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SHORT COMMUNICATIONS

Appearance of "β-Like" Circular Dichroism Spectra on Protein Aggregation That Is not Accompanied by Transition to β-Structure

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Abstract—CD spectra in the 200 to 250 nm spectral region for small ordered aggregates (trimers-pentamers) of tobacco mosaic virus (TMV) coat protein (CP) and for long virus-like helical aggregates of TMV CP were compared. It was found that small (4S) TMV CP aggregates have a CD spectrum typical of a protein with high α-helix content, which agrees well with results of X-ray diffraction studies. But in the long helical aggregates (and in the TMV virions) TMV CP gives "β-like" CD spectra similar to those of many other aggregated proteins. From X-ray diffraction data, it is well known that TMV CP subunits do not change their secondary or tertiary structure on assembly into virions or the helical repolymerized protein. Thus, the change in the shape of 200 to 250 nm CD spectra cannot be employed as the sole criterion of the conversion of a protein to β-structure in the course of aggregation.

Key words: tobacco mosaic virus coat protein, aggregation, circular dichroism

In recent years the process of protein aggregation has started to attract much attention [1-3] because of its role in prion infections [4], in amyloid disease progression [5, 6], and in protein crystallization [7], as well as in formation of inclusion bodies during the expression of recombinant proteins in bacteria [1]. In many cases it was shown that the process of protein aggregation is accompanied (or induced) by transition to a form with high β-structure content [1, 2, 8-13]. The specific changes in the 200 to 250 nm region of the CD spectrum are often used as one of the main arguments in favor of such conversion [1, 8-12]. Here we show that similar changes in the CD spectrum are observed on polymerization of tobacco mosaic virus (TMV) coat protein (CP) into helical rods or virions that is not accompanied by any changes in the protein secondary or tertiary structure.

MATERIALS AND METHODS

TMV purification and TMV CP preparation. Wild-type (strain U1) TMV was propagated in *Nicotiana tabacum* var. Samsun plants and purified by standard methods [14]. TMV CP was prepared by the acetic acid method [15] and stored at concentration of about 5 mg/ml in 5 mM phosphate buffer (PB), pH 8.0, at 4°C.

20S-aggregates and repolymerized protein preparation. For CP 20S-aggregate preparation, 1/9 volume of 0.5 M PB, pH 7.0, was added to a TMV CP solution (3-5 mg/ml) in 5 mM PB, pH 8.0, and the mixture was incubated overnight at 20°C and clarified by centrifugation for 20 min at 16,000*g* in a JA-21 rotor of a Beckman J-21C centrifuge. To obtain helical repolymerized protein (RP) preparation, 1/9 volume of 0.5 M PB, pH 5.0, was added to a TMV CP preparation in 5 mM PB, pH 8.0, at 0°C, and the mixture was kept for 2 h at 20°C. Then RP was pelleted by centrifugation for 90 min at 105,000*g* in a 50Ti rotor of a Beckman L5-50 ultracentrifuge. RP was dissolved overnight at 4°C in 50 mM PB, pH 5.6, and clarified by centrifugation for 20 min at 16,000*g* in a JA-21 rotor of the Beckman J-21C centrifuge.

UV spectroscopy. Absorption spectra were measured in 0.5 or 1 cm cells in a Specord UV-VIS (Carl Zeiss, Germany) spectrophotometer. The concentrations of intact virus, 4S-protein, 20S-aggregates, and RP were determined by UV spectroscopy using the following coefficients: $E_{260}^{0.1\%} = 2.30$ for intact virus and $E_{280}^{0.1\%} = 1.30$ for 4S-protein, 20S-aggregates, and RP [16]. For light-scattering suspensions of intact virus and RP, true values of

Abbreviations: TMV) tobacco mosaic virus; CP) coat protein; RP) repolymerized protein; PB) phosphate buffer.

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absorption determined by the extrapolation method [16] were used for calculations.

CD spectroscopy. CD spectra in the 200 to 250 nm region were measured in a modified Jobin-Ivon Mark V (France) dichrograph interfaced with an IBM-compatible PC using the RDA and Wtest programs developed in our laboratory. Measurements were performed at 20°C in 1 mm cells at TMV CP concentration of 200 μg/ml.

