THE INTERACTION OF POLYNUCLEOTIDES WITH CATIONS*

GARY FELSENFELD AND SYLVIA HUANG

*Department of Biophysics***, University of Pittsburgh, Pa. (U.S.A.)*

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SUMMARY

The interaction with divalent cations of the synthetic polynucleotides, polyadenylic acid and polyuridylic acid, has been studied by conductiometric titration. It is shown that further binding of divalent cation by polynucleotide ceases when one equivalent of cation has been added per mole of polymer phosphate, and that the titration curve is the same for both polymers. This result is compared with that obtained previously for DNA in distilled water, and it is shown that the anomalous end-point for DNA is eliminated if sufficient points are taken in the course of the titration.

The interaction of polynucleotides with polylysine has also been investigated, both spectrophotometrically and by conductiometric titration. It is shown that a stoichiometrically well defined complex is formed, and that in the course of the reaction the polylysine is capable of displacing an equivalent amount of divalent cation.

INTRODUCTION

The ability of the synthetic polynucleotides, polyadenylic acid and polyuridylic acid, to react with one another in solution is strongly dependent upon the ionic composition of the solution[1-3]. This behavior is particularly interesting because the two-stranded complex formed by poly A and poly U has properties resembling those of natural DNA[2,4,5].

In this paper we describe studies of the interaction of polynucleotides with divalent cations and with the polycation, polylysine. It will be shown that the evidence from conductiometric titrations and spectrophotometry indicates the formation of stoichiometrically well defined complexes in each case.

METHODS

Spectrophotometric studies were carried out in the Cary Model 14 recording spectrophotometer. Conductiometric titrations were performed using a bridge constructed from resistance and capacitance components supplied by General Radio Co., Cambridge, Mass. All resistors used are stated by the manufacturer to be accurate to

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Abbreviations: poly A, polyadenylic acid; poly U, polyuridylic acid; DNA, deoxyribonucleic acid.

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± 0.05% at 1000 cycles A.C. The source of the 1000 cycle signal was a General Radio 1214-A oscillator, and the balance point was determined with a General Radio 1212-A amplifier-null detector. A simple dipping-type conductiometric cell with cell constant 0.110 cm^{-1} was employed with electrodes unplatinized to avoid problems of polymer adsorption on the electrode surface. The current across the cell was kept low—of the order of 100 μA at balance.

Addition of titrant solutions was made with a pipette, always delivering between the same two marks on the pipette, or in the case of small volumes of material (0.02 ml or less) by means of a Gilmont Micropipette (total capacity 1 ml). Temperature was kept constant at 27.2° in most cases to ± 0.02° with a water bath and Bronwill constant temperature regulator.

The polynucleotides were prepared by the authors from polynucleotide phosphorylase isolated by the authors from *Azotobacter agile* according to a procedure kindly provided by Dr. S. OCHOA. The mean sedimentation coefficients of the polymers were 8.5 for poly A and 5.4 for poly U. The polymers were dialyzed successively against ethylenediamine tetra-acetic acid, sodium chloride, and triple-distilled water before freeze-drying.

Polylysine hydrobromide, with an average chain length of 50 residues, was provided by Dr. MICHAEL SELA.

In certain cases indicated in the text, notably the polylysine titrations, a linear correction has been applied to the data to take account of the dilution of the original solution during the course of the titration. This correction becomes important when the conductivity of the initial solution is high and the concentration of titrant is low. The correction alters slopes, but not the position of changes in slope.

Redistilled water was used in all experiments.

The DNA, isolated from calf thymus, was supplied by the Sigma Chemical Company.

Polylysine concentrations were determined by a modified Nessler method, which is accurate to about 5%.

