky plots was obtained for the TMV assembly at 15℃ and at 25℃ (data not shown).

Another index is, distant distribution ($p(r)$) function represents a statistical distribution of a pair of points being

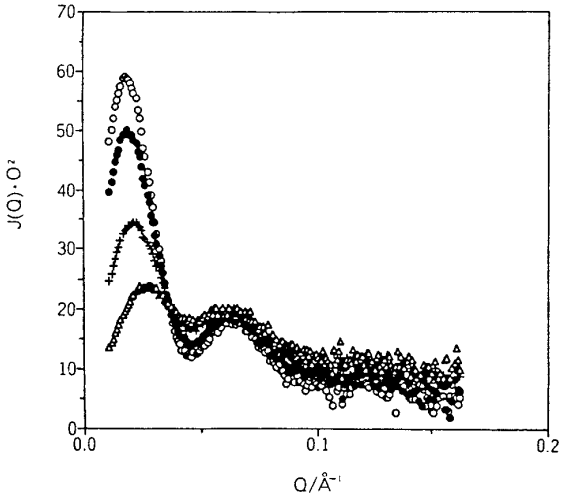

Figure 2. Time variation of the Kratky plots during TMV assembly at 20℃. (Δ) 1 min, (+) 5 min, (●) 11 min and (○) 25 min incubation time.

separated by a distance of rÅ within a molecule. The $p(r)$ function changes with the time during TMV assembly at 20℃ for 1, 5, 11 and 25 min after mixing of TMV-RNA with the protein. A relative ratio of $p(r)$ at around 100Å to that at around 60Å was found to increase gradually with the incubation time. A similar incubation-time dependence in the function was observed during TMV assembly at 15℃ and 25℃ and 25℃ (data not shown).

The results calculated from the SAXS measurements on TMV assembling solutions indicate that at the incubation of TMV-RNA and TMV-protein at 20℃ the reconstitution of TMV starts immediately after mixing, and proceeds fast enough to form a long rod within 10 to 20 min. The full length of TMV particle seems to be formed in 40 to 60 min in this condition. Simulated curves of the Guinier plot, the Kratky plot and the $p(r)$ function from four types of models qualitatively reproduce the experimental curves.

Time course of the Rg,z increases for the TMV assembly system at 20℃ is considerably similar to that obtained from the electron microscopic studies.

The present study proves that low-temperature quenching is useful technique to trace biological phenomena in order of minutes by SAXS or SANS.

References

1. Durham A C H and Klug A, *Nature New Biol.*, **229**, 42-46 (1971).