THE INTERACTION OF T-4 PHAGE PARTICLES AND MERCURIC ION

A report on a Ch 80 project of 36 units, carried out between December 28, 1962 and June, 1963, under the guidance of Dr. Norman Davidson, by Thomas T. Bopp.

SUMMARY

The interaction of T-4 phage particles and mercuric ion has been studied by ultraviolet spectrophotometry. The changes in the ultraviolet absorption spectrum upon the addition of Hg(II) are very similar to the changes observed by Yarane and Davidson and others for free DNA.

GENERAL BACKGROUND

Deoxyribonucleic Acid (DNA) is known to consist of the nucleotides adenine, cytosine, guanine, and thymine, linked to the sugar deoxyribose. These sugars are in turn joined to a phosphate "backbone" in long chains in a hydrogen-bonded double helix. The absorption spectrum of DNA in the ultraviolet has a maximum around 260 mμ. The nucleotides separately have similar absorption spectra, and it is clear that the DNA absorption in this region (210-320 mμ) is due to a contribution to absorption by the nucleotides in the DNA molecule.

One approach to the problem of the structure of DNA is that of studying the metal ion complexes of the nucleotides, synthetic polynucleotides, and of DNA itself.

In the case of Mg^{++}, Ca^{++}, or Mn^{++}, conductometric titrations, the absence of significant spectral shifts, and other considerations indicate that these ions are bound to the negative phosphate groups by electrostatic forces.

The behavior of Hg^{++} is qualitatively different.[1] The addition of $HgCl_2$ or $Hg(ClO_4)_2$ to a dilute DNA solution is accompanied by a drastic decrease in the intrinsic viscosity of the solution, a shift in the ultraviolet absorption, and the release of protons.

As Hg^{++} is added, one type of complex with a characteristic spectrum forms up to a ratio of one Hg^{++} to two bases. With

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1. Yamane, Tetsuo and Davidson, Norman, J.A.C.S., 83, 2599, (1961)

excess Hg^{++}, a second higher complex forms, accompanied by the disappearance of the isosbestic point in the ultraviolet absorption spectrum characteristic of the lower complex.

Upon the addition of chloride ion or other complexing agents for Hg^{++}, the spectrum becomes that of uncomplexed DNA, and the DNA can be recovered. Davidson and Yamane were unable to propose a structure for the complexes that was consistant with all their observations, but it is clear that some sort of direct interaction of the Hg^{++} with the base moieties is involved.

THE PROBLEM

Phage particles are known to consist of a small packet of DNA surrounded by a protein coat. It would be interesting to ascertain in what ways the DNA inside a phage particle behaves like free DNA in a solution, and in what ways its behavior differs. One clue to this would be to study the binding of Hg^{++} to phage particles.

Inside a phage particle, the DNA molecules are tightly packed together. It is conceivable that in this state the binding of Hg^{++} would be different than for molecules in solution. Possibly the protein coat would entirely shield the DNA from the Hg^{++}.

Another reason for studying the Hg^{++}-phage interaction is the possibility that Hg^{++} could be used as a DNA-specific stain for electron microscopy. Mercury is a heavy metal, and is fairly opaque to a beam of electrons. If Hg^{++} does form a DNA complex in spite of the protein coat of the phage particles, the information may have definite application toward looking at the DNA inside a phage with the electron microscope.

For these reasons, and because the problem is intrinsically interesting in itself, a study of the interaction of phage particles with mercuric ion was undertaken. Since a preparation of T-4 phage (a certain strain) was available, and since T-4 is also suitable for electron microscopy, this strain of phage was selected for study.

RESULTS

The most direct means of studying this interaction is ultraviolet absorption spectrophotometry. When sufficiently dilute and purified, so that scattering due to aggregates is negligible, the absorption of the DNA is observable. A crude calculation of the absorption in this region due to the protein coat itself at these concentrations predicts that the contribution to the absorption due to the protein is less than 1% of the absorption due to the DNA.

The observation is that on the basis of spectrophotometric data (Fig.1), T-4 phage particles and DNA behave exactly the same as regards formation of complexes with mercuric ion. Two complexes seem to be formed. The spectrum of the complex formed at low Hg^{++} has a isosbestic point at 262 mμ. This is in the center of the range 261.5-262.5 mμ observed by Yamane and Davidson for the isosbestic points of the mercury complexes of calf thymus and various bacterial DNA's. At ratios of Hg^{++} to phosphate of greater than 0.5, the isosbestic point is seen to disappear. This was observed by Yamane and Davidson for free DNA.

Experiments are currently underway to ascertain the reversibility of this reaction. Chloride and glutathione are known to form stable complexes with Hg^{++}, and would be expected to compete with the DNA for Hg^{++}. This process should be accompanied by a return of the spectrum to that of unmercurated phage.

If it should turn out that the Hg^{++} can indeed be removed from the DNA and the mercury-free phage particles recovered, the question remains as to whether the protein coat has been

damaged by the process. This could be partially ascertained by a study of the viability of such "reclaimed" phages, i.e., comparing their ability to attack bacteria such as E. coli with the attacking ability of phages which have never been mercurated.

Consistant results from the reversibility experiment with chloride ion are not yet available, although it is hoped that they will be complete by the end of the year.