I. DIVALENT CATIONS

RESULTS

The conductiometric titration of DNA with divalent cations was first performed by SHACK[6], who found that in distilled water there appeared to be a sharp end-point to the titration at about 0.8 equivalent of Mg^{++} or Ca^{++} per mole of polymer phosphate. Under similar conditions involving the same number of experimental points, poly U in distilled water appears to have the same end-point when titrated with manganese chloride. However, if the number of experimental points is increased sufficiently, it becomes apparent that the titration curve consists in the first approximation of three regions rather than two (Fig. 1). The intermediate region, which appears to be satisfactorily approximated, except at its extremes, by a straight line, was not obvious in earlier studies because in these studies only one or two experimental points fell in this region. The straight lines formed by the points on either side of the intermediate region intersect at about 0.7 equiv. of Mn^{++}, the value observed in our earlier experiments. On the other hand, if one draws a straight line through the intermediate set of

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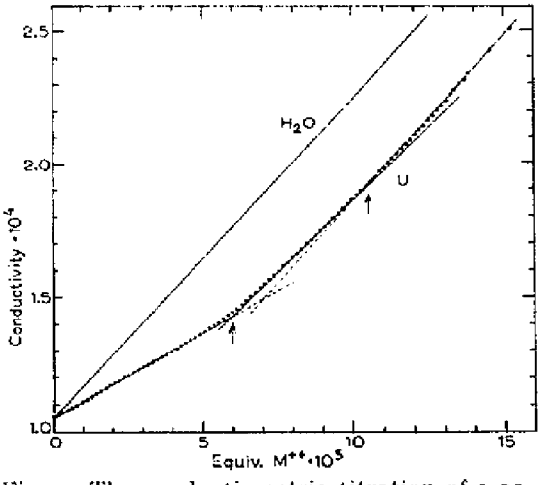

Fig. 1. The conductiometric titration of $1.00 \cdot 10^{-4}$ *M* polyuridylic acid with $2 \cdot 10^{-3}$ *M* $MnCl_2$. The arrows show the breaks in the experimental curve, the dotted lines show the end-point which would be obtained if a smaller number of experimental points were taken. The upper straight line, marked "H_2O", shows the curve for titration of water with $MnCl_2$.

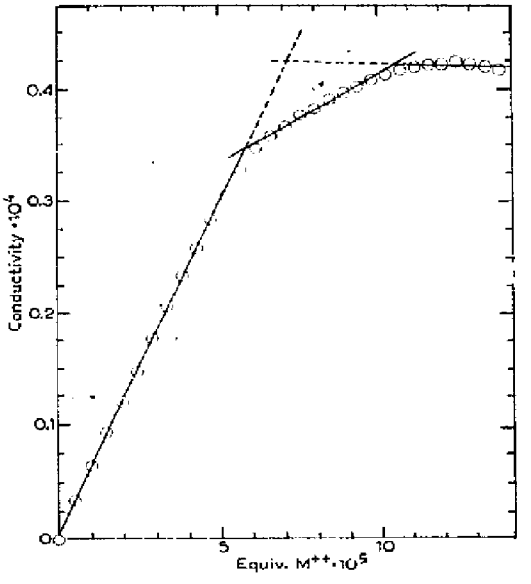

Fig. 2. The difference between the curve marked "H_2O" and the curve marked "U" in Fig. 1, showing the double break.

points there are now two "end-points", one at about 0.6 equiv. and the other almost exactly at 1.0 equiv. of divalent cation.

The slope of the second straight line region is about 1.7 times the first, while that of the third straight line region is about 1.15 times that of the second. All of these differences are far beyond the range of experimental error. The same titration curve is observed over a 10-fold range of poly U concentration. The titration curve of poly U can be replotted (Fig. 2) to show the double break in the curve even more distinctly.

We will use this simplified description of the titration data because, although the points (particularly those in the intermediate region) could be thought of as lying on a continuous slightly non-linear curve, our approximation permits us to obtain an accurate description of the shape of the curve in terms of E_1 and E_2, the two "breaks" in the curve, and λ_1, λ_2 and λ_3, the slopes of the three best straight lines through the points (Table I). If the values of these parameters changed with concentration there would be considerable question concerning the desirability of the approximation, since the simplest explanation of concentration-dependent curvature near the end-point is a partial dissociation of the complex being formed, and we might better conclude, as did SHACK, that we were dealing with a single end-point at about 0.7 equiv. But the fact that the parameters remain constant over at least a 10-fold concentration range suggests that this is not the proper interpretation (see DISCUSSION) and the description in terms of these parameters, particularly E_2, seems a meaningful one.

