CIRCULAR DICHROISM OF TWO TMV MUTANTS WITH IDENTICAL SINGLE AMINO ACID REPLACEMENT IN DIFFERENT POSITION IN POLYPEPTIDE CHAIN

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SUMMARY. The coat proteins of two TMV mutants carrying proline → leucine replacement in different positions were shown by circular dichroism to have different conformation. Great dissimilarity observed in near UV CD spectra furnish evidence for considerable changes in the environment of some aromatic side chains. Far UV CD spectra suggest some increase in α-helicity in the mutants as compared with wild-type TMV protein.

The protein coat of tobacco mosaic virus (TMV) is composed of identical subunits each of which contain 158 amino acid residues in a well known sequence. Among the many chemically induced mutants of TMV are those with replacements of one or two and more rarely three amino acid residues in the coat protein (2,8). Of the one-residue-replacement mutants, two strains have been isolated in which proline has been replaced by leucine: the first in position 20 (Ni118) and the second in position 156 (Ni1927) (6,7). While strain Ni1927 is temperature-resistant in that like the wild-type TMV it can multiply at a wide variety of temperatures, strain Ni118 is temperature-sensitive and cannot produce whole progeny above 30°. This property is believed to be due to the thermolability of its coat protein (4) thus preventing the specific encoating of the viral nucleic acid

The proteins of these two viruses offer remarkable material for structural studies. They have identical amino acid composition, the same sequence through 156 residues, and differ only in the position where the single amino acid residue was replaced. The present experiments were initiated to investigate how much protein conformation is affected when identical amino acid replacement occurs in different parts of the polypeptide chains.

MATERIALS AND METHODS. *Nicotiana tabacum* L. var. Samsun was used as the host. Plants infected with Ni118 were kept at 19° in a growth chamber; those infected with Ni1927 or with wild-type *vulgare* TMV were maintained in an ordinary greenhouse. Purified virus was prepared from infected tobacco leaves by differential centrifugation. Coat protein was isolated by the acetic acid method (1). Temperature dependence of denaturation rate was measured by an aggregation test (5); results shown in Fig. 1 are in accordance with published report (5).

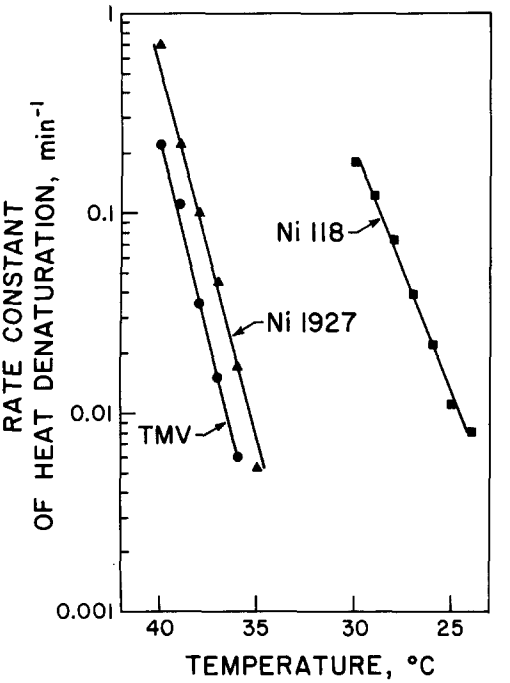

Fig. 1. Temperature dependence of denaturation rates of TMV, Ni1927 and Ni118 proteins. The proteins were heated for different time, at different temperatures, at low ionic strength, pH 7.5 and 10^{-3} M mercaptoethanol. The denatured protein was precipitated at pH 5.0 and removed. The amount of protein left in supernatant was plotted versus time and the rate constant was calculated (for details see ref. (5)). These rate constants are plotted on the graph versus temperature.

Cary Model 60 recording spectropolarimeter with a circular dichroism (CD) attachment and a thermostated jacketed cell holder was used. The optical path in the near UV region was 1.0 cm and in the far UV spectral region below 240 nm was 0.1 or 0.01 cm. Absorption of the protein solutions in 0.02 M sodium

phosphate buffer pH 8.0 was about 1.5 as measured in a 1.0 cm cell at 281 nm. The mean residue weight of 110.6 was used and the data were corrected for the dispersion of the refractive index using published tabulated values (3).

RESULTS AND DISCUSSION. It has been found in this laboratory that TMV protein gives in near UV a complex CD spectrum. Six well resolved peaks were observed at 252, 257, 265, 274, 281 and 291 nm, a deep trough at 296 nm and a crossover at 242 nm (Fig. 3). The spectrum of Nil927 protein appears much alike both at 4° and 25°. In comparison with spectrum of TMV protein ellipticity at 252 and 257 nm is the same but other bands show a slight decrease.

Nil18 protein displays quite a different spectrum. At 4° it has all the bands observed in the other mutant, but significantly less pronounced than in Nil927. The crossover point is shifted to 245 nm. At 25°, however, the same solution gains very faint bluish turbidity and shows pronounced changes in the spectrum. The deep trough at 296 nm disappears, peaks at 291, 281 and 274 nm are not resolved, peak at 252 nm is no more visible and crossover moves to 255 nm.

