M-PM-J1 THE FOLDING OF RIBOSOMAL RNA IS DETERMINED BY THE RIBONUCLEOTIDE SEQUENCE
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The mechanism in which ribosomal 5S, 16S, and 23S RNAs acquire their native conformation has been investigated by unfolding and refolding studies using circular dichroism, hydrodynamic measurements, and thermal denaturation to monitor the changes in conformation, hydrodynamic shape, and stability, respectively. Furthermore, the refolded RNAs were examined for their protein binding properties and *in vitro* reconstitution behavior. The results showed that ribosomal RNAs can be denatured by 5M urea and EDTA (molar ratio of EDTA to RNA: 3.25×10^3) to an unfolded state which is consistent with a single stranded highly helically stacked polyribonucleotide. Removal of the denaturant (urea and EDTA) by exhaustive dialysis against native and reconstitution buffers results in refolded RNAs whose conformation and stability are indistinguishable from the native RNAs. Sedimentation velocity and intrinsic viscosity measurements demonstrated that the refolded RNAs have the same hydrodynamic properties as the native RNAs under the same buffer conditions. Difference two-dimensional gel electrophoresis showed that both refolded 16S and 23S RNAs bind their corresponding ribosomal proteins to form reconstitution particles which are very similar to those reconstituted from their corresponding native RNAs. However, some subtle but significant differences were observed in comparing the steps of *in vitro* reconstitution of the refolded RNAs and those of the native RNAs. These results indicate that the folding of the ribosomal RNAs into their native conformations free in solution is determined solely by their ribonucleotide sequences.

M-PM-J2 DEMONSTRATION OF ORDERED RNA STRUCTURE AND RNA-CAPSID INTERACTIONS IN COWPEA CHLOROTIC MOTTLE VIRUS BY DIFFERENCE RAMAN SPECTROSCOPY. B.J.M. Verduin, Dept. of Virology, Agricultural Univ., Wageningen, The Netherlands; B. Prescott and G.J. Thomas, Jr., Dept. of Chemistry Southeastern Mass. Univ., N. Dartmouth, MA 02747.

Laser Raman spectra have been recorded for the following states of the bromovirus CCMV and its components in aqueous solution: native virus particles, swollen virus particles which retain RNA, empty capsids which are devoid of RNA, coat protein dimers which result from capsid disassembly, and coat protein core molecules which lack a 25 residue fragment of the N-terminus. The Raman spectrum of encapsidated viral RNA has also been generated by computer subtraction of the spectra of whole virus and empty capsids or subunits. The data reveal a number of structural characteristics of the viral genome and coat subunits, including the following: (i) CCMV RNA manifests a highly ordered secondary structure in the native virion, which is perturbed slightly by pH-induced swelling of the capsid; (ii) conversion of the native virus (pH 5.0) to swollen particles (pH 7.5) causes elimination of specific RNA base interactions; (iii) the secondary structure of the coat protein is predominantly a β-sheet structure in all assembly states mentioned above; (iv) the secondary structure of the N-terminal fragment does not differ appreciably from that of the coat protein. Raman difference spectroscopy also reveals that amino acid side chains of the capsid subunit are involved in the structure transition which accompanies swelling of the virion.

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M-PM-J3 THE STRUCTURE AND STABILITY OF A DOUBLE-STRAND HELIX WITH AN EXTRA CYTIDINE. (Kathleen M. Morden and Ignacio Tinoco, Jr., Chemistry Department, University of California, Berkeley, CA 94720.)

Three deoxyribo-oligonucleotides have been compared to determine the effects of a perturbation on the structure and dynamics of a double-stranded DNA helix: I) $dCA_6G + dCT_6G$, II) $dCA_3CA_3G + dCT_6G$, and III) $dCA_5G + dCT_5G$. Thermodynamic parameters have been measured from optical melting curves. The extra cytidine in helix II significantly destabilizes the double strand compared to helix I; an increase in free energy of 2.9 Kcal/mol at 25°C is found. Double-strand formation in the perturbed helix II is destabilized by 1.7 Kcal/mol at 25°C relative to helix III with one less base pair. Nuclear magnetic resonance has been used to study the conformation of these three helices in solution. The temperature dependence of the chemical shifts has been investigated for the aromatic base protons in each of the double helices as well as in the single strands. The base-pairing imino protons from helix II have been observed in H_2O and show that one of the A·T imino resonances has an upfield shift of more than 0.4 ppm relative to helix III. The properties of the three helices are compared to determine their conformations and the effects of the extra cytidine.