Polyadenylic acid in distilled water adjusted to about pH 7.6 has the same titration curve as poly U (Fig. 3), with a second end-point at 1.0 equivalent of Mn^{++} per mole of phosphate. However, if the pH is lowered to 5.8 before starting the titration, most of the combining groups on the polymer appear to be rendered inactive

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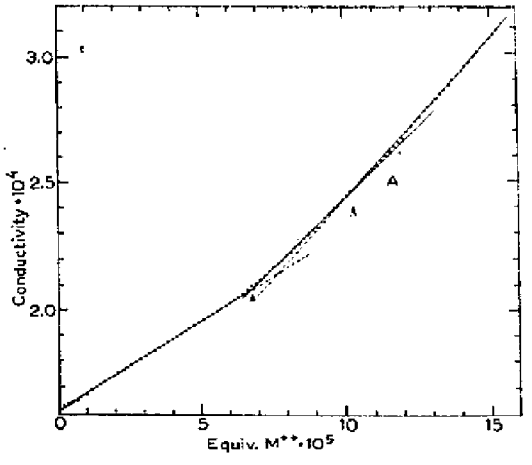

Fig. 3. Titration of $1.06 \cdot 10^{-4}$ *M* polyadenylic acid with $2 \cdot 10^{-3}$ *M* $MnCl_2$. The polymer solution was adjusted to pH 7.6 before the titration.

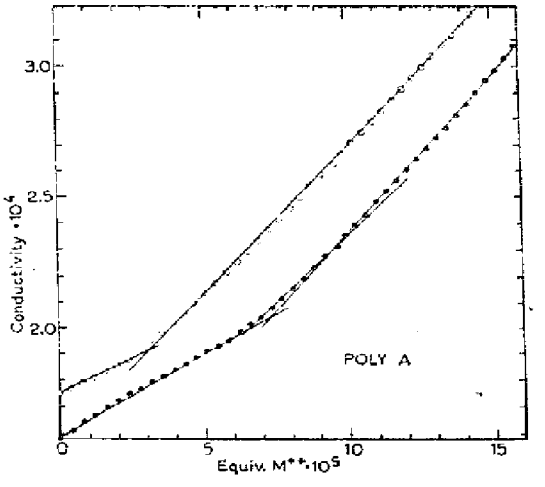

Fig. 4. ●, Titration of $1.00 \cdot 10^{-4}$ *M* polyadenylic acid with 10^{-3} *M* $MgCl_2$. Initial pH about 8.0. ○, Titration of $1.03 \cdot 10^{-4}$ *M* polyadenylic acid with 10^{-3} *M* $MnCl_2$. Initial pH about 5.8.

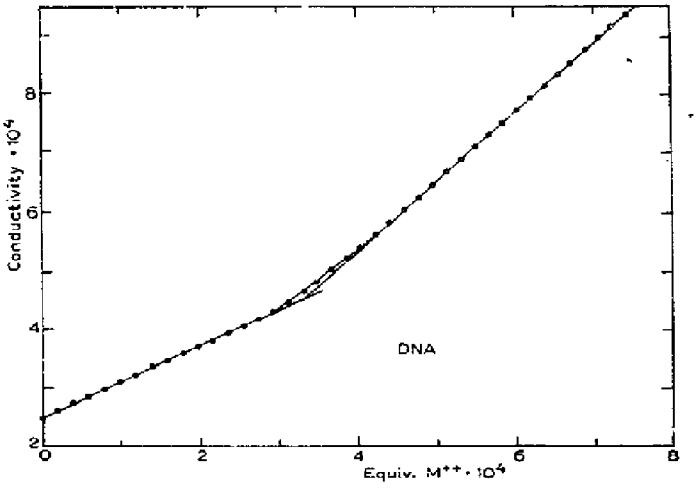

Fig. 5. Calf thymus DNA in distilled water, titrated with $MnCl_2$. O.D. (260) = 4.0.

(Fig. 4). This is consistent with the finding of FRESCO AND DOTY[7] regarding transition of poly A to a new form below pH 6.5 in distilled water, and with the suggestion of BEERS AND STEINER[8] that the phosphate groups in the new structure are involved in internal "salt linkages" with positively charged adenine groups in such a manner as to reduce the affinity of the phosphate groups for divalent cations.

Fig. 4 also shows that the shape of the titration curve is essentially the same for titration with Mg^{++} as it is with Mn^{++}.

In view of the preceding experiments it is not surprising that calf thymus DNA in distilled water (Fig. 5) shows the same behavior as poly A and poly U, under conditions of concentration similar to those employed by SHACK.