RESULTS AND DISCUSSION

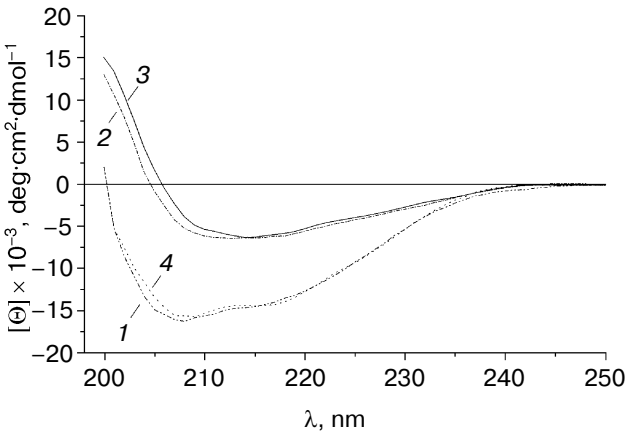
It is well known that depending on conditions (pH, temperature, ionic strength, protein concentration) TMV CP forms in solution several types of specific ordered aggregates [17, 18]. In particular, at pH ≥ 8.0 and low ionic strength TMV CP exists in the form of a dynamic mixture of two-layer trimers and pentamers called 4S- or A-protein. At pH near 7.0 and ionic strength of about 0.1 M, TMV CP is transformed into 20S-aggregate forms (two-layer disk or short helix) with an admixture of 4S-protein, and at pH ≤ 6.0 the protein produces long virus-like helical aggregates called RP [17, 18].

The CD spectrum in the 200 to 250 nm region of TMV CP 4S-aggregates is shown in the figure (curve *1*). This spectrum has been measured in several works [19, 20], and similar results were obtained. The spectrum with a negative band at 208 nm ($[\Theta]_{max}$ = −16,300 deg·cm^2·dmol^{-1}) and a shoulder at about 222 nm is typical of a predominantly α-helical protein. Estimation of α-helix content using the Greenfield and Fasman equation [21] gives the value about 45%, in excellent agreement with the X-ray diffraction results [22-24].

RP formation drastically changes the 200 to 250 nm CD spectrum of TMV CP (figure, curve *2*). The total intensity is strongly decreased, the negative maximum is shifted to about 215 nm, and $[\Theta]_{208}$ drops to about −5,200 deg·cm^2·dmol^{-1}. This spectrum is rather similar to spectra of aggregated forms of many different proteins [8-12].

The CD spectrum of intact TMV virions, containing 95% CP and 5% RNA (figure, curve *3*), was rather close to the spectrum of RP, but $[\Theta]_{215}$ was even a bit lower (−3,800 deg·cm^2·dmol^{-1}). This small difference may be due to a small positive contribution of intravirus RNA, somewhat more tight packing of subunits in the virions, or the presence of an admixture of some 4S-protein in RP preparations. CD spectra of intact TMV did not change over the pH 5.6 to pH 8.0 interval.

We also measured CD spectra of 20S-aggregates, which correspond either to short (35-40 subunits) fragments of TMV helix [25] or to double-layer "disks" built of 34 TMV CP subunits [26]. These 20S-aggregates were shown to initiate TMV self-assembly *in vitro* and *in vivo* [17, 18], and high-resolution X-ray diffraction data for TMV CP were obtained on the double-layer disk crystals



CD spectra of different aggregation forms of TMV CP and of intact virus: *1*) 4S-protein (10 mM PB, pH 8.0); *2*) RP (50 mM PB, pH 5.6); *3*) intact TMV (10 mM PB, pH 8.0); *4*) 20S-aggregates (50 mM PB, pH 7.0)

[22, 24]. We found that to the limits of the accuracy of the method, the TMV 20S-aggregate CD spectrum (figure, curve *4*) does not differ from the spectrum of 4S-protein (figure, curve *1*). Thus, the formation of short ordered TMV CP aggregates does not produce any changes in the 200-250 nm CD spectrum.

From X-ray diffraction data it is definitely known that the TMV CP subunits assembly into RP [27] or into the virions [22-24] is not accompanied by any changes in their secondary or tertiary structure. Thus, in the case of TMV CP polymerization the characteristic change in the shape of 200 to 250 nm CD spectrum should be determined not by conversion to β-structure, but by some other reasons. Among such reasons interactions between closely packed α-helices from different subunits or local electric field alterations upon RP (or virion) formation or some differential light scattering-related effects may be mentioned. In any case, these effects should by quite large, as the absolute value of $[\Theta]_{max}$ drops about threefold on TMV CP polymerization from trimers-pentamers or "disks" into long helical rods (with or without intravirus RNA).

Of course, this does not mean that the same is true for all other protein aggregation systems. In many cases cross-β-structure formation in protein aggregates was confirmed by other methods such as infrared spectroscopy [9, 10], NMR [8, 11, 12], X-ray fiber diffraction [13], and so on. But this does mean that the change in the 200 to 250 nm CD spectrum shape cannot be used as a sole criterion of the conversion of a protein to β-structure during aggregation.

We ourselves observed similar CD spectral changes on heat-induced unordered TMV CP aggregation [20]. In

that work formation of aggregates with sedimentation coefficient of 5,000S did not result in " β-like" changes in CD spectra in the 200-250 nm region, while formation of 50,000S-aggregates did induce such changes.

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