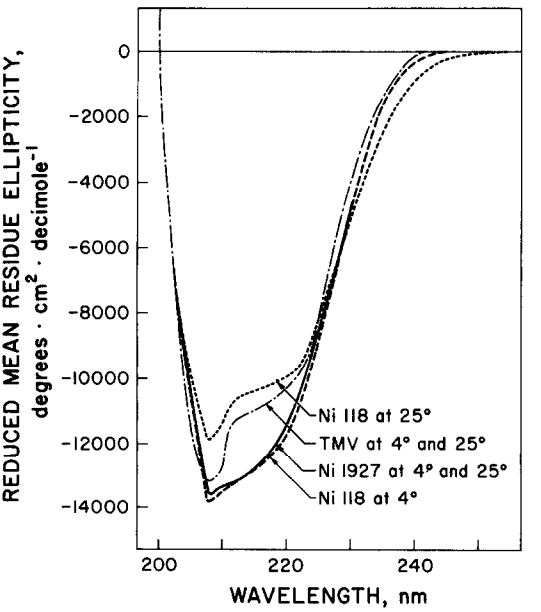

Fig. 2. Far UV CD spectra of TMV, Nil18 and Nil927 proteins measured at 4° and 25°. Protein concentration was 1.1 mg/ml, solvent—0.02 M sodium phosphate buffer pH 8.0, optical path—0.1 or 0.01 cm.

The replacement of proline by leucine is not expected to manifest itself directly in the whole CD spectrum for there is no contribution to optically active absorption bands above 240 nm and no change in the number of peptide groups. Removing of proline, an α-helix breaker, and substitution by leucine, an α-helix promoter, may, however, induce some additional twisting of polypeptide chain. Far UV CD spectra did indeed show some differences. It was found (Fig. 2) that $[\theta']_{222}$ amounts to: -9700, -10500, -10800 and -9600 degrees·cm^2·decimole^{-1} for TMV, Ni1927, Ni118 at 4° and Ni118 at 25° respectively. $[\theta']_{208}$ was about -13500 for TMV, Ni1927 and Ni118 at 4° but -11900 for Ni118 at 25°. This means that the replacement of proline by leucine has a direct influence on the conformation of polypeptide chain. This influence seems to be the same on both Ni118 and Ni1927 proteins since similar slight increase in α-helicity has been found.

The observed differences in near UV CD spectra of the two mutants suggest that the replacement induces also indirect alterations in the environment and conformation of some aromatic side chains. The amino acid replacement in Ni1927 is localized in an external and hydrophylic region of the protein molecule. Apparently the tertiary structure of this region is less important for the function of the protein which is the specific polymerization into a superhelical hollow rod. Consequently, properties of Ni1927 protein resemble those of TMV protein.

In contrast, the amino acid replacement in Ni118 protein occurs in a hydrophobic area which very probably is involved in the polymerization of subunits. Distortion of the secondary and tertiary structures in this area probably propagates perturbation on other parts of the protein molecule so that some changes in the environment of phenylalanine, tyrosine and tryptophane can be demonstrated in near UV CD. This distortion may bring about sensitivity to heat denaturation (Fig. 1). Ni118 protein denatured at 25° displays not only further dissolution of specific orientation of aromatic side chains but also some loss of ordered secondary structure of the polypeptide chain.

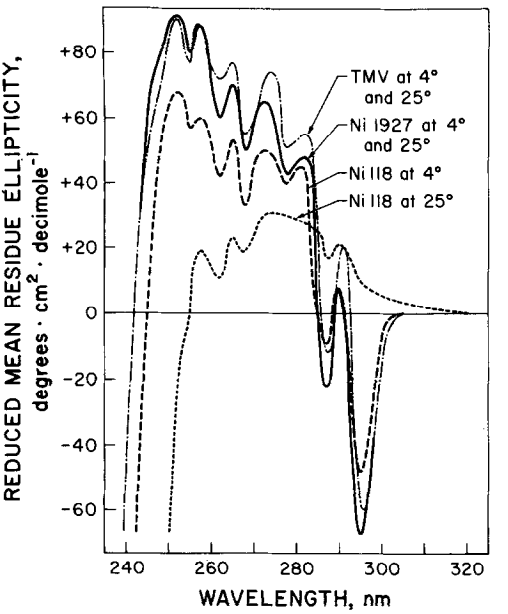

Fig. 3. Near UV CD spectra of TMV, Ni118 and Ni1927 proteins measured at 4° and 25°. Protein concentration was 1.1 mg/ml, solvent—0.02 M sodium phosphate buffer pH 8.0, optical path—1.0 cm.

The question still remains open whether the abnormality of strain Ni118 is due solely to the thermolability of its coat protein. It is not known whether the one-residue-replacement mutants are really of one hit type. The impaired infectivity may arise also from other additional mutations in the TMV-RNA.

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