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DISCUSSION

The titration data indicate that it is necessary to revise previous conclusions about the binding of divalent cations to polynucleotides. The point at which the titration curve finally becomes parallel to that obtained for the addition of divalent cation to water is the point at which one equivalent of cation has been added for each mole of phosphate. There is, in other words, some binding of cation up to this point, and none beyond it. As we have mentioned in the previous section, it does not seem likely that the shape of the titration curve can be explained in terms of partial dissociation of the complex between the divalent cation and the polynucleotide. The invariance in the shape of the curves over a 10-fold range of polymer concentration is not consistent with this viewpoint. A calculation shows that easily measurable changes should occur with increasing polymer concentration if this model were correct. The second "end-point" in any case represents the point of saturation of binding sites on the polynucleotide. The simplest remaining interpretation is that this saturation value is not at 0.7 to 0.8 equiv. but that 1 equiv. of cation is bound strongly for each equiv. of negative charge.

The reason for the sharp increase in slope at 0.6 equiv. also remains in question. It is possible that this is the point at which sodium ion, about half of which is probably held tightly in the vicinity of untitrated polynucleotide[9, 11], begins to be replaced with higher efficiency by divalent cation, or the break may represent a major shape change in the polynucleotide with attendant change in the conductivity increment of neutralization of the polymer. It is known that the viscosity of solutions of charged polymer is strongly depressed by small amounts of divalent ion. In any event, there is interaction between divalent cation and polynucleotide until the equivalence point is reached.

It is interesting to note that the data of SHACK dealing with the effect of divalent cations on the optical density of DNA in distilled water are consistent with the titration curves reported here. Though SHACK[6] chose the end-point of the spectrophotometric titration by extrapolation and obtained the value 0.80 equiv., it appears equally valid to choose the point at which further optical density changes cease. SHACK's studies show that this occurs at about 1.1 equiv. The discontinuity of his spectrophotometric titration curve at 0.6 equiv. is also consistent with our present observations.

Whatever the interpretation given to the shape of the titration curves, it seems clear that these data do not support the suggestion[10] that purines in a polynucleotide chain are involved in the binding of divalent cations, or that cations are bound more strongly at sites involving a purine than they are at sites involving a pyrimidine. The identical behavior of polyadenylic and polyuridylic acids at neutral pH indicates that the binding properties of the polypurine and the polypyrimidine are the same, at least within the limitations of the present conditions and techniques. (The titration method cannot detect chelation, for example, if the affinity of divalent cation for the phosphate groups of the backbone is in itself very great, so that the chelation does not increase the amount of bound ion measurably.) The hypothesis that the purines are specially involved in the binding of divalent cations has arisen from the previously anomalous end-point of the DNA titration. ZUBAY AND DOTY[10] point out that DNA in distilled water has a large part of its hydrogen bonded system disrupted, so that bases would be available for interaction with metal ions. They explain the observed

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TABLE I

Titration	*"Endpoints"* $\left(\frac{\text{Equiv. Titrant}}{\text{Equiv. Phosphate}}\right)$		*Slopes (Mhos cm^{-1} Equiv.$^{-1}$ l) Numbers in parentheses uncorrected for dilution*		
	E_1	E_2	λ_1	λ_2	λ_3
Poly U ($1.00 \cdot 10^{-4}$ *M*) + Mn^{++} *	0.585	1.05	0.071 (0.069)	0.118 (0.110)	0.134 (0.132)
Poly U ($1.10 \cdot 10^{-4}$ *M*) + Mn^{++}	0.595	1.03	0.067 (0.065)	0.108 (0.106)	0.141 (0.139)
Poly U ($1.07 \cdot 10^{-3}$ *M*) + Mn^{++}	0.585	1.04	0.073 (0.071)	0.112 (0.108)	0.131 (0.127)
Poly A ($1.06 \cdot 10^{-4}$ *M*) + Mn^{++} *	0.645	1.01	0.077 (0.075)	0.118 (0.116)	0.136 (0.134)
Poly (A + U) + Polylysine	1.13		0.138 (0.018)	0.080 (−0.040)	
H_2O + Mn^{++}			0.140		
$\cdot 10^{-4}$ *M* NaCl + Polylysine			0.080 (−0.045)		

* Least squares result.

end-point at 0.7 equiv. in terms of a relatively weak contribution to binding by those sites involving pyrimidines. Our present experiments indicate that the end-point of the titration of "denatured" DNA occurs at 1 equiv., so that the anomaly is eliminated. Finally, it should be noted that the evidence from titration of poly A and poly U does not appear to support the suggestion of ZUBAY[12] that polyadenylic acid can form complexes which involve chelation of divalent cations by adenine. It is of course not possible to determine the correctness of ZUBAY's proposed structure for the three-stranded poly (A + 2U) molecule on this basis, but it does not appear that titration data provide any evidence in favor of a structural requirement involving divalent cation chelation by adenine.