EXPERIMENTAL

Concentrated solutions of T-4 phage were obtained from Dr. J. Vinograd. These were diluted to an absorbancy (at 260 mμ, 1 cm. path) of about 10, and were exhaustively dialyzed at 4° C. against a solvent of 0.100 F $NaClO_4$, 0.005 F $Mg(ClO_4)_2$, 0.001 F Cacodylic acid buffer, pH 7.0 . The Mg^{++} is necessary to stabilize the protein of the phage particles. It should not interfere with the Hg^{++} reaction.

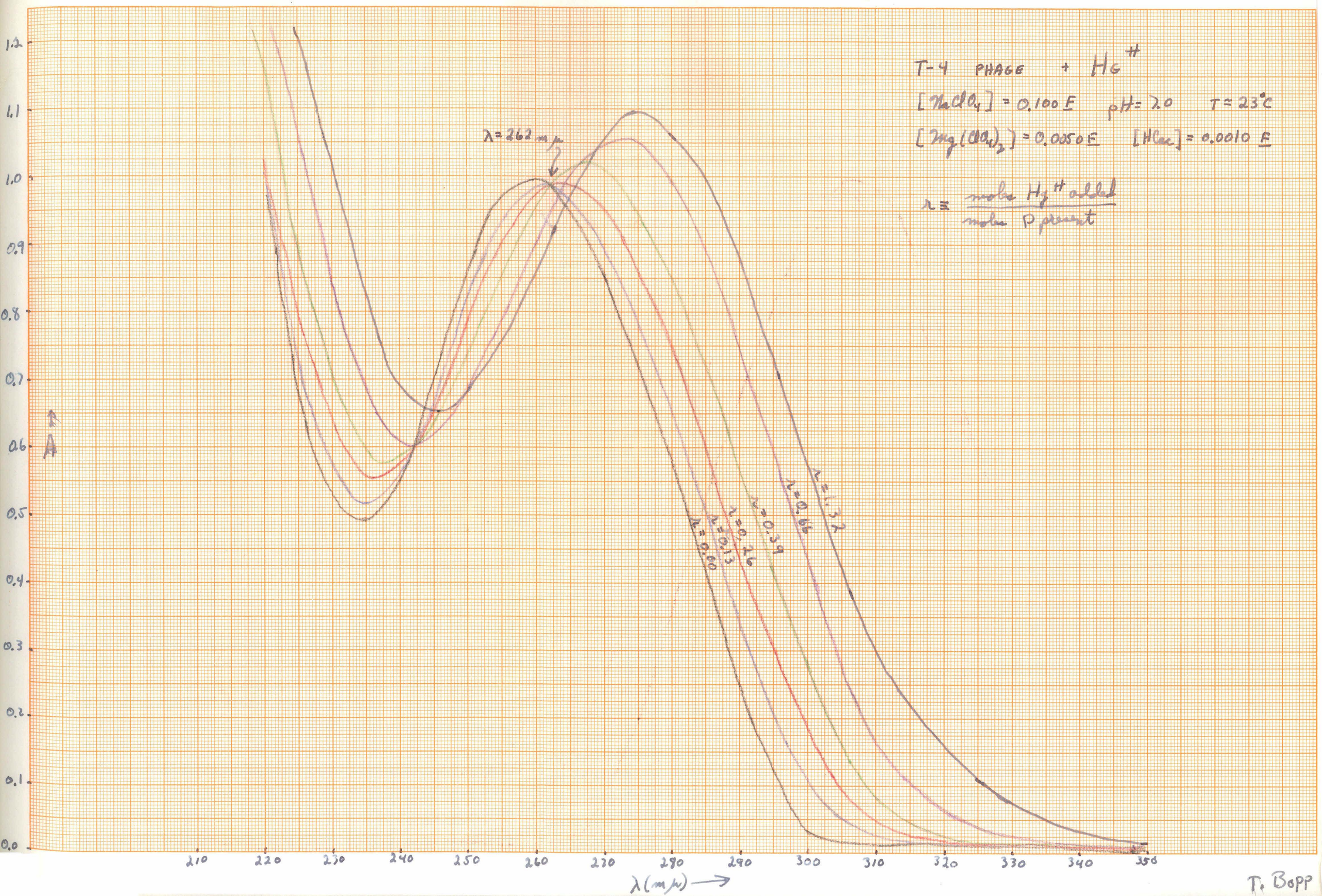
The resulting cloudy solution was centrifuged at 20,000 rpm in a Beckman Model L ultracentrifuge in an SW 39 rotor for 20 minutes to remove aggregates which would cause extensive scattering. The resulting clear solution was siphoned off and used as stock solution, to be diluted 10:1 with the above solvent to obtain solutions for experiments.

Much of the time involved in the project to date was in working out the above procedure for obtaining satisfactory phage solutions.

Spectra were taken on a Cary Model 14 recording spectrophotometer. Corrections for volume changes and blank changes due to Hg^{++} were made when applicable in calculating all spectra.

Phosphate concentrations of DNA in phage particles were determined spectrophotometrically, assuming $\epsilon_{260} = 6.60 \times 10^3$ liter-mole $P^{-1}cm^{-1}$.

FIG 1 ← TwO 0107



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