PROTEIN-RNA INTERACTIONS IN TOBACCO MOSAIC VIRUS

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INTRODUCTION

Tobacco mosaic virus (TMV) is a rod-shaped virus, 3,000 Å long and 180 Å in diameter. It has helical symmetry, with 49 coat protein subunits in three turns. A single strand of RNA follows the basic helix at 40 Å radius, with three nucleotides bound to each protein subunit. See Caspar (1963) for a review of early work.

Using x-ray diffraction from oriented gels and a technique analogous to crystallographic isomorphous replacement (but requiring more heavy atom derivatives) Stubbs et al. (1977) solved the structure of the intact virus to a resolution of 4 Å. The electron density map they produced has been improved by applying an envelope function to the map, back-transforming, and applying the resulting phases to the observed data. This is commonly used in protein crystallography, and is a much more powerful constraint in fiber diffraction. The interpretation of the map has been improved by consideration of maps of the protein helix, which is structurally almost identical to the virus, except for the absence of the RNA. The RNA model has been improved by computer model-building techniques as described below.

METHODS

Fiber Diffraction

Oriented gels, 15–20% TMV, diffract x-rays to give a high quality fiber diffraction pattern. (See Holmes et al. (1975) for references.) Patterns were recorded photographically in a Guinier camera using a point-focused beam from two bent quartz or germanium crystals and an Elliot fine focus rotating anode generator (GX6 or GX13). Films were scanned with a computer controlled flat bed densitometer.

Model Building

The RNA triplet was built to conform to five basic criteria: (*a*) it should fit the electron density map; (*b*) bond lengths and angles should be reasonable; (*c*) sugar conformations should resemble those observed in small molecules; (*d*) close contacts should be avoided; (*e*) the helical symmetry of the virus should be preserved, that is, adjacent (and therefore covalently connected) RNA triplets should have the same structure.

Reasonable bond lengths and angles were achieved using a model-fitting program based on one designed for proteins by Dodson et al. (1976), and adapted for nucleic acids. Corrections to the structure to conform to the other four criteria were alternated with the model fitting.

RESULTS

The starting model for the RNA was obtained by considering a difference map between the virus and a form of the coat protein which is isomorphous to the virus (Mandelkow et al., 1976). This model was then fitted to the corresponding electron density in the virus map.

After 23 rounds of model fitting, a structure conforming to the five criteria was found. It is broadly similar to the unrefined structure described by Stubbs et al. (1977), but with two significant differences: (*a*) all three sugar rings have the 3′-endo conformation, and (*b*) one of

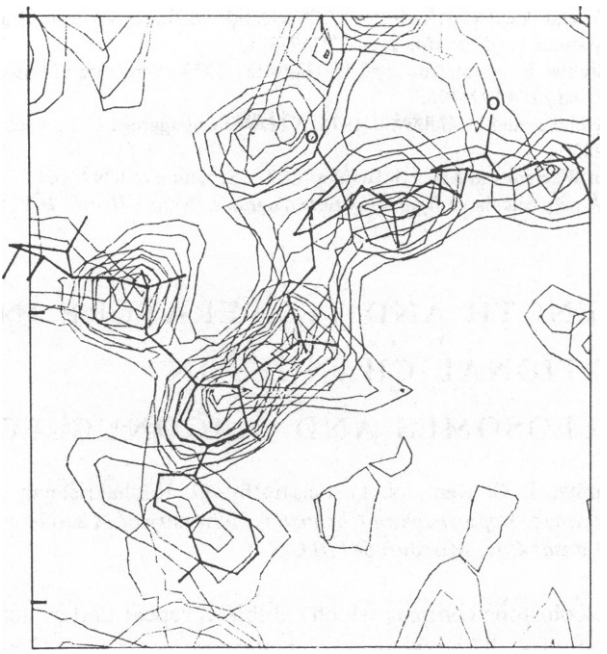

Figure 1 Three sections of electron density with the main chain and one base of the RNA after 23 rounds of model fitting. Hydroxyls which could be forming hydrogen bonds are marked "O."

the bases was found to fit the density much better in the *syn* conformation than in the *anti* conformation. Although unusual, this conformation has now been observed in several polynucleotides in other laboratories, and was considered possible, at least for purines, in the analysis of Haschemeyer and Rich (1967).

The final model superimposed on the electron density map is shown in Fig. 1.

DISCUSSION

We observe three types of binding of the trinucleotide to the virus coat protein: (*a*) The phosphate groups form ion pairs with arginines 90 and 92, and probably with Arg 41 or 113. (*b*) The RNA ribose 2'OH on residues 1 and 3 could well hydrogen bond to a protein side chain. In each case, the hydroxyl group points into protein density. It is not yet possible to identify unambiguously the side chains involved. (*c*) All three bases lie flat against methyl or methylene groups in the side chains of the left radial α-helix in the coat protein.

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IONIC STRENGTH AND TEMPERATURE INDUCED CONFORMATIONAL CHANGES IN MONONUCLEOSOMES AND OLIGONUCLEOSOMES

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Chromatin is a nucleohistone complex which exhibits a repeat unit structure as inferred from nuclease digestion studies. The repeat unit, or nucleosome, is defined as ~200 base pairs of DNA wrapped about the surface of an octameric histone complex (two copies each of the histones H2A, H2B, H3, and H4). We report in this communication preliminary studies on the conformation of chromatin mononucleosomes and oligonucleosomes as a function of temperature and ionic strength. The methods used were conductivity, fluorescence of bound proflavine, and quasielastic light scattering.

Chicken erythrocyte chromatin was digested at 37°C with micrococcal nuclease as described by Rill et al. (1978). The sample was placed on an A5m column (200–400 mesh) and the fractions designated by tube number (200 drops/tube).

The monomer pool from the A5m column was rechromatographed on a second column (A0.5m,100–200 mesh) and the tube with maximum absorption at 260 nm was designated as sample D. The tubes on either side were designated D–1 and D+1. Polyacrylamide gel electrophoresis indicated that no proteolysis of the histones occurred and that the distribution of DNA lengths of the monomer was biomodal (144–153,162–181 base pairs). Some dimer contamination was observed in sample D-1 but not in sample D+1. The conductivity increment (C'-C)/C for mononucleosomes in 1 mM cacodylate was determined for selected fractions at several temperatures. (cf. Fig. 1*A* caption for definitions of terms). A decrease in (C'-C)/C with temperature from 5° to 35°C suggests the mononucleosomes absorb ions from solution, which is then followed by an apparent release of ions at higher temperatures. These data suggest at least one conformational change occurs at 35°C. Proflavine was used as a probe to study the thermally induced conformational change of mononucleosomes in 1 mM cacodylate and reported as the function (D/P)(F'-F)/F. (cf. Fig. 1*B* caption for definitions of terms). These data suggest conformational changes may occur at 30°, 35°, 37°, and 42°C.

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