Naturally, these results apply only to the case of polynucleotides or DNA in distilled water. There is good reason to believe[13] that at physiological concentrations[9] of sodium chloride, for example, a smaller amount of divalent cation is held by denatured DNA than is held in distilled water. Undenatured DNA, as ZUBAY AND DOTY have shown[10] has a titration curve entirely different from that of denatured DNA. The relative rigidity of the undenatured material should have a marked effect upon its binding properties[10,14], so that these properties may differ from those shared by the single-stranded polynucleotides and denatured DNA.

II. POLYLYSINE

RESULTS

If a small excess of Mn^{++} is added to a mixture of poly A and poly U in 1:1 molar ratio, the complete formation of the poly (A + U) two-stranded structure occurs[2]. If we now perform a continuous variation experiment with a solution of polylysine* which has the same concentration (residues of lysine = moles of polymer phosphate) as the poly (A + U), keeping the total concentration of (phosphate + lysine) constant and varying the ratio of nucleotide to amino acid, the series of absorption curves shown in Fig. 5 is obtained. Polylysine at this concentration has negligible absorption in the region 230–300 mμ, and at most ratios of polynucleotide to polylysine the absorption curve shows a linear dilution effect upon the poly (A + U) spectrum.

* Polylysine hydrobromide.

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However, in the range of ratios around 1:1, there is a general elevation of the entire curve on the optical density scale. This may be attributed to the formation of aggregates with great scattering power; the aggregates will precipitate over a period of 24 h. The range in which such aggregation occurs is limited under these conditions to several percent on either side of the 50% point.

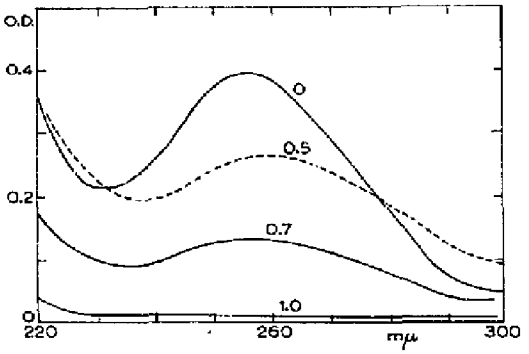

Fig. 5. Absorption spectra of mixtures of poly (A + U) and polylysine. The numbers adjacent to the curves show the fraction of the total concentration (base + amino acid) which is polylysine.

These results show that there is interaction between the polynucleotide and the polypeptide, but one question which is not answered is that of the intimacy of the lysine–phosphate linkage. Are the positive charges located fairly close to the negative charges along the entire lengths of the chains? In Fig. 6 are given the results of an experiment in which a solution of poly (A + U) which had been brought together with a small excess of Mn^{++} was titrated with a polylysine solution. The first part of the curve is parallel to the line obtained by adding Mn^{++} to water, while the second part is parallel to the line obtained by adding polylysine to a dilute NaCl solution. The results show that for every mole of lysine residue added, an equiv. of metal ion is displaced. The end-point, which occurs at 1 equiv. of polylysine within the experimental error, is consistent with the formation of a stoichiometric poly (A + U)–polylysine complex*.

It should be noted that poly A or poly U alone, to which excess Mn^{++} has been added, show similar titration behavior but somewhat different spectral behavior in the presence of polylysine. Details of these studies will be discussed in another paper.

DISCUSSION

Though the result of the spectrophotometric study is not particularly surprising, since it reflects the relative insolubility of a molecule at the isoelectric point, it at least suggests that non-matted, only loosely cross-linked structures are being formed. There

* It is possible to interpret the slightly high value of the end-point polylysine concentration in terms of one of the irreversible models for polymer interaction proposed in ref. [5]. However, the errors in determining the concentrations of the several compounds involved in the titration are sufficient to account for the results. The small discrepancies in slope must be viewed in the light of the rather large dilution correction which has been applied in this case (see METHODS and Table I).

