Title
STEM MASS MEASUREMENTS OF POLYOMA CAPSIDS AND VIRIONS

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T-AM-G10 EARLY STAGE POLYMERIZATION OF E66 TMV PROTEIN. Ragaa A. Shalaby and Max A. Lauffer. Biophysical Laboratory, Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh,PA 15260

As previously reported, the loading concentration, C, at which the first trace of 20S component appears in the ultracentrifuge has been used to investigate the effect of temperature, pH and ionic strength, μ, on the polymerization of TMV coat protein. From these measurements, ΔH, ΔS and ΔW_{el} per mole of A protein and the salting-out constant, K_s', were determined. The same procedure has now been followed for obtaining these parameters for the protein of E66, a mutant of TMV, differing only in the substitution of lysine for asparagine at position 140. This results in a less hydrophobic protein with one less net negative charge per subunit at pH 7. Prior to each sedimentation run, the protein at the desired pH, μ and concentration was warmed slowly to the desired temperature and incubated in the centrifuge at that temperature for at least 6 hours. To investigate the effect of μ, experiments were carried out at 12°C and pH 6.7 with values of μ between 0.01 and 0.15. For studying the effect of temperature, experiments were carried out at pH 6.7 and μ of 0.10. The effect of pH was determined by sedimentation measurements at constant T and μ. The results show that ΔH and ΔS and the binding of hydrogen ions are nearly the same as for the polymerization of TMV protein. However, the electrical work contribution, ΔW_{el}, and the salting-out constant, K_s', both determined from the effect of ionic strength, are each approximately one-half the values previously obtained for TMV protein, in nearly quantitative agreement with expectation from the difference in amino acid composition. This result provides strong support for the appropriateness of this method of analysis. (Supported by NIH Grant GM 21619.)

T-AM-G11 ION BINDING BY TMV, TMV PROTEIN AND TMV-RNA. Warren H. Gallagher, H. H. Gastfriend and Max A. Lauffer. Biophysical Laboratory, Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

Calcium ion titrations were carried out on TMV and TMV coat protein and both calcium ion and potassium ion titrations on TMV-RNA using ion-specific electrodes. Scatchard analysis showed that near neutrality the virus has two Ca^{++} binding sites, treated as non-identical, independently titrating centers, with apparent stability constants, β_{Ca}', greater than 10^4 M^{-1}. The higher affinity site for TMV titrated in water has a value of $\log\beta_{Ca}'$ which varies from 8.5 at pH 8.5 to 3.9 at pH 5. For Ca^{++} titration of TMV in 0.01 M KCl, the value is between 6.2 at pH 8.0 and 3.7 at pH 5.5. The higher affinity site for TMV in water binds up to two competing H^+ ions, one with an apparent pK_H greater than 8.5 and the other with a value which varies from 6.0 at pH 5.5 to 7.3 at pH 8.0. For TMV in 0.01 M KCl, only the competing H^+ ion binding with pK_H greater than 8.5 remains. TMV coat protein is incapable of binding Ca^{++} under equilibrium conditions at pH values above its isoionic point, but they are bound under nonequilibrium conditions. RNA in water bound 0.45 Ca^{++} ions per nucleotide with an apparent stability constant of $\log\beta_{Ca}$ = 6.03. 0.24 H^+ ions were released per Ca^{++} bound. Less Ca^{++} was bound in 0.01 M KCl solution and no H^+ ions were released. K^+ titrations in water solution showed that this ion is bound to the extent of 0.25 per nucleotide with $\log\beta_K$ of 2.96 and very little H^+ released. These results explain the fact that TMV and polymerized TMV coat protein have identical electrophoretic mobilities. (Supported by NIH Grant GM 21619.)

T-AM-G12 STEM MASS MEASUREMENTS OF POLYOMA CAPSIDS AND VIRIONS. T.S. Baker, I. Rayment, D.L.D. Caspar and W.T. Murakami, Rosenstiel Basic Medical Sciences Research Center and Department of Biochemistry, Brandeis University, Waltham, MA and J. Wall and J. Hainfeld, Biology Department, Brookhaven National Laboratory, Upton, NY.

The unexpected discovery by Rayment et al. (Nature 295, 110, 1982) that the 72 capsomeres of the polyoma virus capsid are all pentamers shows that bonding specificity is not conserved among the protein subunits in this icosahedrally symmetric assembly. Physicochemical characterization of polyoma virus particles has been limited to a few studies, most tending to support the 360 subunit model revealed by X-ray crystallography. Electron scattering measurements on unstained, unfixed particles in the scanning transmission electron microscope (STEM) provide a unique procedure for measuring the molecular weight of individual particles and, thereby, the weight distribution of the population. Preliminary analysis of mass measurements of capsids (899 particles; M_r=16.1±1.4x10^6 daltons) and virions (440 particles; M_r=20.7±1.0x10^6 daltons) are in complete agreement with the 360 VP1 subunit model. The virion mass is about 10% lower than previously measured using physical-chemical methods. The possibility that this low value may result from systematic errors in the STEM measurements is being explored. Alternatively, the composition of the complete virion may be somewhat different than expected from other measurements. Examination of developmental intermediates, including minichromosomes, previrions and tubular polymorphic aggregates by STEM, conventional electron microscopy and image analysis techniques will provide more detailed information about their composition, interrelation and role in the control of virus assembly. Supported by NIH grant CA15468 (DLDC).