The use of the hydrobromide of polylysine rather than the hydrochloride should affect the conductivity increment only slightly.

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is good reason to expect such behavior from polylysine–polynucleotide mixtures in the work of CHARGAFF and his collaborators[15] and others[16]. The stoichiometric solubility dependence is very much like that observed in another manner by SPITNIK, LIPSHITZ AND CHARGAFF for DNA-polylysine[15].

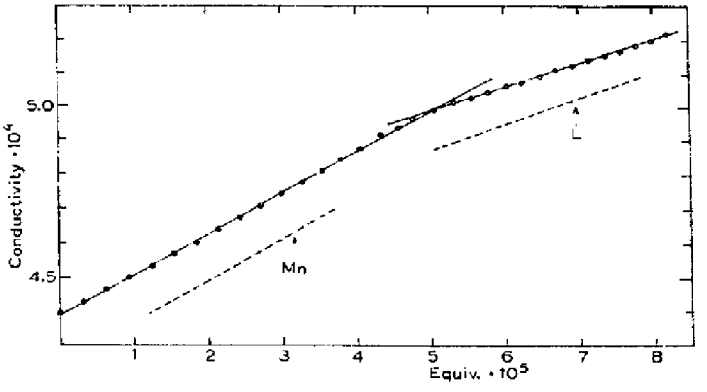

Fig. 6. Titration of $4.8 \cdot 10^{-5}$ *M* poly (A + U), [total base concentration] brought together with $MnCl_2$, with $4.0 \cdot 10^{-4}$ *M* polylysine. M is the curve for addition of $MnCl_2$ to water. L is the curve for addition of polylysine to $4 \cdot 10^{-4}$ *M* NaCl solution. All curves are corrected for dilution (see METHODS).

The titration of poly (A + U) with polylysine demonstrates that a polycation is capable of completely replacing strongly bound divalent metal ions under the conditions of the experiment. The fact that the curve remains parallel to the line representing addition of Mn^{++} to water until the end-point is reached is confirmation that 1 equiv. of manganese ion is bound by the polynucleotides for each mole of phosphate. Recent spectrophotometric studies by the authors also indicate that one equivalent of divalent ion must be bound to complete the formation of poly (A + U), and that excess divalent ion is required to drive this reaction to completion. Two-stranded rigid poly (A + U) thus does appear to have a lower affinity for divalent ions than do the single-stranded polynucleotides, though this difference is not apparent in the titration experiments described here.

The fact that there is only one break in the curve, and that this occurs at the 1-equiv. point, is consistent with the idea of a stoichiometric polylysine–polynucleotide complex. On the other hand, it is possible that there is binding of polylysine to polynucleotide beyond the equivalence point, and that this is undetected in the titration because the conductivity increment is the same whether the additional polylysine binds to the electrically neutral polynucleotide–polypeptide complex or remains free in solution. This excess binding can only occur if sites are available because of "overlapping" attachment of polylysine to the poly (A + U) (see ref. [5]). It does not seem likely that this overlapping occurs to a great extent, since in that case the displacement of manganese ion in the first part of the curve would presumably be much smaller. However, as much as 10% of overlapping and excess binding could occur undetected, and as mentioned in the footnote of the preceding section, might account for the slightly high end-point. It might also account for the narrow range of ratios which produce aggregation in the spectrophotometric studies, though this may be a matter of a relatively insensitive technique.

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The charge-for-charge replacement suggests that each of the positively charged lysine residues is capable of coming close to a negatively charged phosphate. This is particularly interesting because the structural studies of WILKINS[17] indicate that the lysine-rich fraction of histone can be combined with DNA to give a compound which has an X-ray diffraction pattern like that of nucleoprotamine, so that it is likely that the lysine-rich histone lies in the small groove of DNA in a regular manner. It seems reasonable that in the case of the two-stranded poly (A +U) molecule a similar structure should form. The titration results are consistent with this structure, but the fact that poly A and poly U separately exhibit the same behavior suggests that the method establishes only a necessary condition. Further investigation, including structural studies, will be necessary to determine whether we are dealing with a synthetic analog of natural nucleoprotein.

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